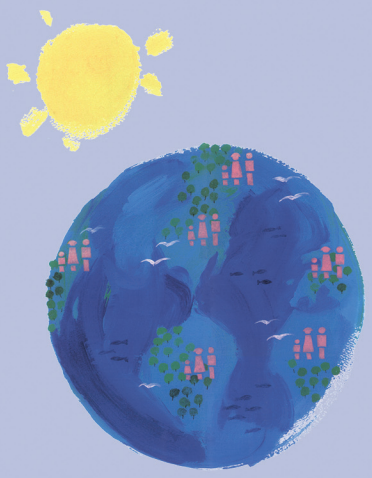
NIH Publication No. 08-6529



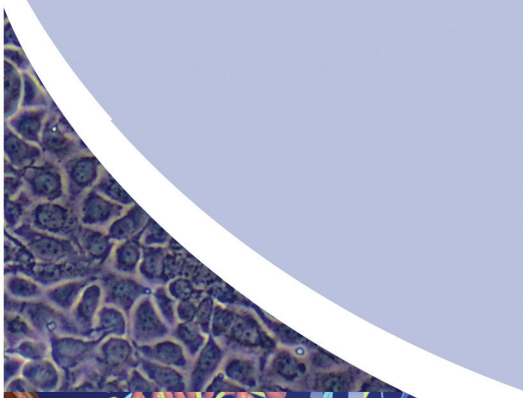
Biennial Progress Report

Interagency Coordinating Committee on the Validation of Alternative Methods

2006-2007

National Toxicology Program
Interagency Center for the Evaluation
of Alternative Toxicological Methods

U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences
U.S. Public Health Service



About the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

In 1997, the National Institute of Environmental Health Sciences (NIEHS), one of the National Institutes of Health (NIH), established ICCVAM to:

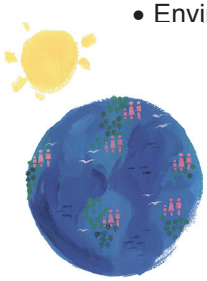
- Coordinate interagency technical reviews of new and revised toxicological test methods, including alternative test methods that reduce, refine, or replace the use of animals
- Coordinate cross-agency issues relating to validation, acceptance, and national and international harmonization of new, modified, and alternative toxicological test methods

On December 19, 2000, the ICCVAM Authorization Act (Public Law 106-545, 42 U.S.C. 285*l*-3) established ICCVAM as a permanent interagency committee of NIEHS under NICEATM.

ICCVAM consists of representatives from 15 U.S. Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability. ICCVAM promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety or hazards of chemicals and products and that reduce, refine (decrease or eliminate pain and distress), and/or replace animal use. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. More information about ICCVAM and NICEATM can be found on the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov) or obtained by contacting NICEATM (telephone: [919] 541-2384, e-mail: niceatm@niehs.nih.gov).

The following Federal regulatory and research agencies are ICCVAM members:

- Consumer Product Safety Commission
- Department of Agriculture
- Department of Defense
- Department of Energy
- Department of Health and Human Services
  - Centers for Disease Control and Prevention
    - Agency for Toxic Substances and Disease Registry
    - National Institute of Occupational Safety and Health
  - Food and Drug Administration
  - National Institutes of Health
    - Office of the Director
    - National Cancer Institute
    - National Institute of Environmental Health Sciences
    - National Library of Medicine
- Department of the Interior
- Department of Labor
  - Occupational Safety and Health Administration
- Department of Transportation
- Environmental Protection Agency



The NICEATM-ICCVAM graphic symbolizes the important role of new and alternative toxicological methods in protecting and advancing the health of people, animals, and our environment.

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National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences
U.S. Public Health Service

This document is available electronically at:
http://iccvam.niehs.nih.gov/about/ICCVAMrpts.htm

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List of Figures and Tables

List of Acronyms and Abbreviations

3T3	BALB/c 3T3 mouse fibroblast cells
AR	Androgen receptor
BCOP	Bovine corneal opacity and permeability
BRD	Background review document
CCi	Certi-Chem Inc.
DNT	Developmental neurotoxicity
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
EPAA	European Partnership for Alternative Approaches to Animal Testing
ER	Estrogen receptor
ESAC	ECVAM Scientific Advisory Committee
FDA	U.S. Food and Drug Administration
FR	*Federal Register*
GHS	U.N. Globally Harmonized System of Classification and Labelling of Chemicals
HET-CAM	Hen's egg test-chorioallantoic membrane
ICCR	International Cooperation on Cosmetics Regulation
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
IL-1	Interleukin-1
IL-6	Interleukin-6
IRE	Isolated rabbit eye
ISO	International Organization for Standardization
JaCVAM	Japanese Center for the Validation of Alternative Methods
JSAAE	Japanese Society for Alternatives to Animal Experiments
LD_{50}	Lethal Dose 50
LLNA	Murine local lymph node assay
MCF-7	Breast cancer cell line
NHK	Normal human keratinocyte
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Ocular Toxicity Working Group
PBMC	Peripheral blood mononuclear cell
RPT	Rabbit pyrogen test
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SOT	Society of Toxicology
SPSF	Standard Project Submission Form
TA	Transcriptional activation
UDS	Unscheduled DNA synthesis
U.S.	United States
U.N.	United Nations
VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products
WC6	6th World Congress on Alternatives and Animal Use in the Life Sciences
WHO/IPCS	World Health Organization/International Programme on Chemical Safety
XDS	Xenobiotic Detection Systems, Inc.

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

The table below lists individuals who served as designated agency representatives during the 2006-2007 reporting period. Unless otherwise noted, individuals were serving as of January 2008.

★ Principal agency representative ◊ Alternate principal agency representative

Agency for Toxic Substances and Disease Registry
★Moiz Mumtaz, Ph.D.

Consumer Product Safety Commission
★Marilyn L. Wind, Ph.D. (Chair)
Patricia Bittner, M.S. (through 12/07)
◊ Kristina Hatlelid, Ph.D.
Kailash C. Gupta, D.V.M., Ph.D. (through 8/06)

Department of Agriculture
★Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
◊ Elizabeth Goldentyer, D.V.M.

Department of Defense
★Robert E. Foster, Ph.D.
◊ Patty Decot
Peter J. Schultheiss, D.V.M., D.A.C.L.A.M.
Harry Salem, Ph.D.
John M. Frazier, Ph.D. (through 8/06)

Department of Energy
★Michael Kuperberg, Ph.D.
◊ Marvin Stodolsky, Ph.D.

Department of the Interior
★Barnett A. Rattner, Ph.D.
◊ Sarah Gerould, Ph.D.

Department of Transportation
★George Cushmac, Ph.D.
◊ Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Science Coordination and Policy
★Karen Hamernik, Ph.D.
Office of Research and Development
◊ Julian Preston, Ph.D.
Suzanne McMaster, Ph.D.
Harold Zenick, Ph.D. (through 2/06)
OECD Test Guidelines Program
Jerry Smrchek, Ph.D.
Maurice Zeeman, Ph.D. (through 2/06)
Office of Pesticides Programs
Amy Rispin, Ph.D.
Deborah McCall

Food and Drug Administration
Leonard M. Schechtman, Ph.D. (Chair through 1/07)
Office of Science and Health Coordination
★Suzanne Fitzpatrick, Ph.D., D.A.B.T.
Center for Drug Evaluation and Research
◊ Abigail C. Jacobs, Ph.D.
Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D.
Raju Kammula, D.V.M., Ph.D., D.A.B.T. (through 8/06)
Center for Biologics Evaluation and Research
Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.
Center for Food Safety and Nutrition
David G. Hattan, Ph.D.
Robert L. Bronaugh, Ph.D.
Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.
National Center for Toxicological Research
William T. Allaben, Ph.D.
Paul Howard, Ph.D.
Office of Regulatory Affairs
Lawrence A. D'Hoostelaere, Ph.D.

National Cancer Institute
★Alan Poland, M.D.
◊ T. Kevin Howcroft, Ph.D.

National Institute of Environmental Health Sciences
★William S. Stokes, D.V.M., D.A.C.L.A.M.
◊ Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., D.A.B.T.
Jerrold J. Heindel, Ph.D.
John R. Bucher, Ph.D., D.A.B.T. (through 6/07)

National Institute for Occupational Safety and Health
★Paul Nicolaysen, V.M.D.
◊ K. Murali Rao, M.D., Ph.D.

National Institutes of Health
★Margaret D. Snyder, Ph.D.

National Library of Medicine
◊ Jeanne Goshorn, M.S.
Vera Hudson, M.S. (through 6/07)

Occupational Safety and Health Administration
★Surender Ahir, Ph.D.

National Toxicology Program
Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences

Rear Admiral William Stokes, D.V.M., D.A.C.L.A.M.
Director; Project Officer

Raymond Tice, Ph.D.
Deputy Director

Deborah McCarley
Special Assistant; Assistant Project Officer

NICEATM Support Contract Staff (Integrated Laboratory Systems, Inc.)

David Allen, Ph.D., Principal Investigator

Thomas Burns, M.S.

Patricia Ceger, M.S.

Frank Deal, M.S.

Elizabeth Lipscomb, Ph.D.

Linda Litchfield

Michael Paris

Eleni Salicru, Ph.D.

Catherine Sprankle

Judy Strickland, Ph.D., D.A.B.T.

James Truax, M.A.

Douglas Winters, M.S.

Preface

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was formally authorized and designated as a permanent interagency committee by the ICCVAM Authorization Act of 2000 (Public Law 106-545, 42 U.S.C. 285*l*-3). The Act required ICCVAM to prepare an annual progress report on its first anniversary in December 2001, and every other year thereafter. This document is the third ICCVAM biennial progress report and covers the period from January 2006 to December 2007.

The ICCVAM Authorization Act directs ICCVAM to coordinate interagency technical evaluations of new, revised, and alternative testing methods, develop test recommendations based on those evaluations, and forward the test method recommendations to U.S. Federal agencies for their consideration. ICCVAM also coordinates interagency issues and provides guidance on alternative toxicological test method development, validation, regulatory acceptance, and national and international harmonization. ICCVAM's overall goal is to achieve the regulatory acceptance of scientifically valid toxicological tests that protect human and animal health and the environment, while reducing, refining (decreasing pain and distress), and replacing animal use where scientifically feasible.

This report summarizes the progress and activities of ICCVAM, working in conjunction with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), during the 2006-2007 reporting period. ICCVAM evaluated and developed test method recommendations on non-animal (*in vitro*) test methods for: (1) assessing pharmaceuticals and other products for their potential to produce fever and inflammation (pyrogenicity), (2) estimating starting doses for testing to determine whether substances can result in poisoning (acute oral toxicity), and (3) identifying substances that may cause permanent or serious eye damage (ocular corrosives and irritants). Consideration and use of these *in vitro* methods where appropriate will reduce the number of animals required for testing for these purposes. ICCVAM also began an international validation study on an *in vitro* test method to detect substances with the potential to disrupt normal hormonal function (endocrine disruptors), and sponsored a workshop that identified current options and research priorities to reduce, refine, and replace the use of animals in botulinum toxin testing.

In 2007, ICCVAM and NICEATM, in conjunction with the 15 ICCVAM member Federal agencies, developed a five-year plan that identifies priorities and activities to advance alternative test methods of high scientific quality to protect and advance the health of people, animals and the environment. ICCVAM and NICEATM also launched a new version of the NICEATM-ICCVAM website. Interaction and cooperation with our international counterparts, the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM), continue to be a priority for ICCVAM. This report describes the increased collaborations and cooperative activities between ICCVAM and our international counterparts, and other outreach activities over the last two years.

On behalf of ICCVAM, we want to take this opportunity to gratefully acknowledge several ICCVAM representatives who departed the committee during the past two years. First, we want to recognize the outstanding leadership and contributions of Dr. Leonard Schechtman, who served as the chairman of ICCVAM from 2002 until his retirement from the Food and Drug Administration in December 2006. He was a member of the original ad hoc ICCVAM established in 1994, and served as the principal FDA representative on ICCVAM from 1999-2006. Dr. Schechtman worked tirelessly and enthusiastically on behalf of ICCVAM during this time. He was instrumental in building stronger collaborations and cooperation with ECVAM and JaCVAM and achieving international harmonization of guidance on validation and the use of alternative methods. Dr. Schechtman exhibited an unwavering commitment to high quality science and animal welfare,

and the principle that new alternative methods must provide equivalent or better protection of people, animals, and the environment. He exhibited the highest level of professionalism and unselfish service during his 13 years on ICCVAM.

We also thank Dr. Kailash Gupta, an agency representative for the Consumer Product Safety Commission, for his dedicated service on the original ad hoc ICCVAM beginning in 1994 until his retirement in December 2006. Dr. Gupta, a veterinarian and toxicologist, was a tireless advocate of new alternative methods. He was one of the charter members and chair of the Interagency Regulatory Alternatives Group, an interagency group organized in the 1980s to advocate for scientifically valid alternatives for regulatory testing. Dr. Gupta was a devoted member of several ICCVAM Working Groups, and contributed significantly to the technical evaluation of several alternative methods that were subsequently adopted by regulatory agencies.

We also acknowledge the dedicated service of Ms. Vera Hudson, who served as the ICCVAM Principal Agency Representative for the National Library of Medicine (NLM) since the inception of the ad hoc ICCVAM in 1994 until her death in June 2007. Ms. Hudson was an ardent supporter of humane animal care and use and the development and validation of alternatives for research and testing. She served a key role in development of the NLM's Bibliography on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing to aid scientists in identifying available alternatives for their studies. We are thankful for the opportunity to have known and worked with such a wonderful person, and will dearly miss her.

Finally, we want to acknowledge the many other individuals whose contributions and enthusiastic support have been essential to ICCVAM's success. These include the ICCVAM agency representatives and working group members, NICEATM and its contract support staff, the Scientific Advisory Committee on Alternative Toxicological Methods, numerous international experts and Peer Review Panel members, and many other interested stakeholders. With continued national and international cooperation and collaboration, we are confident that we will make even greater progress in the next few years to develop, validate, and gain regulatory acceptance of safety evaluation methods that will reduce, refine, and replace animal use while ensuring the continued or improved protection of people, animals, and the environment.

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM

Rear Admiral William S. Stokes, D.V.M., D.A.C.L.A.M.
Assistant Surgeon General, U.S. Public Heath Service
Director, NICEATM
Executive Director, ICCVAM

Highlights

ICCVAM 2006-2007 Biennial Report

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established to facilitate the review and adoption of scientifically valid safety testing methods that can be used to safeguard the health of people, animals, and the environment while refining, reducing, and replacing animal use where scientifically feasible. Since ICCVAM's establishment in 1997, through December 2007, Federal regulatory agencies have approved or endorsed 16 alternative methods. ICCVAM and its member agency scientists have contributed to the review of all of these alternative methods, including formal review and technical evaluations on eight of the test methods. The alternative methods accepted by Federal agencies include 10 non-animal methods and six methods that use fewer animals and reduce the potential for discomfort. Most of the *in vitro* methods do not require the use of animals when positive results are obtained.

This report describes test method evaluations and other activities conducted by ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Methods (NICEATM) during the 2006-2007 reporting period. Selected highlights of the report are presented below. More detailed information is provided in the corresponding sections of the report.

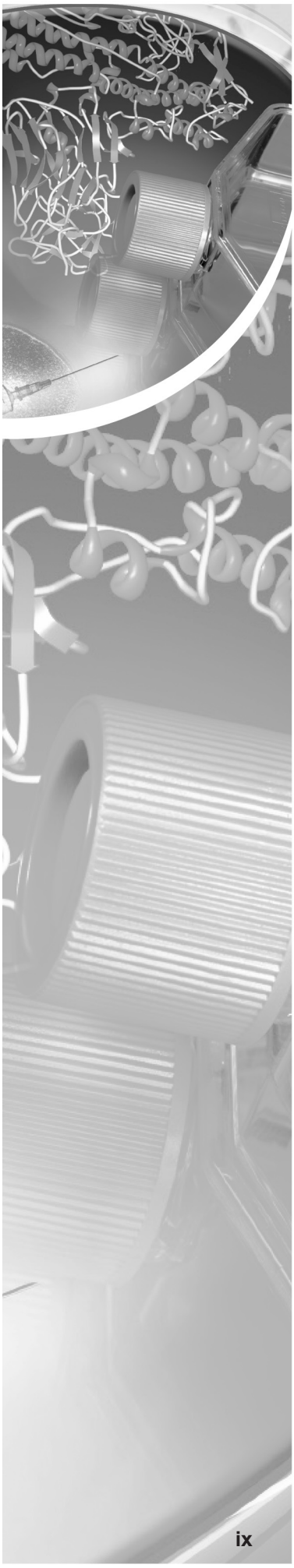
ICCVAM Test Method Evaluations and Related Activities

Ocular Safety Testing

- ICCVAM completed technical evaluations of four *in vitro* ocular safety testing methods and transmitted recommendations to Federal agencies in October 2007. ICCVAM recommended two methods for use as screening tests to identify substances that can cause irreversible and severe eye damage, such as blindness. ICCVAM further recommended that these methods should always be considered before conducting animal testing, and used when determined appropriate. Use of these tests where appropriate will avoid the need to test positive substances in animals, thereby reducing and refining animal use for ocular safety testing.
- NICEATM analyzed data showing that pretreatment with topical anesthetics can minimize or avoid discomfort during ocular safety testing without significantly altering ocular hazard classification. The results of this study are expected to support the routine use of topical anesthetics for ocular safety testing when animals must be used. NICEATM presented this data at national and international meetings.

In Vitro *Endocrine Disruptor Screening Methods*

- ICCVAM published an updated list of 78 reference substances for the validation of *in vitro* endocrine disruptor screening methods, such as assays to evaluate estrogen receptor (ER) and androgen receptor (AR) transcriptional activation (TA) and receptor binding. All recommended substances are commercially available at reasonable cost.
- NICEATM completed standardization studies for an ER TA protocol and began an international validation study using this protocol. This is the first collaborative international validation study conducted by NICEATM



with both the European Centre for the Validation of Alternative Methods (ECVAM) and the recently established Japanese Center for the Validation of Alternative Methods (JaCVAM). Laboratories sponsored by NICEATM-ICCVAM, JaCVAM, and ECVAM are taking part in the study. During the first phase of the study, the participating laboratories demonstrated proficiency with the assay protocols, and initial intra-laboratory repeatability and intra- and inter-laboratory reproducibility.

- NICEATM conducted a preliminary evaluation of a second proposed assay for estrogenic activity in October 2006. ICCVAM recommended a high priority for additional validation studies with this assay, which measures a cell proliferation response to chemicals. Adequately validated *in vitro* endocrine disruptor screening methods may reduce the numbers of animals needed to identify substances with the potential to disrupt normal hormonal function.

In Vitro *Pyrogenicity Test Methods*

- ICCVAM completed evaluation of five *in vitro* test methods using human blood cells and a human cell line proposed for assessing pyrogenicity (fever-inducing) potential of injectable pharmaceuticals and other products. The evaluation included a 2007 review by an independent scientific Peer Review Panel* of a comprehensive draft background review document (BRD) and draft ICCVAM test method recommendations. ICCVAM developed final recommendations on these test methods, which will be published and transmitted to Federal agencies in 2008.

In Vitro *Cytotoxicity Test Methods for Estimating Acute Oral Systemic Toxicity (Poisoning)*

- ICCVAM completed evaluation of two *in vitro* test methods for estimating starting doses for acute oral systemic toxicity tests. An independent scientific Peer Review Panel evaluated the draft BRD and draft ICCVAM test method recommendations in May 2006. ICCVAM developed final recommendations of the use of these methods, which will be transmitted to Federal agencies in early 2008. ICCVAM recommends that the test methods should be considered before using animals for acute toxicity testing, and that the methods should be used where determined appropriate in a weight-of-evidence approach to estimate starting doses for acute oral toxicity protocols. For some types of substances, this approach will reduce the numbers of animals needed for testing by up to 50%. For highly toxic substances, this approach may also reduce the number of animals that die or need to be humanely killed.
- ICCVAM and NICEATM completed planning for a scientific workshop on acute chemical safety testing, to be co-sponsored with ECVAM and JaCVAM and held in February 2008. The workshop objectives are to:
  - Identify and standardize procedures for collecting mechanistic information from acute oral toxicity studies necessary to support the development of predictive *in vitro* test methods that can further reduce animal use and eventually replace animals for acute toxicity testing
  - Identify more humane predictive endpoints that may be used to terminate studies earlier in order to further reduce pain and distress

* “Peer Review Panel” throughout this text refers to an ICCVAM-sponsored independent scientific peer review panel.

Assessing the Validation Status of New Modifications and Applications of the Murine Local Lymph Node Assay (LLNA), a Test Method for Identifying Skin Sensitization Potential of Chemicals

- ICCVAM completed draft LLNA performance standards, draft BRDs and ICCVAM draft test method recommendations for six new versions and applications of the LLNA. These will be released in early 2008 for a Peer Review Panel meeting scheduled for March 2008. These evaluations are based on an ICCVAM nomination from the U.S. Consumer Product Safety Commission requesting assessment of the validation status of new versions and applications of the LLNA for determining whether substances may cause allergic contact dermatitis. These include three modified LLNA protocols that do not require the use of radioactive markers, and a limit dose procedure that reduces the number of mice required by 40%.

Alternative Methods for Assessing Potency of Botulinum Toxin

- In November 2006, ICCVAM and NICEATM held a scientific workshop, co-sponsored by ECVAM, to review the state-of-the-science and current knowledge of alternatives that may reduce, replace, and refine (reduce pain and distress) the use of mice for botulinum toxin testing. This workshop was organized in response to a nomination by the Humane Society of the United States, and identified priorities for research, development, and validation efforts needed to advance the use of alternative methods for this purpose.

ICCVAM Outreach and Cooperative Activities

Collaborations with ECVAM and JaCVAM

- ICCVAM and NICEATM continued to increase collaborations with ECVAM and JaCVAM to evaluate alternative test methods and to conduct validation studies. The aims of this extensive cooperation are to (1) make the best use of resources and scientific expertise, (2) maximize efficiency of evaluation and validation efforts, (3) minimize duplication of effort, and (4) ensure an early exchange of information concerning test method validation in order to promote international recognition, acceptance, and implementation of scientifically valid test methods. This cooperation promotes the international adoption of validated alternative methods by providing standardized and adequately validated test method protocols that can be readily adopted as international test guidelines by organizations such as the Organisation for Economic Co-operation and Development (OECD).
- NICEATM, ECVAM, and JaCVAM jointly designed and began a multiphased international validation study to evaluate an *in vitro* test method for assessing endocrine disruptor activity. This is the first joint validation study sponsored by all three validation organizations and represents an important milestone in international cooperation in validating alternative methods.
- ICCVAM and NICEATM are also collaborating with ECVAM and JaCVAM on a JaCVAM-sponsored effort to evaluate *in vitro* and *in vivo* comet assays to assess the potential of substances to cause genetic damage.
- Other cooperative activities include ECVAM co-sponsorship of the November 2006 workshop on alternative methods to the mouse LD_{50} assay for botulinum toxin testing, ECVAM and JaCVAM co-sponsorship of the upcoming workshop on acute chemical safety testing, and liaison attendance at each other's advisory committee meetings.

ICCVAM Participation in National and International Workshops, Conferences, and Meetings

- ICCVAM representatives presented or participated in national and international meetings on developmental neurotoxicity testing, genotoxicity testing, and skin sensitization.
- Representatives of ICCVAM and NICEATM made presentations at the 2006 meeting of the Japanese Society for Alternatives to Animal Experiments. ICCVAM and NICEATM were also active participants in the 6th World Congress on Alternatives and Animal Use in the Life Sciences, contributing to 12 platform and 11 poster presentations. ICCVAM and NICEATM also participated in both the 2006 and 2007 annual meetings of the Society of Toxicology, contributing to seven poster presentations in 2006 and to one platform and 12 poster presentations in 2007.

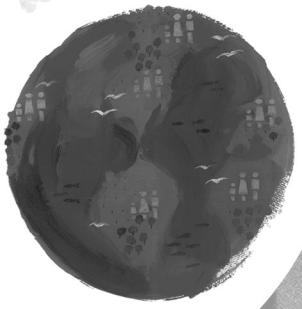
NICEATM-ICCVAM Five-Year Plan

- ICCVAM and NICEATM prepared a five-year plan to promote research, development, validation, and translation of alternative methods. The plan addresses ICCVAM's vision to play a leading role in fostering and promoting the development, validation, and regulatory acceptance of scientifically sound alternative test methods. Implementing this plan involves four key challenges:
  - Identifying priorities and conducting and facilitating alternative test method activities
  - Incorporating new science and technology
  - Fostering regulatory acceptance and appropriate use of alternative methods
  - Developing partnerships and strengthening interactions with ICCVAM stakeholders

  The draft NICEATM-ICCVAM Five-Year Plan was made available in May 2007, and the final plan will be made publicly available in February 2008 at a Ten-Year Anniversary Symposium.

NICEATM-ICCVAM Communications

- NICEATM staff launched a redesigned version of the NICEATM-ICCVAM website in March 2007. The new website provides user-friendly access to the latest information on validation processes on validation criteria and processes, and the current review status of all test method nominations and submissions to ICCVAM.
- In March 2007, NICEATM published a brochure summarizing the purpose, duties, and accomplishments of ICCVAM and NICEATM. This will be distributed at national and international scientific conferences to increase awareness of alternative test methods.
- ICCVAM and NICEATM published 21 *Federal Register* notices and 13 documents and reports during 2006 and 2007. ICCVAM members and NICEATM staff also contributed to 55 scientific articles and poster and platform presentations, and helped or led development of international test guidelines for 10 test methods. A full list of these publications and documents can be found in **Chapter 6.**



Biennial Progress Report of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM): 2006-2007

This report begins with a brief overview of the history of ICCVAM, ICCVAM's mission and vision, and ICCVAM's evaluation process for test methods. This is followed by descriptions of the activities carried out since the last ICCVAM Biennial Report (2004-2005), including test method evaluations, national and international collaborations, meetings, workshops, and conferences.

Chapter 1

History and Organization of ICCVAM

History of ICCVAM

The Director of the National Institute of Environmental Health Sciences (NIEHS) established an ad hoc ICCVAM committee in September 1994 to develop a report to respond to requirements in the National Institutes of Health (NIH) Revitalization Act of 1993.[1] This Act required NIEHS to establish criteria for the validation and regulatory acceptance of alternative toxicological testing methods. The Act also required NIEHS to recommend a process to achieve the regulatory acceptance of scientifically valid alternative test methods. The ad hoc ICCVAM committee consisted of representatives from the 15 United States (U.S.) Federal agencies now represented on ICCVAM.

In 1997, the ad hoc ICCVAM committee published its final report, *Validation and Regulatory Acceptance of Toxicological Test Methods* (ICCVAM 1997). In the same year, NIEHS established a standing ICCVAM committee to implement a process by which new test methods of agency interest could be evaluated, and to coordinate interactions among agencies related to the development, validation, acceptance, and national and international harmonization of toxicological test methods.

[1]Public Law 103-43, 42 U.S.C. 285*l*-1 and 42 U.S.C. 283*e*, which can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/about_docs/pl103_43.pdf.

The ICCVAM Authorization Act of 2000

The ICCVAM Authorization Act of 2000,[2] signed into law in December 2000, established ICCVAM as a permanent interagency committee of NIEHS under the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The law was enacted

> "to establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness."

The Act defines the composition of ICCVAM as the heads, or their designees, of 15 U.S. Federal agencies (see **Appendix A**).

The Act states that the purposes of ICCVAM are to:

- Increase the efficiency and effectiveness of U.S. Federal agency test method review
- Eliminate unnecessary duplication of effort and share experience among U.S. Federal regulatory agencies
- Optimize use of scientific expertise outside the U.S. Federal government
- Ensure that new and revised test methods are validated to meet the needs of U.S. Federal agencies
- Reduce,[3] refine,[4] or replace[5] the use of animals in testing where feasible

ICCVAM's Duties

The ICCVAM Authorization Act directs ICCVAM to carry out the following duties:

- Coordinate the technical review and evaluation of new and revised test methods
- Submit ICCVAM test recommendations to appropriate U.S. Federal agencies
- Foster interagency and international harmonization of test protocols that encourage reducing, refining, and replacing animal test methods (an approach to developing test methods referred to as the "3R's")
- Assist with and provide guidance on validation criteria and processes
- Promote the acceptance of scientifically valid test methods
- Promote awareness of accepted test methods
- Consider petitions from the public for reviewing and evaluating new and revised test methods that have evidence of scientific validity
- Make ICCVAM final test recommendations available to the public
- Prepare reports on ICCVAM progress and accomplishments under the Act and make these available to the public

[2] Public Law 106-545, 42 U.S.C. 285*l*-3, which can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf.
[3] Reduction alternative: A new or modified test method that reduces the number of animals required.
[4] Refinement alternative: A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.
[5] Replacement alternative: A new or modified test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one.

NICEATM

NICEATM is a Center in the NTP, which is a component of NIEHS, with headquarters in Research Triangle Park, NC.

NICEATM was established in 1998 to:

- Administer ICCVAM
- Provide technical and scientific support and coordination for ICCVAM and ICCVAM working groups, Peer Review Panels, expert panels, workshops, validation efforts, and the scientific advisory committee
- Organize committee-related activities, such as peer reviews and workshops for test methods of interest to U.S. Federal agencies
- Provide a mechanism for communication among agencies as well as between agencies and test method developers

NICEATM provides a wide range of scientific and operational support for ICCVAM test method evaluations. Examples include:

- Evaluating new submissions and nominations of test methods for adherence to the *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods* (ICCVAM 2003a)
- Assessing the completeness of background review documents (BRDs) for test methods submitted for ICCVAM evaluation
- Determining whether and to what extent new, revised, and alternative test methods proposed for ICCVAM evaluation are applicable to regulatory safety testing
- Assembling information about current best practices for the humane care and use of animals in toxicological research and testing

ICCVAM and NICEATM also carry out activities applicable to provisions of the NIH Revitalization Act of 1993 (Public Law 103-43) that direct NIEHS to develop and validate improved testing methods for acute and chronic toxicity, including methods that will reduce or replace animal use. As resources allow, NICEATM conducts and coordinates international validation studies to evaluate potential new alternative test methods that may reduce, replace, or refine animal use for toxicity testing and that may provide improved safety assessments for people, animals, and the environment.

ICCVAM's Mission and Vision

ICCVAM developed mission and vision statements at a 2004 strategic planning meeting. The committee also developed a strategic direction for ICCVAM for the next three to five years, consistent with its purposes and duties in the ICCVAM Authorization Act of 2000.

Mission[6]

ICCVAM's mission is to promote development, validation, and regulatory acceptance of new and revised regulatory test methods that reduce, refine, or replace the use of animals in testing while maintaining and promoting scientific quality and protecting human health, animal health, and the environment.

Vision

ICCVAM will:

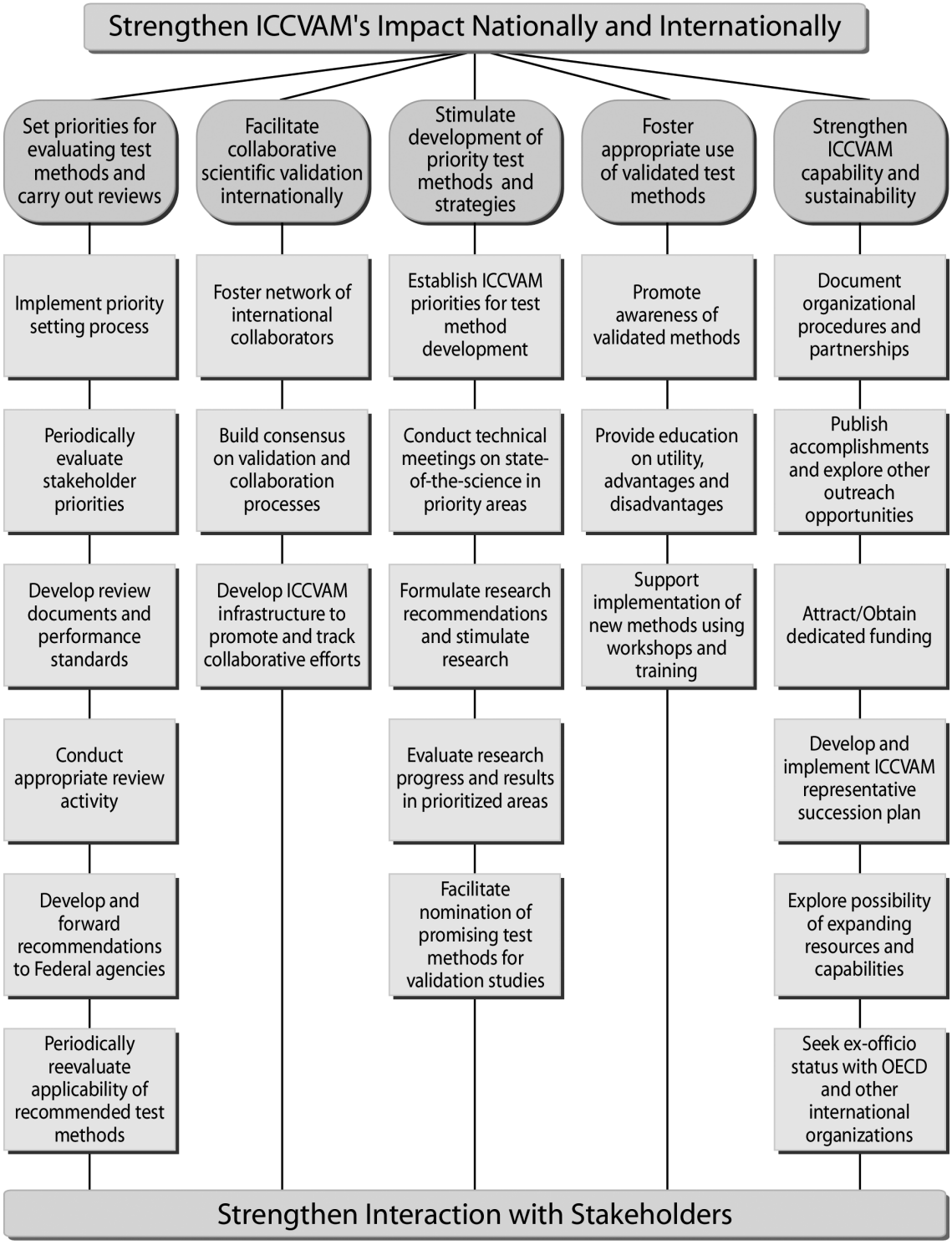
- Be recognized as a leading authority on test method development and validation both within the U.S. Federal government and internationally
- Play a leading role in:
  - Promoting high-quality science as the basis for national and international regulatory policy
  - Setting and harmonizing international standards for scientifically validating test methods
  - Promoting and facilitating the development of priority alternative test methods
  - Identifying key alternative test methods and strategies and facilitating their validation and acceptance
  - Fostering humane and ethical approaches to testing that replace, reduce, and refine the use of animals
  - Promoting awareness and adoption of scientifically validated test methods by regulatory agencies both nationally and internationally
- Develop the internal and collaborative capacity to:
  - Ensure the scientific quality and integrity of its work
  - Implement reliable processes and operating procedures that are credible, effective, and efficient
  - Build national and international partnerships with governmental and non-governmental groups, including academia, industry, advocacy groups, and other key stakeholders
  - Secure the human and financial resources needed to effectively carry out its mission

Figure 1 maps the six strategic priorities that ICCVAM considers necessary to strengthen its impact both nationally and internationally. Each strategic priority is accompanied by a brief rationale and list of the strategic objectives that should be pursued.

ICCVAM then developed a specific plan to implement each of these strategic priorities. These plans are reviewed and updated periodically to track the progress toward meeting the objectives. **Chapter 5** discusses further development of these strategic priorities in the draft 2008-2012 NICEATM-ICCVAM Five-Year Plan.

[6] ICCVAM's activities are grounded in the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples).

Figure 1. ICCVAM Strategic Map



Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

In accordance with the ICCVAM Authorization Act, SACATM was established in 2002 to advise the NIEHS Director, ICCVAM, and NICEATM regarding statutorily mandated ICCVAM functions. SACATM also provides advice to NIEHS and NICEATM on NICEATM activities.

In compliance with the Act, SACATM was established with the following members:

- At least one member from each of the following stakeholders:
  - The personal care, pharmaceutical, industrial chemicals, or agriculture industry
  - Any other industry regulated by one of the ICCVAM agencies
  - A national animal protection organization

- Additional representatives selected from the following:
  - Academic institutions
  - State government agencies
  - An international regulatory body or any corporation developing or marketing new or revised or alternative test methodologies, including contract laboratories

The SACATM charter, related *Federal Register* (*FR*) notices, meeting minutes, and future meeting announcements, are on the NTP website.[7] Lists of SACATM members and meetings held during 2006-2007 can be found in **Appendix C**.

[7] Available at http://ntp.niehs.nih.gov/index.cfm?objectid=720165EC-BDB7-CEBA-F517D1DEE4D7D129.

Chapter 2

ICCVAM Processes

ICCVAM and NICEATM serve an important role in evaluating the usefulness and limitations of new and revised test methods and promoting the regulatory acceptance of those methods found to be scientifically valid for their intended purpose. The interagency cooperation fostered by ICCVAM and NICEATM provides an efficient and effective mechanism for Federal review of test methods and helps to promote adoption of scientifically valid new and revised test methods that meet the needs of relevant Federal regulatory agencies while reducing, refining, and replacing the use of animals in testing where scientifically feasible. However, ICCVAM and NICEATM do not have authority to approve new or revised testing regulations or guidelines, and have no regulatory authority to require the use of a test method for a particular purpose. Only Federal agencies can approve new test methods, and determine whether data from new test methods can be accepted and how they might be used in their respective programs.

ICCVAM Guidelines for Nomination and Submission

ICCVAM published revised *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods* (*ICCVAM Guidelines*) in 2003 (NIH Publication No. 03-450; ICCVAM 2003a). The original version of this document was published in May 1998 and revised in October 1999. The second revision in 2003 reflects experience gained by ICCVAM since 1999. The document provides guidance to sponsors and nominators of test methods on the information needed by ICCVAM to evaluate the validation status of new or revised test methods at any stage, from development through completion of validation studies. It also includes a framework outline for organizing the information and data in submissions necessary to support the validity of a proposed test method.

ICCVAM Test Method Nomination and Submission Process

ICCVAM has an established process for considering nominations and submissions of test methods and prioritizing them for review and evaluation. An overview of this process is depicted in **Figure 2**. Submissions should be accompanied by all requested information; the *ICCVAM Guidelines* summarize information that should be provided in submissions and nominations. Although there is no mandatory minimum requirement for information to be provided with nominations, providing as much of the requested information as possible will expedite ICCVAM's consideration of a proposed test method. Areas where the requested information is unavailable or incomplete should be indicated, along with the scientific approach, or approaches, planned to gather or generate those data.

The Director of NICEATM solicits and tracks the status of nominations and submissions of test methods, provides updates to ICCVAM, and arranges for a preliminary evaluation of nominations and submissions by NICEATM, as resources permit. Preliminary evaluations summarize the extent to which nominations or submissions of test methods address the following ICCVAM prioritization criteria:

- The extent to which the proposed test method is:
  - Applicable to regulatory testing needs
  - Applicable to multiple agencies or programs
  - Warranted, based on the extent of expected use or application and impact on human, animal, or ecological health

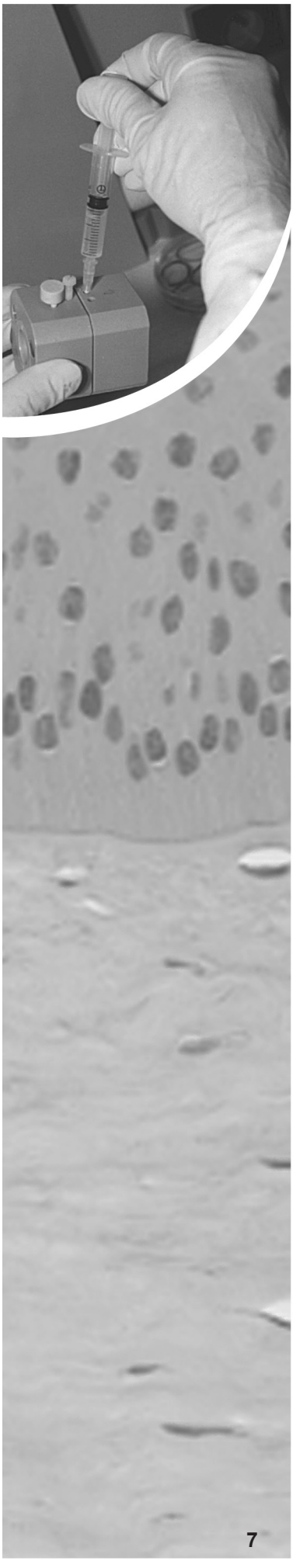
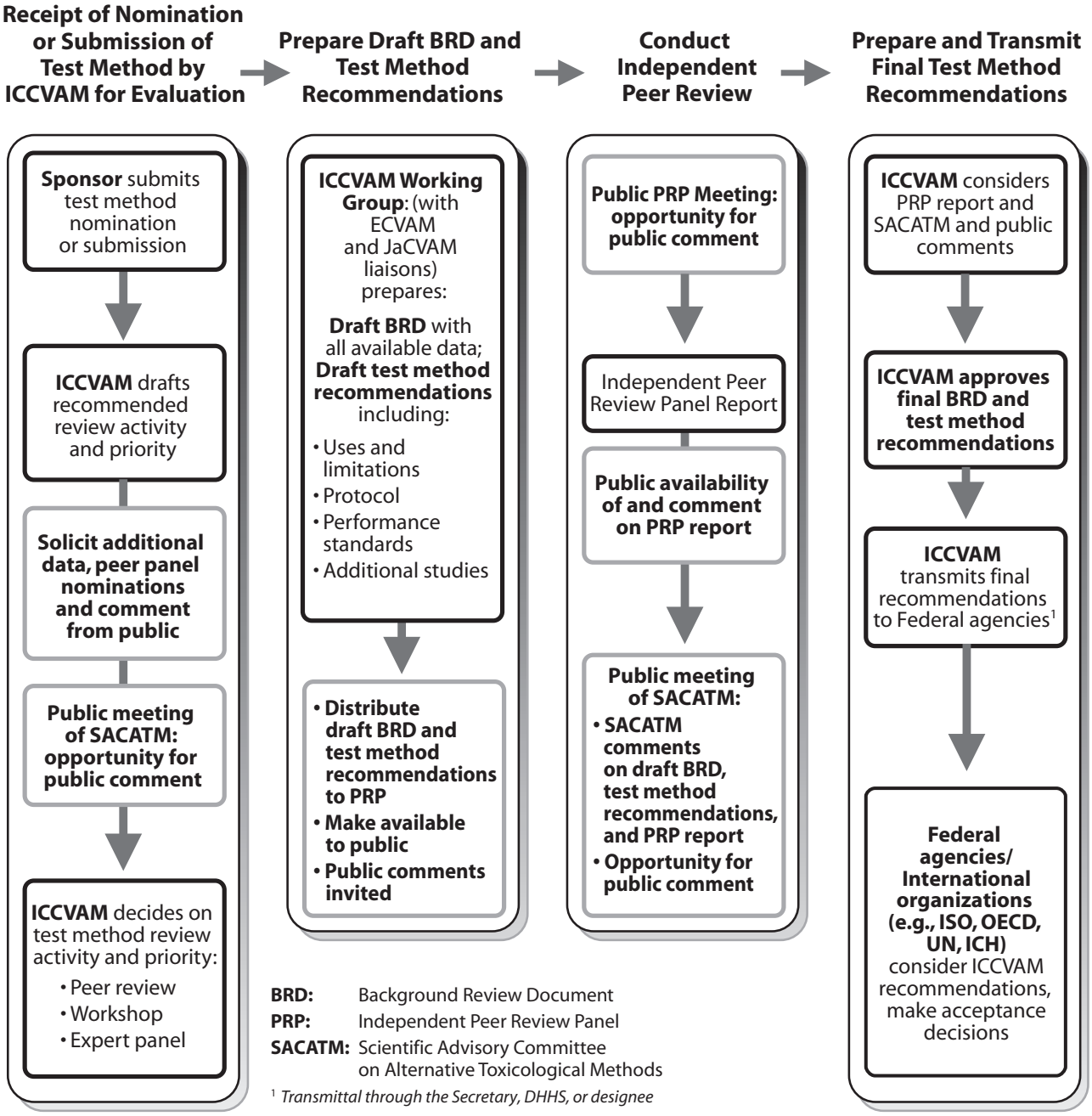


Figure 2. ICCVAM Test Method Evaluation Process



- The potential for the proposed test method to refine, reduce, or replace animal use, compared to current test methods accepted by regulatory agencies
- The potential for the proposed test method to improve predictions of adverse health or environmental effects, compared to current methods
- The extent to which the test method provides other advantages (e.g., reduced cost and performance time) compared to current methods
- The completeness of the nomination or submission with regard to ICCVAM test method submission guidelines

The Director of NICEATM provides the results of NICEATM's preliminary evaluations to ICCVAM, including recommendations for further evaluations (e.g., workshop, expert panel meeting, peer review meeting, peer review meeting, and expedited review process) or validation studies. ICCVAM then:

- Reviews the NICEATM preliminary evaluation report
- Determines whether the test method is of sufficient interest (e.g., applicable to one or more agencies, or would be extensively employed) to warrant further evaluation
- Develops draft recommendations for evaluation priority, validation studies needed, and other activities associated with the test method nomination or submission

In addition, the Director of NICEATM provides SACATM with:

- A status report on submissions and nominations of test methods
- Results of ICCVAM's preliminary evaluation of nominations and submissions of test methods
- Draft recommendations for evaluation priority, validation studies needed, and other activities associated with a nomination or submission of a test method
- Public comments specific to these various activities

SACATM comments on draft recommendations regarding future ICCVAM priorities and activities. ICCVAM also seeks comments from the public via public meetings, electronic methods (e.g., ICCVAM e-mail lists and the NICEATM-ICCVAM website), and printed materials and publications such as the *Federal Register*. ICCVAM then considers comments from SACATM and the public, develops final recommendations for future activities for the nomination or submission, and prioritizes these activities.

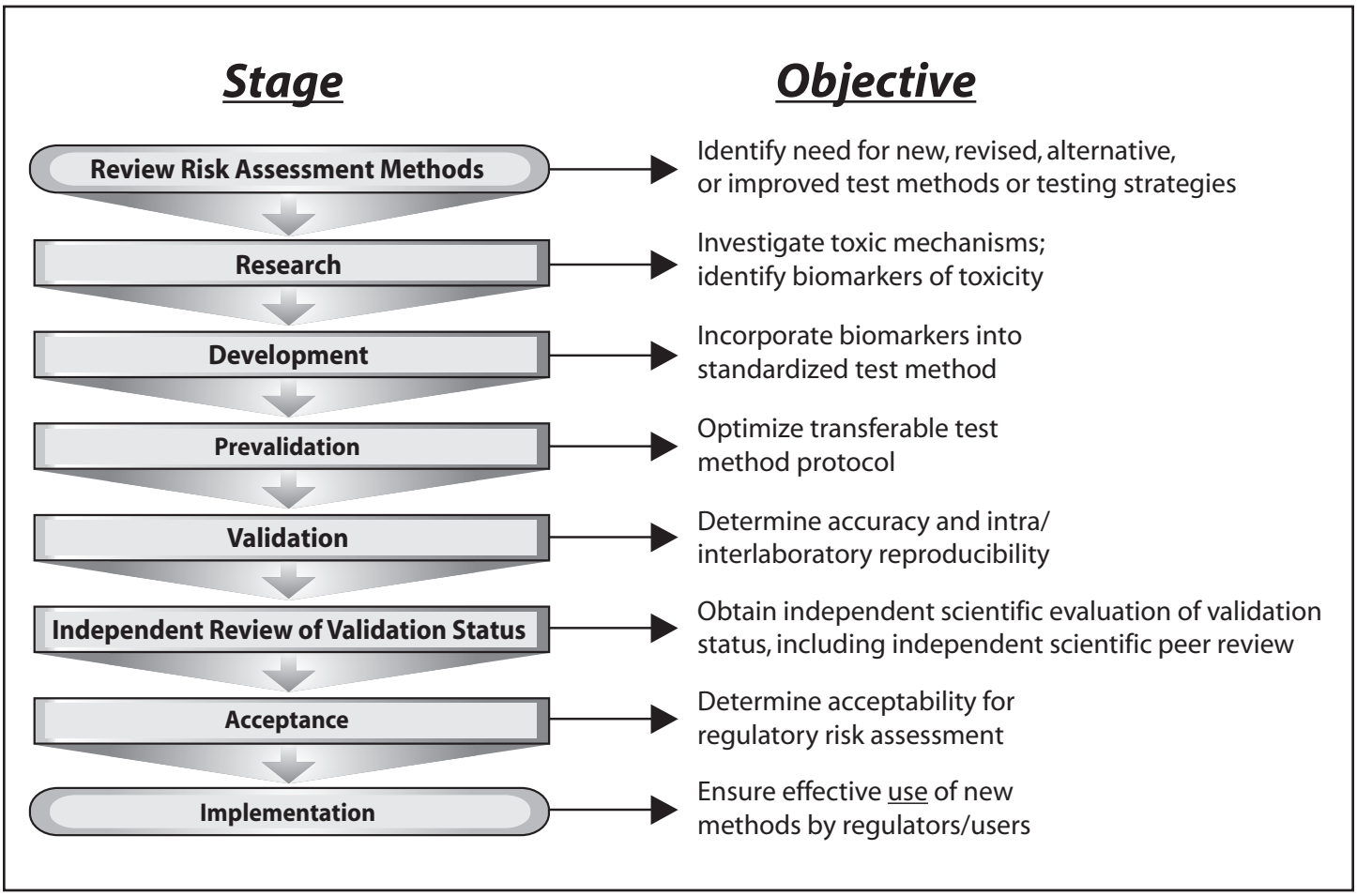
The Director of NICEATM estimates resource requirements for proposed evaluations and/or validation studies and forwards these, along with recommendations from SACATM and ICCVAM, to the Associate Director of NTP. The NTP Associate Director considers ICCVAM priorities, activities, and needed resources, and forwards a recommendation to the Director of NIEHS, who also serves as the Director of the NTP. Based on this information, the NIEHS Director determines priorities and makes resource allocations for approved activities.

The Director of NICEATM informs ICCVAM of the availability of funding from NIEHS, other ICCVAM agencies, or other stakeholders that can be used to support the recommended activities (e.g., workshops, expert panel meetings, independent peer reviews, expedited reviews, and validation studies). When resources are available to support a recommended activity, NICEATM, in collaboration with ICCVAM and the appropriate working group, organizes the activity.

ICCVAM Test Method Evaluation Process

ICCVAM conducts interagency technical reviews of proposed new, revised, and alternative test methods and coordinates issues across agencies relating to the validation, acceptance, and national and international harmonization of toxicological test methods. ICCVAM places priority on test methods that may improve predictions of adverse human, animal, or ecological effects and to those that may reduce, refine, or replace animal use. Advancement of a proposed test method from concept to regulatory acceptance involves a number of stages (**Figure 3**). The ICCVAM Test Method Evaluation Process addresses the critical stage of independent evaluation

Figure 3. Development, Evaluation, and Acceptance of Test Methods



of the validation status (i.e., the usefulness and limitation of the test method for a specific purpose), including independent scientific peer review (**Figure 2**).

The submission of a proposed test method to ICCVAM for consideration and review is the first step in the evaluation process. NICEATM, on behalf of ICCVAM, receives nominations and submissions of proposed test methods and communicates with test method sponsors and nominators. Typically, the ICCVAM evaluation of the nomination or submission involves:

- An initial assessment of the adequacy and completeness of the nomination or submission of a test method
- Prioritization of the proposed test method review activity by ICCVAM
- Consideration of comments and recommendations from the public and from SACATM on the review priority and activity
- ICCVAM decision on final priority for evaluation

Once a test method has been accepted for evaluation, ICCVAM assembles an interagency working group of government scientists with appropriate scientific and regulatory expertise to evaluate the test method in collaboration with NICEATM (see **Figure 2**).

To facilitate the evaluation, the working group works with NICEATM to prepare a comprehensive draft BRD on the test method under consideration. This draft BRD provides the rationale and scientific basis for use of the test method, components of the test method protocol, and descriptions of substances used to evaluate the test method and comparable *in vivo* reference

data. The draft BRD contains information submitted by the sponsor in support of the nomination or submission. It may also contain relevant data submitted by other interested parties, information on use of the test method obtained from searches of the scientific literature, analyses of accuracy and reliability of the test method, and discussions of animal welfare considerations and other parameters, such as time, cost and infrastructure requirements, to be considered when putting the test method into practice. The ICCVAM working group, working with NICEATM, considers the draft BRD as it develops draft recommendations on the use of the test method.

Once they are completed, the draft BRD and draft test method recommendations are posted on the NICEATM-ICCVAM website, and an *FR* notice is published to announce that the documents are available for public review and comment. The documents are then evaluated at a meeting of an independent scientific Peer Review Panel. The members of this Panel are research scientists, clinicians, test method developers, statisticians, and other professionals with relevant expertise, drawn from industry, academia, animal welfare organizations and regulatory entities that are normally other than those represented on ICCVAM. ICCVAM's goal is for its Peer Review Panels to have an international scientific composition representing the viewpoints of all interested parties in consideration of the test method.

The Peer Review Panel meets in public session, and comments from the public are welcomed during the meeting. The conclusions and recommendations of the Panel are contained in an independent report that is issued shortly after the Panel meeting. This document is also posted on the NICEATM-ICCVAM website, and an *FR* notice is published to announce availability of the report.

In preparing the ICCVAM test method evaluation report, ICCVAM considers the independent Peer Review Panel review of the ICCVAM draft BRD and draft recommendations, as well as comments received from the public and SACATM on the draft documents.

The evaluation report for a test method includes the ICCVAM recommendations regarding the regulatory applicability of the method and the demonstrated usefulness and limitations for specific proposed hazard and safety assessments. In addition to these recommendations, the report will usually also include the following:

- *Recommended standardized test method protocol:* A recommended protocol is developed from information gathered during the test method evaluation. This protocol specifies details on how to conduct the method and includes, for example, information about the purpose and applicability of the test method, study design, data evaluation, decision criteria, and study report preparation. Use of the recommended standardized protocol promotes the generation of consistent uniform data that can be used to expand the test method's validation database.
- *Recommended performance standards (if applicable):* Performance standards communicate the basis by which the new test methods can be determined to have sufficient accuracy[8] and reliability[9] for specific testing purposes. Performance standards are discussed in more detail below.
- *Recommendations for future studies:* ICCVAM may identify and recommend additional research, development, and/or validation studies that have the potential to improve or broaden the applicability of the test method.

[8] Accuracy is a measure of test method performance and one aspect of "relevance." Accuracy refers to (a) how closely a test method's results agree with accepted reference values, and (b) the proportion of correct outcomes of a test method. "Accuracy" is often used interchangeably with "concordance." Accuracy strongly depends on how many true positive results are possible in the population being studied.

[9] Reliability is a measure of how well a test method produces the same results within the same laboratory and across different laboratories over time. It is assessed by calculating intra- and inter-laboratory reproducibility and intralaboratory repeatability.

Performance Standards

Performance standards communicate the basis by which new proprietary (i.e., copyrighted, trademarked, or registered) and nonproprietary test methods have been determined to have sufficient accuracy and reliability for specific testing purposes. ICCVAM develops and recommends performance standards as part of its evaluation of new proposed test methods. Once a proposed test method has been accepted by regulatory agencies, these performance standards can be used to evaluate the reliability and accuracy of other test methods that are based on similar scientific principles and that measure or predict the same biological or toxic effect. The ICCVAM process for developing performance standards for new test methods is described below:

- NICEATM and the appropriate ICCVAM working group develop proposed performance standards for consideration during the ICCVAM evaluation process. If a test method sponsor proposes performance standards, ICCVAM considers them at this stage. Generally, the performance standards are based on information provided in the test method submission or other available applicable data.
- The NICEATM-ICCVAM Peer Review Panel evaluates the proposed performance standards for completeness and appropriateness when it evaluates the validation status of the proposed test method. The proposed performance standards and the test method submission are made available to the public for comment before and during the Peer Review Panel meeting.
- The appropriate ICCVAM working group, with assistance from NICEATM, prepares final recommended performance standards for ICCVAM approval, taking into consideration the recommendations of the Peer Review Panel and public comments.

ICCVAM includes recommended performance standards in test method evaluation reports, which are transmitted to U.S. Federal agencies and made available to the public. Regulatory authorities can then reference the ICCVAM performance standards when they accept a new test method. Regulatory authorities can also include or reference the performance standards in new or revised test method guidelines. Availability of ICCVAM test method evaluation reports are announced in the *Federal Register*, NTP newsletters, and NICEATM-ICCVAM e-mail lists.

Regulatory Acceptance of ICCVAM-Recommended Test Methods

ICCVAM was established to promote the review and adoption of scientifically valid safety testing methods that protect human health, animal health and the environment while refining, reducing or replacing animal use where scientifically feasible. As illustrated in **Figures 2 and 3**, the final step in the ICCVAM test method evaluation process is the acceptance of ICCVAM-recommended methods by Federal agencies for regulatory testing.

Since ICCVAM's establishment through December 2007, Federal regulatory agencies have approved or endorsed 16 alternative methods, including 10 alternative test methods based on comprehensive technical evaluations by ICCVAM. These are summarized in **Figure 4**. The alternative methods recommended by ICCVAM and accepted by Federal agencies include 10 non-animal methods and six methods that use fewer animals and reduce the potential for discomfort. Several of the methods can be used for the most commonly conducted safety tests, including tests to identify substances that can cause allergic skin reactions and irritation and chemical burns to the skin. Recommendations for two *in vitro* methods to help reduce animal use for acute oral toxicity (poisoning) and recommendations on five *in vitro* methods to test for pyrogens (fever-causing substances) will be transmitted to Federal agencies in 2008. Use of these methods is expected to further reduce and refine the use of animals for regulatory testing.

Figure 4. Acceptance of Alternative Methods by U.S. Regulatory Agencies, 1998-2007

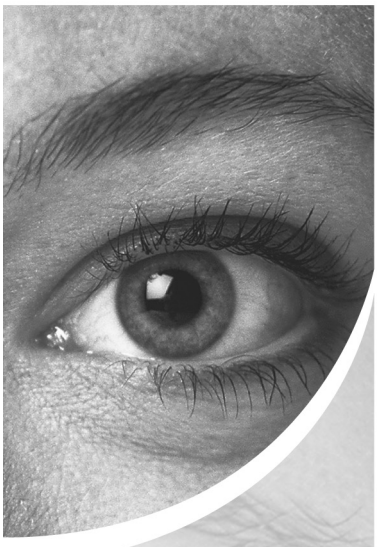
No.	Method	ICCVAM Evaluation	U.S. Regulatory Acceptance/ Endorsement	OECD Adoption	EU Regulatory Acceptance/ Endorsement*
1	CORROSITEX® Skin Corrosivity Test	ICCVAM peer review and report; recommended in 1999	Yes, accepted by U.S. in 2000; as OECD Test Guideline (TG) 435 in 2006	OECD TG 435 (2006)	Yes, via OECD
2	Local Lymph Node Assay for skin sensitization	ICCVAM peer review and report; recommended in 1999	Yes, accepted by U.S. in 1999; as OECD TG 429 in 2002	OECD TG 429 (2002)	Yes, via OECD
3	Up and Down Procedure (UDP)	ICCVAM peer review and report; recommended in 2001	Yes, accepted by U.S. in 2003; as OECD TG 425 in 2003	OECD TG 425 (2001)	Yes, via OECD
4	Fixed Dose Procedure (FDP)	ICCVAM Working Group (WG) contributed to TG development	Yes, as OECD TG 420 in 2001	OECD TG 420 (2001)	Yes, via OECD
5	Acute Toxic Class Method (ATC)	ICCVAM WG contributed to TG development	Yes, as OECD TG 423 in 2001	OECD TG 423 (2001)	Yes, via OECD
6	EPISKIN® Skin Corrosivity Test	ICCVAM review and report; recommended in 2002	Yes, as OECD TG 431 in 2004	OECD TG 431 (2004)	67/548/EEC
7	EpiDerm™ Skin Corrosivity Test	ICCVAM review and report; recommended in 2002	Yes, as OECD TG 431 in 2004	OECD TG 431 (2004)	67/548/EEC
8	SkinEthic™ Skin Corrosivity Test	ICCVAM contributed to U.S. OECD TG review	Yes, within context of OECD TG 431	OECD TG 431 (2004)	Yes, via OECD
9	Rat TER Skin Corrosivity Test	ICCVAM review and report; recommended in 2002	Yes, as OECD TG 430 in 2004	OECD TG 430 (2004)	67/548/EEC
10	3T3 NRU Phototoxicity Test	ICCVAM contributed to U.S. OECD review	Yes, as OECD TG 432 in 2004	OECD TG 432 (2004)	67/548/EEC
11	3T3 NRU Phototoxicity Test: Application to UV Filter Chemicals	ICCVAM contributed to U.S. OECD review	Yes, as OECD TG 432 in 2004	OECD TG 432 (2004)	Yes, via OECD
12	*In vitro* dermal absorption methods	ICCVAM contributed to U.S. OECD TG review, expert consultation meetings	Yes, as OECD TG 428 in 2004	OECD TG 428 (2004)	Yes, via OECD
13	Use of Humane Endpoints in Animal Testing of Biological Products	ICCVAM agency initiative	Yes, 9 CFR 117.4e		
14	Rabies Vaccine, Humane Endpoints	ICCVAM agency initiative	Yes, 9 CFR 117.4e		
15	Acute Toxicity *In Vitro* Dose Procedure, 3T3 cells	ICCVAM 2001 report; 2006 peer review and report; to be recommended in 2008	Yes		
16	Acute Toxicity *In Vitro* Dose Procedure, NHK cells	ICCVAM 2001 report; 2006 peer review and report; to be recommended in 2008	Yes		

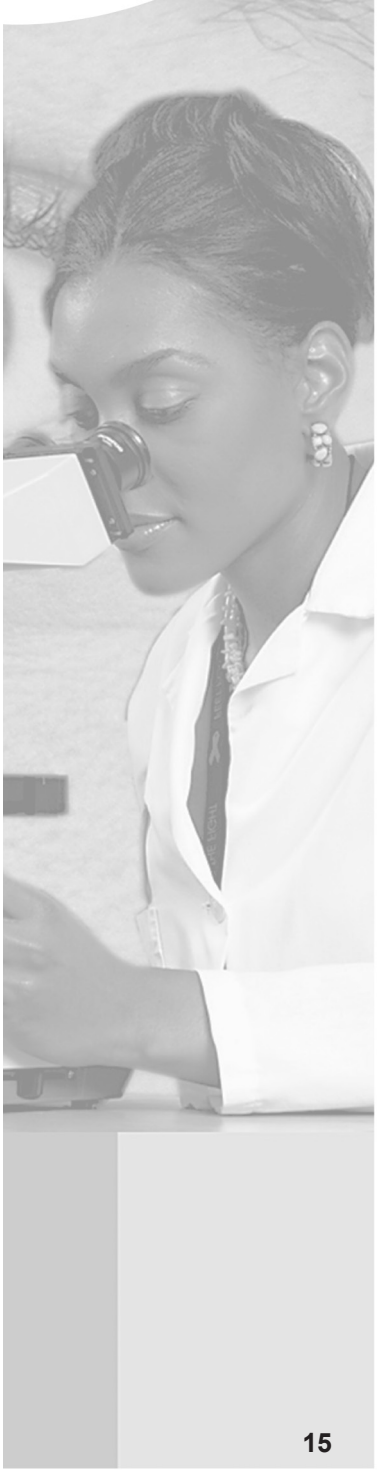
* Information provided by Executive Secretary, ESAC (European Centre for the Validation of Alternative Methods Scientific Advisory Committee), April 13, 2008.

Chapter 3

ICCVAM Test Method Evaluations and Related Activities 2006-2007

This chapter provides an update on ICCVAM test method evaluations and related activities conducted during 2006-2007 and their status as of December 2007. The reports and information identified in this chapter are available electronically on the Internet (see footnotes for locations of specific reports or website pages) or in hardcopy from NICEATM (NIEHS, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709; telephone: 919-541-2384; fax: 919-541-0947; e-mail: niceatm@niehs.nih.gov).





Ocular Safety Testing

During this reporting period, ICCVAM and NICEATM continued to devote significant effort and resources to alternative test methods for ocular safety testing. These activities address nominations from the U.S. Environmental Protection Agency (EPA) that include:

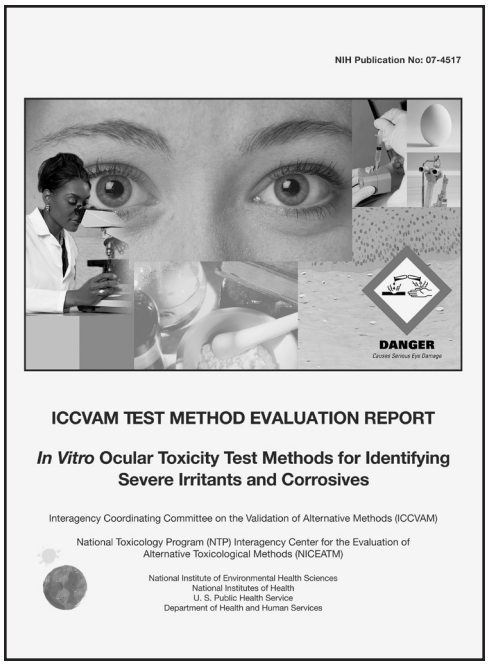
1. Review the validation status of four *in vitro* ocular test methods with the potential to screen chemicals for ocular corrosion or severe irritation:
   - The Bovine Corneal Opacity and Permeability (BCOP) assay
   - The Isolated Rabbit Eye (IRE) assay
   - The Isolated Chicken Eye (ICE) assay
   - The Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) assay
2. Review the state-of-the-science for other *in vitro* methods for assessing moderate or mild eye irritation
3. Obtain good-quality reference data for *in vivo* eye irritation/corrosion
4. Review ways to alleviate pain and suffering that might arise from current *in vivo* eye irritation testing

ICCVAM and SACATM endorsed these nominated activities as a high priority. The following sections describe ICCVAM progress during 2006-2007.

In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants*

Summary

In October 2007, ICCVAM forwarded its first recommendations for the use of *in vitro* methods for ocular safety testing to Federal agencies. ICCVAM recommended that the BCOP and ICE test methods can be used, in appropriate circumstances and with certain limitations, as screening tests for detecting ocular corrosives and severe irritants in a tiered-testing strategy as part of a weight-of-evidence approach. The report also recommends that the four *in vitro* test methods should be considered before using animals for ocular testing and used when determined appropriate. Used in this manner, these test methods will reduce the number of animals used for ocular safety testing, since positive results do not require testing in animals. ICCVAM recommended that the protocols and decision criteria for the IRE and HET-CAM test methods need further optimization and validation before their use for regulatory testing to identify ocular corrosives and severe irritants.



In October 2007, ICCVAM forwarded its first recommendations for the use of in vitro *methods for ocular safety testing to Federal agencies*

Background Information

In March 2006, NICEATM published final BRDs for the four *in vitro* ocular test methods listed above.[10] Prepared in conjunction with the ICCVAM Ocular Toxicity Working Group (OTWG), these BRDs contain comprehensive summaries of available data and related information used to characterize the usefulness and limitations of each test method, including what is known about their relevance[11] and reliability and the range of substances tested. Each final BRD incorporated comments and suggestions submitted on earlier draft versions during March and September 2005 meetings of an independent Peer Review Panel and by the general public during the public peer review process.[12]

ICCVAM considered the BRDs, the reports of the Peer Review Panel, and comments from SACATM and the public, and prepared the report: *Test Method Evaluation Report:* In Vitro *Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives*.[13] As noted above, the report recommended the use of the BCOP and ICE as screening tests for detecting ocular corrosives and severe irritants in a tiered-testing strategy, as part of a weight-of-evidence approach. None of the four *in vitro* test methods evaluated can be considered complete replacements for the *in vivo* Draize ocular irritation test conducted in rabbits. However, these *in vitro* methods, when used appropriately, should reduce the number and severity of chemicals that need to be tested in rabbits, thereby reducing and refining animal use for ocular safety testing.

ICCVAM recommended several future studies, which were then carried forward as ICCVAM-nominated activities to SACATM at their November 2006 meeting. One of these activities involves evaluating the optimal corneal holder and vehicle for the BCOP test method.[14] Separate studies have been proposed to evaluate how the following modifications to the protocol will affect the accuracy and/or reliability of the BCOP:

- Using an alternative corneal holder that maintains normal curvature (e.g., the corneal mounting system designed by Ubels et al. 2002)
- Using 0.9% sodium chloride instead of distilled water as the diluent

[10] All four ocular BRDs can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd.htm.
[11] Relevance is the extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance includes considering the accuracy (concordance) of a test method.
[12] Information about the Peer Review Panel meetings, and the panel's report, can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_report.htm.
[13] The ICCVAM test method evaluation report can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm.
[14] The nomination can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocudocs/CornealSACATM26ct06F.pdf.

The remaining ICCVAM-nominated activities are more broadly applicable to *in vitro* ocular toxicity test methods;[15] and include:

- Creating a reference atlas of histopathology for chemical-induced ocular lesions
- Creating a standardized scoring scheme to be used in conjunction with the histopathology atlas to identify decision criteria that could be used in hazard classification
- Developing a targeted research grants program to focus on areas identified as high priority at the 2005 Symposium on Mechanisms of Chemically-Induced Ocular Injury and Recovery

In Vitro *Methods for Assessing Moderate or Mild Eye Irritation, and Expansion of the* In Vivo *Eye Irritation/Corrosion Reference Database*

In addition to the evaluation discussed above, the EPA nomination included review of the validation status of *in vitro* methods for identifying non-severe ocular irritants (i.e., those that induce reversible ocular damage) and nonirritants. ICCVAM and NICEATM are collaborating with ECVAM (the European Centre for the Validation of Alternative Methods) on this activity. ICCVAM and NICEATM have taken the lead on reviewing BCOP, HET-CAM, ICE, and IRE for this purpose, while ECVAM is the lead on the other relevant *in vitro* ocular toxicity test methods.

In June 2007, NICEATM published an *FR* notice (Vol. 72, No. 109, pp. 31582-31583, June 7, 2007)[16] requesting data from substances tested for ocular irritancy in humans, rabbits, and/or *in vitro* test systems. These data, in addition to other relevant data submitted in response to earlier requests, will be compiled and added to a database that will eventually be made publicly available for all interested stakeholders. This searchable, web-based database will contain data from *in vivo* Draize ocular irritation tests, collected on a wide range of substances from a variety of public and private sources. These data can be used for comparison with *in vitro* test results and will provide a valuable resource for the development and validation of alternative ocular testing methods.

[15] The nomination can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocudocs/OcularActivities26Oct06F.pdf.

[16] Full titles of all *FR* notices, as well as links to the notices in PDF format on the NICEATM-ICCVAM website, are listed in Chapter 6.

Minimizing Pain and Distress in Ocular Toxicity Testing

NICEATM evaluated data to assess the effect of using a topical anesthetic pretreatment (0.5% [weight/volume] tetracaine hydrochloride) on classification of the irritancy potential of 97 proprietary formulations. This study considered the effect of this anesthetic pretreatment on (a) the hazard classification category of the observed ocular irritation, (b) agreement between pretreated and untreated rabbits tested with the same formulation, and (c) the number of days required for an ocular lesion to clear. Results from these analyses were presented at the 46th Annual Meeting of the Society of Toxicology (SOT), Baltimore, MD (March 2007),[17] and at the 6th World Congress on Alternatives and Animal Use in the Life Sciences (WC6), Tokyo, Japan (August 2007).[18] In most of the formulations tested, topical anesthetic pretreatment had no statistically significant impact on (a) irritancy classification category, (b) the variability in rabbit ocular hazard classification responses, or (c) the number of days required for an ocular lesion to clear. The results indicated that topical treatment with tetracaine hydrochloride had no significant impact on hazard classification for the EPA, Globally Harmonized System of Classification and Labelling of Chemicals (GHS; United Nations 2003), and European Union classification systems. These results support its use as a topical pretreatment in the *in vivo* ocular irritation test, to avoid or lessen potential pain or discomfort that might occur during or shortly after test article application.

To obtain additional data to further support the use of pain prevention and alleviation strategies, NICEATM requested the submission of data from completed *in vivo* ocular irritancy studies that included the use of topical anesthetics and/or systemic analgesics via an *FR* notice (Vol. 72, No. 89, p. 26396, May 9, 2007). Although no data were received, NICEATM will continue to seek data that might be used to evaluate topical anesthetics and/or analgesics for their usefulness for *in vivo* ocular toxicity testing and their potential impact on ocular hazards classification. NICEATM also requested information and data on other *in vivo* methods, procedures, and/or testing strategies that may reduce or eliminate the potential pain and distress associated with current *in vivo* eye irritation methods.

[17] The poster can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/meetings/SOT07/posters/SOT07Choksi.pdf.
[18] The poster can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/meetings/6thWC/posters/ChoksiPretreat.pdf.

In Vitro Endocrine Disruptor Screening Methods

The report *ICCVAM Evaluation of* In Vitro *Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays* (ICCVAM 2003b) identified a need for adequately validated *in vitro* assays to screen for endocrine disruptors. The following sections describe ICCVAM activities during 2006-2007 to follow up on this finding.

Updated List of Reference Substances for Validation of In Vitro *Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Test Methods*

The report *ICCVAM Evaluation of* In Vitro *Test Methods for Detecting Potential Endocrine Disruptors* included a list of 78 substances proposed for standardization and validation of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) test methods. The report defined a subset of 53 of these substances considered essential for adequately evaluating ER-based assays, and 44 essential substances for AR-based assays. To prepare for an international validation study of an ER TA assay (see next section), NICEATM reassessed the commercial availability and cost of these substances. NICEATM determined that four of these substances either were not commercially available or had limited availability, and another six were considered to be possibly cost-prohibitive (i.e., more than $2,500/500 mg). ICCVAM considered this information and made the following revisions:

- Four substances were chosen to replace the substances with limited or no commercial availability. The replacement substances were judged to have similar ER or AR binding or agonist TA activity profiles, or to be similarly concordant for antagonist TA activity across studies.
- Two of the six relatively expensive substances were replaced with less expensive alternatives with similar ER or AR binding or agonist TA activity profiles, or that are similarly concordant for antagonist TA activity across studies. The other four relatively expensive substances were retained because their physicochemical properties were considered unique and necessary for inclusion on the reference list.

NICEATM published an *FR* notice (Vol. 71, No. 51, pp. 13597-13598, March 16, 2006) requesting public comment on ICCVAM's recommended revisions. No public comments were received, and ICCVAM approved the proposed revisions. The updated reference substance list was published in September 2006 as an Addendum to the report *ICCVAM Evaluation of* In Vitro *Test Methods for Detecting Potential Endocrine Disruptors*.[19]

[19] The report and addendum can both be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/endocrine/end_TMER.htm.

Protocol Standardization and Validation of the LUMI-CELL® ER Assay

ICCVAM received nominations of two *in vitro* methods for validation studies. Both methods detect ER agonist/antagonist activity, and both met the ICCVAM recommendations that such test methods should avoid the use of receptors that must be obtained from euthanized animals and that require the use of radioactive materials.

ICCVAM received the first nomination from Xenobiotic Detection Systems, Inc. (XDS), for LUMI-CELL, a stably transfected recombinant cell-based ER TA test method. The LUMI-CELL ER assay measures whether a substance induces or inhibits TA activity via ER-mediated pathways, and if so, by how much.

In 2006, NICEATM conducted protocol standardization studies for the LUMI-CELL assay.[20] These studies:

- Standardized procedures for using the assay to identify ER agonists and antagonists
- Standardized procedures for quantitatively assessing cell viability for use with the agonist and antagonist assays
- Established a historical database for reference standards and controls used in the agonist and antagonist assay protocols
- Demonstrated the adequacy of the standardized protocols for detecting ER agonists and antagonists using eight substances covering a range of ER agonist and antagonist activities

In 2007, ICCVAM initiated an international validation study of the LUMI-CELL ER assay in conjunction with ECVAM and the Japanese Center for the Validation of Alternative Methods (JaCVAM) to:

- Evaluate how well the standardized LUMI-CELL ER assay (agonist and antagonist) protocols developed at XDS can be transferred to other laboratories, including one sponsored by ECVAM and another by JaCVAM
- Provide an opportunity between phases of the validation study to further optimize the protocol to enhance accuracy and reproducibility
- Test the 78 coded ICCVAM-recommended test substances
- Evaluate assay accuracy and intra- and inter-laboratory reproducibility

Phase I of the international validation study began in all participating laboratories in November 2007. The goal of phase I is for the participating laboratories to demonstrate proficiency with agonist and antagonist protocols, demonstrate initial intralaboratory repeatability and intra- and inter-laboratory reproducibility, establish historical positive and vehicle control databases to be used as quality controls for later study phases, and modify test plate configurations to improve test throughput.

Phase II will be started at all participating laboratories in early 2008. In this phase, each laboratory will test a total of 12 coded substances, with each compound tested three times for both ER agonist

[20] The standardized protocols can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/endocrine/end_eval.htm.

and antagonist activity. The 12 compounds represent a subset of the ICCVAM-recommended list of 78 ER reference substances.[21] Intra- and inter-laboratory reproducibility will be assessed, and accuracy determined by comparing LUMI-CELL ER assay results against established reference values. Excessive variation will be investigated and protocols modified accordingly.

In phase III, the performance of the optimized test method protocol will be evaluated by having each laboratory test each of the remaining 41 reference substances in the ICCVAM minimum list of 53 reference substances. The remaining 25 ICCVAM-recommended substances will be tested during phase IV in the lead laboratory only. By identifying and resolving sources of variability early in the validation process, this multiphased approach should produce a standardized protocol for a reproducible and sensitive ER agonist/antagonist TA assay that is suitable for international regulatory use.

Evaluation of the Certi-Chem Inc. MCF-7 Cell Proliferation Assay of Estrogenic Activity

In June 2005, NICEATM received a nomination from Certi-Chem Inc. (CCi) for validation studies on a cell-based ER TA assay. This assay evaluates the potential for substances to disrupt endocrine activity by measuring whether a substance induces cell proliferation in MCF-7 breast cancer cells via ER-dependent pathways, and if so, by how much. In support of this nomination, CCi submitted a BRD to NICEATM in January 2006, titled *Test Method Nomination: MCF-7 Cell Proliferation Assay of Estrogenic Activity.* In accordance with the ICCVAM nomination process, NICEATM conducted a prescreen evaluation of the CCi BRD.

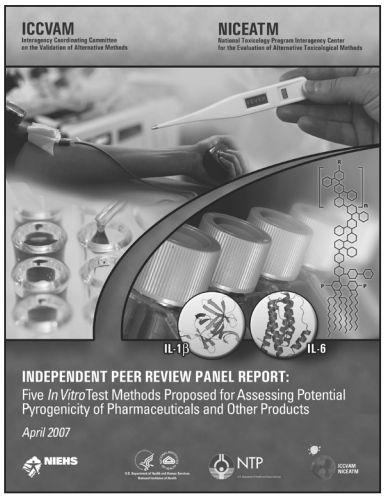
The prescreen evaluation was made available to the public in October 2006 via an *FR* notice (Vol. 71, No. 199, pp. 60748-60749, October 16, 2006), which also invited comments on whether the CCi MCF-7 cell proliferation assay should be considered for additional validation studies. Eight public comments were received in response to the *FR* notice, most of which encouraged ICCVAM to proceed with such studies.[22] After considering public comments and recommendations from SACATM, ICCVAM recommended that the assay be given a high priority for additional validation.[23] Adequately validated *in vitro* methods for screening endocrine disruptors may reduce the numbers of animals needed to identify substances with the potential to disrupt normal hormonal function.

[21] The full list of reference substances can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/endo_docs/EDAddendFinal.pdf.
[22] Comments in response to this *FR* notice may be viewed at http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm?ftitle=E6-17134.
[23] The prescreen evaluation of the CCi protocol can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/endocrine/end_eval-CChem.htm.

In Vitro Pyrogenicity Test Methods

The U.S., European, and Japanese Pharmacopeias recognize two test methods for pyrogen testing, the rabbit pyrogen test (RPT) and the *in vitro* bacterial endotoxin test, also referred to as the *Limulus* amebocyte lysate test. In 2005, ECVAM submitted BRDs on the following five *in vitro* pyrogenicity tests, based on human blood cells, to ICCVAM for consideration as replacements for the RPT:

- The Human Peripheral Blood Mononuclear Cell/Interleukin-6 *In Vitro* Pyrogen Test (PBMC/IL-6)
- The Human Whole Blood/Interleukin-1 (IL-1) *In Vitro* Pyrogen Test
- The Human Whole Blood/IL-1 *In Vitro* Pyrogen Test: Application of Cryopreserved Human Whole Blood
- The Human Whole Blood/IL-6 *In Vitro* Pyrogen Test
- An Alternative *In Vitro* Pyrogen Test Using the Human Monocytoid Cell Line MONO MAC-6



These test methods are similar to each other in that they measure cytokine levels in human blood, isolated PBMCs, or a human monocytoid cell line as a biomarker of a pyrogenic response. In each assay, cytokine levels are measured with an enzyme-linked immunosorbent assay, or ELISA.

After a NICEATM prescreen evaluation of the ECVAM BRDs, ICCVAM and its Pyrogenicity Working Group determined that they had enough data to warrant an independent evaluation of the relevance and reliability of each of the five *in vitro* pyrogenicity test methods. NICEATM prepared a comprehensive BRD that provided information and data from the validation studies for each of the five *in vitro* test methods. A draft BRD was released in December 2006.

An independent Peer Review Panel meeting was convened in February 2007 to evaluate the validation status of the five test methods (announced in *FR* Vol. 71, No. 238, pp. 74533-74534, Dec. 11, 2006). Four public comments on the ICCVAM recommendations were received in response to this notice.[24] The Peer Review Panel reviewed the BRD and evaluated the extent to which established validation and acceptance criteria had been addressed for the methods. The Peer Review Panel also commented on draft ICCVAM recommendations on test method uses, future studies, and test method protocols. The Peer Review Panel's conclusions and recommendations are included in the *Independent Peer Review Panel Report: Five* In Vitro *Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products*, which was published in April 2007.[25]

ICCVAM considered the conclusions and recommendations of the Peer Review Panel, as well as comments from the public and SACATM, before finalizing the BRD and test method recommendations. ICCVAM recommends these methods be considered for use to detect Gram-negative endotoxin in human parenteral drugs on a case-by-case basis, subject to validation for that specific product to demonstrate equivalence to the RPT, in accordance with applicable U.S. Federal regulations (e.g., U.S. Food and Drug Administration [FDA]).[26,27] Although none of these test methods can be considered a complete replacement for the RPT for all testing situations for detecting Gram-negative endotoxin, these and other *in vitro* alternative test methods should be considered before *in vivo* pyrogenicity testing, and used where determined appropriate for a specific testing situation. When used in this manner, these methods should reduce the number of animals needed for pyrogenicity testing. A final BRD and ICCVAM test method evaluation report, including ICCVAM recommendations, will be published in 2008.[28]

[24] Public comments in response to this notice can be viewed at http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm?ftitle=E6-21038.

[25] The Peer Review Panel's report can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/pyrogen/PrRevPanFinRpt.pdf.

[26] The use of any recommended method will be subject to product-specific validation to demonstrate equivalence as recommended by the FDA (e.g., U.S. Code of Federal Regulations (CFR) 21 CFR 610.9 and 21 CFR 314.50(d)(1)(ii)(a)). Mechanisms exist for test method developers to qualify their method on a case-by-case basis.

[27] Substances other than endotoxin may induce the cellular release of IL-1 and/or IL-6. For this reason, users of these test methods should be aware that the presence of these types of substances might interfere with the interpretation of the assay.

[28] The final BRD and ICCVAM test method recommendations will be posted on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/pyrogen/pyrogen.htm.

In Vitro Cytotoxicity Test Methods for Estimating Acute Oral Systemic Toxicity

Validation Study of In Vitro *Cytotoxicity Text Methods*

At the request of the EPA Office of Pesticides, Prevention and Toxic Substances, ICCVAM reviewed the validation status of *in vitro* methods for estimating acute oral toxicity in 2000. The review confirmed a correlation between *in vitro* cellular toxicity (cytotoxicity) and *in vivo* acute oral toxicity (ICCVAM 2001). This correlation suggested that an *in vitro* cytotoxicity assay might be used to more accurately determine a starting dose for the LD_{50} acute oral toxicity test, which could reduce the number of animals needed for each assay. ICCVAM assigned a high priority to evaluating *in vitro* cytotoxicity test methods for this purpose. NICEATM, working in collaboration with ECVAM, designed a validation study using three different laboratories to evaluate the performance of two standardized *in vitro* cytotoxicity test methods using 72 reference substances. The validation study used two mammalian cell types, primary normal human keratinocytes (NHK) and BALB/c 3T3 mouse fibroblasts (3T3), with neutral red dye uptake to indicate numbers of surviving cells.[29]

After completing the validation study, NICEATM, in conjunction with ICCVAM and the ICCVAM Acute Toxicity Working Group, prepared a comprehensive BRD containing the study results and analyses. ICCVAM then convened an independent Peer Review Panel meeting on May 23, 2006. The meeting was announced in an *FR* notice (Vol. 71, No. 54, pp. 14229-14231, Mar. 21, 2006). Nearly 300 public comments were received in response to the *FR* notice, mostly encouraging ICCVAM to increase efforts to identify nonanimal methods for acute toxicity testing.

The Peer Review Panel reviewed the BRD and evaluated the extent to which established validation and acceptance criteria had been addressed for the two cytotoxicity test methods. The Peer Review Panel also commented on draft ICCVAM recommendations on test method uses and limitations, future studies, test method protocols, and performance standards. The report from this meeting, *Peer Review Panel Report: The Use of* In Vitro *Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing*, was published in July 2006.[30]

ICCVAM considered the Peer Review Panel report, public comments, and SACATM comments. ICCVAM's final test method recommendations are provided in the report *ICCVAM Test Method Evaluation Report:* In Vitro *Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests*.[31] These recommendations will be forwarded to ICCVAM member agencies for consideration in early 2008.

The ICCVAM recommendations include the following:

- The 3T3 and NHK methods are not sufficiently accurate to predict acute oral toxicity for the purpose of regulatory classification of hazards, but they may be used in a weight-of-evidence approach to determine the starting dose for current acute oral toxicity protocols.
- The test methods should be considered and used where appropriate before acute oral toxicity testing is conducted using animals. For some types

[29] Details about the validation study can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_announce.htm.

[30] The Peer Review Panel report can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_scpeerrev.htm.

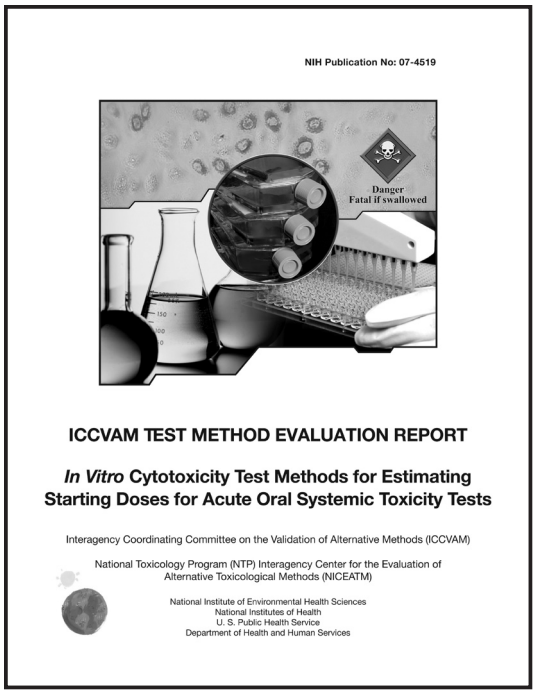
[31] The ICCVAM test method evaluation report can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_tmer.htm.

of substances, this approach will reduce the numbers of animals needed for testing, and may also reduce the number of animals that die or need to be humanely killed.

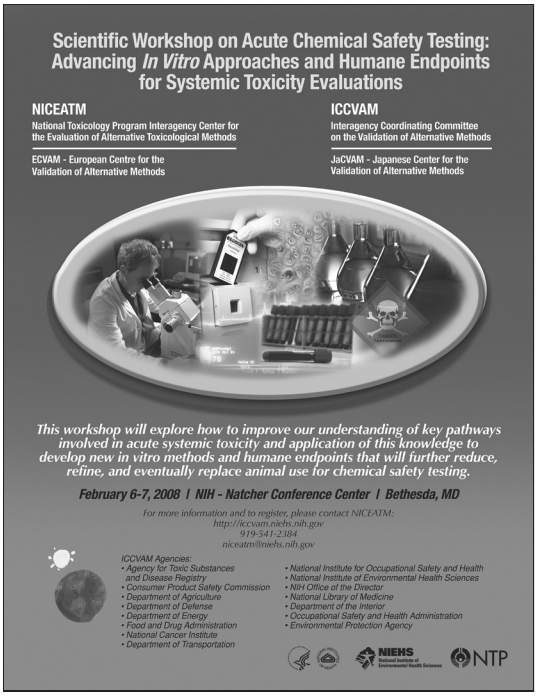
- Of the two test methods, the 3T3 method appears to be less labor-intensive and less expensive to conduct. Although it was slightly less reproducible than the NHK method, the 3T3 method was slightly higher in animal savings and accuracy in predicting GHS acute oral toxicity category.

ICCVAM also recommended standardized test method protocols and performance standards for the NHK and 3T3 methods, along with future studies, including:

- Evaluating the usefulness and limitations of the 3T3 method for predicting the rodent acute oral toxicity of chemical mixtures
- Collecting high-quality comparative *in vitro* basal cytotoxicity data when rat acute oral toxicity testing is conducted, to supplement the high-quality *in vitro* cytotoxicity validation database begun during this study
- Standardizing procedures to collect *in vivo* measurements and observations on lethality in rat acute oral toxicity studies
- Identifying additional *in vitro* tests and other methods to achieve more accurate acute oral hazard classification



Workshop on Acute Chemical Safety Testing



One recommendation of the Peer Review Panel was that ICCVAM consider convening a working group to explore mechanisms of action of acute toxicity, and approaches to acquiring additional information on acute toxic mechanisms when conducting required *in vivo* acute toxicity testing. SACATM supported this recommendation.

In response to this recommendation, NICEATM and ICCVAM will hold an international workshop on February 6-7, 2008.[32] ECVAM and JaCVAM are co-sponsors of the workshop, titled *Scientific Workshop on Acute Chemical Safety Testing: Advancing* In Vitro *Approaches and Humane Endpoints for Systemic Toxicity Evaluations*. The workshop objectives are to:

- Identify predictive and more humane endpoints that may be used to end studies earlier, which can be expected to further reduce or avoid pain and distress
- Identify and standardize procedures for collecting mechanistic information from acute oral toxicity testing that will help develop batteries of predictive *in vitro* test methods that can further reduce and perhaps eventually replace animals for acute toxicity testing

[32] More information on the workshop can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/meetings/AcuteToxWksp08/AcuteToxWksp08.htm.

Assessing the Validation Status of New Modifications and Applications of the Murine Local Lymph Node Assay (LLNA), a Test Method for Identifying Skin Sensitization Potential of Chemicals

The LLNA was the first alternative toxicity test method evaluated by ICCVAM. The LLNA is a method for assessing the potential of substances to induce allergic contact dermatitis, i.e., to be skin sensitizers. In 1999, ICCVAM concluded that the LLNA is a valid alternative to currently accepted guinea pig skin sensitization test methods, and that the LLNA reduces the number of animals required for testing and, for most testing situations, avoids animal pain and distress. The LLNA was later incorporated into national and international test guidelines (OECD Test Guideline 429 [OECD 2002]; International Organization for Standardization [ISO] 10993-10: Tests for irritation and delayed-type hypersensitivity [ISO 2002]; EPA Health Effects Test Guideline OPPTS 870.2600 on skin sensitization [EPA 2003]).

In January 2007, the U.S. Consumer Product Safety Commission requested that NICEATM and ICCVAM assess the validation status of:

- The LLNA as a stand-alone assay for determining potency for classification purposes
- Three alternative LLNA protocols using non-radiolabeled probe chemicals
- The LLNA limit test[33]
- The use of the LLNA to test mixtures, aqueous solutions, and metals

ICCVAM reviewed the nomination, assigned it a high priority, and proposed that NICEATM, in conjunction with the ICCVAM Immunotoxicity Working Group, prepare comprehensive BRDs summarizing the available data and relevant information. Because the concept of performance standards did not exist at the time of the original review, ICCVAM also recommended that performance standards be developed as part of the evaluation.

An *FR* notice was issued (Vol. 72, No. 95, pp. 27815-27816, May 17, 2007) requesting public comment on these activities, nominations for the independent Peer Review Panel, and submissions of relevant data. Among the responses received were data submissions from seven sources and nominations of potential members for the Peer Review Panel.[34]

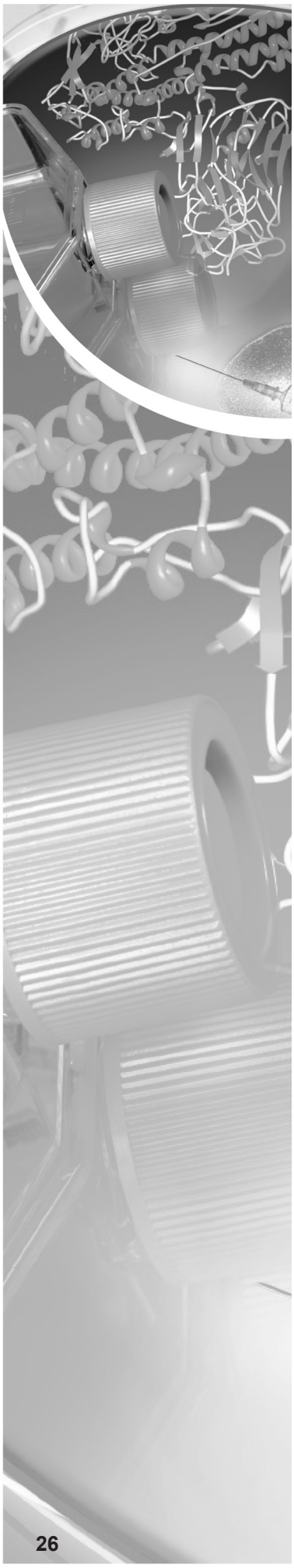
Draft LLNA performance standards were published in September 2007; the availability of this document for comments was announced in an *FR* notice (Vol. 72, No. 176, pp. 52130-52131, Sept. 12, 2007). Four public comments on the draft performance standards were received in response to the *FR* notice.[35] Draft BRDs will be prepared and made available to the Peer Review Panel and the public in early 2008. These documents will be considered at the Peer Review Panel meeting, scheduled for March 4-6, 2008.[36] This evaluation is expected to support broader use of the LLNA for regulatory testing of chemicals and products for their potential to produce allergic contact dermatitis, which should further reduce and refine animal use for this purpose.

[33] The LLNA limit test is also known as the "reduced LLNA."

[34] Comments and nominations received in response to the *FR* notice can be viewed at http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm?ftitle=E7-9544.

[35] Comments on the draft LLNA performance standards can be viewed at http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm?ftitle=E7-18011.

[36] All materials associated with this meeting can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm.



Alternative Methods for Assessing Potency of Botulinum Toxin

On November 13-14, 2006, ICCVAM and NICEATM, in conjunction with ECVAM, held a workshop titled *Scientific Workshop on Alternative Methods to Refine, Reduce, and Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing*. The workshop was held in response to a nomination from the Humane Society of the United States requesting that alternative test methods to the mouse LD_{50} assay for botulinum toxin potency testing be assessed and prioritized for prevalidation and validation efforts.[37]

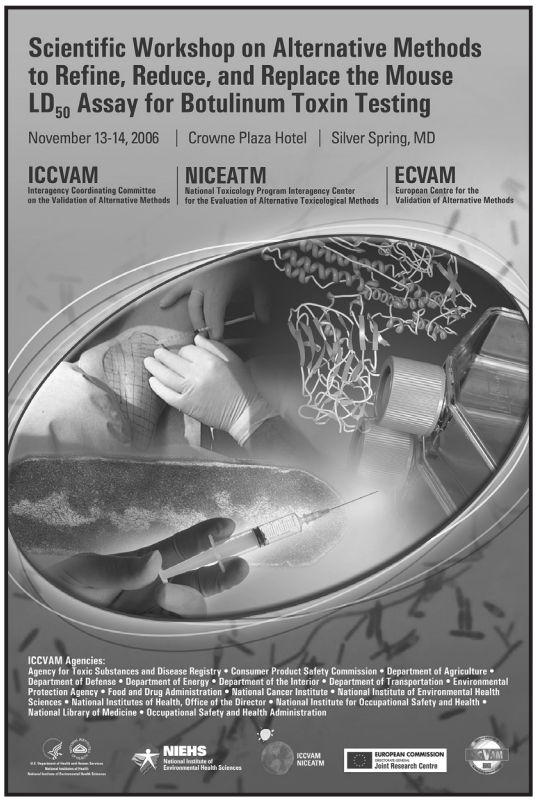
The goals of the workshop were to:

- Review the state-of-the-science and current knowledge of alternatives that may reduce, replace, and refine (decrease pain and distress) the use of mice for botulinum toxin testing
- Identify priorities for research, development, and validation efforts needed to advance the use of alternative methods

Specific objectives of the workshop included:

- Review the public health needs for botulinum toxin testing, including the need to determine the safety and efficacy of products containing botulinum toxin
- Review the current state-of-the-science, identify knowledge gaps regarding botulinum toxin structural aspects, mechanisms, and modes of action that are important to developing alternative methods for *in vivo* botulinum toxin tests, and prioritize future research initiatives that would address these knowledge gaps
- Review current development and/or validation status of alternative test methods for *in vivo* botulinum toxin tests and their potential to reduce, refine (decrease pain and distress), or replace the use of the mouse LD_{50} assay

[37] The Humane Society nomination can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/biologics/botdocs/HSUSnomLD50.pdf.



- Identify alternative methods that should have the highest priority for future development and validation studies to assess potency/toxicity of botulinum toxin

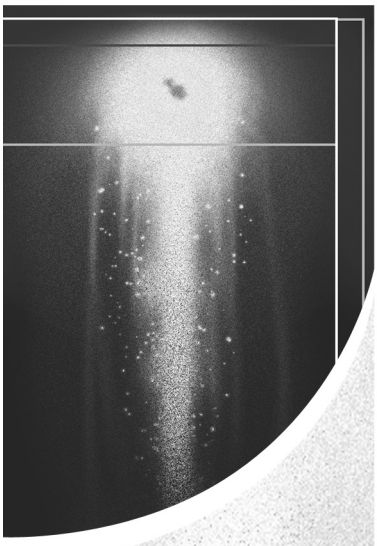
In general, the consensus of the workshop participants was that the reviewed methods could be used, in specific circumstances or in a tiered-testing strategy, to reduce and refine the use of mice in the current botulinum toxin test method. However, none of the reviewed methods can currently be used as a complete replacement for the botulinum toxin test method. The workshop participants felt that, with additional development and validation efforts, one or more of the reviewed methods might be useful as a replacement for the current botulinum toxin test methods in the near future. Participants noted that most methods needed additional validation studies, including comparing results to *in vivo* outcomes and validation of methods for specific applications.

Some best practices discussed to decrease the number of animals tested for studies included:

- Use of reference standards to minimize the number of replicate animals needed
- Use of standardized methodologies
- Reduction in the number of doses tested for assays where confirmation of potency is being evaluated

Proceedings of the workshop can be viewed on the NICEATM-ICCVAM website. A workshop report is currently in preparation and will be published in 2008.[38]

[38] Proceedings of the workshop can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/biologics/bot_workshop.htm.



Chapter 4

ICCVAM Outreach and Cooperative Activities

Collaborations with ECVAM and JaCVAM

ICCVAM and NICEATM continue to cooperate with ECVAM and JaCVAM on the validation and evaluation of alternative test methods, and the depth and frequency of these interactions have steadily increased during 2006 and 2007. The aim of this extensive cooperation is to (a) make the best use of resources and scientific expertise, (b) maximize efficiency of evaluation/validation efforts and minimize duplication of effort, (c) ensure an early exchange of information concerning planned test method validation studies and evaluations of test methods, (d) develop harmonized recommendations on the demonstrated usefulness and limitations of new test methods, and (e) to promote the international acceptance and implementation of scientifically valid test methods in Europe, Japan, and the United States. These interactions are also expected to promote and expedite the development of OECD test guidelines by providing harmonized standardized protocols supported by detailed test method evaluation reports of their scientific validity.

International Validation Study on In Vitro *Test Methods to Detect ER-Interacting Substances*

As discussed in **Chapter 3**, NICEATM, ECVAM, and JaCVAM jointly designed and began a multiphased international validation study to evaluate the reproducibility and accuracy of the LUMI-CELL® ER TA test method, using three laboratories, one each in the United States, Europe, and Japan. The study began in 2007 and is expected to be completed in 2008, with peer review panel evaluation in 2009. This is the first joint validation study sponsored by all three validation organizations, and represents an important milestone in international cooperation in validating alternative methods.

International Comet Assay Validation Study

The comet assay validation effort sponsored by JaCVAM is the second validation activity involving ICCVAM and NICEATM collaborating with ECVAM and JaCVAM. The initial focus is on validation of an *in vivo* rodent comet assay test method, using liver and stomach as the target organs, as a potential alternative for the *in vivo* rat hepatocyte unscheduled DNA synthesis (UDS) assay. The UDS assay is currently accepted by regulatory agencies as an *in vivo* genotoxicity test for hazard identification. However, the comet assay can be integrated into other testing protocols such as subacute tests, potentially reducing animal use, and can be used to assess genotoxicity in multiple tissues, as opposed to liver only for the UDS test. Also being evaluated is an *in vitro* comet assay for detecting genotoxic substances using the human B lymphoblastoid TK6 cell line.

Key accomplishments on this project during 2006 and 2007 included developing a standardized *in vivo* comet assay protocol, evaluating data from inter-laboratory reproducibility studies, considering the number and types of chemicals to be used in the validation effort, and issuing an open invitation for experienced laboratories to participate.

Other Collaborations

Other activities where ICCVAM and NICEATM have collaborated or cooperated with ECVAM and/or JaCVAM during this reporting period include the following:

- The Chair of ICCVAM and the Director of NICEATM continued to participate in ECVAM Scientific Advisory Committee (ESAC) meetings as official observers. ESAC meetings were held in March and November 2006, and in April and October 2007.
- The Head of ECVAM and the Director of JaCVAM continued to participate in SACATM meetings as ex officio non-voting liaison members.
- ICCVAM members and ICCVAM-nominated experts participated on ECVAM taskforces, workshops, and validation management teams in 2006-2007. These activities included:
  - ECVAM workshop on the "Consistency of Production Approach and Its Potential to Reduce Animal Tests in the Quality Control of Vaccines" held in Ispra, Italy, in May 2006. A report of the workshop was published in the journal *Biologicals* (Hendriksen et al. 2008).
  - ECVAM Workshop on Reference Standards in Validation in January 2006
- ICCVAM evaluated five *in vitro* test methods proposed for assessing potential pyrogenicity of pharmaceuticals and other products (see **Chapter 3**) in response to an ECVAM submission.
- Each ICCVAM working group active during the reporting period (see **Chapter 3**) also included a liaison from ECVAM and JaCVAM.
- ICCVAM requested nominations of experts from ECVAM and JaCVAM for the LLNA and pyrogenicity test methods international independent scientific peer review panels.
- ICCVAM, NICEATM, and ECVAM co-sponsored the *Scientific Workshop on Alternative Methods to Refine, Reduce, and Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing* (see **Chapter 3**).
- ICCVAM Vice-Chair Dr. Jodie Kulpa-Eddy (U.S. Department of Agriculture) and ICCVAM member Dr. Devaraya Jagannath (FDA Center for Veterinary Medicine) presented information on ICCVAM-NICEATM/ECVAM/JaCVAM collaborations at the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Steering Committee meeting on January 25, 2007, in Washington, D.C. VICH is a trilateral (European Union, Japan, U.S.) program consisting of regulatory and industry representatives, aimed at harmonizing technical requirements for veterinary product registration.
- ICCVAM, NICEATM, ECVAM, and JaCVAM are jointly organizing the *Scientific Workshop on Acute Chemical Testing: Advancing* In Vitro *Approaches and Humane Endpoints for Systemic Toxicity Evaluations,* to be held in February 2008 (see **Chapter 3**).

Important interactions among ICCVAM, NICEATM, ECVAM, and JaCVAM include:

- *Joint validation study on* in vitro *test methods to detect substances that interact with estrogen receptors*
- *Joint validation study on the use of the comet assay to identify substances that may cause genetic damage*
- *Co-sponsorship of a workshop in 2006 and a workshop scheduled for 2008*

ICCVAM Participation in National and International Workshops, Conferences, and Meetings

ICCVAM and NICEATM members participated in numerous international workshops, conferences, and meetings in 2006 and 2007. Brief descriptions of selected events are provided below.

Please note that any conclusions and recommendations outlined below are those of the respective workshop participants, and the inclusion of these conclusions and recommendations in this report should not be interpreted as an endorsement by ICCVAM or any of its member agencies.

Third Annual Conference of the European Partnership for Alternative Approaches to Animal Testing (EPAA)

The Third Annual Conference of the EPAA took place in Brussels, Belgium, on November 5, 2007. Dr. William Stokes, Director of NICEATM, participated on a Panel Discussion on "International Aspects of Validation and Acceptance," and Dr. Abigail Jacobs spoke on "Regulatory Acceptance of Alternative Methods: Pharmaceuticals in the U.S." The EPAA is a joint initiative of the European Commission and a number of companies and trade federations active in various industrial sectors. The EPAA's work focuses on mapping existing research, developing new alternative approaches and strategies, and promoting communication, education, validation and acceptance of alternative approaches.

International Cooperation on Cosmetics Regulation (ICCR)

The first meeting of the ICCR took place in Brussels, Belgium, on September 26-28, 2007. Alternatives to animal testing was one of five agenda items. Dr. William Stokes participated as part of the U.S. delegation to provide information about ICCVAM-NICEATM progress, activities, and plans. ICCR is a voluntary international group of cosmetics regulatory authorities from the U.S., Japan, the European Union, and Canada. The group was created to identify ways to remove obstacles among the regions in the area of cosmetics regulations, while maintaining the highest level of global consumer protection.

ECVAM Workshop on Alternative Endpoints for the Local Lymph Node Assay (LLNA)

On September 25-27, 2007, an ECVAM-sponsored workshop was held in Ispra, Italy, to review the status of modified LLNA protocols using alternative endpoints, including their suitability for the purpose of identifying skin sensitizers, and to make recommendations for additional studies necessary to adequately demonstrate their usefulness for this purpose. Dr. William Stokes and Dr. Joanna Matheson attended the workshop and presented an overview on ICCVAM LLNA activities and the draft LLNA performance standards published by ICCVAM for public comment several weeks before the workshop.

Workshop on Reducing False Positive Results with In Vitro *Genotoxicity Testing*

The *in vitro* genetic toxicology tests used for regulatory purposes measure DNA damage (gene mutations and chromosomal changes) induced by tested compounds and help predict the carcinogenic potential of test substances. The high false-positive rate of the established *in vitro* mammalian cell tests means that many compounds are subjected to earlier and additional *in vivo* genotoxicity testing. Because part of the NTP testing program is for genotoxicity, efforts to reduce the false-positive rate for *in vitro* tests could have a significant impact on how the NTP testing program is conducted.

To address the high rate of false-positive results, ECVAM hosted and sponsored a workshop in Ispra, Italy, April 26–28, 2006. More than 20 experts from academia, government, and industry were invited to contribute. This meeting considered ways to improve the selection or conduct of existing mammalian cell genotoxicity assays, identified new test methods that show promise as candidates for mammalian cell genotoxicity testing, and prioritized future activities that may reduce animal use in genotoxicity testing. Dr. Raymond Tice, Deputy Director of NICEATM, attended the meeting and served as co-chair of one of three breakout groups. For more details of the workshop, please see Kirkland et al. (2007).

WHO/IPCS Workshop on Skin Sensitization in Chemical Risk Assessment

A workshop on skin sensitization in chemical risk assessment, sponsored by the World Health Organization's International Programme on Chemical Safety (WHO/IPCS), was conducted in Berlin, Germany, October 17-18, 2006. This workshop focused on skin sensitization arising from exposure to chemicals. It aimed to evaluate experimental techniques for both identifying and classifying hazards. The ultimate goal was to evaluate how well these techniques produce data to inform risk assessment, including providing information about dose response and sensitive subpopulations. The meeting also explored whether methods that identify skin sensitizers could be applied to develop methods to identify chemicals that could sensitize the respiratory tract. Dr. Marilyn Wind, Chair of ICCVAM, and Dr. Abigail Jacobs participated in the workshop.

ICCVAM and NICEATM participated in the following national and international meetings:

- *Third Annual Conference of the European Partnership for Alternative Approaches to Animal Testing*
- *International Cooperation on Cosmetics Regulation*
- *ECVAM workshop on* in vitro *genotoxicity testing*
- *ECVAM workshop on LLNA performance standards*
- *WHO/IPCS workshop on skin sensitization*
- *20th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments*
- *6th World Congress on Alternatives and Animal Use in the Life Sciences*
- *45th and 46th Annual Meetings of the Society of Toxicology*
- *TestSmart DNT symposium on developmental neurotoxicity testing*

20th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments (JSAAE)

Dr. Raymond Tice and former ICCVAM Chair Dr. Leonard Schechtman attended the 20th Annual Meeting of the JSAAE, held December 8-9, 2006, in Tokyo, Japan. This meeting was primarily a forum for Japanese scientists to present advances in developing and validating alternative test methods. It also served as a venue for representatives from China, India, Korea, European Union (ECVAM), and the United States (NICEATM-ICCVAM) to present and discuss the current state of affairs with regard to developing, validating, and implementing alternative test methods and the 3Rs in their respective countries or regions. The upcoming formation of a Korean Center for the Validation of Alternative Methods (KoCVAM), and plans for China to organize a similar institution, were announced at this meeting. Dr. Schechtman gave a presentation summarizing the current practices of ICCVAM, and Dr. Tice reviewed the purpose and activities of NICEATM.

6th World Congress on Alternatives and Animal Use in the Life Sciences (WC6)

NICEATM and ICCVAM were active participants in WC6, held in Tokyo, Japan, August 21-25, 2007. This international biennial conference included sessions on developing, validating, and evaluating alternative test methods that might be used to reduce, refine, and/or replace the use of animals in regulatory testing strategies. The specific objectives of WC6 were to:

- Review progress made toward reduction, refinement, and replacement in education, research, and testing
- Develop a realistic understanding of the status of alternatives
- Create an understanding that in research, animal studies, together with clinical studies and *in vitro* methods, advance science
- Contribute to our basic understanding of biology and diseases
- Encourage constructive discussions among animal protection groups and scientific communities

Dr. William Stokes and Dr. Raymond Tice both served on the WC6 Organizing Committee, and served on the Scientific Program Committee along with Dr. Margaret Snyder. Dr. Stokes co-chaired scientific sessions on "Validation" and "Research, Development and Evaluation of Alternatives for Ocular Toxicity." Dr. Tice co-chaired the sessions "Comet Assay" and "Alternatives for Acute Systemic Toxicity." Two ICCVAM committee members, Dr. Richard McFarland and Dr. Margaret Snyder, co-chaired sessions on "Achievement in Other Biologicals Quality Control" and "Public Participation on Decision-Making on Animal Use in Different Cultural Contexts," respectively. Twelve NICEATM and ICCVAM scientists from seven Federal agencies attended WC6, contributing to 12 platform and 11 poster presentations. Titles and authors of these presentations are included in **Chapter 6** of this report.[39]

45th and 46th Annual Meetings of the Society of Toxicology (SOT)

ICCVAM and NICEATM participated in the 2006 and 2007 annual meetings of the SOT during the reporting period.

The 45th Annual SOT Meeting was held on March 5-9, 2006, in San Diego, CA. Nine members of ICCVAM and NICEATM contributed to seven poster presentations. Titles and authors of these presentations are included in **Chapter 6** of this report.[40]

The 46th Annual SOT Meeting was held on March 25-29, 2007, in Charlotte, NC. Nineteen members of ICCVAM and NICEATM contributed to one platform and 12 poster presentations. Titles and authors of all presentations are included in **Chapter 6** of this report.[41]

TestSmart DNT: Creating a Humane and Efficient Approach to Developmental Neurotoxicity Testing

A symposium on alternative approaches to developmental neurotoxicity (DNT) testing, organized by the Johns Hopkins University Center for Alternatives for Animal Testing and co-sponsored by NIEHS/NTP, was held on March 13-15, 2006, in Reston, VA. Current methods for DNT testing are complex and expensive in terms of scientific resources, time, and animal use. TestSmart DNT is a long-term program aimed at identifying methods for DNT testing that meet government requirements, enhance decision-making, and promote humane science. This symposium was the first of a series bringing together leading stakeholders to develop the DNT testing methods of the future.[42] Dr. Marilyn Wind spoke to the symposium on "ICCVAM: Successes and Future Vision." Dr. William Stokes gave a presentation on "Alternative Approaches to Validation."

[39] More detailed information, including abstracts and posters, can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/meetings/6thWC/6WCablst.htm.

[40] More detailed information, including abstracts and posters, can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/meetings/SOT06/sotablst.htm.

[41] More detailed information, including abstracts and posters, can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/meetings/SOT07/sotablst.htm.

[42] Further information on the symposium can be found at http://caat.jhsph.edu/programs/workshops/testsmart/dnt/proceedings/index.htm.

Chapter 5
NICEATM-ICCVAM Five-Year Plan

In response to requests from the Appropriations Committees of the U.S. House of Representatives and U.S. Senate, ICCVAM and NICEATM prepared a five-year plan to describe goals and objectives for the years 2008 through 2012. This plan was developed in conjunction with Federal agency program offices, and describes how NICEATM and ICCVAM will promote the research, development, translation, validation, and regulatory acceptance of alternative test methods that reduce, refine, and replace the use of animals in testing while maintaining scientific quality and protecting human health, animal health, and the environment.

A draft plan was provided to the public on May 7, 2007 (announced in an *FR* notice: Vol. 72, No. 83, pp. 23832-23833, May 1, 2007). The plan identified four key challenges:

- Identifying priorities and conducting and facilitating alternative test method activities
- Incorporating new science and technology
- Fostering regulatory acceptance and appropriate use of alternative methods
- Developing partnerships and strengthening interactions with ICCVAM stakeholders

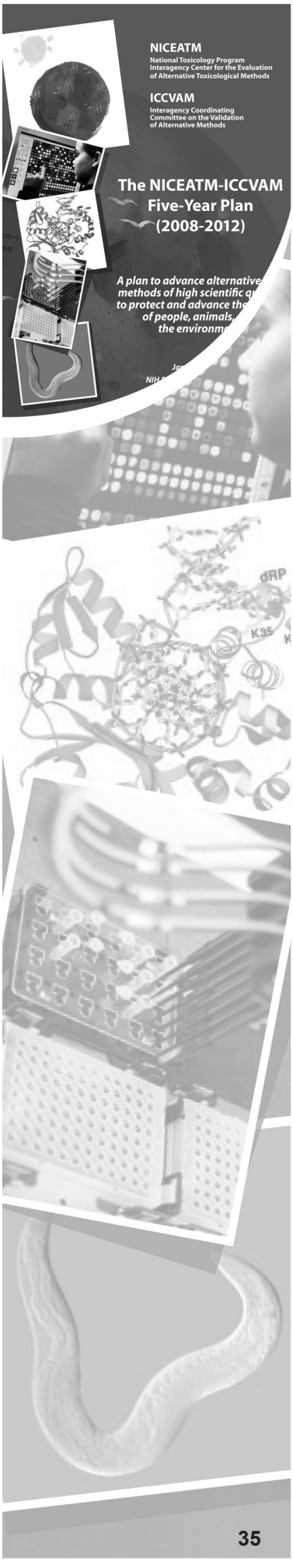
ICCVAM and NICEATM organized a Town Hall meeting on June 11, 2007, to obtain additional comments on the Draft Plan from interested stakeholders. The complete, final NICEATM-ICCVAM Five-Year Plan will be released at a Ten-Year Anniversary Symposium to be held in February 2008 (announced in an *FR* notice: Vol. 72, No. 245, pp. 72727-72729, Dec. 21, 2007) and will be available on the NICEATM-ICCVAM website after the Symposium.[43]

Identifying Priorities and Conducting and Facilitating Alternative Test Method Activities

The first key challenge for ICCVAM and NICEATM is to identify priority areas and conduct and facilitate alternative test method activities in these areas. ICCVAM identified priorities that focus on alternatives for those regulatory test methods that potentially use large numbers of animals and involve significant animal pain and distress. The four highest-priority testing areas are currently ocular (eye) toxicity, dermal (skin) toxicity, acute systemic toxicity (poisoning), and biologics. Other high-priority testing areas include immunotoxicity, endocrine disruptors, pyrogenicity, reproductive and developmental toxicity, and chronic toxicity and carcinogenicity. Neurotoxicity testing is also an area of interest. Although these represent current priorities and interests, ICCVAM and NICEATM recognize that planning must be flexible in order to take advantage of advances in science and technology and to respond to new testing needs. Integrated testing approaches will be emphasized to effectively address the inherent complexity of human and animal responses to toxicants while maximizing the impact of new testing alternatives to reduce, refine, and replace animal use. Critical knowledge and data gaps will also be identified to more effectively promote high-priority research, development, translation, and validation of alternative test methods.

Incorporating New Science and Technology

ICCVAM and NICEATM will identify and promote research and technologies that have a high potential to yield new test methods and approaches that will reduce, refine or replace the use of animals. Although many of these new test methods will require several years to develop and validate, some may be ready for use more quickly. To maximize the efficiency of this process, ICCVAM and NICEATM are working with Federal agencies and other stakeholders to link research and development activities to the standardization and validation of alternative test methods applicable to regulatory testing.



[43] The Five-Year Plan can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/5yearplan.htm. Information about the Ten-Year Anniversary Symposium can be found at http://iccvam.niehs.nih.gov/meetings/10thAnnivSymp/10thAnnivSymp.htm.

Fostering Regulatory Acceptance and Appropriate Use of Alternative Methods

ICCVAM and NICEATM will foster regulatory acceptance and appropriate use of alternative test methods by promoting active communication and outreach efforts with both government and non-governmental stakeholders. ICCVAM and NICEATM will provide high-quality comprehensive BRDs and test method evaluation reports, based on independent scientific peer reviews, to expedite acceptance decisions on these test methods by regulatory agencies and the international community. Once an alternative test method has been accepted, ICCVAM will work to promote the use of the test method by sponsoring and participating in training workshops for interested stakeholders who may generate or review data from the test method.

Developing Partnerships and Strengthening Interactions with ICCVAM Stakeholders

ICCVAM and NICEATM will further develop partnerships and strengthen interactions with stakeholders while considering advice from SACATM. The overall aims of these partnerships are to make the best use of existing resources and scientific expertise, maximize the efficiency of test method validation and evaluation processes, minimize duplication of effort, and ensure early exchange of information concerning test method validation. This will promote and expedite national and international recognition, acceptance, and implementation of scientifically valid alternative test methods.

The Five-Year Plan Development Process

Consistent with all ICCVAM and NICEATM document publications, the process of producing the report allowed for transparency and several opportunities for public comment consistent with the deadline for providing the report (see **Figure 5**). This process provided six separate opportunities for public comments: two opportunities during the early, planning stages of the report, and four opportunities for comment on the draft report to be considered before it was finalized. The timeline also included two opportunities for review and comment on the plan by SACATM: one during the planning process and one after the draft report was released.

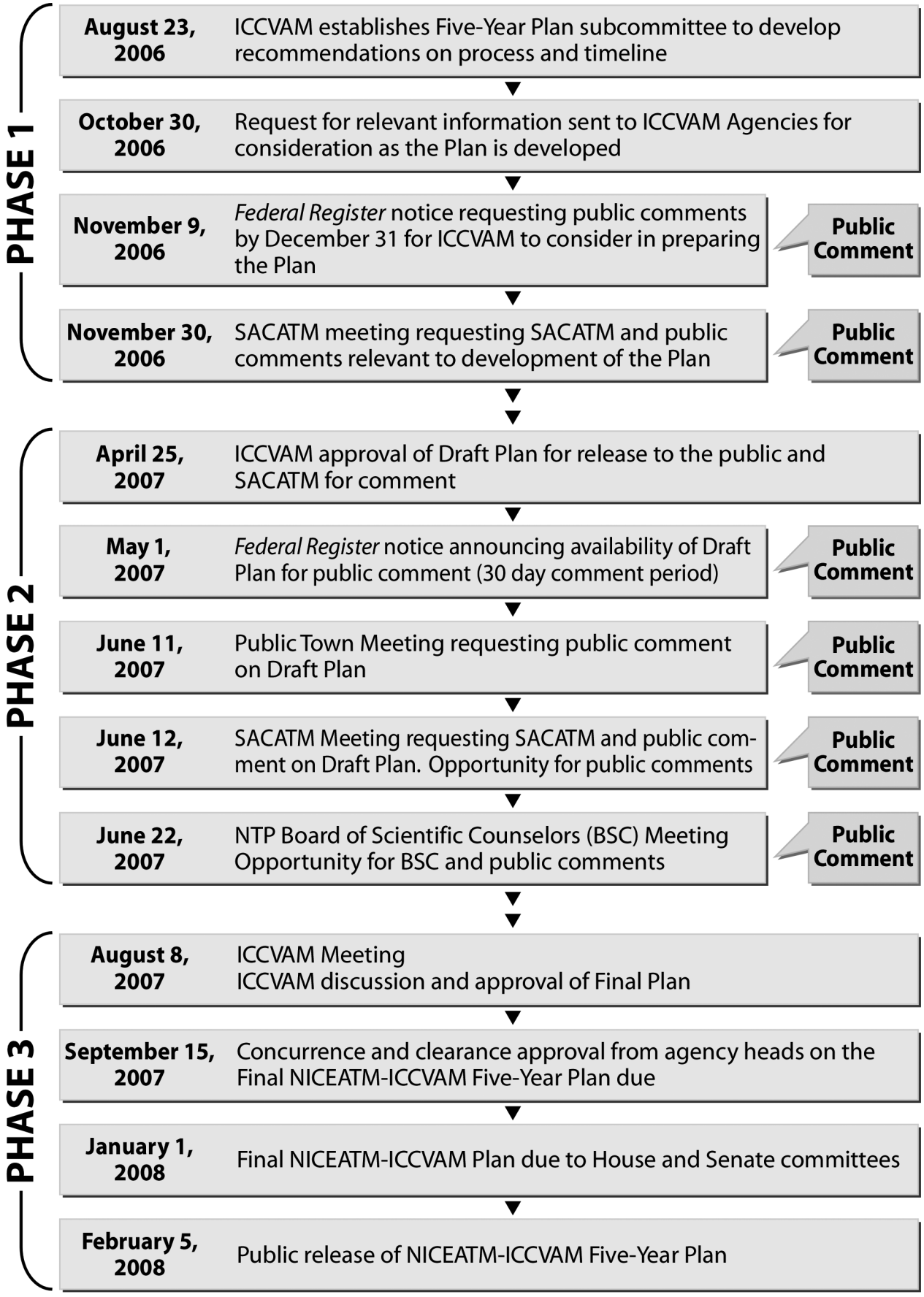
The plan was developed in three phases. The first phase involved soliciting and receiving input from all 15 of the ICCVAM agencies regarding priorities and ongoing activities for developing and implementing alternative testing methods. An *FR* notice (Vol. 71, No. 218, pp. 66172-66173, Nov. 9, 2006) was also published soliciting comments and suggestions from the public. Nine responses to this notice were received and considered in developing the draft plan.

During the second phase, the ICCVAM Five-Year Plan Subcommittee considered the comments and suggestions received from agencies and the public. In conjunction with NICEATM, the subcommittee prepared an initial draft of the plan for review and comment by the full ICCVAM committee and the 15 ICCVAM agencies. After this review, comments and suggestions were incorporated into a draft plan released to the public for comment. Availability of the draft plan and a Town Meeting to allow an opportunity for public comment were announced in an *FR* notice (Vol. 72, No. 83, pp. 23832-23833, May 1, 2007). More than 300 comments were received in response to this announcement, offering both specific comments on the draft plan and general encouragement to ICCVAM to increase efforts to identify non-animal methods for toxicity testing. The Town Meeting was held on June 11, 2007 at NIH in Bethesda, MD. SACATM met on June 12 to consider and comment on the draft plan and consider comments from the Town Meeting.

The final phase of the Five-Year Plan development process focused on ICCVAM and NICEATM finalizing the draft plan, taking into consideration public and SACATM comments. The finalized plan was sent to ICCVAM agency heads, who indicated unanimous concurrence. NIEHS forwarded the plan to NIH and the Department of Health and Human Services for clearance and transmittal to the U.S. House of Representatives and U.S. Senate Appropriations Committees. In February 2008, the plan will be released to the public and will be available at the NICEATM-ICCVAM website.[44]

[44] The Five-Year Plan can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/5yearplan.htm.

Figure 5. Development of the NICEATM-ICCVAM Five-Year Plan



Chapter 6

NICEATM-ICCVAM Communications

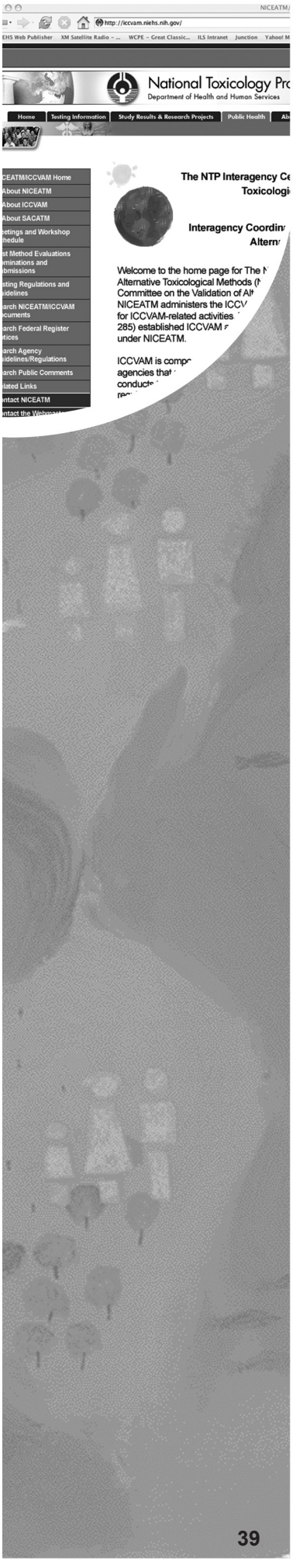
NICEATM-ICCVAM Website

The NICEATM-ICCVAM website is a vital tool for effective communication with stakeholders. The site receives approximately 15,000 visits per month, indicating a high level of public interest. Recognizing that the Internet represents a primary source of information for ICCVAM stakeholders, NICEATM launched a redesigned and enhanced version of the website (http://iccvam.niehs.nih.gov/) in March 2007.

The new website provides four searchable databases that contain *FR* notices, public comments, NICEATM-ICCVAM publications, and guidelines and regulations. Additional searchable databases containing information for specific test method areas are currently under development. Visitors to the website can also obtain up-to-date information on test method evaluation activities, relevant guidelines and regulations, and meeting and workshop information. Procedures for making nominations and submissions to ICCVAM and background information concerning ICCVAM and NICEATM are also available. Online forms enable visitors to submit public comments to NICEATM and register for NICEATM meetings and workshops. Persistent menus (identical menus appearing on every page of the site) and a site map enable easy navigation throughout the website, and all information is presented in a manner that is consistent with U.S. Federal Government Plain Language Guidelines. The revised NICEATM-ICCVAM website will play a key role in promoting effective communication between NICEATM-ICCVAM and its stakeholders.

NICEATM-ICCVAM Color Brochure

In March 2007, NICEATM published a brochure summarizing the purpose, duties, and accomplishments of ICCVAM and NICEATM. This color brochure is distributed at scientific meetings attended by ICCVAM and NICEATM (e.g., SOT and WC6) and at public meetings sponsored by ICCVAM and NICEATM.[45]



[45] The brochure can be viewed on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/about_docs/NICEATMTriFold.pdf.

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ICCVAM Contributions to OECD Test Guidelines and Guidance Documents

During 2006 and 2007, ICCVAM actively participated in the development and national review of OECD guidelines for the testing of chemicals. OECD test guidelines represent internationally agreed testing methods that can be used by government, industry, and independent laboratories in the 30 member countries to determine the safety of chemicals and chemical preparations.[46]

In 2006-2007, NICEATM and ICCVAM participated in the following OECD Test Guidelines Program activities:

- Drafted and submitted OECD Standard Project Submission Forms for test methods under evaluation by ICCVAM:
  - Stably Transfected Transcriptional Activation Assay for the Detection of Estrogen Receptor Agonists and Antagonists
  - BCOP and ICE ocular toxicity test methods.
- Reviewed and provided comments on draft test guidelines (TG) for:
  - The Uterotrophic Bioassay in Rodents: A Short-Term Screening Test for Oestrogenic Properties
  - The Hershberger Bioassay in Rats
  - Updated TG 407, Repeated Dose 28-Day Oral Toxicity Study in Rodents
  - Stably Transfected Transcriptional Activation Assay for Detecting Estrogenic Activity of Chemicals
  - Draft TG 433: Acute Inhalation Toxicity—Fixed Concentration Procedure
  - Draft TG 487: *In Vitro* Micronucleus Test
- Reviewed and commented on the OECD Standard Project Submissions for:
  - Development of a Detailed Review Paper on Availability of *In Vitro* Receptor Assays in Fish for Screening of Endocrine Modulating Activities of Environmental Chemicals
  - New Mammalian Level 5 Test Involving Various Life Stages

[46] See OECD Test Guidelines Home Page: http://www.oecd.org/document/40/0,2340,en_2649_34377_37051368_1_1_1_1,00.html.

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Appendix A

The ICCVAM Authorization Act of 2000 (Public Law 106-545, December 19, 2000)

PUBLIC LAW 106–545—DEC. 19, 2000 114 STAT. 2721

Public Law 106–545 106th Congress 42 U.S.C. 285*l*-3

An Act

To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE.

This Act may be cited as the ‘‘ICCVAM Authorization Act of 2000’’.

SEC. 2. DEFINITIONS.

In this Act:

(1) ALTERNATIVE TEST METHOD.—The term ‘‘alternative test method’’ means a test method that—

(A) includes any new or revised test method; and

(B) (i) reduces the number of animals required;

(ii) refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being; or

(iii) replaces animals with non-animal systems or one animal species with a phylogenetically lower animal species, such as replacing a mammal with an invertebrate.

(2) ICCVAM TEST RECOMMENDATION.—The term ‘‘ICCVAM test recommendation’’ means a summary report prepared by the ICCVAM characterizing the results of a scientific expert peer review of a test method.

SEC. 3. INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS.

(a) IN GENERAL.—With respect to the interagency coordinating committee that is known as the Interagency Coordinating Committee on the Validation of Alternative Methods (referred to in this Act as ‘‘ICCVAM’’) and that was established by the Director of the National Institute of Environmental Health Sciences for purposes of section 463A(b) of the Public Health Service Act, the Director of the Institute shall designate such committee as a permanent interagency coordinating committee of the Institute under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods. This Act may not be construed as affecting the authorities of such Director regarding ICCVAM that were in effect on the day before the date of the enactment of this Act, except to the extent inconsistent with this Act.

(b) PURPOSES.—The purposes of the ICCVAM shall be to—

(1) increase the efficiency and effectiveness of Federal agency test method review;

(2) eliminate unnecessary duplicative efforts and share experiences between Federal regulatory agencies;

(3) optimize utilization of scientific expertise outside the Federal Government;

(4) ensure that new and revised test methods are validated to meet the needs of Federal agencies; and

(5) reduce, refine, or replace the use of animals in testing, where feasible.

(c) COMPOSITION.—The ICCVAM shall be composed of the heads of the following Federal agencies (or their designees):

(1) Agency for Toxic Substances and Disease Registry.

(2) Consumer Product Safety Commission.

(3) Department of Agriculture.

(4) Department of Defense.

(5) Department of Energy.

(6) Department of the Interior.

(7) Department of Transportation.

(8) Environmental Protection Agency.

(9) Food and Drug Administration.

(10) National Institute for Occupational Safety and Health.

(11) National Institutes of Health.

(12) National Cancer Institute.

(13) National Institute of Environmental Health Sciences.

(14) National Library of Medicine.

(15) Occupational Safety and Health Administration.

(16) Any other agency that develops, or employs tests or test data using animals, or regulates on the basis of the use of animals in toxicity testing.

(d) SCIENTIFIC ADVISORY COMMITTEE.—

(1) ESTABLISHMENT.—The Director of the National Institute of Environmental Health Sciences shall establish a Scientific Advisory Committee (referred to in this Act as the ‘‘SAC’’) to advise ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods regarding ICCVAM activities. The activities of the SAC shall be subject to provisions of the Federal Advisory Committee Act.

(2) MEMBERSHIP.—

(A) IN GENERAL.—The SAC shall be composed of the following voting members:

(i) At least one knowledgeable representative having a history of expertise, development, or evaluation of new or revised or alternative test methods from each of—

(I) the personal care, pharmaceutical, industrial chemicals, or agriculture industry;

(II) any other industry that is regulated by the Federal agencies specified in subsection (c); and

(III) a national animal protection organization established under section 501(c)(3) of the Internal Revenue Code of 1986.

(ii) Representatives (selected by the Director of the National Institute of Environmental Health Sciences) from an academic institution, a State government agency, an international regulatory body, or any corporation developing or marketing new or revised or alternative test methodologies, including contract laboratories.

(B) NONVOTING EX OFFICIO MEMBERS.—The membership of the SAC shall, in addition to voting members under subparagraph (A), include as nonvoting ex officio members the agency heads specified in subsection (c) (or their designees).

(e) DUTIES.—The ICCVAM shall, consistent with the purposes described in subsection (b), carry out the following functions:

(1) Review and evaluate new or revised or alternative test methods, including batteries of tests and test screens, that may be acceptable for specific regulatory uses, including the coordination of technical reviews of proposed new or revised or alternative test methods of interagency interest.

(2) Facilitate appropriate interagency and international harmonization of acute or chronic toxicological test protocols that encourage the reduction, refinement, or replacement of animal test methods.

(3) Facilitate and provide guidance on the development of validation criteria, validation studies and processes for new or revised or alternative test methods and help facilitate the acceptance of such scientifically valid test methods and awareness of accepted test methods by Federal agencies and other stakeholders.

(4) Submit ICCVAM test recommendations for the test method reviewed by the ICCVAM, through expeditious transmittal by the Secretary of Health and Human Services (or the designee of the Secretary), to each appropriate Federal agency, along with the identification of specific agency guidelines, recommendations, or regulations for a test method, including batteries of tests and test screens, for chemicals or class of chemicals within a regulatory framework that may be appropriate for scientific improvement, while seeking to reduce, refine, or replace animal test methods.

(5) Consider for review and evaluation, petitions received from the public that— (A) identify a specific regulation, recommendation, or guideline regarding a regulatory mandate; and (B) recommend new or revised or alternative test methods and provide valid scientific evidence of the potential of the test method.

(6) Make available to the public final ICCVAM test recommendations to appropriate Federal agencies and the responses from the agencies regarding such recommendations.

(7) Prepare reports to be made available to the public on its progress under this Act. The first report shall be completed not later than 12 months after the date of the enactment of this Act, and subsequent reports shall be completed biennially thereafter.

SEC. 4. FEDERAL AGENCY ACTION.

(a) IDENTIFICATION OF TESTS.—With respect to each Federal agency carrying out a program that requires or recommends acute or chronic toxicological testing, such agency shall, not later than 180 days after receiving an ICCVAM test recommendation, identify and forward to the ICCVAM any relevant test method specified in a regulation or industry-wide guideline which specifically, or in practice requires, recommends, or encourages the use of an animal acute or chronic toxicological test method for which the ICCVAM test recommendation may be added or substituted.

(b) ALTERNATIVES.—Each Federal agency carrying out a program described in subsection (a) shall promote and encourage the development and use of alternatives to animal test methods (including batteries of tests and test screens), where appropriate, for the purpose of complying with Federal statutes, regulations, guidelines, or recommendations (in each instance, and for each chemical class) if such test methods are found to be effective for generating data, in an amount and of a scientific value that is at least equivalent to the data generated from existing tests, for hazard identification, dose-response assessment, or risk assessment purposes.

(c) TEST METHOD VALIDATION.—Each Federal agency carrying out a program described in subsection (a) 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.

(d) REVIEW.—Not later than 180 days after receipt of an ICCVAM test recommendation, a Federal agency carrying out a program described in subsection (a) shall review such recommendation and notify the ICCVAM in writing of its findings.

(e) RECOMMENDATION ADOPTION.—Each Federal agency carrying out a program described in subsection (a), or its specific regulatory unit or units, shall adopt the ICCVAM test recommendation unless such Federal agency determines that—

(1) the ICCVAM test recommendation is not adequate in terms of biological relevance for the regulatory goal authorized by that agency, or mandated by Congress;

(2) the ICCVAM test recommendation does not generate data, in an amount and of a scientific value that is at least equivalent to the data generated prior to such recommendation, for the appropriate hazard identification, dose-response assessment, or risk assessment purposes as the current test method recommended or required by that agency;

(3) the agency does not employ, recommend, or require testing for that class of chemical or for the recommended test endpoint; or

(4) the ICCVAM test recommendation is unacceptable for satisfactorily fulfilling the test needs for that particular agency and its respective congressional mandate.

SEC. 5. APPLICATION.

(a) APPLICATION.—This Act shall not apply to research, including research performed using biotechnology techniques, or research related to the causes, diagnosis, treatment, control, or prevention of physical or mental diseases or impairments of humans or animals.

(b) USE OF TEST METHODS.—Nothing in this Act shall prevent a Federal agency from retaining final authority for incorporating the test methods recommended by the ICCVAM in the manner determined to be appropriate by such Federal agency or regulatory body.

(c) LIMITATION.—Nothing in this Act shall be construed to require a manufacturer that is currently not required to perform animal testing to perform such tests. Nothing in this Act shall be construed to require a manufacturer to perform redundant endpoint specific testing.

(d) SUBMISSION OF TESTS AND DATA.—Nothing in this Act precludes a party from submitting a test method or scientific data directly to a Federal agency for use in a regulatory program.

Approved December 19, 2000.

LEGISLATIVE HISTORY—H.R. 4281 (S. 1495):

HOUSE REPORTS: No. 106–980 (Comm. on Commerce).

SENATE REPORTS: No. 106–496 accompanying S. 1495 (Comm. on Health, Education,

Labor, and Pensions).

CONGRESSIONAL RECORD, Vol. 146 (2000):

Oct. 17, considered and passed House.

Dec. 6, considered and passed Senate.

Appendix B

NIH Revitalization Act, Sections 1301 and 205

Public Law 103-43
42 U.S.C. 285*l*-1 and 42 U.S.C. 283*e*:
A bill to amend the Public Health Service Act to revise and extend the programs of the National Institutes of Health, and for other purposes.

Item 81: (34) TITLE XIII--NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
Item 82: (32) SEC. 1301. APPLIED TOXICOLOGICAL RESEARCH AND TESTING PROGRAM

(a) In General.--Subpart 12 of part C of title IV of the Public Health Service Act (42 U.S.C. 2851) is amended by adding at the end the following section:

APPLIED TOXICOLOGICAL RESEARCH AND TESTING PROGRAM

Sec. 463A. (a) There is established within the Institute a program for conducting applied research and testing regarding toxicology, which program shall be known as the Applied Toxicological Research and Testing Program.

(b) In carrying out the program established under subsection(a), the Director of the Institute shall, with respect to toxicology, carry out activities--

(1) to expand knowledge of the health effects of environmental agents;

(2) to broaden the spectrum of toxicology information that is obtained on selected chemicals;

(3) to develop and validate assays and protocols, including alternative methods that can reduce or eliminate the use of animals in acute or chronic safety testing;

(4) to establish criteria for the validation and regulatory acceptance of alternative testing and to recommend a process through which scientifically validated alternative methods can be accepted for regulatory use;

(5) to communicate the results of research to government agencies, to medical, scientific, and regulatory communities, and to the public; and

(6) to integrate related activities of the Department of Health and Human Services.'

(b) Technical Amendment.--Section 463 of Public Health Service Act (42 U.S.C. 2851) is amended by inserting after 'Sciences' the following: '(in this subpart referred to as the Institute)'.

S.1 As finally approved by the House and Senate (Enrolled)

Item 35: (55) SEC. 205. PLAN FOR USE OF ANIMALS IN RESEARCH.

SEC. 205. PLAN FOR USE OF ANIMALS IN RESEARCH.

(a) In General - Part A of Title IV of the Pubic Health Service Act, as amended by section 204 of this Act, is amended by adding at the end the following new section:

PLAN FOR THE USE OF ANIMALS IN RESEARCH

SEC. 404C. (a) The Director of NIH, after consultation with the committee established under subsection (e), shall prepare a plan

(1) for the National Institutes of Health to conduct or support research into

(A) methods of medical research and experimentation that do not require the use of animals;

(B) methods of such research and experimentation that reduce the number of animals used in such research;

(C) methods of such research and experimentation that produce less pain and distress in such animals; and

(D) methods of such research and experimentation that involve the use of marine life (other than marine mammals);

(2) for establishing the validity and reliability of the methods described in paragraph (1);

(3) for encouraging the acceptance by the scientific community of such methods that have been found to be valid and reliable; and

(4) for training scientists in the use of such methods that have been found to be valid and reliable.

(b) Not later than October 1, 1993, the Director of NIH shall submit to the Committee on Energy and Commerce of the House of Representatives, and to the Committee on Labor and Human Resources of the Senate, the plan required in subsection (a) and shall begin implementation of the plan.

(c) The Director of NIH shall periodically review, and as appropriate, make revisions in the plan required under subsection (a). A description of any revision made in the plan shall be included in the first biennial report under section 403 that is submitted after the revision is made.

(d) The Director of NIH shall take such actions as may be appropriate to convey to scientists and others who use animals in biomedical or behavioral research or experimentation information respecting the methods found to be valid and reliable under section (a)(2).

(e)(1) The Director of NIH shall establish within the National Institutes of Health a committee to be known as the Interagency Coordinating Committee on the Use of Animals in Research (in this subsection referred to as the 'Committee').

(2) The Committee shall provide advice to the Director of NIH on the preparation of the plan required in subsection (a).

(3) The Committee shall be composed of--

(A) the Directors of each of the national research institutes and the Director of the Center for Research Resources (or the designees of such Directors); and

(B) representatives of the Environmental Protection Agency, the Food and Drug Administration, the Consumer Product Safety Commission, the National Science Foundation, and such additional agencies as the Director of NIH determines to be appropriate, which representatives shall include not less than one veterinarian with expertise in laboratory-animal medicine.

(b) Conforming Amendment. Section 4 of the Health Research Extension Act of 1985 (Public Law 99-158; 99 Stat. 880) is repealed.

Appendix C

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

This appendix lists all SACATM members during 2006 and 2007; ending dates of appointments are indicated.

Daniel Acosta, Jr., Ph.D.
Dean, College of Pharmacy
University of Cincinnati
Cincinnati, OH
Appointment ended: 6/30/2006

Frank Barile, Ph.D.
Assoc. Professor
College of Pharmacy & Allied Health Professions, St. John's University
Jamaica, NY
Appointment ends: 6/30/2009

Richard A. Becker, Ph.D.
Sr. Director, Public Health Team
American Chemistry Council
Arlington, VA
Appointment ends: 6/30/2008

June A. Bradlaw, Ph.D.
Science Advisor
International Foundation for Ethical Research
Rockville, MD
Appointment ends: 6/30/2010

Marilyn J. Brown, D.V.M.
Exec. Director, Animal Welfare & Training
Charles River Laboratories
East Thetford, VT
Appointment ends: 06/30/2009

Grantley D. Charles, Ph.D.
Sr. Scientist, Toxicology & Drug Safety Evaluation
Allergan, Inc.
Irvine, CA
Appointment ends: 6/30/2009

Mary Jane Cunningham, Ph.D.
Director, Toxicology Programs, Environment Group
Assoc. Director, Life Sciences and Health
Houston Advanced Research Center
The Woodlands, TX
Appointment ends: 6/30/2008

George L. DeGeorge, Ph.D.
Director of Toxicology
MB Research Laboratories
Spinnerstown, PA
Appointment ends: 6/30/2008

Helen E. Diggs, D.V.M.
Director, Office of Laboratory Animal Care
University of California
Berkeley, CA
Appointment ends: 6/30/2010

Michael H. Dong, Ph.D.
Staff Toxicologist, Worker Health & Safety Branch
California Department of Pesticide Regulation
Sacramento, CA
Appointment ends: 6/30/2008

Marion F. Ehrich, Ph.D.
Professor, Dept. of Biomedical Sciences & Pathobiology/ Lab. for Neurotoxicity Studies
VA-MD Regional College of Veterinary Medicine
Blacksburg, VA
Appointment ends: 6/30/2010

Nancy Flournoy, Ph.D.
Professor, Department of Statistics
University of Missouri-Columbia
Columbia, MO
Appointment ended: 6/30/2006

Donald A. Fox, Ph.D.
Professor, Dept. of Pharmacological & Pharmaceutical Sciences
College of Optometry, University of Houston
Houston, TX
Appointment ends: 6/30/2009

James Freeman, Ph.D. (Chair)
Section Head, Global Product Stewardship and Regulatory Affairs
ExxonMobil Biomedical Sciences, Inc.
Annandale, NJ
Appointment ends: 6/30/2010

Daniel S. Marsman, D.V.M., Ph.D.
Section Head, Animal Welfare and Alternatives
Procter & Gamble
Cincinnati, OH
Appointment ends: 6/30/2009

Nancy A. Monteiro-Riviere, Ph.D.
Professor, Department of Clinical Sciences
College of Veterinary Medicine
Center for Cutaneous Toxicology
North Carolina State University
Raleigh, NC
Appointment ended: 6/30/2006

Roger O. McClellan, D.V.M.
Consultant
Albuquerque, NM
Appointment ends: 6/30/2008

Annie (Peiyong) Qu, Ph.D.
Assoc. Professor, Department of Statistics
Oregon State University
Corvallis, OR
Appointment ends: 6/30/2010

Stephen H. Safe, Ph.D.
Distinguished Professor
Department of Veterinary Physiology and Pharmacology
Texas A&M University
College Station, TX
Appointment ended: 6/30/2006

Jacqueline H. Smith, Ph.D.
Chesapeake Consulting Team
Royal Oak, MD
Appointment ended: 6/30/2006

Martin L. Stephens, Ph.D.
Vice President for Animal Research
The Humane Society of the United States
Washington, DC
Appointment ended: 6/30/2006

Peter Theran, V.M.D.
Consultant, Massachusetts Society for
the Prevention of Cruelty to Animals
Novato, CA
Appointment ended: 6/30/2006

Calvin C. Willhite, Ph.D.
Toxicologist
Department of Toxic Substances Control
State of California
Berkeley, CA
Appointment ended: 6/30/2006

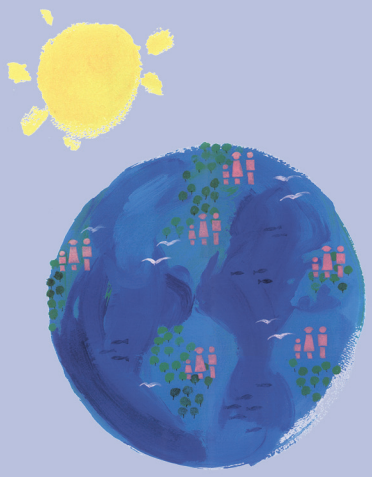
SACATM Meetings

SACATM held three meetings during 2006 and 2007:

August 3, 2006, teleconference, announced in *Federal Register* Vol. 71, No. 132, pp. 39121-39122, July 11, 2006

November 30, 2006, in Research Triangle Park, NC, announced in *Federal Register* Vol. 71, No. 206, pp. 62480-62481, October 25, 2006

June 12, 2007, in Bethesda, MD, announced in *Federal Register* Vol. 72, No. 83, pp. 23831-23832, May 1, 2007



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