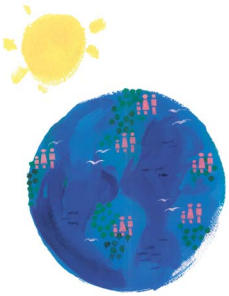
Biennial Progress Report 2008-2009

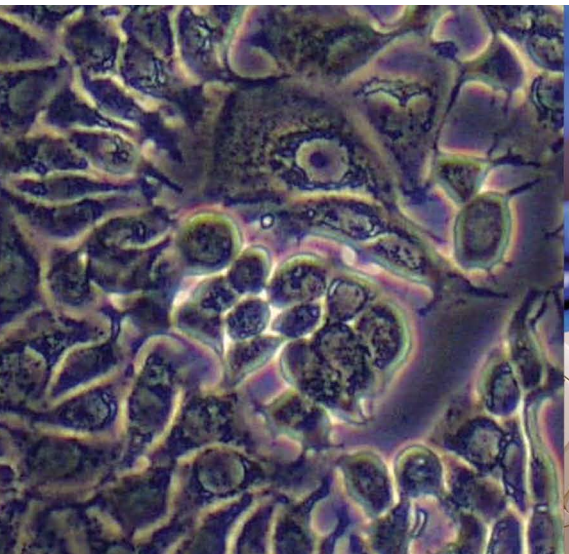


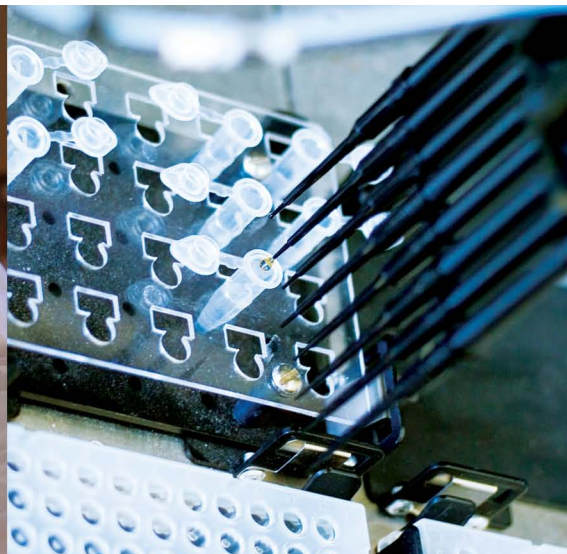
Interagency Coordinating Committee on the Validation of Alternative Methods

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

NIH Publication No. 10-7612





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

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, 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.

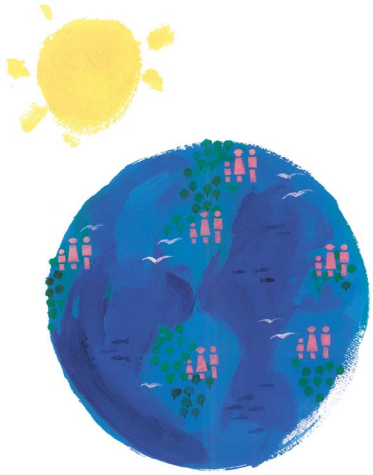
ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability. ICCVAM promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety or hazards of chemicals and products and that reduce, 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 NICEATM and ICCVAM 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).

ICCVAM is an interagency committee with representatives from the following 15 U.S. Federal regulatory and research member agencies that require, use, generate, or disseminate toxicological information:*

- *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*

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

* *Italics indicate those agencies represented on ICCVAM, as specified in the ICCVAM Authorization Act.*



Biennial Progress Report

2008-2009

Interagency Coordinating
Committee on the Validation of
Alternative Methods

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

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

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Table of Contents

The ICCVAM Member Agencies
page 3

A Message from the Director of the NIEHS and NTP
page 4

A Message from NICEATM and ICCVAM
page 5

2008-2009 Highlights
page 6

Introduction: History and Organization of NICEATM and ICCVAM
page 12

ICCVAM Test Method Evaluations and Related Activities 2008-2009
page 22

ICCVAM Outreach and Cooperative Activities
page 54

NICEATM-ICCVAM Communications
page 68

References Cited in this Report
page 72

Appendices:

Appendix A: ICCVAM Agency Representatives 2008-2009
page 74

Appendix B: ICCVAM Test Method Evaluation Process
page 75

Appendix C: NICEATM and ICCVAM Publications 2008-2009
page 82

Appendix D: List of Abbreviations and Acronyms
page 88

Appendix E: The ICCVAM Authorization Act
page 90

Appendix F: Scientific Advisory Committee on Alternative Toxicological Methods
page 94

Appendix G: NIH Revitalization Act, Sections 1301 and 205
page 96

The ICCVAM Member Agencies

The ICCVAM Authorization Act (Public Law 106-545, 42 U.S.C. 285*l*-3), directs that the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is composed of the heads (or their designees) of 15 specific Federal agencies, as well as "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." The Federal research and regulatory agencies specified in the ICCVAM Authorization Act are listed below and highlighted in italics. A complete list of designated member-agency representatives during 2008-2009 is provided as **Appendix A.**

- *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*

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

A Message from the Director of the NIEHS and NTP

The mission of the National Institute of Environmental Health Sciences (NIEHS) is to discover how substances in our environment cause or contribute to injury and disease, and to use this knowledge to reduce and prevent injuries and disease that can result from such exposures. The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) contribute to this mission by translating advances in science and technology into safety assessment tools that can be used to protect the health of people, animals, and the environment.

NICEATM and ICCVAM have made significant progress during the 2008-09 reporting period, as described in this fourth *ICCVAM Biennial Progress Report*. This progress includes achieving U.S. and international adoption of several new alternative test methods for regulatory safety testing and forging an agreement for enhanced international cooperation in the validation and evaluation of alternative test methods for our global community.

The *NICEATM-ICCVAM Five-Year Plan*, published in February 2008, describes how NICEATM and ICCVAM will advance alternative test methods of high scientific quality to better protect the health of people, animals, and the environment. The *NICEATM-ICCVAM Five-Year Plan* identifies specific goals relevant to advancing new test methods in specific priority areas. Other goals address fostering technology development, encouraging acceptance of validated test methods, developing partnerships, and strengthening interactions with stakeholders. I am pleased to note that the accomplishments described in the *2008-2009 ICCVAM Biennial Progress Report* reflect achievement of many of the goals outlined in the *Five-Year Plan*. Test methods recommended by ICCVAM over the last two years will provide better protection from potential hazards of eye irritation, acute poisoning, and allergic contact dermatitis, while reducing, refining, and replacing the use of animals.

To promote enhanced international cooperation and coordination of the scientific validation of alternative toxicity testing methods, I signed a Memorandum of Cooperation with our counterparts in Japan, Europe, and Canada. This agreement, the International Cooperation on Alternative Test Methods, arose from an ICCVAM initiative and will further reduce animal use in safety testing worldwide, while ensuring that the validation studies and scientific peer reviews of new test methods are of the highest quality. NICEATM and ICCVAM are also actively supporting interagency research collaborations to evaluate high throughput screening approaches that may yield new safety testing methods.

The dedication and cooperation exhibited by the ICCVAM committee members make ICCVAM one of the most effective interagency organizations in the Federal government. The activities described in this report promise to better protect public health and the environment while improving animal welfare, both now and in years to come.

Linda S. Birnbaum, Ph.D. DABT, ATS
Director, National Institute of Environmental Health Sciences
and National Toxicology Program

A Message from NICEATM and ICCVAM

Marilyn Wind, Ph.D.

William S. Stokes, D.V.M., DACLAM

On behalf of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), we are pleased to present the *ICCVAM Biennial Progress Report* for the 2008-09 reporting period. The ICCVAM Authorization Act directs ICCVAM to coordinate interagency technical evaluations of alternative test methods, develop test method recommendations based on their scientific validity for regulatory safety testing, and forward these recommendations to U.S. Federal agencies for their consideration. ICCVAM also coordinates interagency collaboration and provides guidance on alternative toxicological test method development, validation, regulatory acceptance, and national and international harmonization. These activities focus on test methods that will protect or improve the health of people, animals, and the environment, and that will reduce, refine (decrease or eliminate pain and distress), and replace animal use whenever scientifically feasible.

Since its inception, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and ICCVAM have now contributed to the national and international adoption and endorsement of 31 alternative test methods. These methods have and will continue to provide for significant reduction, refinement, and replacement of animal use for regulatory safety testing. This report summarizes NICEATM and ICCVAM test method evaluation activities in 2008 and 2009.

ICCVAM also has fostered stronger collaborations with its international partners. The United States, together with the European Union, Canada, and Japan, signed the International Cooperation on Alternative Test Methods to promote enhanced international cooperation and coordination among national validation organizations. This agreement provides a framework for ICCVAM and NICEATM to work cooperatively with these organizations on validation studies, independent peer reviews, and development of harmonized test method recommendations in order to speed the adoption of new test methods.

On behalf of ICCVAM, we gratefully acknowledge the contributions of the representatives from the 15 ICCVAM member agencies. Their commitment to high-quality science and animal welfare was instrumental to the progress made during this reporting period. In particular, we would like to recognize some of the representatives who have been active participants and that retired or moved to other responsibilities in their agencies in 2008-2009. From the Food and Drug Administration, they include Dr. Melvin Stratmeyer of the Center for Devices and Radiological Health and Dr. William Allaben of the National Center for Toxicological Research. From the Environmental Protection Agency, they include: Dr. Karen Hamernik of the Office of Science Coordination and Policy; Drs. Susanne McMaster, Stephanie Padilla, and Julian Preston of the Office of Research and Development; Dr. Amy Rispin of the Office of Pesticide Programs; and Dr. Jerry Smrchek, who served as the U.S. National Coordinator for the OECD Test Guidelines Programme. We would also like to recognize the service of Dr. Alan Poland of the National Cancer Institute.

Finally, we want to acknowledge the many other individuals whose contributions and enthusiastic support have been essential to ICCVAM's success. These include scientists serving on ICCVAM interagency working groups, our international partners, NICEATM and its contract support staff, members of the Scientific Advisory Committee on Alternative Toxicological Methods, our international experts and peer review panel members, and many other stakeholders.

With continued national and international cooperation and collaboration, we expect to make even greater progress in gaining regulatory acceptance of new, modified, and alternative test 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., DACLAM
National Institute of Environmental Health Sciences
Assistant Surgeon General, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

2008-2009 *Highlights*

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)[1] was established in 1997 to conduct interagency technical evaluations of new safety testing methods, including alternative testing methods that will reduce, refine, and replace the use of animals. ICCVAM was also established to coordinate cross-agency issues relating to development, validation, acceptance, and national and international harmonization of new, modified, and alternative toxicological test methods.

ICCVAM and its member agencies have contributed to the evaluation of 31 alternative methods that have now been approved or endorsed by Federal regulatory agencies and international test guideline organizations. Eighteen are *in vitro* methods that either replace or reduce animal use, while the other 13 are *in vivo* methods that significantly reduce the number of animals used or further reduce or avoid potential for pain, distress or discomfort.

This report describes test method evaluations and other activities that ICCVAM conducted in 2008 and 2009 in conjunction with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). Selected highlights follow.

Ocular Safety Testing

- Based on ICCVAM's reviews and recommendations, the bovine corneal opacity and permeability (BCOP) and isolated chicken eye (ICE) test methods can now be used worldwide in place of live animals for hazard identification of most substances that can cause severe and painful eye injuries resulting in temporary or permanent blindness. These are the first scientifically valid alternative methods to gain regulatory acceptance for ocular safety testing that do not use live animals.
- ICCVAM evaluated additional alternative methods and strategies that will further reduce animal use or reduce the potential for distress and discomfort during ocular safety testing. ICCVAM will forward the following test method recommendations to Federal agencies in 2010:
  - Procedures to update existing test guidelines to incorporate the routine use of a comprehensive pain management plan when *in vivo* ocular safety testing is still necessary. The plan includes the integrated use of topical anesthetics, systemic analgesics, and earlier humane endpoints, and is expected to further reduce or eliminate any pain and distress that might occur.
  - Use of the *in vitro* Cytosensor® Microphysiometer test method to identify certain types of products and substances that may not require hazard labeling for eye irritation, thereby significantly reducing the number of animals used for ocular safety testing of these types of minimally or nonirritating substances.

[1] A full list of acronyms and abbreviations used in this report can be found in **Appendix D**.

– A recommendation for additional studies for the BCOP, Cytosensor Microphysiometer, and EpiOcular™ test methods, which make up an *in vitro* testing strategy proposed to assess the eye irritation potential of antimicrobial cleaning products. The results from these studies will allow a more complete evaluation of the usefulness and limitations of the proposed testing strategy.

– A recommendation to discontinue using the low volume eye test for prospective *in vivo* ocular safety testing.

See page 23

Acute Oral Systemic Toxicity

- Federal agencies accepted ICCVAM's February 2008 recommendation to always consider using one of two *in vitro* basal cytotoxicity test methods to estimate starting doses for acute oral systemic toxicity testing. Using these test methods in a weight-of-evidence approach for determining starting doses for *in vivo* studies can reduce animal use by up to an additional 50%.
- NICEATM and ICCVAM prepared a draft Organisation for Economic Co-operation and Development (OECD) guidance document describing how to use these two *in vitro* test methods to estimate starting doses for acute oral systemic toxicity tests. ICCVAM forwarded the document to the OECD for consideration in July 2009.
- During a 2008 NICEATM and ICCVAM sponsored workshop on acute chemical safety testing, participants concluded that systematic collection of mechanistic data from required *in vivo* studies could help identify predictive biomarkers of systemic toxicity. These biomarkers could be used as earlier, more humane endpoints to further reduce or avoid pain and distress in test animals. Participants also recommended ways to collect data to identify key toxicity pathways for acute oral systemic toxicity that could then be used to target the development of alternative predictive *in vitro* test methods.

See page 29

Dermal Safety Testing

- In 2009, several scientists from the ICCVAM Dermal Corrosivity and Irritation Working Group participated in OECD Expert Consultation meetings to evaluate several *in vitro* test methods that may be useful for reducing the number of animals used for skin irritation testing.
- NICEATM is conducting a study to determine how *in vitro* skin irritation test methods will classify corrosive substances incorrectly identified as noncorrosives by *in vitro* corrosivity test methods. These data will be used to ensure that any limitations associated with an *in vitro* testing strategy for skin corrosivity and irritation are adequately identified.

See page 33

Biologics Testing

- In 2008, NICEATM and ICCVAM published a report on a scientific workshop on alternatives to the mouse LD_{50} assay for botulinum toxin testing. The workshop was cosponsored by NICEATM-ICCVAM and the European Centre for the Validation of Alternative Methods (ECVAM). Workshop participants identified methods that could be used in specific circumstances to reduce or refine the use of mice in the current mouse LD_{50} assay for botulinum toxin potency testing, and they identified additional development and validation efforts necessary for methods that might eventually replace the use of animals. The workshop participants also recommended best practices that could reduce and refine animal use required in the currently used animal test.
- NICEATM-ICCVAM, ECVAM, the Japanese Center for the Validation of Alternative Methods (JaCVAM), and Health Canada are organizing an international workshop on alternative methods for vaccine potency and safety testing to take place in September 2010. This workshop will review the state of the science of alternative methods that are currently available and/or accepted that reduce, refine, and replace the use of animals in vaccine potency and vaccine safety testing. Workshop participants will also discuss ways to promote the implementation of such methods.
- NICEATM and ICCVAM are planning an evaluation of an *in vitro* test method that could replace the use of animals for potency testing of vaccines used to protect pets and livestock from the bacterial disease leptospirosis.

See page 35

Immunotoxicity Testing: Allergic Contact Dermatitis

- NICEATM and ICCVAM evaluated several new versions and applications of the murine local lymph node assay (LLNA), an alternative test method that can be used in place of the traditional guinea pig tests for assessing the allergic contact dermatitis hazard potential of chemicals and products.
  - ICCVAM recommended an updated LLNA test method protocol that provides for a 20% reduction in the number of required animals.
  - ICCVAM recommended that the reduced LLNA test method should be used routinely to determine the allergic contact dermatitis hazard potential of chemicals and products before conducting the traditional LLNA, unless a substance is expected to produce positive results and dose-response information is needed. The rLLNA can reduce animal use by 40% for each test.
  - Working in conjunction with ECVAM and JaCVAM, ICCVAM developed performance standards for the LLNA. The performance standards will enable more rapid and efficient evaluation of the validity of new versions of the LLNA that are mechanistically and functionally similar to the LLNA.
  - Based on these evaluations, NICEATM and ICCVAM forwarded a proposal to update the OECD test guideline for the LLNA to reflect the internationally harmonized LLNA performance standards, incorporate the updated LLNA test method protocol using fewer animals, and allow use of the reduced LLNA where appropriate. The updated guideline, when OECD formally adopts it in 2010, will result in worldwide acceptance of these important revisions.

- ICCVAM evaluated the validity of three modified nonradiolabeled versions of the LLNA to identify substances as potential skin sensitizers or as nonsensitizers. ICCVAM will forward recommendations on the use of nonradiolabeled LLNA methods to Federal agencies in 2010.
- The ICCVAM recommendations on the new versions and applications of the LLNA will reduce animal use for identification of chemicals and products that could cause allergic contact dermatitis. They will also allow more institutions to take advantage of the animal welfare benefits afforded by the LLNA.

- NICEATM and ICCVAM are evaluating the application of *in vitro* methods and integrated decision strategies to reduce, refine, and replace the use of animals for identification of substances that could potentially cause allergic contact dermatitis.

See page 38

Endocrine Disruptor Testing

- NICEATM is coordinating validation studies of two *in vitro* test methods that could reduce the number of animals used to detect estrogenic and anti-estrogenic activities: the LUMI-CELL® estrogen receptor (ER) assay developed by Xenobiotic Detection Systems, Inc., and the MCF-7 cell proliferation assay developed by CertiChem, Inc. An independent scientific peer review panel will evaluate the results from international validation studies for these test methods in 2011.

See page 44

Pyrogen Testing

- All applicable Federal agencies, including the Food and Drug Administration, accepted or endorsed ICCVAM's 2008 recommendations on the use of five *in vitro* test methods to assess the potential pyrogenicity of pharmaceuticals and other products. The test methods may now be used instead of animal tests in specific circumstances to detect Gram-negative endotoxin that can cause fever reactions in people in human parenteral drugs.

See page 46

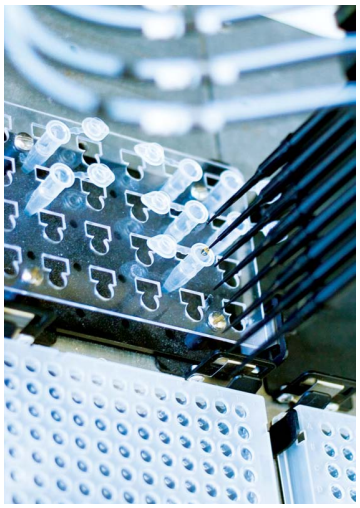
Genetic Toxicity Testing

- In 2009, the ICCVAM Genetic Toxicity Working Group provided comments on cytotoxicity evaluation procedures and study design for the draft OECD Test Guideline 487 for the *in vitro* micronucleus test. This test is intended to reduce the number of animals used to identify substances that may cause genetic damage that can lead to cancer and other adverse health effects.
- The Genetic Toxicity Working Group commented on the proposed study plan, protocol, and reference substances for a JaCVAM-led international validation study of the comet assay.

- The working group nominated experts to an ECVAM-sponsored peer review panel for an ongoing validation of a cell transformation assay that uses cultured mouse and hamster cells to detect genotoxic and nongenotoxic carcinogens. This test method is also intended to reduce the number of animals used to detect substances that may cause cancer.

See page 48

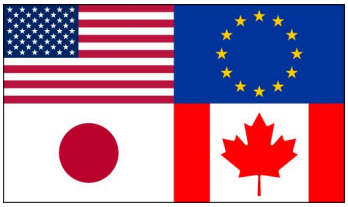
New Technologies Supporting Alternative Methods Development



- NICEATM and ICCVAM are collaborating with member-agency scientists involved in cooperative research initiatives to speed the translation of research advances and new technologies into scientifically valid safety testing methods that will further reduce, refine, and replace animal use. Similar collaborations with our international partners are also ongoing.
  - ICCVAM established a Research and Development Working Group (RDWG) to collaborate with the ICCVAM Five-Year Plan Implementation Subcommittee. The RDWG includes scientists that are integrally involved in ICCVAM member-agency research programs. Their participation on the RDWG will help ICCVAM identify promising test methods for referral to appropriate ICCVAM working groups for evaluations or other activities.
  - NICEATM and ICCVAM are participating in an interagency collaboration between the National Toxicology Program (within the National Institute of Environmental Health Sciences), the Environmental Protection Agency, and the National Institutes of Health Chemical Genomics Center to evaluate defined high throughput screening approaches. This initiative is expected to yield candidate methods and approaches with potential applicability to regulatory testing. Promising methods and approaches will then be reviewed by ICCVAM, who will forward recommendations on appropriate use to Federal agencies.
    - In 2009, NICEATM nominated nearly 1000 chemicals for inclusion in a 10,000-chemical library that will be used to evaluate each *in vitro* assay nominated and accepted for this initiative.
- The RDWG and Five-Year Plan Implementation Subcommittee are planning an implementation workshop that will describe best practices for the consideration and use of alternative methods that have been accepted for regulatory use. This workshop will bring together potential users of accepted alternative test methods and representatives of regulatory agencies in an effort to facilitate broader use of accepted alternative methods that can reduce, refine, or replace the use of animals for specific safety testing purposes.

See page 50

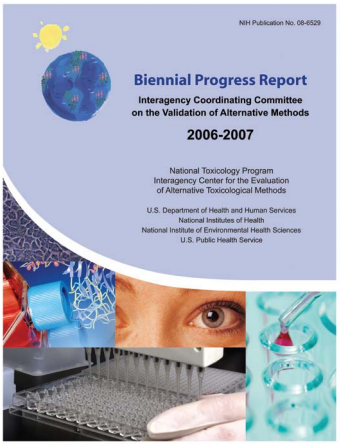
ICCVAM Outreach and Cooperative Activities



- In April 2009, an ICCVAM initiative fostered an international agreement between the United States, Canada, Japan, and the European Union that is expected to further reduce animal use in product toxicity testing worldwide. The agreement involves globally coordinated high-quality validation studies and peer reviews executed using a transparent process that should speed the international harmonization and adoption of alternative toxicity testing methods.
- NICEATM is coordinating an international validation study of an assay to identify potential endocrine disruptor activity. NICEATM and ICCVAM provided recommendations and/or liaisons to the management teams for four other international validation studies.
- NICEATM and ICCVAM prepared, commented on, or otherwise contributed to the development of over 20 OECD new test guidelines, revisions of existing test guidelines, and guidance documents.
- United States and international organizations recognized ICCVAM members and NICEATM staff with five awards for their activities in support of ICCVAM's mission.
- ICCVAM members and NICEATM staff gave presentations at meetings of the Society of Toxicology in 2008 and 2009, and at the Seventh World Congress on Alternatives and Animal Use in the Life Sciences in 2009. Representatives of NICEATM and ICCVAM also attended and gave presentations at eight other international meetings and conferences.

See page 54

NICEATM-ICCVAM Communications



- NICEATM and ICCVAM organized a symposium in February 2008 to commemorate the tenth anniversary of the establishment of ICCVAM and to announce the release of the *NICEATM-ICCVAM Five-Year Plan.*
- New pages were added to the NICEATM-ICCVAM website to enable easy access to key information about NICEATM and ICCVAM activities and progress of NICEATM-ICCVAM projects.
- ICCVAM publications in 2008 and 2009 included test method evaluation reports, background review documents, workshop reports, peer review panel reports, and the *NICEATM-ICCVAM Five-Year Plan (2008-2012)*. NICEATM published 16 *Federal Register* notices and ICCVAM members or NICEATM staff were authors of 23 manuscripts and abstracts describing NICEATM-ICCVAM activities.

See page 68

Introduction: History and Organization of NICEATM and ICCVAM

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 promoting scientific quality and protecting human health, animal health, and the environment.

Above: NICEATM Director Dr. William Stokes, Ocular Peer Review Panel Chair Dr. Wallace Hayes, and ICCVAM Chair Dr. Marilyn Wind listen to presentations at the May 2009 meeting of the independent scientific peer review panel evaluating the validation status of alternative ocular safety testing methods and approaches.

Development of a Process for Evaluation of Alternative Test Methods

U.S. regulatory agencies are charged with protecting human and animal health and the environment. As part of this mission, agencies need to determine the safety of, or possible hazards presented by, substances such as pesticides, consumer products, workplace chemicals, and so on. Toxicological testing is performed to assess the safety or hazards presented by such substances. Many of the test methods currently accepted for this purpose use laboratory animals. Alternative test methods are test methods that reduce, refine (decrease or eliminate pain or distress), or replace animal use in regulatory toxicity testing.

The Director of the National Institute of Environmental Health Sciences (NIEHS)[1] created an *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in September 1994 to respond to directives in the National Institutes of Health (NIH) Revitalization Act of 1993.[2] This Act required NIEHS to establish criteria for the validation and regulatory acceptance of alternative toxicological test methods. The Act also required NIEHS to recommend a process through which alternative methods could be accepted for regulatory use once their usefulness and limitations for a specific proposed purpose were demonstrated through appropriate validation studies. The *ad hoc* ICCVAM committee consisted of representatives from the 15 U.S. Federal agencies still represented on ICCVAM today.

In 1997, the *ad hoc* ICCVAM committee published its final report, *Validation and Regulatory Acceptance of Toxicological Test Methods* (ICCVAM 1997). That same year, NIEHS established a standing ICCVAM committee to (1) implement a process to evaluate new test methods of cross-agency interest and (2) coordinate agency interactions related to the development, validation, acceptance, and national and international harmonization of toxicological test methods.

Establishment of NICEATM and ICCVAM

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) is a Center in the NTP, which has headquarters at NIEHS. NICEATM was established in 1998 to:

- Administer ICCVAM and its scientific advisory committee
- 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
- Conduct independent validation studies for priority alterative test methods that may reduce, refine (decrease or eliminate pain and distress), or replace animal use for regulatory safety testing.

[1] A full list of acronyms and abbreviations used in this report can be found in **Appendix D**.

[2] The excerpt of Public Law 103-43 (42 U.S.C.285*l*-1 and 42 U.S.C. 283*e*) relevant to the establishment of NICEATM and ICCVAM is included in this report as **Appendix G**. It can also 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[3] established ICCVAM as a permanent interagency committee of NIEHS under NICEATM. The ICCVAM Authorization Act 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 states that the purposes of ICCVAM are to:

- Increase the efficiency and effectiveness of Federal agency test method review
- Eliminate unnecessary duplicative efforts and share experiences between Federal regulatory agencies
- Optimize utilization of scientific expertise outside the Federal Government
- Ensure that new and revised test methods are validated to meet the needs of Federal agencies
- Reduce, refine, or replace the use of animals in testing, where feasible

Duties and Activities of NICEATM and ICCVAM

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

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

ICCVAM Agencies

The ICCVAM Authorization Act defines the composition of ICCVAM as the heads, or their designees, of the following 15 U.S. Federal agencies:

Agency for Toxic Substances and Disease Registry
Consumer Product Safety Commission
Department of Agriculture
Department of Defense
Department of Energy
Department of the Interior
Department of Transportation
Environmental Protection Agency
Food and Drug Administration
National Institute for Occupational Safety and Health
National Institutes of Health
National Cancer Institute
National Institute of Environmental Health Sciences
National Library of Medicine
Occupational Safety and Health Administration

[3] The ICCVAM Authorization Act (Public Law 106-545, 42 U.S.C. 285*l*-3) is included in this report as **Appendix E**. It can also be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf.

NICEATM is a Center in the National Toxicology Program, which has headquarters at the National Institute of Environmental Health Sciences, located in Research Triangle Park, NC

The ICCVAM test method evaluation process is described in detail in **Appendix B** of this report.

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

- Evaluating new test method submissions and nominations for adherence to the *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods* (ICCVAM 2003b)
- Assessing the completeness of background review documents 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

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

Scientific Advisory Committee on Alternative Toxicological Methods

In accordance with the ICCVAM Authorization Act, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) was established in 2002 to advise the NIEHS Director, ICCVAM, and NICEATM about Federally mandated ICCVAM functions and ICCVAM activities.

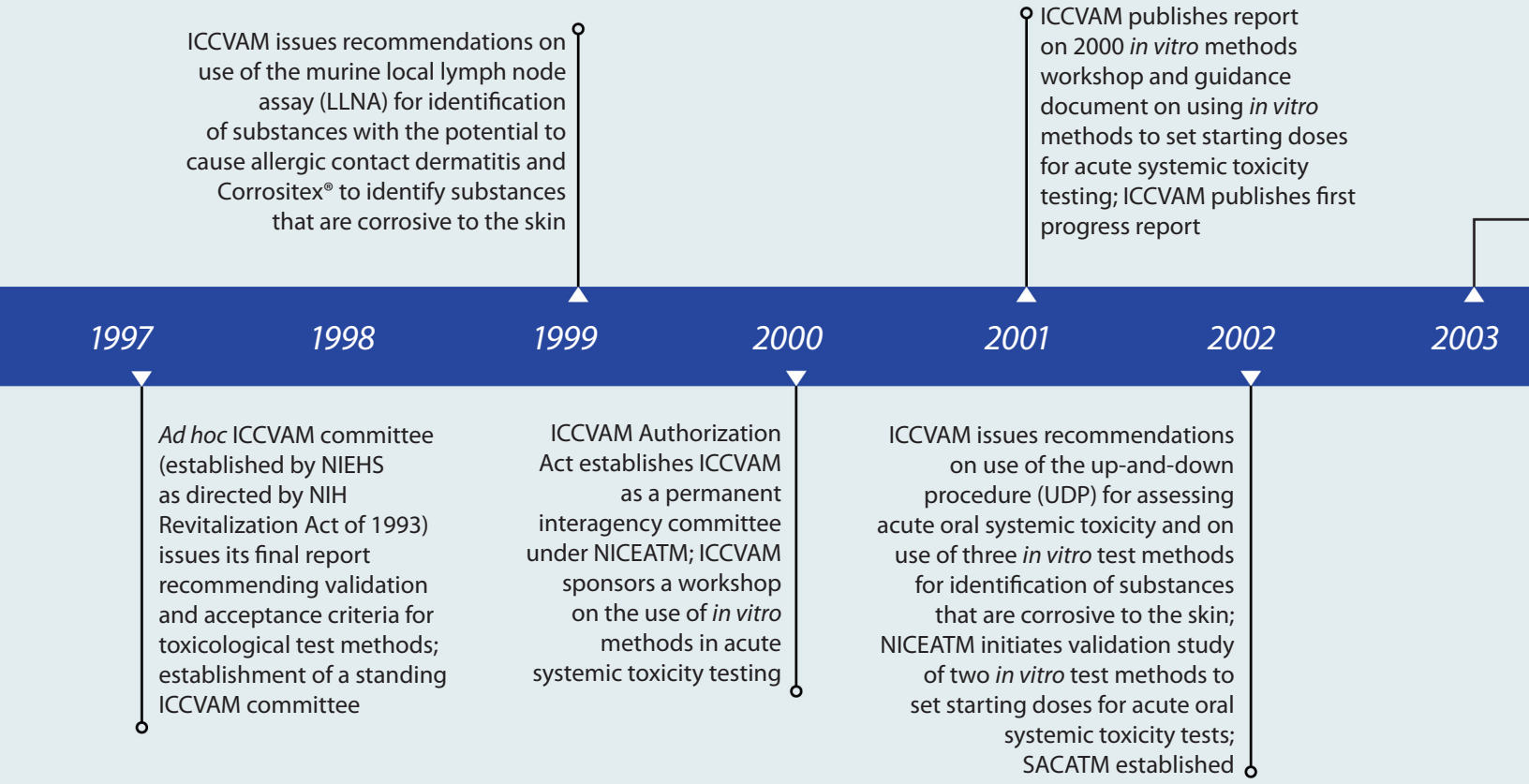
The ICCVAM Authorization Act states that SACATM will include:

- 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

(continued on page 18)

NICEATM-ICCVAM

Advancing Public Health and Animal Welfare with Sound Science for Over Ten Years



Why Do Toxicological Test Methods Require Validation?

New regulatory safety test methods must protect public health and the environment as well as or better than currently accepted methods. Test method validation must demonstrate that an alternative test method is scientifically valid and as protective as currently accepted methods. The process evaluates proposed alternative test methods to determine their usefulness and limitations for a specific safety testing application.

During the test method validation process, the test method must be assessed for its reliability and relevance. *Reliability* is the degree to which a test method produces consistent results within and among laboratories over time. *Relevance* is the extent to which a test method correctly predicts or measures the biological effect of interest. The evaluation of a test method's relevance takes into consideration its accuracy, or the test method's proportion of outcomes that agree with an accepted reference value. Test methods must be reliable and relevant enough that the data can be used in regulatory safety testing to realistically predict a tested substance's effect on human health, animal health, or the environment.

Determining the usefulness and limitations of a new test method for a specific regulatory purpose includes collecting and evaluating all available data concerning the reliability and relevance of the proposed method. The data are compared to data from the currently approved test method(s) and to any relevant human toxicity data. If the available data are insufficient, validation studies may be needed to generate data to specifically test the reliability and relevance of the new test method. Additional details on the criteria for adequate test method validation can be found in the ICCVAM report, *Validation and Regulatory Acceptance of Toxicological Test Methods* (ICCVAM 1997).

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.

2004

ICCVAM publishes results of its evaluation of the validation status of *in vitro* methods to identify potential endocrine disruptors and recommendations on procedures for future validation studies; revised guidelines for nominations and submissions published; EPA nominates ocular test methods for evaluation

2005

ICCVAM holds strategic planning meeting and develops a "Mission, Vision, and Strategic Priorities" document; ICCVAM publishes performance standards for skin corrosion test methods; two *in vitro* test methods to identify potential endocrine disruptors nominated for NICEATM-sponsored validation studies

2006

ICCVAM issues recommendations on use of *in vitro* test methods to identify ocular corrosives and severe irritants. NICEATM initiates validation study of *in vitro* test method to identify potential endocrine disruptors. ICCVAM workshop on alternative botulinum toxin testing methods; OECD test guidelines based on ICCVAM evaluations of Corrositex and UDP accepted

2007

ICCVAM issues recommendations on use of *in vitro* test methods to set starting doses for acute oral toxicity tests; improved NICEATM-ICCVAM website launched; CPSC nominates new versions and applications of LLNA for evaluation

2008

ICCVAM releases *Five-Year Plan* at Ten-Year Aniversary Symposium; ICCVAM issues recommendations on the use of *in vitro* methods for identification of pyrogens; ICCVAM workshop on *in vitro* approaches and humane endpoints for systemic toxicity evaluations

2009

ICCVAM issues recommendations on use of the reduced LLNA test method for identification of sensitizers; ICCVAM publishes LLNA performance standards; OECD test guidelines based on ICCVAM evaluation of ocular test methods accepted

SACATM includes a representative from a national animal protection organization, a representative from the personal care, pharmaceutical, industrial chemicals, or agriculture industry, and representatives from any other industry regulated by one of the ICCVAM agencies.

Photo of the SACATM membership and the NICEATM and ICCVAM leadership at the June 2008 meeting of SACATM in Research Triangle Park, NC.

Scientific Advisory Committee on Alternative Toxicological Methods (cont'd)

- Additional representatives are 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 may be found on the NTP website (http://ntp.niehs.nih.gov/go/167). The SACATM charter[4] directs SACATM to meet once per fiscal year. Times and locations of the 2008-2009 SACATM meetings, as well as a list of SACATM members during 2008–2009, can be found in **Appendix F**.

At these meetings, the Director of NICEATM provides SACATM with the following:

- A status report on nominations and submissions of test methods
- Results of ICCVAM's preliminary evaluation of test method nominations and submissions
- 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 activities

SACATM also receives updates from Federal agencies, liaisons from international validation organizations, and other individuals on topics relevant to ICCVAM activities.

The NICEATM–ICCVAM Five-Year Plan (2008-2012)

In February 2008, at a symposium to commemorate its tenth anniversary, ICCVAM released *The NICEATM–ICCVAM Five-Year Plan (2008-2012): A Plan to Advance Alternative Test Methods of High Scientific Quality to Protect and Advance the Health of People, Animals, and the Environment*. ICCVAM prepared the plan in response to requests from the Appropriations Committees of the U.S. House of Representatives and U.S. Senate. The *NICEATM-ICCVAM Five-Year Plan* describes goals and objectives for the years 2008 through 2012 that support ICCVAM's purposes as outlined in the ICCVAM Authorization Act. It also 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, as outlined in the 2004 *ICCVAM Mission, Vision, and Strategic Priorities*.[5]

[4] The SACATM charter is available on the NTP website at http://ntp.niehs.nih.gov/ntp/About_ntp/sacatm/2009/SACATMCharter120709s.pdf.
[5] The *ICCVAM Mission, Vision and Strategic Priorities* document is available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/docs/about_docs/MisVisStrat.pdf

The *NICEATM-ICCVAM Five-Year 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

The ICCVAM Five-Year Plan Implementation Subcommittee was established to coordinate activities and monitor progress toward achieving the Plan's goals. The subcommittee prepared an Implementation Plan and released it in June 2009. This working document describes how the strategies outlined in the *NICEATM-ICCVAM Five-Year Plan* are being implemented. The Implementation Plan also explains how those activities address the four key challenges. It includes goals, specific objectives, planned activities, and progress toward the goals and objectives outlined in the *NICEATM-ICCVAM Five-Year Plan*. ICCVAM will periodically update the working document to reflect achievement of key milestones.

Definitions of Key Terms

Accuracy: the closeness of agreement between a test method result and an accepted reference value, or the test method's proportion of correct outcomes

Nomination: a proposal to ICCVAM to conduct activities to review or advance the current validation status of test method(s), such as gathering additional information, conducting workshops and peer reviews, and performing validation studies

Reduction alternative: a new or modified test method that reduces the number of animals required

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

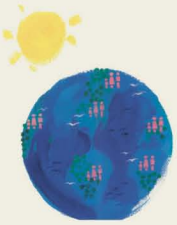
Relevance: the extent to which a test method correctly predicts or measures the biological effect of interest

Reliability: the degree to which a test method produces consistent results within and among laboratories over time

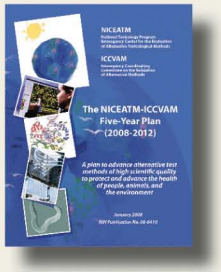
Replacement alternative: a new or modified test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one

Submission: a test method proposed to ICCVAM for review and evaluation with adequate validation studies completed and a submission package prepared according to the *ICCVAM Guidelines*

Five-Year Plan Implementation



Descriptions of NICEATM and ICCVAM activities will note how these activities relate to the goals and objectives outlined in the *NICEATM-ICCVAM Five-Year Plan* and the Implementation Plan.



Regulatory Acceptance of Test Methods Evaluated by ICCVAM

Test methods that can accurately detect whether chemicals and products can cause injury or disease are vital to prevention. Improved prevention of injury and disease requires effective translation of new knowledge into better test methods.

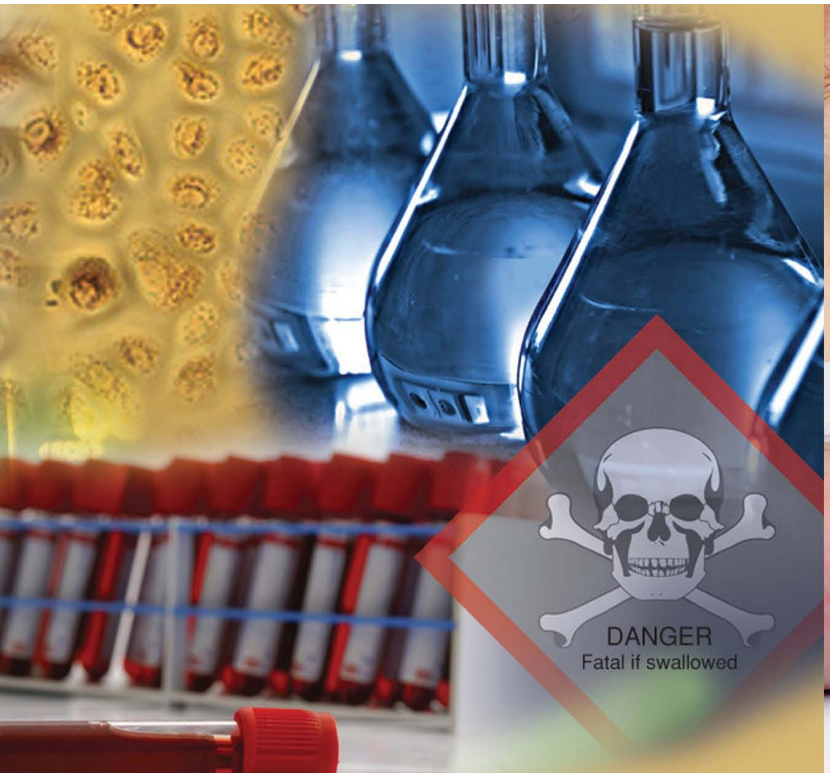
The most commonly conducted safety tests used to fulfill regulatory requirements for establishing the safety of regulated substances assess the following potential hazards:

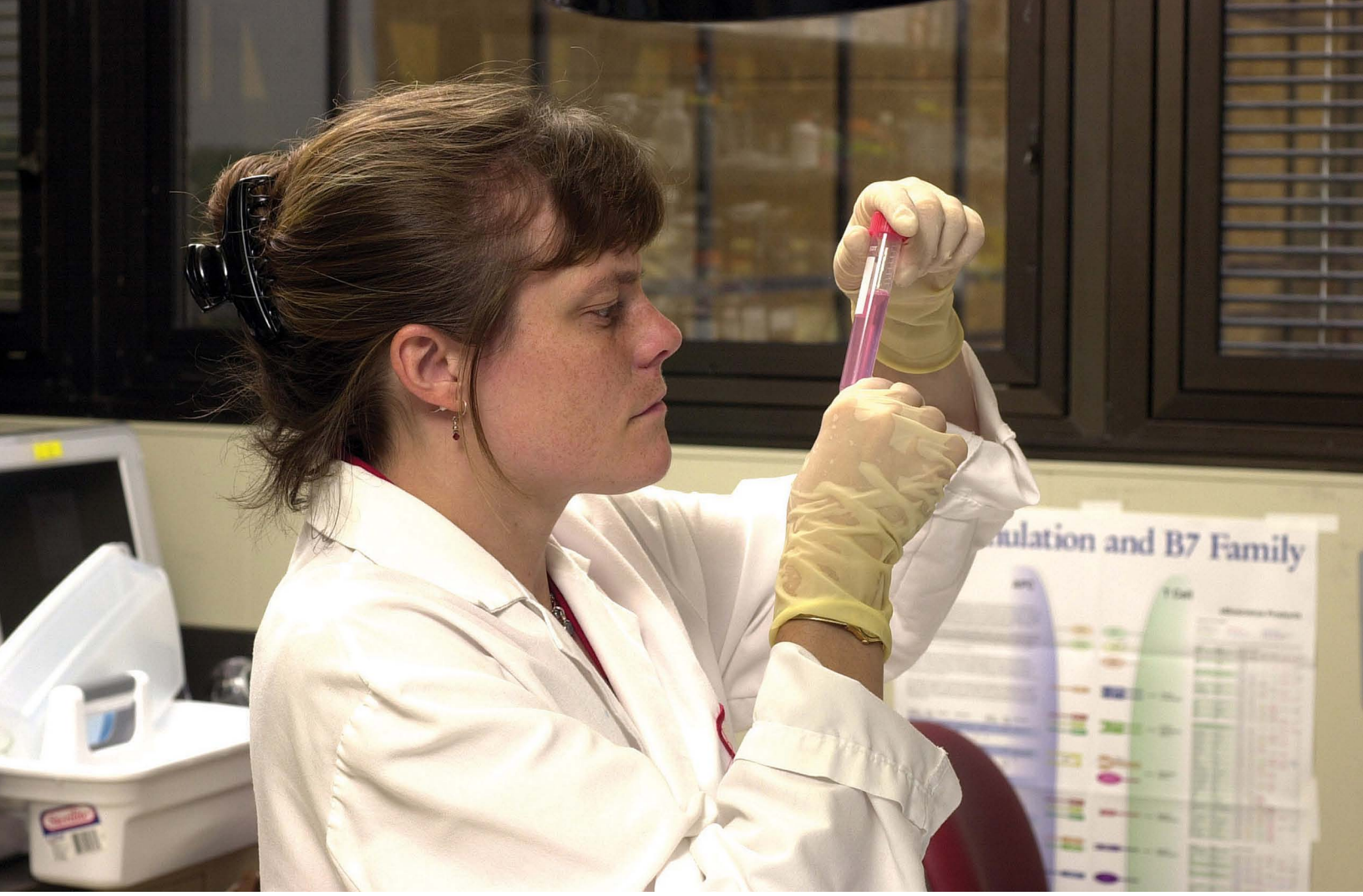
- Acute oral toxicity (risk of poisoning when swallowed)
- Acute inhalation toxicity (risk of poisoning when inhaled)
- Acute dermal toxicity (risk of poisoning when absorbed through the skin)
- Eye irritation or burns
- Skin irritation or burns
- Dermal sensitization (risk of inducing allergic contact dermatitis upon repeated skin exposure)

ICCVAM's goal in evaluating new test methods is to translate research advances and new technologies into scientifically valid safety testing methods for regulatory use. Since its establishment, ICCVAM has contributed to the evaluation of 31 alternative test methods that have been accepted or endorsed by Federal regulatory agencies (see table in **Appendix B** for list). NICEATM and ICCVAM have coordinated comprehensive technical evaluations of 15 of these methods. Of the alternative methods accepted by Federal agencies, 18 are *in vitro* methods that either replace animal use or reduce the number of animals required for testing. The remaining 13 are either modifications of existing *in vivo* test methods or new alternative *in vivo* test methods that reduce the number of animals required or refine animal use to reduce the potential for distress or discomfort.

The 31 alternative methods that have been accepted or endorsed by Federal regulatory agencies provide alternatives for five of the test method areas listed above: skin and eye irritation or burns, dermal sensitization, or poisoning when swallowed or inhaled. ICCVAM is currently developing additional recommendations on methods that reduce, refine, or replace animal use for identification of substances that may cause allergic contact dermatitis or eye irritation. ICCVAM will forward these recommendations to Federal agencies in early 2010. NICEATM and ICCVAM will also propose an up-and-down procedure to further reduce animal use for acute dermal systemic toxicity testing.

ICCVAM has contributed to the evaluation of 31 alternative test methods that have been accepted or endorsed by Federal regulatory agencies. These methods provide alternatives for five of the most commonly conducted safety tests: skin and eye irritation or burns, dermal sensitization, or poisoning when swallowed or inhaled.





ICCVAM Test Method Evaluations and Related Activities 2008-2009

During 2008-2009, ICCVAM conducted evaluations, made test method recommendations, and sponsored workshops and validation studies for alternative test methods for eight types of toxicity tests, including the four most commonly used toxicity tests.

Above: A scientist examines resuspended cells in a test tube. ICCVAM test method recommendations include a recommended test method protocol, describing in detail best practices for laboratory personnel conducting the test method in accordance with GoodLaboratory Practices.

ICCVAM test method evaluation activities are prioritized after considering the following factors:

- The potential of a proposed test method to provide improved prediction of adverse health or environmental effects
- The potential impact that an alternative test method may have on reducing, refining, or replacing animals used in testing
- The potential for an alternative test method to apply to testing required by multiple agencies[6]

This chapter provides an update on ICCVAM test method evaluations and related activities during 2008 and 2009. Test method evaluation activities are presented in an order that reflect the prioritization criteria described above.[7]

Ocular Safety Testing ..... page 23
Acute Oral Systemic Toxicity ..... page 29
Dermal Safety Testing ..... page 33
Biologics Testing ..... page 35
Immunotoxicity Testing: Allergic Contact Dermatitis ..... page 38
Endocrine Disruptor Testing ..... page 44
Pyrogen Testing ..... page 46
Genetic Toxicity Testing ..... page 48
New Technologies Supporting Alternative Methods Development ..... page 50

Ocular Safety Testing

Public Health and Animal Welfare Perspective

In the 1930s, more than a dozen women were blinded and one woman died from using a permanent mascara called Lash Lure. Since then, U.S. laws have required that chemicals and products be tested for ocular safety. Accidents involving common household products such as oven cleaner and bleach cause about 125,000 eye injuries each year (American Academy of Ophthalmology 2009). Substances that may cause temporary or permanent damage to the eyes must be identified so that they can be appropriately packaged, labeled, and handled.

Multiple regulatory agencies require ocular safety testing, which is one of the four most commonly required product safety tests. The primary method currently accepted by U.S. and international regulatory agencies for assessing ocular safety is the Draize rabbit eye test (Draize et al. 1944). This test is conducted by applying a small amount of a test substance into the conjunctival sac of the rabbit eye and evaluating the presence and severity of any injuries to the cornea, conjunctiva, and the iris for up to 21 days. Ocular safety testing can involve large numbers of animals, and animals used in tests to identify

[6] Additional information about the ICCVAM Test Method Evaluation Process, including criteria for prioritization of nominations and submissions, is available in **Appendix B**.

[7] Documents mentioned in this chapter that were published during 2008-2009 are listed in **Appendix C**. All ICCVAM documents are available electronically on the NICEATM–ICCVAM website (see footnotes for locations of specific reports or website pages). They are also available in hard copy from NICEATM (see inside back cover for contact information).

ICCVAM evaluated the use of nine alternative methods and strategies for ocular safety testing in 2008 and 2009, and will forward test method recommendations resulting from their evaluation to Federal agencies in early 2010.

Members of the ICCVAM-sponsored independent scientific peer review panel to evaluate the validation statue of alternative ocular safety testing methods and approaches listen to presentations during their May 2009 meeting.

ocular hazards can experience pain and distress when eye injuries occur. Therefore, development of alternative *in vitro* test methods and approaches to the Draize rabbit eye test is a high priority for NICEATM–ICCVAM.

Highlights of ICCVAM Activities

- Based on ICCVAM's reviews and recommendations, the bovine corneal opacity and permeability (BCOP) and isolated chicken eye (ICE) test methods can now be used worldwide in place of live animals for hazard identification of most substances that can cause severe and painful eye injuries resulting in temporary or permanent blindness. These are the first scientifically valid alternative methods to gain regulatory acceptance for ocular safety testing that do not use live animals.
- ICCVAM evaluated additional alternative methods and strategies that will further reduce animal use or reduce the potential for distress and discomfort during ocular safety testing. ICCVAM will forward the following test method recommendations to Federal agencies in early 2010:
  - Procedures to update existing test guidelines to incorporate the routine use of a comprehensive pain management plan when *in vivo* ocular safety testing is still necessary. The plan includes the integrated use of topical anesthetics, systemic analgesics, and earlier humane endpoints, and is expected to further reduce or eliminate any pain and distress that might occur.
  - Use of the *in vitro* Cytosensor® Microphysiometer test method to identify certain types of products and substances that may not require hazard labeling for eye irritation, thereby significantly reducing the reduce the number of animals used for ocular safety testing of these types of minimally or nonirritating substances.
  - A recommendation for additional studies for the BCOP, Cytosensor Microphysiometer, and EpiOcular™ test methods, which make up an *in vitro* testing strategy proposed to assess the eye irritation potential of antimicrobial cleaning products. The results from these studies will allow a more complete evaluation of the usefulness and limitations of the proposed testing strategy.
  - A recommendation to discontinue using the low volume eye test for prospective *in vivo* ocular safety testing.

National Acceptance of In Vitro *Test Methods to Identify Ocular Corrosives and Severe Irritants*

In 2007, ICCVAM forwarded its first recommendations for the use of *in vitro* methods for ocular safety testing to U.S. Federal agencies. NICEATM and ICCVAM evaluated the following four *in vitro* test methods for their usefulness in detecting ocular corrosives and severe irritants:

- Bovine corneal opacity and permeability (BCOP)
- Hen's egg test–chorioallantoic membrane (HET-CAM)
- Isolated chicken eye (ICE)
- Isolated rabbit eye (IRE)

Of these, the BCOP and the ICE test methods were found to be useful, in appropriate circumstances and with specific limitations, to screen for ocular corrosives and severe irritants. If either of these alternative methods yields a positive response, the product can be labeled as an ocular corrosive or severe irritant, and no live animal testing is required.

In June 2008, Federal agencies accepted the ICCVAM recommendations for use of the BCOP and ICE test methods for these purposes, allowing these methods to be used in regulatory testing in the United States.[8]

International Acceptance of In Vitro *Test Methods to Identify Ocular Corrosives and Severe Irritants*

NICEATM and the ICCVAM Ocular Toxicity Working Group then prepared draft test guidelines for the use of the BCOP and ICE test methods to detect ocular corrosives and severe irritants. Liaisons from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) participated.

The BCOP and ICE test guidelines were submitted to the Test Guidelines Programme of the Organisation for Economic Co-operation and Development (OECD), which formally adopted them in 2009. The BCOP and ICE test methods can now be used worldwide to identify substances as ocular corrosives and severe irritants (Test Guideline 437 and Test Guideline 438, respectively). Positive results in these *in vitro* tests can be used for hazard classification, thereby avoiding the pain and distress that may have resulted from testing in animals.

[8] Information on the evaluation of the *in vitro* ocular test methods, and the letters from Federal agencies accepting the ICCVAM recommendations, can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox.htm.

Definitions of Key Terms

Antimicrobial cleaning product (AMCP): a commercially available household cleaning product that kills or inhibits the growth of bacteria and viruses

Histopathology evaluation: microscopic evaluation of cells or tissues for signs of disease or other changes

Humane endpoint: predetermined criteria used to evaluate whether a study should be discontinued early to stop or minimize test animal pain or distress

Ocular corrosives: substances that cause irreversible tissue damage in the eye following application

Severe irritants (ocular): substances that cause tissue damage in the eye that is not reversible within 21 days of application or cause serious physical decay of vision

Systemic analgesic: an agent that acts throughout the body to reduce or alleviate pain

Topical anesthetic: an agent applied directly to cause temporary loss of sensation, including the feeling of pain

Guidance Document for Test Guidelines 437 and 438

In November 2009, ICCVAM sent the OECD a guidance document for use with Test Guidelines 437 and 438. This document (1) promotes histopathology evaluation as an additional endpoint for ocular safety testing and (2) provides specific guidance on using the BCOP and ICE test methods to expand their respective databases of test results and optimize the test methods' use for identifying all hazard categories.

The guidance document provides detailed protocols to help with routine collection of tissues for histopathology evaluation. Histopathology evaluations are necessary to build a database of reference data that may support broader uses of the BCOP and ICE test methods. The guidance document also recommends decision criteria for the BCOP and ICE test methods to identify moderate and mild irritants and substances not labeled as eye irritants.

Evaluation of Approaches to Avoid and Minimize Pain and Distress During In Vivo *Testing*

In 2005, NICEATM-ICCVAM cosponsored a symposium entitled *Minimizing Pain and Distress in Ocular Toxicity Testing*.[9] Expert participants recommended that ICCVAM consider the use of anesthetics, analgesics, and humane endpoints to minimize animal pain and distress during *in vivo* ocular safety testing. NICEATM and ICCVAM requested data and compiled information on the use of topical anesthetics and/or systemic analgesics during ocular safety testing in rabbits. They then prepared a draft background review document and recommendations on the use of topical anesthetics, systemic analgesics, and humane endpoints. In May 2009, an independent scientific peer review panel reviewed the draft documents. ICCVAM will consider the panel's recommendations, along with comments from the public and from SACATM, as it develops its final recommendations.

In the *ICCVAM Test Method Evaluation Report on Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing*,[10] ICCVAM recommends that pain-relieving drugs be routinely administered both prior to and during ocular safety testing in rabbits. The recommended topical anesthetics are commonly used in human eye surgery procedures. The test method evaluation report includes a pain management plan detailing specific pain-relieving drugs and a schedule of administration to effectively avoid or minimize pain and discomfort. The report also recommends that rabbits be routinely evaluated for clinical signs of pain and/or distress, and provides examples of earlier humane endpoints that should be used to end a study early and thereby avoid or minimize pain and distress experienced by test animals.

ICCVAM will forward recommendations to U.S. Federal agencies in 2010. Adoption and use of these recommendations will result in the routine consideration and use of topical anesthetics, systemic analgesics, and earlier humane endpoints whenever the Draize rabbit eye test is conducted for regulatory safety testing unless pain response monitoring is required (e.g., pharmaceutical tolerability testing). Use of these modified procedures for *in vivo* ocular safety testing will reduce or eliminate pain and distress experienced by test animals.

[9] Information on the ocular toxicity symposia cosponsored by NICEATM and ICCVAM can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/meetings/ocumeet/sympinfo.htm.

[10] The report (NIH publication number 10-7514) will be available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/OcuAnest-TMER.htm once it is published in 2010.

Evaluation of In Vitro *Test Methods for Identifying Nonsevere Ocular Irritants*

The 2006 ICCVAM evaluation of *in vitro* ocular safety test methods was limited to their usefulness and limitations for identifying ocular corrosives and severe irritants. In 2008, NICEATM and ICCVAM expanded the evaluation. They prepared draft recommendations on the current validation status of five test methods to identify moderate and mild irritants and substances not considered to be eye irritants (Not Labeled):

- Bovine Corneal Opacity and Permeability (BCOP)
- Cytosensor® Microphysiometer (CM)
- Hen's egg test–chorioallantoic membrane (HET-CAM)
- Isolated chicken eye (ICE)
- Isolated rabbit eye (IRE)

The independent international peer review panel reviewed ICCVAM's draft recommendations in May 2009. ICCVAM considered the panel's comments, as well as comments from the public and SACATM, as it developed its final recommendations.

Based upon performance statistics, ICCVAM did not recommend the BCOP, HET-CAM, and ICE test methods for distinguishing substances not labeled as irritants from all other hazard categories. Additional studies and potential improvements were recommended for the IRE test method to identify moderate and mild ocular irritants.

ICCVAM concluded that the CM test method may be used on water-soluble substances to identify ocular corrosives and severe irritants. The CM test method may also be used to identify substances not labeled as irritants among water-soluble surfactant (surface-active) chemicals and certain types of surfactant-containing formulations, such as cosmetics and personal-care products. The *ICCVAM Test Method Evaluation Report: Current Validation Status of* In Vitro *Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products*[11] will present ICCVAM's recommendations on use of the evaluated test methods. It includes recommended test method protocols and suggests future studies.

ICCVAM will forward recommendations to U.S. Federal agencies in 2010. If Federal agencies accept the CM test method for these purposes, it will represent the first *in vitro* test method available to identify substances not labeled as irritants. Use of the CM test method can be expected to reduce animal use for ocular toxicity testing, while continuing to support the protection of human health.

ICCVAM members and NICEATM staff are also participating in an ECVAM-sponsored evaluation of four cell function-based *in vitro* test methods:

- Cytosensor® Microphysiometer
- Fluorescein leakage
- Neutral red release
- Red blood cell hemolysis

[11] The report (NIH publication number 10-7553) will be available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm once it is published in 2010.

These four test methods are being evaluated for their ability to identify (1) substances that can cause mild to moderate ocular irritation and (2) substances that do not require hazard labeling. ICCVAM provided recommendations to an ECVAM Scientific Advisory Committee for peer review. ICCVAM members and NICEATM staff are serving on the validation management group.

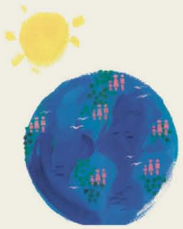
Evaluation of a Non-animal Approach for Assessing Eye Irritation Potential of Antimicrobial Cleaning Products

In January 2008, the Institute for *In Vitro* Sciences, Inc., requested that NICEATM and ICCVAM evaluate a non-animal testing strategy to assess the potential of antimicrobial cleaning products (AMCPs) to cause eye irritation. The Institute for In Vitro Sciences, Inc., developed the AMCP testing strategy along with the Environmental Protection Agency (EPA) and a group of U.S. consumer product companies. The testing strategy uses the BCOP, CM, and EpiOcular® *in vitro* test methods to determine a substance's EPA toxicity category. The EPA toxicity category is then used to ensure that that an AMCP is labeled accurately for users' safety.

NICEATM and ICCVAM evaluated the proposed testing strategy and developed draft recommendations, which an independent scientific peer review panel reviewed in May 2009. ICCVAM's final recommendations take into consideration the opinions of the panel as well as comments from the public and SACATM.

ICCVAM recommends additional studies to further characterize the proposed AMCP testing strategy and *in vitro* test methods. The results from these studies will allow a more complete evaluation of the usefulness and limitations of the proposed testing strategy. ICCVAM provides test method recommendations for the proposed AMCP testing strategy in the *ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed* In Vitro *Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial*

Five-Year Plan Implementation: Ocular Safety Testing



- ICCVAM evaluated the use of the following *in vitro* test methods for identifying moderate and mild irritants and substances not considered to be eye irritants:
  - Bovine corneal opacity and permeability
  - Hen's egg test—chorioallantoic membrane
  - Isolated chicken eye
  - Isolated rabbit eye
  - Cytosensor® Microphysiometer
- ICCVAM evaluated an *in vitro* testing strategy to determine the ocular irritation potential of antimicrobial cleaning product formulations.
- ICCVAM reviewed the use of topical anesthetics and systemic analgesics for reducing pain and distress during *in vivo* ocular safety testing.
- ICCVAM provided input to the European Centre for the Validation of Alternative Methods on:
  - The design of a prospective validation study of the use of reconstructed human tissue models to distinguish nonirritants from irritants
  - The use of cell-function based assays for ocular hazard identification
- ICCVAM is promoting the evaluation of ocular histopathology for its potential to improve test method predictivity. Toward that end, ICCVAM drafted an OECD guidance document for use with the test guidelines for using the bovine corneal opacity and permeability and isolated chicken eye test methods.

Cleaning Products.[12] ICCVAM will forward this document to Federal agencies for consideration in 2010. Agency responses are expected later in the year.

Evaluation of the Low Volume Eye Test

ICCVAM also reviewed the validation status of the *in vivo* low volume eye test (LVET), because data from the LVET were used to support the validity of the *in vitro* test methods in the proposed AMCP testing strategy. ICCVAM does not consider the LVET a valid replacement for the Draize rabbit eye test and, therefore, does not recommend it for prospective *in vivo* ocular safety testing. If animals must be used for ocular safety testing, ICCVAM recommends using the modified Draize rabbit eye test, which includes routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize animal pain and distress.

These recommendations consider the opinions of the peer review panel as well as comments from the public and SACATM. They are presented in the *ICCVAM Test Method Evaluation Report on the Low Volume Eye Test: Recommendation to Discontinue Use for Further Ocular Safety Testing*.[13]

Other Ocular Test Method Evaluation Activities

ECVAM is planning validation studies of two *in vitro* test methods to identify ocular corrosives and severe irritants. The EpiOcular™ and SkinEthic HCE™ test methods are based on reconstructed human tissue models. ICCVAM provided input on the study design, and NICEATM and ICCVAM have liaisons on the ECVAM-led study management team.

Acute Oral Systemic Toxicity

Public Health and Animal Welfare Perspective

Death rates from accidental poisoning have more than tripled over the past 20 years. In the United States, accidental poisonings are now second only to motor vehicle crashes as the leading cause of accidental deaths. In some U.S. states, overdoses have surpassed motor vehicle crashes to become the leading cause of unintentional death (National Safety Council 2009). The majority of poisonings in the United States are due to accidental drug overdoses; however, household chemicals, pesticides, and environmental agents such as lead and carbon monoxide also present poisoning risks. It is essential that substances that could be poisonous when ingested are accurately identified in order to adequately protect human and animal health.

Acute oral systemic toxicity testing is the most commonly performed safety test worldwide. The administration of poisonous substances can cause significant pain and distress to test animals; therefore, it is important to identify alternatives to reduce and refine the use of animals for this purpose.

Methods such as the *in vivo* up-and-down procedure, which was accepted by U.S. regulatory authorities in 2003 after an ICCVAM evaluation and recommendation, have reduced the number of animals required for acute oral systemic toxicity testing. However, identification of *in vitro* methods to further reduce the need for animal testing remains a top priority for NICEATM and ICCVAM.

[12] The report (NIH publication number 10-7513) will be available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/OcuAnest-TMER.htm once it is published in 2010.
[13] The report (NIH publication number 10-7515) will be available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/LVET.htm once it is published in 2010.

Definitions of Key Terms

Acute systemic toxicity: the immediate or near-immediate effect of a toxic substance after it is absorbed and distributed throughout the body. Different acute systemic toxicities are distinguished by the route of exposure (oral, dermal, or inhalation).

Basal cytotoxicity: the ability of substances to harm cells by interfering with the structures or processes essential for cell survival, proliferation, and/or function

Biomarker: a distinctive characteristic that can be measured or evaluated as an indicator of toxicity or disease

Cytotoxicity: the ability of a substance to kill or harm cells

Endpoint: the biological process, response, or effect assessed by a test method

High throughput screening: the use of rapid, automated test methods to screen large numbers of substances for their potential biological activity

Key toxicity pathway: a chain of cellular responses that, when disrupted, may cause an adverse health effect

Mode of action: the chemical or physical interactions by which a substance produces an effect

Weight-of-evidence approach: the use of the strengths and weaknesses of collected information to form a conclusion that may not be evident from the individual data

Highlights of ICCVAM Activities

- Federal agencies accepted ICCVAM's February 2008 recommendation to always consider using one of two *in vitro* basal cytotoxicity test methods to estimate starting doses for acute oral systemic toxicity testing. Using these test methods in a weight-of-evidence approach for determining starting doses for *in vivo* studies can reduce animal use by up to an additional 50%.
- NICEATM and ICCVAM prepared a draft Organisation for Economic Co-operation and Development (OECD) guidance document describing how to use these two *in vitro* test methods to estimate starting doses for acute oral systemic toxicity tests. ICCVAM forwarded the document to the OECD for consideration in July 2009.
- During a 2008 NICEATM and ICCVAM sponsored workshop on acute chemical safety testing, participants concluded that systematic collection of mechanistic data from required *in vivo* studies could help identify predictive biomarkers of systemic toxicity. These biomarkers could be used as earlier, more humane endpoints to further reduce or avoid pain and distress in test animals. Participants also recommended ways to collect data to identify key toxicity pathways for acute oral systemic toxicity that could then be used to target the development of alternative predictive *in vitro* test methods.

Evaluation of In Vitro *Test Methods to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests*

In vitro test methods that use mammalian cell cultures and various cytotoxicity endpoints have been proposed as alternatives to *in vivo* acute oral systemic toxicity tests that use rodents. *In vitro* test methods that measure basal cytotoxicity are not regarded as suitable replacements for rodent acute oral systemic toxicity tests. However, validated *in vitro* test methods can be used to estimate starting doses for *in vivo* tests, thereby reducing the number of animals needed.

Over one hundred scientists from five countries attended the ***Scientific Workshop on Acute Chemical Safety Testing: Advancing In Vitro Approaches and Humane Endpoints for Systemic Toxicity Evaluations***

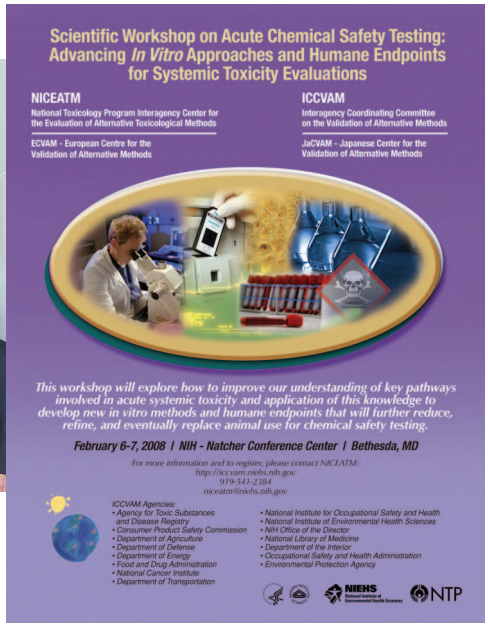


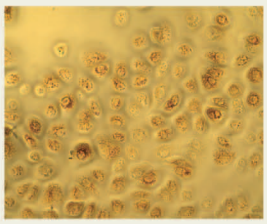
Photo shows workshop panelists and the NICEATM and ICCVAM leadership at the Natcher Conference Center at National Institutes of Health.

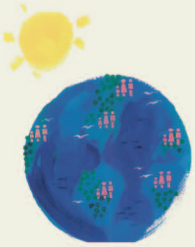
NICEATM and ECVAM cosponsored a validation study of two *in vitro* basal cytotoxicity test methods: the neutral red uptake endpoint assay using (1) 3T3 murine fibroblasts or (2) normal human epidermal keratinocytes. Based on the results, in March 2008, ICCVAM forwarded recommendations to Federal agencies on the use of the two test methods to estimate starting doses for acute oral systemic toxicity tests. ICCVAM recommended that these test methods be considered and used where appropriate before animals are used for acute oral systemic toxicity testing. These recommendations were accepted by applicable Federal agencies. Data from these test methods can now be used in a weight-of-evidence approach to determine starting doses for *in vivo* studies, which can reduce the number of animals required for each toxicity test.

NICEATM and ICCVAM prepared a draft guidance document that describes use of these two *in vitro* test methods for estimating starting doses for acute oral systemic toxicity tests. The draft guidance document was forwarded for consideration by the Test Guidelines Programme of the Organisation for Economic Co-operation and Development (OECD). Acceptance by OECD will facilitate worldwide use of these test methods to set starting doses for acute oral systemic toxicity tests, reducing the number of animals required for this testing.

Five-Year Plan Implementation: Acute Oral Systemic Toxicity Testing

- NICEATM and ICCVAM organized an international workshop to:
  - Identify standardized procedures for collecting mechanistic information from acute toxicity testing
  - Seek more predictive and humane endpoints that may be used to terminate studies earlier to reduce pain and distress
- NICEATM and ICCVAM are participating in the study management group of an ECVAM-sponsored *in vitro* validation study on biotransformation enzyme induction using HepaRG cells and cryopreserved human hepatocytes.





Workshop on Acute Chemical Safety Testing

In its evaluation of *in vitro* cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity tests (ICCVAM 2006), ICCVAM recommended that standardized procedures to collect information pertinent to an understanding of toxicity mechanisms be identified. These procedures would be included in future *in vivo* acute oral systemic toxicity studies. To that end, NICEATM and ICCVAM, in collaboration with ECVAM and JaCVAM, organized a *Scientific Workshop on Acute Chemical Safety Testing: Advancing* In Vitro *Approaches and Humane Endpoints for Systemic Toxicity Evaluations*.

More than 100 people from five countries attended the February 2008 workshop. Participants included representatives of U.S. Federal agencies, academia, industry, international organizations, and the animal welfare community. Attendees participated in breakout group discussions to address goals, objectives, and questions relating to the following themes:

- Key pathways for acute systemic toxicity
- Current acute systemic toxicity injury and toxicity assessments
- Identification of earlier humane endpoints for acute systemic toxicity testing
- Application of *in vivo* mode of action and mechanistic information to the development and validation of *in vitro* methods for assessing acute systemic toxicity
- Industry involvement in test method development, validation, and use

The workshop participants recommended data collection practices to identify key pathways for acute systemic toxicity. This mechanistic information could then be used to develop predictive *in vitro* alternative test methods. Mechanistic data collected from required *in vivo* studies would help identify biomarkers that may predict systemic toxicity. Predictive biomarkers could be used as earlier, more humane endpoints during *in vivo* tests, further avoiding or reducing pain and distress. A report of the workshop proceedings is available on the NICEATM-ICCVAM website.[14]

Other Activities Supporting Development of Alternative Methods for Acute Oral Systemic Toxicity Testing

Members of the ICCVAM Acute Toxicity Working Group and NICEATM staff are participating in the study management group of an ECVAM-sponsored *in vitro* validation study of biotransformation enzyme induction using HepaRG cells (a human hepatoma cell line) and cryopreserved human hepatocytes. The aims of this study are to (1) expose the two cell types to compounds that may induce cytochrome p450 enzymes, (2) produce metabolites from added enzyme substrates, and (3) measure metabolite production. This study is intended to validate an *in vitro* model for assessing the metabolism and toxicity of drugs, but it could lead to a novel *in vitro* platform for assessing metabolism and toxicity of additional substances.

NICEATM and ICCVAM continue to identify test methods for potential use in high throughput screening strategies that may help increase the accuracy of *in vitro* methods for predicting *in vivo* toxicity. High throughput screening involves the development and use of rapid, mechanism-based assays that can be used to screen large numbers of substances for their potential biological activity. The National Toxicology Program's High Throughput Screening Program, in collaboration with the Environmental Protection Agency and the National Institutes of Health Chemical Genomics Center, recently evaluated 13 cytotoxicity test methods for predicting acute oral systemic toxicity (Xia et al. 2008). The screening of 1,408 compounds produced robust and reproducible results, which allowed cross-compound, cross-cell type, and cross-species comparisons and may lead to prediction of *in vivo* biological responses.

[14] The workshop report is available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/acutetox/toxwksp-rpt.htm.

Public Health and Animal Welfare Perspective

Injuries to the skin, also called dermal injuries, fall into two categories. Skin corrosion is permanent damage that occurs when contact with a substance kills or destroys skin cells. Skin irritation is reversible damage that occurs when a chemical injures skin cells. In 2007, the American Association of Poison Control Centers reported nearly 150,000 injuries due to chemical burns from agents such as acids, alkalis, peroxides, bleach, and phenols or phenol products (Bronstein et al. 2008). Many of these injuries were caused by household products. For this reason, regulatory agencies require testing to determine whether substances may present skin corrosion or irritation hazards. The agencies then classify and label corrosive or irritant chemicals so that consumers and workers can take appropriate precautions to prevent injury. Test results are also used to determine appropriate packaging that will minimize hazardous spills during transport.

Dermal corrosion and irritation testing is typically done by applying a test substance to the skin of a laboratory animal. As an alternative, ICCVAM has evaluated and recommended four *in vitro* corrosivity test methods for use in a weight-of-evidence approach in an integrated testing scheme for dermal corrosion and irritation. Positive responses can be used to classify and label skin irritants without animal testing. Similar test methods to identify dermal irritants can further reduce the number of animals needed.

Highlights of ICCVAM Activities

- In 2009, several scientists from the ICCVAM Dermal Corrosivity and Irritation Working Group participated in OECD Expert Consultation meetings to evaluate several *in vitro* test methods that may be useful for reducing the number of animals used for skin irritation testing.
- NICEATM is conducting a study to determine how *in vitro* skin irritation test methods will classify corrosive substances incorrectly identified as noncorrosives by *in vitro* corrosivity test methods. These data will be used to ensure that any limitations associated with an *in vitro* testing strategy for skin corrosivity and irritation are adequately identified.

Definitions of Key Terms

Integrated testing strategy: an approach that combines different types of data and information (e.g., *in vivo*, *in vitro*, and physicochemical) in an overall decision-making process

Performance standards: standards, based on a validated test method, that provide a basis for comparing a similar proposed test method

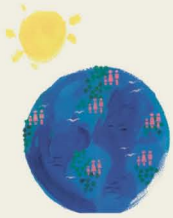
Skin corrosion: permanent damage that occurs when a substance kills skin cells

Skin irritation: reversible damage that occurs when a substance injures skin cells

Tiered testing: an approach based on sequential assessments, in which the results of tests in one tier are used to determine the tests to use in the next tier

Five-Year Plan Implementation: Dermal Safety Testing

- NICEATM and ICCVAM are evaluating alternative dermal irritation test methods for their usefulness and limitations in U.S. regulatory testing
- NICEATM and ICCVAM are contributing to revision of OECD test guidelines



Revisions to OECD Test Guidelines 430 and 431

In 2004, the Organisation for Economic Co-operation and Development (OECD) adopted test guidelines describing two *in vitro* methods for identifying substances corrosive to the skin, the rat transcutaneous electrical resistance and the reconstructed human epidermis test method (Test Guidelines 430 and 431, respectively; OECD 2004a, 2004b). At the time, there were no performance standards that could be used to evaluate the accuracy and reliability of other test methods that were (1) based on similar scientific principles and (2) measured or predicted the same biological or toxic effects. Also in 2004, ICCVAM published recommended performance standards for *in vitro* methods for identifying skin corrosives (ICCVAM 2004).

In 2006, the OECD adopted Test Guideline 435 for the *in vitro* membrane barrier test method for skin corrosion (OECD 2006). Unlike the earlier test guidelines, Test Guideline 435 included test method performance standards.

In 2009, the ICCVAM Dermal Corrosivity and Irritation Working Group and NICEATM staff drafted updates to OECD Test Guidelines 430 and 431 that included information contained in the ICCVAM-recommended performance standards. These updates are consistent with OECD Test Guideline 435. The OECD member countries are currently reviewing the ICCVAM updates to Test Guidelines 430 and 431.

Review of Proposed OECD Test Guidelines

NICEATM and ICCVAM are participating in the review of an OECD draft test guideline for skin irritation testing, *In Vitro Skin Irritation: Reconstructed Human Epidermis (RhE) Test Method.*

The OECD is currently reviewing the LabCyte™ EPI-MODEL24 *In Vitro* Human Skin Irritation Test Method. ICCVAM and the Dermal Corrosivity and Irritation Working Group nominated experts for an OECD peer review panel and reviewed the validation report and supporting documents.

Evaluation of Potential False Negative Corrosives in Proposed **In Vitro** *Dermal Irritation Assays*

Any testing strategy for dermal corrosivity and irritation must be able to accurately identify corrosive substances. These substances can cause permanent injuries and even death from severe chemical burns. The four *in vitro* corrosivity test methods evaluated by ICCVAM for dermal safety testing failed to identify an estimated 12% to 18% of dermal corrosives. Therefore, substances that test negative for corrosivity in these *in vitro* tests must be tested *in vivo* for dermal irritation as part of a weight-of-evidence tiered testing strategy. Corrosive substances that incorrectly test negative would be identified correctly by the *in vivo* test.

NICEATM is currently conducting a study to determine how *in vitro* skin irritation test methods will classify corrosive substances that were incorrectly identified as noncorrosives (false negatives) by *in vitro* corrosivity test methods. (The *in vitro* dermal irritation and dermal corrosivity test methods are slight modifications of the same test system.) This study will also confirm how much the false negative rate in corrosivity tests may be reduced by using a procedure to identify substances that affect a chemical reaction used in the test methods.

Biologics Testing

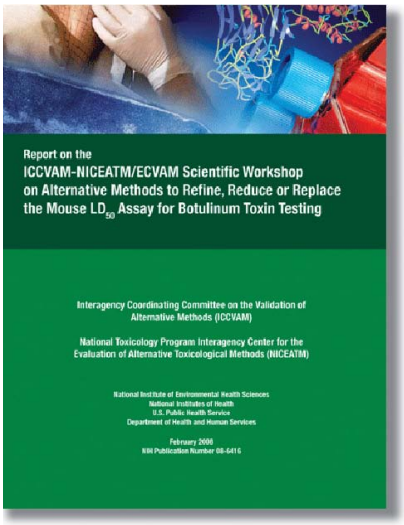
Public Health and Animal Welfare Perspective

Biological products, referred to as biologics, are derived from living organisms. They include vaccines, blood and blood components, tissues, antibodies, and other substances used to treat or protect against disease in humans and animals. Regulatory agencies such as the Food and Drug Administration and the U.S. Department of Agriculture require testing of biologics to ensure safety and potency and for labeling and lot release purposes.

Testing of biologics can require large numbers of animals. The animals used may experience significant pain and distress during testing. Therefore, identification of *in vitro* methods that would reduce the need for animal testing for biologics is a high priority for NICEATM and ICCVAM.

Highlights of ICCVAM Activities

- In 2008, NICEATM and ICCVAM published a report on a scientific workshop on alternatives to the mouse LD_{50} assay for botulinum toxin testing. The workshop was cosponsored by NICEATM-ICCVAM and the European Centre for the Validation of Alternative Methods (ECVAM). Workshop participants identified methods that could be used in specific circumstances to reduce or refine the use of mice in the current mouse LD_{50} assay for botulinum toxin potency testing, and they identified additional development and validation efforts necessary for methods that could eventually replace the use of animals. The workshop participants also recommended best practices that could decrease the number of animals required in the currently used animal test.
- NICEATM-ICCVAM, ECVAM, the Japanese Center for the Validation of Alternative Methods, and Health Canada are organizing an international workshop on alternative methods for vaccine potency and safety testing to take place in September 2010. This workshop will review the state of the science of alternative methods that are currently available and/or accepted for use that reduce, refine, and replace the use of animals in vaccine potency and vaccine safety testing. Workshop participants will also discuss ways to promote the implementation of such methods.
- NICEATM and ICCVAM are planning an evaluation of an *in vitro* test method that could replace the use of animals for potency testing of vaccines used to protect pets and livestock from the bacterial disease leptospirosis.



A report on a workshop cosponsored by NICEATM-ICCVAM and ECVAM that reviewed alternative methods for potency testing of therapeutic formulations of botulinum toxin was published in 2008.

Scientific Workshop on Alternative Methods to Refine, Reduce, or Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing

In response to a nomination from the Humane Society of the United States, NICEATM-ICCVAM and ECVAM cosponsored a November 2006 *Scientific Workshop on Alternative Methods to Refine, Reduce, or Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing*. The goals of the workshop were (1) to review the state of the science and current knowledge of alternatives that may reduce, refine (decrease or eliminate pain and distress), and replace the use of mice for botulinum toxin testing and (2) to identify priorities for research, development, and validation efforts needed to advance the use of alternative methods.

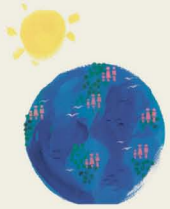
Workshop participants identified methods that could be used in specific circumstances to reduce or refine the use of mice in the mouse LD_{50} assay for botulinum toxin potency testing, and they identified additional development and validation efforts necessary for methods that could eventually replace the use of animals. The workshop participants also recommended best practices that could decrease the number of animals required in the currently used animal test. Complete conclusions and recommendations from the workshop may be found in the workshop report, which ICCVAM published in early 2008.[15]

International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing

Vaccines represent a vital and cost-effective tool to prevent many infectious diseases. The increasing occurrence of antibiotic-resistant bacteria, the emergence of novel viral illnesses, and the priority given by the World Health Organization to the eradication of a number of diseases all underscore the importance of vaccines. Currently, animal tests are used in various stages of vaccine manufacturing, testing, and quality control. Some of the tests require large numbers of animals, many of which experience unrelieved pain and distress. Accordingly, efforts have increased in recent years to develop alternative methods that reduce, refine, and replace the use of animals for vaccine potency and safety testing.

Five-Year Plan Implementation: Biologics Testing

- NICEATM and ICCVAM will sponsor a workshop to evaluate alternative methods and testing strategies for vaccine safety and potency testing.
- NICEATM and ICCVAM are planning an evaluation of *in vitro* potency tests for vaccines to prevent leptospirosis.



[15] The workshop report (NIH publication number 08-6416) and other information about the workshop are available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/biologics/biologics.htm.

NICEATM-ICCVAM, ECVAM, JaCVAM, and Health Canada are organizing a workshop that will bring together an international group of scientific experts representing relevant stakeholder organizations. Workshop participants will:

- Review the state of the science of alternative methods that are currently available and/or accepted for use that reduce, refine and/or replace the use of animals in vaccine potency and safety testing; and discuss ways to promote their use
- Identify knowledge and data gaps that need to be addressed in order to develop alternative methods that can further reduce, refine, and replace the use of animals in vaccine potency and safety testing
- Identify and prioritize the corresponding research, development, and validation efforts needed to advance the use of alternative methods while ensuring the protection of human and animal health

The workshop will be open to the public and will be held September 14–16, 2010, at the William H. Natcher Conference Center at the National Institutes of Health in Bethesda, Maryland. More information about the workshop will be posted on the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov/meetings/BiologicsWksp-2010/BiologicsWksp.htm) as it becomes available.

Evaluation of In Vitro *Potency Tests for Veterinary Vaccines*

Vaccine potency tests ensure that the active components of a vaccine are present at a concentration and in a formulation shown to be effective in preventing the target disease. The U.S. Department of Agriculture (USDA), an ICCVAM member agency, is collaborating with Michigan State University to conduct a study of *in vitro* potency tests for vaccines to prevent leptospirosis, a disease against which pets and livestock are commonly vaccinated. The USDA is reviewing the study data and NICEATM and ICCVAM are planning an evaluation of the test method.

The USDA is also investigating biomarkers that could be used to refine the animal tests currently used to verify the potency of rabies vaccine.

A September 2010 NICEATM-ICCVAM workshop will bring together an international group of scientific experts to review the current state of the science, availability, and future need for alternative methods that can reduce, refine, and replace the use of animals for vaccine potency and safety testing.

Public Health and Animal Welfare Perspective

Occupational skin diseases, including allergic contact dermatitis, are the second most common type of occupational disease, with estimated annual costs exceeding $1 billion (NIOSH 2009).

Allergic contact dermatitis (ACD) is a skin reaction characterized by localized redness, swelling, blistering, or itching after direct contact with a skin allergen. It frequently develops in workers and consumers exposed to skin-sensitizing chemicals and products. ACD results in lost workdays and can significantly diminish quality of life (Hutchings et al. 2001; Skoet et al. 2003). Because the prognosis for ACD is poor, prevention is imperative.

To protect workers and consumers, U.S. regulatory agencies require the testing of chemicals and products to determine their potential to cause ACD. Potential sensitizers include (1) chemicals such as formaldehyde; (2) formulations such as pesticides, dyes, and natural complex substances; and (3) metals such as nickel.

Historically, tests using guinea pigs (the guinea pig maximization test and the Buehler test) were the traditional test methods used to detect the ACD hazard potential of chemicals. These tests use a qualitative visual assessment of redness and swelling at the challenge site which can result in significant discomfort to the test animal. Based on a 1998 evaluation (ICCVAM 1999), ICCVAM recommended the murine local lymph node assay (LLNA) as a valid alternative to these guinea pig tests for most testing situations, a test that virtually eliminates pain and distress experienced by the test animal. More recently, ICCVAM has evaluated new applications and versions of the LLNA that should allow for its more widespread use.

Highlights of ICCVAM Activities

- NICEATM and ICCVAM evaluated several new versions and applications of the murine local lymph node assay (LLNA), an alternative test method that can be used in place of the traditional guinea pig tests for assessing the allergic contact dermatitis hazard potential of chemicals and products.
  - ICCVAM recommended an updated LLNA test method protocol that provides for a 20% reduction in the number of required animals.
  - ICCVAM recommended that the reduced LLNA test method should be used routinely to determine the allergic contact dermatitis hazard potential of chemicals and products before conducting the traditional LLNA, unless a substance is expected to produce positive results and dose-response information is needed. The rLLNA can reduce animal use by 40% for each test.
  - Working in conjunction with European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods, ICCVAM developed performance standards for the LLNA. The performance standards will enable more rapid and efficient evaluation of the validity of new versions of the LLNA that are mechanistically and functionally similar to the LLNA.

- Based on these evaluations, NICEATM and ICCVAM forwarded a proposal to update the test guideline for the LLNA issued by the Organisation for Economic Co-operation and Development (OECD) to reflect the internationally harmonized LLNA performance standards, incorporate the updated LLNA test method protocol using fewer animals, and allow use of the reduced LLNA where appropriate. The updated guideline, when OECD formally adopts it in 2010, will result in worldwide acceptance of these important revisions.
- ICCVAM evaluated the validity of three modified nonradiolabeled versions of the LLNA to identify substances as potential skin sensitizers or as nonsensitizers. ICCVAM will forward recommendations on the use of nonradiolabeled LLNA methods to Federal agencies in 2010.
- The ICCVAM recommendations on the new versions and applications of the LLNA will reduce animal use for identification of chemicals and products that could cause allergic contact dermatitis. They will also allow more institutions to take advantage of the animal welfare benefits afforded by the LLNA.

- NICEATM and ICCVAM are evaluating the application of *in vitro* methods and integrated decision strategies to reduce, refine, and replace the use of animals for identification of substances that could potentially cause allergic contact dermatitis.

Background on the LLNA Evaluation

Traditional test methods for skin sensitization evaluate allergic response in guinea pigs. In contrast, the murine local lymph node assay (LLNA) assesses skin sensitization potential by cell proliferation in lymph nodes near the test substance application site. ICCVAM originally evaluated the scientific validity of the LLNA in 1998 (ICCVAM 1999). ICCVAM recommended that the LLNA was a valid substitute for the traditional guinea pig test methods, and that the LLNA could be used to evaluate most but not all types of substances. ICCVAM also concluded that the LLNA has many advantages over the traditional test methods, including (1) using fewer animals, (2) eliminating potential discomfort from sensitizing substances, and (3) providing information about the relationship between the dose of a test substance and the resulting changes. U.S. Federal agencies accepted the ICCVAM recommendation. The LLNA was subsequently incorporated into national and international test guidelines for the assessment of skin sensitization (OECD 2002; ISO 2002; EPA 2003).

Definitions of Key Terms

Allergic contact dermatitis (ACD): an allergic reaction that results from repeated skin contact with a skin sensitizer. Clinical signs of ACD include redness, swelling, blistering, and itching.

Applicability domain: a range of chemicals and properties for which a test method has been proven useful

Cell proliferation: an increase in the number of cells as a result of cell growth and cell division

Immunotoxicity: adverse effects caused by a chemical or substance that disrupts the normal function of the immune system

Functionally similar: sharing characteristic behaviors in response to a particular reactive unit

Mechanistically similar: sharing similar modes of action and fundamental natural processes in an action, reaction, or other natural phenomenon

Performance standards: standards, based on a validated test method, that provide a basis for comparing a similar proposed test method

Physicochemical property: a property that is both physical and chemical, such as chemical class, molecular weight, solid or liquid, melting point, and boiling point

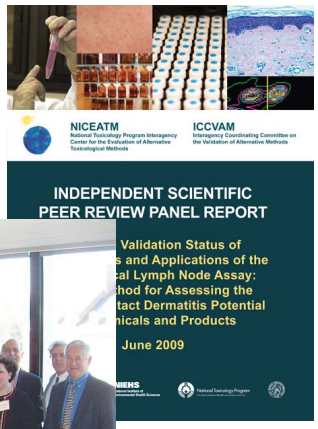
Radioisotope: the radioactive form of an element that can be used to detect and trace substances and chemical reactions by the radiation it releases

Skin sensitization: a hypersensitivity that occurs when a susceptible person comes in direct skin contact with an allergen. Once sensitized, a person may have a secondary immune response when exposed to the same allergen again.

Skin sensitization potency: the relative degree of skin sensitization caused by a chemical

Skin sensitization potential: the likelihood that a substance may cause skin sensitization

ICCVAM test method recommendations on the new versions and applications of the murine local lymph node assay (LLNA), which considered the conclusions of an independent peer review panel, will reduce animal use for identification of chemicals and products that could cause allergic contact dermatitis. They will also allow more institutions to take advantage of the animal welfare benefits afforded by the LLNA.



Members of the independent scientific peer review panel evaluating the validation status of the LLNA gather with the NICEATM and ICCVAM leadership at their March 2008 meeting at U.S. Consumer Product Safety Commisstion Headquarters.

In response to a 2007 nomination by the U.S. Consumer Product Safety Commission (CPSC), NICEATM and ICCVAM evaluated the validation status of the following new versions and applications of the LLNA:

- Three modified versions of the traditional LLNA that do not require the use of radioactive materials:
  - A version of the LLNA that uses the nucleotide analog bromodeoxyuridine (BrdU) in an enzyme-linked immunosorbent assay (ELISA) format to assess lymph node cell proliferation (LLNA: BrdU-ELISA)
  - A version of the LLNA that uses BrdU in a flow cytometry platform to measure lymph node cell proliferation (LLNA: BrdU-FC). Flow cytometry simultaneously measures and then analyzes multiple physical characteristics of cells as they flow in a fluid stream through a beam of light.
  - A version of the LLNA that measures adenosine triphosphate content in the draining lymph nodes of the ears as an indicator of lymph node cell proliferation (LLNA: DA)
- The reduced LLNA (rLLNA; also referred to as the LLNA limit dose procedure), a procedure in which a substance is tested only at a single high dose
- Use of the traditional LLNA to test formulations, metals, and aqueous solutions
- Use of the LLNA to categorize allergic contact dermatitis potency for hazard classification

NICEATM and ICCVAM also developed performance standards for the LLNA, which had not been done as part of the 1998 evaluation.

NICEATM and ICCVAM evaluated the new versions and applications of the LLNA in conjunction with ECVAM and JaCVAM. The evaluations included meetings of an independent international scientific peer review panel in 2008 and 2009. The conclusions and recommendations from the peer review panel meetings are detailed in the following reports:

- *Independent Scientific Peer Review Panel Report. Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products* (May 2008)[16]
- *Independent Scientific Peer Review Panel Report. Updated Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products* (June 2009)[17]

Evaluation of the Reduced LLNA Test Method

The protocol for the rLLNA is almost identical to that of the traditional LLNA. In the rLLNA, however, only a single dose level of each test substance is tested, as compared to three dose levels in the traditional LLNA. This alternative can reduce the number of animals needed for each test by 40%. The dose level tested in the rLLNA is typically the highest dose level of the test substance that does not induce systemic toxicity or excessive skin irritation.

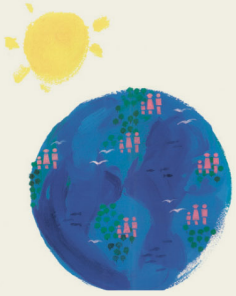
ICCVAM concluded that the rLLNA, when conducted according to the recently updated ICCVAM-recommended LLNA test method protocol, can distinguish between skin sensitizers and nonsensitizers. In light of the reduction of animal use possible by using the rLLNA, ICCVAM recommended that the rLLNA test method should be used routinely to determine the allergic contact dermatitis hazard potential of chemicals and products before conducting the traditional LLNA, unless a substance is expected to produce positive results and dose-response information is needed. The rLLNA can reduce animal use by 40% for each test. The ICCVAM recommendations on the rLLNA, included in the *ICCVAM Test Method Evaluation Report — The Reduced Murine Local Lymph Node Assay: An Alternative Test Method Using Fewer Animals to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products*, were forwarded to U.S. Federal agencies in September 2009. All agency responses should be received by March 2010.[18]

Development of LLNA Performance Standards

Before a new test method is accepted for regulatory applications, validation studies assess the test method's ability to (1) yield the same results when tested by different laboratories or by the same laboratory at different times and (2) correctly predict or measure the biological effect of interest. Performance standards define and explain how validated test methods achieve these objectives for a specific testing purpose. These performance standards can then be used as criteria to evaluate the accuracy and reliability of new test methods that are functionally and mechanistically similar to the accepted test method.

Five-Year Plan Implementation: Immunotoxicity Testing

- ICCVAM evaluated whether the LLNA can be used as a stand-alone method to determine the potency of potential sensitizers and evaluated the possible expansion of the scope of substances for which it may be used.
- ICCVAM evaluated modifications to the LLNA that may further reduce the number of animals used or eliminate the need to use radioisotopes:
  - Evaluation of the reduced LLNA
  - Evaluation of three modified nonradiolabeled versions of the LLNA
  - Development of updated LLNA test method protocol
- ICCVAM developed performance standards for the LLNA in collaboration with international validation organizations
- ICCVAM is participating on the management team of a study to evaluate *in vitro* methods to detect potential skin sensitizers



[16] The peer review panel report and other information about the 2008 peer review panel meeting are available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel08.htm.

[17] The peer review panel report and other information about the 2009 peer review panel meeting are available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm.

[18] Letters to agencies, agency responses, and a link to the *ICCVAM Test Method Evaluation Report* are available at http://iccvam.niehs.nih.gov/methods/immunotox/rLLNA.htm.

ICCVAM recommended the reduced murine local lymph node assay (LLNA) be used routinely to determine the allergic contact dermatitis potential of chemicals and products before conducting the traditional LLNA. This practice can reduce animal use by 40% for each test. ICCVAM developed performance standards for the LLNA that will enable more rapid and efficient validation of new versions of the LLNA, as well as further reducing animal use.

NICEATM and ICCVAM developed LLNA performance standards in conjunction with the test method evaluations conducted in response to the 2007 CPSC nomination. ECVAM also independently drafted LLNA performance standards in 2007. JaCVAM initiated two validation studies of modified LLNA test methods using a list of proposed reference substances to evaluate their validity. To facilitate international acceptance of LLNA performance standards, ICCVAM, ECVAM, and JaCVAM worked together to develop a harmonized document that could be included in an update to Organisation for Economic Co-operation and Development (OECD) Test Guideline 429 for the LLNA (*Skin Sensitization: Local Lymph Node Assay;* OECD 2002).

In 2009, ICCVAM published recommended performance standards that reflect the results of this collaboration in *Recommended Performance Standards: Murine Local Lymph Node Assay*. ICCVAM sent formal LLNA performance standards recommendations to U.S. Federal agencies in September 2009, and agency responses will be received by March 2010. These performance standards can be used to more rapidly and efficiently determine the validity of nonradiolabeled and other modified versions of the LLNA that are mechanistically and functionally similar to the LLNA.[19]

NICEATM and ICCVAM forwarded a proposal to update OECD Test Guideline 429 in July 2009. The proposal reflected the harmonized performance standards and included an updated protocol for the LLNA. The updated protocol requires only four animals in each dose group, resulting in a 20% reduction in the number of animals needed for LLNA testing. The updated Test Guideline 429 also provided for use of the rLLNA procedure, potentially reducing the number of animals needed for LLNA testing by 40%.

[19] Letters to agencies, agency responses, and a link to the ICCVAM *Recommended Performance Standards* are available at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm.

Evaluation of the LLNA Applicability Domain

The original ICCVAM recommendation for the LLNA supported its use for testing a limited range of substances. In the 2007 nomination, the CPSC asked that NICEATM-ICCVAM evaluate the applicability of the LLNA for testing formulations, aqueous solutions, and metals, with the expectation that a wider applicability domain for the LLNA would enable wider use.

Following the conclusion of an independent scientific peer review panel, ICCVAM recommended that the LLNA may be used to test any chemical or product for allergic contact dermatitis hazard potential, including pesticide formulations, natural complex substances, and dyes, unless it has properties associated with it that may interfere with the ability of the LLNA to detect sensitizing substances. The complete ICCVAM recommendations, included in the *ICCVAM Test Method Evaluation Report on Using the Murine Local Lymph Node Assay for Testing Pesticide Formulations, Metals, Substances in Aqueous Solutions, and Other Products,* will be transmitted to U.S. Federal agencies in 2010.[20] Agency responses will be due within 180 days of transmittal. The proposed update to OECD Test Guideline 429 also includes the ICCVAM recommendations on the expanded LLNA applicability domain.

Evaluation of Modified Nonradiolabeled LLNA Test Methods

The traditional LLNA test method uses radioisotopes (^{3}H-methyl thymidine or ^{125}I-iododeoxyuridine) to detect cell proliferation caused by potential sensitizers. Therefore, only laboratories qualified to use radioactive reagents have been able to conduct the LLNA test method. The CPSC nomination requested that NICEATM and ICCVAM also evaluate modified LLNA test methods that do not require the use of radioactive materials. Acceptance of such methods may enable wider use of the LLNA by laboratories that cannot use radioactive reagents, thereby further reducing and refining the use of animals for skin sensitization testing. Furthermore, there are environmental advantages in terms of reduced hazardous waste disposal.

The draft ICCVAM recommendations supported the use of two modified nonradiolabeled versions of the LLNA, the LLNA: BrdU-ELISA and the LLNA:DA, to identify substances as potential skin sensitizers or as nonsensitizers, with certain limitations. ICCVAM deferred recommendations on use of a third modified nonradiolabeled method, the LLNA: BrdU-FC, pending (1) submission of individual animal data for the intralaboratory evaluation and (2) completion of an interlaboratory reproducibility study. ICCVAM recommendations on the LLNA: BrdU-ELISA and the LLNA: DA are being finalized and will be issued in test method evaluation reports, which will be published in 2010.[21,22]

Based on these recommendations, NICEATM and ICCVAM submitted to OECD two new draft test guidelines to facilitate the international acceptance of the LLNA: BrdU-ELISA and the LLNA: DA. The OECD convened an international expert consultation in October 2009 to review these draft test guidelines and updates to Test Guideline 429. The expert consultation meeting was co-hosted by NICEATM–ICCVAM and the CPSC at CPSC Headquarters in Bethesda, Maryland.

NICEATM revised the test guidelines based on discussions and recommendations of the expert consultation. The revised test guidelines were recirculated to member countries for additional comment in early 2010, and will be considered by the OECD Working Group of National Coordinators at its March 2010 meeting.

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[20] The report (NIH publication number 10-7512) will be available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-app/TMER.htm once it is published in 2010.

[21] The ICCVAM test method evaluation report on the LLNA: DA (NIH publication number 10-7551) will be available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/TMER.htm once it is published in 2010.

[22] The ICCVAM test method evaluation report on the LLNA: ELISA (NIH publication number 10-7552) will be available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/TMER.htm once it is published in 2010.

Use of the LLNA to Categorize Skin Sensitization Potency

ICCVAM's 1998 evaluation of the LLNA (ICCVAM 1999) supported its use for classifying substances as potential sensitizers or as nonsensitizers. In the 2007 nomination, CPSC asked that NICEATM and ICCVAM assess the validation status of the LLNA as a stand-alone assay to classify sensitizers according to the relative degree of skin sensitization likely to be caused by a chemical. ICCVAM concluded that the LLNA cannot be used as a stand-alone assay to determine skin sensitization potency for hazard classification. However, ICCVAM encourages the development of integrated decision strategies that use the LLNA along with additional information to determine skin sensitization potency. ICCVAM's evaluation of the LLNA for skin sensitization potency categorization is currently nearing completion, and final ICCVAM recommendations will be forwarded to Federal agencies late in 2010.

Evaluation of In Vitro *Assays to Identify Potential Sensitizers*

NICEATM and ICCVAM are evaluating the use of *in vitro* test methods to assess allergic contact dermatitis hazards. A validation study of a direct peptide reactivity assay and two *in vitro* cell culture-based methods, the human cell line activation test and the myeloid U937 skin sensitization test, is currently in progress. Data from such assays could be used in combination with existing data from *in vivo* tests, physical and chemical properties, and other information to make regulatory decisions about potential sensitizers. ICCVAM members and NICEATM staff are serving as liaisons to the ECVAM-led study management team, which also includes JaCVAM liaisons.

NICEATM is studying how to use data from *in vitro* test methods in combination with information about physical and chemical properties of potential sensitizers to improve regulatory classification of substances with uncertain or borderline *in vivo* test results. NICEATM and ICCVAM will also propose *in vitro* assays that can be used with high throughput screening platforms to increase the accuracy of *in vitro* predictions of allergic contact dermatitis. NICEATM will evaluate the high throughput screening results to identify the most useful assays.

Endocrine Disruptor Testing

Public Health Perspective

The endocrine system is one of the body's main communication networks. Hormones produced by glands throughout the body act as chemical messengers, controlling numerous body functions. Examples of hormones include estrogens, androgens, and thyroid hormones.

Endocrine disruptors mimic or block the action of hormones, interfering with normal hormone function and thereby causing adverse health effects. Evidence suggests that environmental exposure to endocrine disruptors may cause reproductive and developmental problems in humans and wildlife. There is also concern that exposure to endocrine disruptors may increase cancer incidence in humans. Laboratory studies have classified a variety of substances as endocrine disruptors. For example, polycarbonate plastic, such as that used for water bottles and baby formula bottles, contains a chemical called bisphenol A (BPA), a known endocrine disruptor.

In response to early findings, Congress passed the Food Quality Protection Act (Public Law 104-170) in 1996. This law directs the Environmental Protection Agency to screen pesticides and environmental contaminants for their potential to affect

the endocrine systems of humans and wildlife. The Environmental Protection Agency subsequently initiated an Endocrine Disruptor Screening Program and began efforts to standardize and validate test methods to include in the program. In support of this effort, ICCVAM has evaluated the validation status of and conducted validation studies of *in vitro* test methods to identify potential endocrine disruptors.

Highlights of ICCVAM Activities

- NICEATM is coordinating validation studies of two *in vitro* test methods that could reduce the number of animals used to detect estrogenic and anti-estrogenic activity: the LUMI-CELL® estrogen receptor (ER) assay developed by Xenobiotic Detection Systems, Inc., and the MCF-7 cell proliferation assay developed by CertiChem, Inc. An independent scientific peer review panel will evaluate the results from international validation studies for these test methods in 2011.

Validation Study of the LUMI-CELL® ER Assay of Estrogenic Activity

The LUMI-CELL® estrogen receptor (ER) test method uses an immortalized, genetically modified cell line (BG1Luc4E2) that expresses firefly luciferase activity and glows in response to estrogen and estrogen-like substances. The test method measures the light emitted when the cells are exposed to a test substance.

NICEATM is coordinating an international LUMI-CELL® ER validation study at laboratories in Italy, the United States, and Japan. Results from Phase 1 of the study demonstrated acceptable intralaboratory reproducibility and established quality controls for additional testing. Phase 2 was conducted to optimize testing protocols. In Phase 3 of the study, each laboratory used the revised protocol to test the minimum number of reference substances recommended by ICCVAM for the validation of *in vitro* ER test methods. Phase 4 will test the remaining ICCVAM reference substances in the U.S. laboratory only. An independent scientific peer review panel will evaluate the results of the study in 2011.

Definitions of Key Terms

Agonist: a substance that mimics the action of the body's own hormones

Antagonist: a substance that decreases or blocks the action of the body's own hormones

Endocrine disruptor: a natural or man-made substance that may mimic or block the action of hormones, interfering with normal hormonal function and causing adverse health effects

Environmental contaminant: a substance or pollutant released into the environment, causing instability, disorder, or harm to the ecosystem

Estrogen: a class of hormones, produced largely by the ovaries, that serve as the primary female hormones

Estrogen receptor: a protein molecule to which estrogen or estrogen-like substances can attach. This interaction produces a chemical signal or triggers a cellular response.

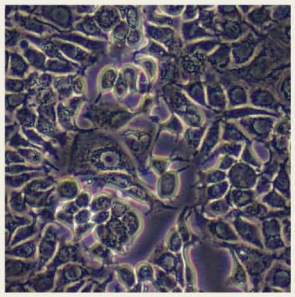
Firefly luciferase: the enzyme in fireflies that undergoes a chemical reaction to produce light (glow) and that scientists can use to detect certain chemical reactions

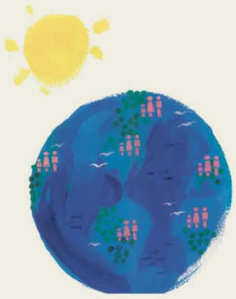
Immortalized cell line: cells that continue to grow and divide indefinitely *in vitro*

Reference substance: a substance used to assess the accuracy and reliability of a test method

Five-Year Plan Implementation: Endocrine Disruptor Testing

- NICEATM-ICCVAM is leading an international study, with the European Centre for the Validation of Alternative Methods and the Japanese Center for the Evaluation of Alternative Methods, to evaluate the usefulness and limitations of the LUMI-CELL® estrogen receptor *in vitro* test method, a test method to identify estrogen-like chemicals. The LUMI-CELL® ER assay does not require animals as donors for test components.





Validation of CertiChem Inc. MCF-7 Cell Proliferation Assay

The CertiChem MCF-7 cell proliferation assay measures cell proliferation as an indicator of estrogenic activity of a test substance. The MCF-7 cell line, an immortalized human cell line derived from human breast cancer cells, includes estrogen receptors and responds to estrogen exposure with cell proliferation. CertiChem conducted validation studies to evaluate the performance of the assay in measuring estrogenic and anti-estrogenic activity. NICEATM worked closely with CertiChem to ensure that ICCVAM recommendations on the conduct of validation studies for endocrine disruptor test methods (ICCVAM 2003a) were followed. NICEATM also provided coded reference substances for testing. NICEATM is now coordinating an interlaboratory validation study of the CertiChem assay.

Other Activities Supporting Development of In Vitro *Assays to Identify Potential Endocrine Disruptors*

The National Toxicology Program is developing a High Throughput Screening Initiative that, among other things, seeks to identify endocrine-disrupting chemicals. NICEATM staff members are monitoring the development and testing of the *in vitro* assays being evaluated for use in the High Throughput Screening Initiative to detect endocrine-disrupting chemicals.

ICCVAM members and NICEATM staff reviewed and commented on the Organisation for Economic Co-operation and Development's (OECD) draft test guidelines and guidance documents for the identification of potential endocrine disruptors. NICEATM and ICCVAM also participated in the OECD Validation Management Group for Non-animal Testing.

Pyrogen Testing

Public Health and Animal Welfare Perspective

Pyrogens cause inflammation and fever when injected or implanted into the body. Sources of pyrogenic materials include bacteria, fungi, and viruses. The inflammatory reaction to these substances can be severe, sometimes leading to multiple organ failure and death. Regulatory agencies such as the Food and Drug Administration require that medical devices and pharmaceutical products intended for administration by injection be free of pyrogen contamination before use in humans and animals.

Definitions of Key Terms

Endotoxin: a harmful chemical or substance that is released from gram-negative bacteria when the bacteria are killed

Gram-negative: a type of bacteria that can react with the immune system, causing inflammation and infection.

Hemolymph extract: proteins and water-binding agents from the blood-like fluid in horseshoe crabs

Monocytes: white blood cells that multiply in response to inflammation and fever

Parenteral: route of administration of a drug or chemical by injection

Pyrogen: a substance that can cause fever

In the past, the U.S. Pharmacopoeia, as well as the European and Japanese Pharmacopeias, recognized two test methods for pyrogen testing. Both methods use animals or animal tissues. The rabbit pyrogen test (RPT) measures the rise in temperature of rabbits after intravenous injection of a test solution. The bacterial endotoxin test (BET) is an *in vitro* assay that measures coagulation of hemolymph extract from the horseshoe crab after exposure to endotoxin. The BET was accepted by the Food and Drug Administration in the 1980s and has substantially decreased rabbit use in the U.S. for pyrogen testing. Other *in vitro* methods are needed, however, for cases where use of the BET is not appropriate.

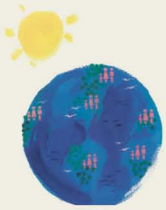
Alternative *in vitro* test systems based on the activation of human monocytes take advantage of the role these cells play in the fever response. In addition to eliminating the need to use live animals for testing in situations where the use of the BET is not appropriate, an assay using human cells could more accurately represent the human response to pyrogens.

Highlights of ICCVAM Activities

- All applicable Federal agencies, including the Food and Drug Administration, accepted or endorsed ICCVAM's 2008 recommendations on the use of five *in vitro* test methods to assess the potential pyrogenicity of pharmaceuticals and other products. The test methods may now be used instead of animal tests in specific circumstances to detect Gram-negative endotoxin that can cause fever reactions in people in human parenteral drugs.

Five-Year Plan Implementation: Pyrogen Testing

- ICCVAM issued recommendations on the current usefulness of five *in vitro* pyrogen test methods and recommendations for future studies that may support their expanded use.



ICCVAM Recommendations and Agency Responses

ICCVAM evaluated five *in vitro* test methods that use human cells to assess the potential of pharmaceuticals and other products to induce fever:

- Human whole blood/interleukin (IL)-1 *in vitro* pyrogen test
- Human whole blood/ IL-1 *in vitro* pyrogen test using cryopreserved human whole blood
- Human whole blood/IL-6 *in vitro* pyrogen test
- *In vitro* pyrogen test using human peripheral blood mononuclear cells
- Alternative *in vitro* pyrogen test using the human monocytoid cell line MONO MAC 6

In 2008, ICCVAM forwarded recommendations to Federal agencies on the use of these test methods in the *ICCVAM Test Method Evaluation Report: Validation Status of Five* In Vitro *Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products*.[23]

ICCVAM concluded that none of these test methods can be considered a complete replacement for the RPT for the detection of Gram-negative endotoxin. However, ICCVAM recommended that they be considered for the detection of Gram-negative endotoxin in human parenteral drugs on a case-by-case basis, subject to validation for each specific product to demonstrate equivalence to the RPT, in accordance with applicable U.S. Federal regulations. In 2009, all applicable Federal agencies, including the Food and Drug Administration, accepted or endorsed the ICCVAM recommendations.

The European Pharmacopeia Commission formally adopted *Monograph 2.6.30, Monocyte Activation Test* (formerly alternative pyrogen test), in March 2009. The monocyte activation test includes components of each of the five *in vitro* test methods recommended by ICCVAM. It will be incorporated into the European Pharmacopeia in 2010.[24]

These methods should now be considered before *in vivo* pyrogen testing and used when appropriate for specific testing situations. These test methods may reduce the number of animals required for pyrogen testing.

Genetic Toxicity Testing

Public Health and Animal Welfare Perspective

Genetic toxicity damages the DNA and/or chromosomes of cells, which may increase the likelihood of birth defects and diseases such as cancer. Genetic toxicity can be caused by (1) physical agents such as radiation and ultraviolet light and (2) chemical substances, including environmental pollutants and compounds in cigarette smoke and certain medicines.

[23] The report (NIH publication number 08-6392) is available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/pyrogen/pyr_tmer.htm.

[24] Reported by the European Directorate for the Quality of Medicine; available at: http://www.edqm.eu/medias/fichiers/133rd_Session_of_the_Eu.pdf.

Definitions of Key Terms

Cytotoxicity: the ability of a substance to kill or harm cells

Genotoxic: describes a substance that harms an organism by damaging its DNA

Reference substance: a substance used to assess the accuracy and reliability of a test method

The Environmental Protection Agency, Food and Drug Administration, and Consumer Product Safety Commission have testing requirements and guidelines to assess the genetic toxicity of regulated products. The Ames test is a bacterial assay that is very effective at identifying genetic toxins, but some substances are only genotoxic after they are metabolized or modified by the body. Because the Ames test and similar assays do not duplicate the body's metabolism, animal tests are also used to identify genetic toxins. NICEATM and ICCVAM work with international collaborators to evaluate genetic toxicity test methods that can reduce, refine, and replace the use of animals for this purpose.

Highlights of ICCVAM Activities

- In 2009, the ICCVAM Genetic Toxicity Working Group provided comments on cytotoxicity evaluation procedures and study design for a draft OECD Test Guideline 487 for the *in vitro* micronucleus test. This test is intended to reduce the number of animals used to identify substances that may cause genetic damage that can lead to cancer and other adverse health effects.
- The Genetic Toxicity Working Group commented on the proposed study plan, protocol, and reference substances for a JaCVAM-led international validation study of the comet assay.
- The working group nominated experts to an ECVAM-sponsored peer review panel for an ongoing validation of a cell transformation assay that uses cultured mouse and hamster cells to detect genotoxic and nongenotoxic carcinogens. This test method is also intended to reduce the number of animals used to detect substances that may cause cancer.

ICCVAM Involvement in Genetic Toxicity Validation Studies

The ICCVAM Genetic Toxicity Working Group provided comments on cytotoxicity evaluation procedures and study design for the Organisation for Economic Co-operation and Development's draft Test Guideline 487 for the *in vitro* micronucleus test. The *in vitro* micronucleus test is a test method that measures a test substance's potential for causing damage to chromosomes. In this method, cultured human or rodent cells are treated with a test substance and then examined for the presence of chromosome fragments known as micronuclei.

The Genetic Toxicity Working Group recommended approval of the draft test guideline based on data from collaborative cytotoxicity studies at European Union and U.S. laboratories. The working group agreed that there was adequate evidence for the alternative measures of cytotoxicity included in the draft test guideline. The group also agreed that either relative population doubling or relative increase in cell counts is sufficient to detect a genotoxic substance, based on comparison to the more traditional measure, relative cell count.

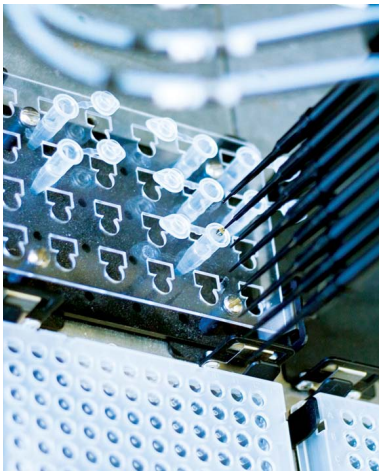
NICEATM and ICCVAM are participating in an international validation study of the comet assay test method led by the Japanese Center for the Validation of Alternative Methods. The ICCVAM Genetic Toxicity Working Group contributed to development of the study plan, test method protocol, and a proposed list of reference substances for Phase IV of the study. The study focuses on validating the comet assay test method, using liver and stomach as the target organs, as an alternative to the rat hepatocyte unscheduled DNA synthesis assay.

The Genetic Toxicity Working Group has also nominated experts for a peer review panel sponsored by the European Centre for the Validation of Alternative Methods on a cell transformation assay. The working group provided technical comments on an ongoing validation of a cell transformation assay that uses mouse BALB/c 3T3 and Syrian hamster embryo cells to detect genotoxic and nongenotoxic carcinogens.

New Technologies Supporting Alternative Methods Development

Public Health and Animal Welfare Perspective

ICCVAM was established to promote the regulatory acceptance of new, scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests. To effectively foster development of new test methods that may improve hazard identification while reducing animal use, NICEATM and ICCVAM must identify research activities relevant to the development of such methods. The *NICEATM-ICCVAM Five-Year Plan* challenged ICCVAM to identify and promote research incorporating new technologies that can be expected to support future test method development. Effective translation of technological advances into new test methods allows better protection of public health while addressing animal use and welfare concerns.



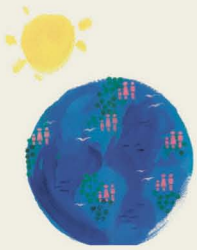
ICCVAM established a Research and Development Working Group to help NICEATM and ICCVAM identify and promote research incorporating new technologies that are expected to support future test method development.

Highlights of ICCVAM Activities

- NICEATM and ICCVAM are collaborating with member-agency scientists involved in cooperative research initiatives to speed the translation of research advances and new technologies into scientifically valid safety testing methods that will further reduce, refine, and replace animal use. Similar collaborations with our international partners are also ongoing.
  - ICCVAM established a Research and Development Working Group (RDWG) to collaborate with the ICCVAM Five-Year Plan Implementation Subcommittee. The RDWG includes scientists that are integrally involved in ICCVAM member-agency research programs. Their participation on the RDWG will help ICCVAM identify promising test methods for referral to appropriate ICCVAM working groups for evaluations or other activities.
  - NICEATM and ICCVAM are participating in an interagency collaboration between the National Toxicology Program (within the National Institute of Environmental Health Sciences), the Environmental Protection Agency, and the National Institutes of Health Chemical Genomics Center to evaluate defined high throughput screening approaches. This initiative is expected to yield candidate methods and approaches with potential applicability to regulatory testing. Promising methods and approaches will then be reviewed by ICCVAM, who will forward recommendations on appropriate use to Federal agencies.
    - In 2009, NICEATM nominated nearly 1000 chemicals for inclusion in a 10,000-chemical library that will be used to evaluate each *in vitro* assay nominated and accepted for this initiative.
- The RDWG and Five-Year Plan Implementation Subcommittee are planning an implementation workshop that will describe best practices for the consideration and use of alternative methods that have been accepted for regulatory use. This workshop will bring together potential users of accepted alternative test methods and representatives of regulatory agencies in an effort to facilitate broader use of accepted alternative methods that can reduce, refine, or replace the use of animals for specific safety testing purposes.

Five-Year Plan Implementation: New Technologies Supporting Alternative Methods Development

- ICCVAM has established a Research and Development Working Group to help NICEATM and ICCVAM identify and promote research incorporating new technologies that can be expected to support future test method development.
- NICEATM and ICCVAM are monitoring the progress of a collaboration between the National Toxicology Program, the Environmental Protection Agency, and the National Institutes of Health Center for Chemical Genomics to develop high throughput technologies for toxicological testing. NICEATM-ICCVAM has nominated nearly 1000 commercially available reference substances for use in this effort.



ICCVAM Research and Development Working Group

NICEATM and ICCVAM are working to maximize the efficiency with which key research advances are translated into new test methods. They are collaborating with member agency scientists involved in cooperative research initiatives to speed the translation of research advances and new technologies into scientifically valid safety testing methods that will further reduce, refine, and replace animal use. Similar collaborations with our international partners are also ongoing.

In 2009, ICCVAM established the Research and Development Working Group to help NICEATM and ICCVAM identify and promote research incorporating new technologies that are expected to support future test method development. The Research and Development Working Group includes scientists from ICCVAM member agencies. These scientists know about the scope of research and development activities within their agencies that are relevant to alternative test methods. Members will be asked to help identify test methods in any phase of development for referral to appropriate ICCVAM working groups, which could then recommend evaluations or other activities to ICCVAM. The Research and Development Working Group will periodically update an inventory of relevant agency research, development, translation, and validation activities.

Implementation Workshop on Best Practices for the Consideration and Use of Alternative Methods

NICEATM and ICCVAM are committed to holding workshops that address the challenges of incorporating alternative methods into regulatory safety testing regimes. NICEATM-ICCVAM workshops also address the difficulty of obtaining high-quality data to validate alternative methods. The Research and Development Working Group and Five-Year Plan Implementation Subcommittee are planning a workshop to describe best practices for the consideration and use of alternative methods that have recently been adopted or endorsed by ICCVAM and U.S. regulatory agencies. This workshop, which will take place in 2011, will provide an excellent forum for discussions of appropriate use of accepted alternative methods with the potential to reduce, refine, or replace animal use in commonly required regulatory safety tests.

High Throughput Screening Technologies

Predictive high throughput assays that identify alterations to key toxicity pathways can make toxicity testing more relevant to human exposures while reducing animal use. In a 2007 report, the National Research Council outlined a vision for development of such assays (NRC 2007). In support of this vision, the National Toxicology Program, in collaboration with the EPA and the NIH Center for Chemical Genomics, is testing many compounds to broadly characterize and define the chemical and biological properties of chemicals with the potential to cause adverse health effects. This is part of an initiative known as "Tox21," for "Toxicity Testing in the 21st Century."

The Tox 21 initiative is expected to yield alternative test methods and approaches with potential applicability to regulatory testing. NICEATM and ICCVAM are participating in this collaboration, and promising methods and approaches will be reviewed by ICCVAM, who will forward recommendations on appropriate use to Federal agencies. NICEATM and ICCVAM have also nominated nearly 1000 commercially available reference substances, for which high-quality reference data are available, for inclusion in this initiative.

NICEATM and ICCVAM are collaborating with member agency scientists involved in cooperative research initiatives to speed the translation of research advances and new technologies into scientifically valid safety testing methods that will further reduce, refine, and replace animal use.

ICCVAM Outreach and Cooperative Activities

NICEATM and ICCVAM cooperate and collaborate with their international counterparts in the evaluation and validation of alternative safety testing methods.

Above: The International Cooperation on Alternative Test Methods was signed on April 27, 2009, by (seated, from left) Dr. Linda Birnbaum, Director of the NIEHS; Dr David Blakey, Director of the Health Canada Environmenatal Science and Research Bureau; Dr. Elke Anklam, Director of the European Union Institute for Health and Consumer Protection; and Dr. Hajime Kojima, Director of JaCVAM. Also present at the signing were (standing, from left) Dr. Marilyn Wind, ICCVAM Chair, Dr. Linda Katz, Director of the FDA Office of Cosmetics and Colors, and Dr. William Stokes, Director of NICEATM.

Highlights of ICCVAM Activities

- In April 2009, an ICCVAM initiative fostered an international agreement between the United States, Canada, Japan, and the European Union that is expected to further reduce animal use in product toxicity testing worldwide. The agreement involves globally coordinated high-quality validation studies and peer reviews executed using a transparent process that should speed the international harmonization and adoption of alternative toxicity testing methods.
- NICEATM is coordinating an international validation study of an assay to identify potential endocrine disruptor activity. NICEATM and ICCVAM provided recommendations and/or liaisons to the management teams for four other international validation studies.
- NICEATM and ICCVAM prepared, commented on, or otherwise contributed to the development of over twenty new test guidelines, revisions of existing test guidelines, and guidance documents being considered by the Organisation for Economic Co-operation and Development.
- United States and international organizations recognized ICCVAM members and NICEATM staff with five awards for their activities in support of ICCVAM's mission.
- ICCVAM members and NICEATM staff gave presentations at meetings of the Society of Toxicology in 2008 and 2009, and at the Seventh World Congress on Alternatives and Animal Use in the Life Sciences in 2009. Representatives of NICEATM and ICCVAM also attended and gave presentations at eight other international meetings and conferences.

Establishment of the International Cooperation on Alternative Test Methods

On April 27, 2009, representatives from four international agencies, including the director of the National Institute of Environmental Health Sciences and National Toxicology Program, signed a Memorandum of Cooperation establishing an International Cooperation on Alternative Test Methods (ICATM). The agreement promotes enhanced international cooperation and coordination on the scientific validation of non- and reduced-animal toxicity testing methods. This cooperation will help translate research advances into more effective tools to protect public health.

The ICATM will speed the adoption of new test methods based on advances in science and technology. New test methods will more accurately predict safety and hazards. Animal welfare will also be improved by prompt national and international acceptance of alternative test methods that reduce, refine, and replace the use of animals.

Background on ICATM

The ICCVAM Authorization Act charges ICCVAM with facilitating international harmonization of test method protocols that encourage the reduction, refinement, and replacement of animals in toxicological testing. This is accomplished via ICCVAM's interactions with international validation organizations such as ECVAM and JaCVAM. Collaboration among these groups has steadily increased during the past 10 years. However, in the past, interaction was coordinated on an *ad hoc*, informal basis.

International Government Organizations Involved in Evaluation of Alternative Test Methods

ICCVAM is an interagency committee of the U.S. government that coordinates technical reviews of alternative test methods and cross-agency issues relating to validation, acceptance, and harmonization of test methods. NICEATM administers ICCVAM and provides scientific support for its activities.

ECVAM (European Centre for the Validation of Alternative Methods) is a unit within the Institute of Health and Consumer Protection in the European Union's Joint Research Centre. ECVAM coordinates the validation of alternative test methods at the European Union level.

JaCVAM (Japanese Center for the Validation of Alternative Methods) is part of the Japanese National Institute of Health Sciences, for which it coordinates the evaluation of alternative test methods.

The Environmental Health Science and Research Bureau within Health Canada coordinates the evaluation of alternative test methods in Canada.

Established in November 2009, KoCVAM (Korean Center for the Validation of Alternative Methods) is part of the National Institute of Food and Drug Safety Evaluation of the South Korean Food and Drug Administration.

The governments of Brazil, Finland, and the People's Republic of China are exploring the establishment of their own centers for the evaluation of alternative test methods.

The International Cooperation on Cosmetic Regulation (ICCR) is a group of cosmetic regulatory authorities from the United States, Japan, the European Union, and Canada. At its first meeting in September 2007, the ICCR recognized the importance of reducing, refining, and replacing animals used in toxicity testing. The group recommended further strengthening international collaboration and communication in the design, execution, and peer review of validation studies for scientific alternatives to animal testing. They noted that such efforts should involve interactions among scientific experts from the regulatory bodies within the participating countries. In response, NICEATM and ICCVAM, ECVAM, JaCVAM, and Health Canada developed a framework to ensure collaboration on this issue.

The ICATM Framework

ICCVAM, ECVAM, JaCVAM, and Health Canada collaborated in developing the framework for ICATM. The goals of this framework are:[25]

- To establish international cooperation in the critical areas of validation studies, independent peer review, and development of harmonized test method recommendations to ensure that alternative methods/strategies are more readily accepted worldwide
- To establish international cooperation necessary to ensure that new alternative test methods/strategies adopted for regulatory use will provide equivalent or improved protection for people, animals, and the environment, while replacing, reducing, or refining (decreasing or eliminating pain and distress) animal use whenever scientifically feasible

The framework addresses three critical areas of cooperation: validation studies on proposed alternative test methods, independent peer review of the validation status of test methods, and development of formal test method recommendations. The ICATM framework has been endorsed by ICCVAM and adopted by the ICCR.

ICCVAM members and NICEATM staff attended four ICATM coordination meetings in 2008 and 2009.

[25] The ICATM Framework is available on the FDA website at: http://www.fda.gov/InternationalPrograms/HarmonizationInitiatives/ucm114518.htm

Collaborations with International Validation Organizations

NICEATM and ICCVAM have collaborated or cooperated with ECVAM, JaCVAM, or KoCVAM on the following additional activities:

- NICEATM and ICCVAM collaborated with ECVAM and JaCVAM in conducting international validation studies. (See next page for list and previous chapter for details.)
- The Head of ECVAM and the Director of JaCVAM continued to participate in SACATM meetings as ex officio nonvoting liaison members.
- ICCVAM collaborated with ECVAM and JaCVAM during 2007 and 2008 to develop internationally harmonized performance standards for the LLNA.
- Dr. Marilyn Wind, Chair of ICCVAM, and Dr. William Stokes, Director of NICEATM, continued to participate in ECVAM Scientific Advisory Committee meetings as official observers. Meetings were held in May and November 2008, and in April and July 2009.
- ECVAM and JaCVAM had liaisons to each ICCVAM working group that was active during the reporting period. Liaisons from ECVAM and JaCVAM provided comments on behalf of their organizations during the development of ICCVAM test method recommendations on ocular safety test methods and new versions and applications of the LLNA.
- ICCVAM requested nominations of experts from ECVAM and JaCVAM for the independent scientific peer review panels on the LLNA and ocular test methods.
- ICCVAM, NICEATM, ECVAM, and JaCVAM cosponsored the February 2008 *Scientific Workshop on Acute Chemical Safety Testing: Advancing* In Vitro *Approaches and Humane Endpoints for Systemic Toxicity Evaluations*.
- ICCVAM nominated experts to participate on ECVAM Scientific Advisory Committee peer reviews for the following test methods:
  - The cell transformation assay
  - Four cell function-based test methods for identifying mild to moderate ocular irritants and substances not labeled as ocular irritants
- Dr. Stokes presented an update on NICEATM-ICCVAM activities at the November 2009 meeting of the scientific advisory committee of JaCVAM.

NICEATM is coordinating the international validation study on the use of the LUMI-CELL® ER assay for identification of potential disruptors of estrogen receptor activity.

NICEATM contract support staff discuss data from the LUMI-CELL® ER assay with members of the international validation study team during a visit to the laboratories of Hiyoshi Ecological Services Co. in Omihachiman, Japan. Pictured from left are Dr. Hajime Kojima (JaCVAM), Ms. Clevita Mascarenkas (Hiyoshi), Mr. Masafumi Nakamura (Hiyoshi), Dr. John Gordon (formerly of XDS, Inc.), Ms. Atomi Nishimura (Hiyoshi), Dr. David Allen (NICEATM), Dr. George Clark (XDS, Inc.), and Mr. Frank Deal (NICEATM).

International Cooperation on Validation Studies

Ocular Safety Testing

- Evaluation of four cell function-based *in vitro* test methods (the fluorescein leakage, neutral red release, Cytosensor® Microphysiometer, and red blood cell hemolysis test methods) to identify substances that can cause mild to moderate ocular irritation and to identify substances not labeled as ocular irritants
  - ICCVAM provided recommendations to an ECVAM Scientific Advisory Committee peer review
  - ICCVAM members and NICEATM staff are serving on the validation management group for this ECVAM-led study; Dr. Raymond Tice, NICEATM Deputy Director (through February 2009) and ICCVAM representative from NIEHS, attended a meeting of the validation management group in Ispra, Italy, in October 2008
- Evaluation of the use of the *in vitro* EpiOcular ™ and SkinEthic HCE ™ test methods for discrimination between irritants and substances that do not cause classifiable ocular irritation
  - NICEATM and ICCVAM have liaison members to the ECVAM-led study management team planning validation studies for these two methods

Acute Oral Systemic Toxicity

- Validation study on *in vitro* biotransformation enzyme induction using HepaRG cells and cryopreserved human hepatocytes
  - ICCVAM members and NICEATM staff are participating in the study management group of this ECVAM-sponsored study

Immunotoxicity Testing: Allergic Contact Dermatitis

- Evaluation of *in vitro* tests for assessing skin sensitization potential of chemicals
  - ICCVAM members and NICEATM staff, along with JaCVAM associates, are serving on the validation management group for this ECVAM-led study
  - NICEATM and ICCVAM submitted comments to the draft study plan

Endocrine Disruptor Testing

- Validation of LUMI-CELL® estrogen receptor assay for identification of potential disruptors of estrogen receptor activity
  - NICEATM is coordinating the international validation study, which includes participating laboratories in the U.S., Italy, and Japan

Genetic Toxicity Testing

- Validation of an *in vivo* rodent comet assay test method as a potential alternative for the *in vivo* rat hepatocyte unscheduled DNA synthesis assay
  - NICEATM and ICCVAM contributed to the development of the study plan, protocol, and list of reference substances for this JaCVAM-led study
- Validation of the cell transformation assay in BALB/c 3T3 and SHE cells to detect genotoxic and non-genotoxic carcinogens
  - ICCVAM provided technical comments for JaCVAM-led study

Carcinogen Testing

- Validation study of the Bhas 42 cell transformation assay to screen chemicals for carcinogenic potential
  - NICEATM and ICCVAM have liaison members to the JaCVAM-led management team for this study; Dr. William Stokes, Director of NICEATM, attended a meeting of the study management team in November 2009

See the previous chapter for details on these activities.

- Dr. Stokes attended the Sixth Congress of the Korean Society for Alternatives to Animal Experimentation and inaugural symposium for the Korean Center for the Validation of Alternative Methods (KoCVAM) in November 2009. He presented on "Validation and Regulatory Acceptance of Alternative Methods for Safety Testing: Recent Progress and Future Directions." Dr. Stokes also met with representatives of KoCVAM to discuss current and future international validation studies. They discussed expanding and strengthening cooperation, collaboration, and communication with NICEATM-ICCVAM on the scientific validation and evaluation of new alternative testing methods.

ICCVAM Contributions to OECD Test Guidelines and Guidance Documents

During 2008 and 2009, ICCVAM actively participated in the development and national review of OECD guidelines for the testing of chemicals. OECD test guidelines represent internationally agreed-upon testing methods that can be used by government, industry, and independent laboratories in the 30 OECD member countries to determine the safety of chemicals and chemical preparations.[26]

Ocular Safety Testing

The ICCVAM Ocular Toxicity Working Group worked with NICEATM to prepare test guidelines for the use of the BCOP and ICE for detection of ocular corrosives and severe irritants. The OECD formally accepted these test guidelines in 2009:

- *Test Guideline 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants* (OECD 2009a)
- *Test Guideline 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants* (OECD 2009b)

NICEATM and ICCVAM have also prepared and submitted a guidance document for use in conjunction with these test guidelines. See the preceding chapter (pages 25-26) for more information about the ICCVAM evaluation of the BCOP and ICE test methods.

Acute Oral Systemic Toxicity

The ICCVAM Acute Toxicity Working Group submitted to the OECD a draft *Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests*. The Acute Toxicity Working Group also provided comments and recommendations on the following OECD documents:

- *Test Guideline 412: Subacute Inhalation Toxicity: 28-Day Study* (OECD 2009c), accepted by OECD in September 2009
- *Test Guideline 413: Subchronic Inhalation Toxicity: 90-Day Study* (OECD 2009d), accepted by OECD in September 2009
- Draft *Guidance Document on Histopathology for Inhalation Toxicity Studies, Supporting Test Guideline 412 (Subacute Inhalation Toxicity: 28-Day) and Test Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)*
- Draft Proposal for a Revised *Test Guideline 403: Acute Inhalation Toxicity* (OECD 2009e), accepted by OECD in September 2009
- Comparison of Test Guideline 403 and C x t Protocols Via Simulation and for Selected Real Data Sets with Comments from the Expert Consultation Meeting in April 2008

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

About the OECD Test Guidelines Programme

The OECD Guidelines for the Testing of Chemicals are a collection of internationally agreed-upon test methods. Government, industry and independent laboratories use them to determine the safety of chemicals and chemical preparations. Data generated using OECD Test Guidelines are subject to the OECD Council Decision on Mutual Acceptance of Data. This means that:

> "data generated in the testing of chemicals in an OECD Member country … in accordance with OECD Test Guidelines and OECD principles of Good Laboratory Practice shall be accepted in other Member countries … for purposes of assessment and other uses relating to the protection of man and the environment."

More information on the OECD Test Guidelines Programme can be found on the OECD website, http://www.oecd.org/. Draft test guidelines currently under consideration can also be found on the OECD website.

- Draft Proposal for a New *Test Guideline 436: Acute Inhalation Toxicity – Acute Toxic Class (ATC) Method* (OECD 2009f), accepted by OECD in September 2009
- *Report on Biostatistical Performance Assessment of the Draft Test Guideline 436 Acute Toxic Class Testing Method for Acute Inhalation Toxicity*
- *OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 39: Draft Guidance Document On Acute Inhalation Toxicity Testing*
- *Guidance Document for the Derivation of an Acute Reference Concentration*
- Draft *Guidance Document on a the Threshold Approach for Acute Fish Toxicity Testing*
- *Proposal for a New Testing Strategy (Step-down approach) to Reduce the Use of Fish in Acute Aquatic Testing (supplement to OECD Test Guideline 203, Fish Acute Toxicity Test)*
- Draft *Guidance for the Derivation of an Acute Reference Dose*
- Draft *Test Guideline 223: Avian Acute Oral Toxicity Test and Draft OECD Report of the Test Method Validation of Avian Acute Oral Toxicity Test*

Dermal Safety Testing

NICEATM and ICCVAM submitted revisions to the following OECD test guidelines:

- *Test Guideline 430:* In Vitro *Skin Corrosion: Transcutaneous Resistance Test (TER)* (OECD 2004a)
- *Test Guideline 431:* In Vitro *Skin Corrosion: Human Skin Model Test* (OECD 2004b)

These revisions incorporate features of ICCVAM performance standards for *in vitro* test methods for skin corrosion (ICCVAM 2004) that were developed after the OECD test guidelines were initially published. NICEATM and ICCVAM have also been reviewing a draft OECD test guideline and participating in OECD Expert Consultation meetings to evaluate *in vitro* skin irritation assays using reconstructed human epidermis models.

Immunotoxicity Testing: Allergic Contact Dermatitis

After ICCVAM evaluated new versions and applications of the LLNA, the ICCVAM Immunotoxicity Working Group and NICEATM staff updated OECD Test Guideline 429 (OECD 2002) for the LLNA to incorporate the ICCVAM-updated protocol, the reduced LLNA procedure, and harmonized performance standards. ICCVAM, in collaboration with JaCVAM, also submitted draft test guidelines to OECD for the LLNA:DA and the LLNA:BrdU-ELISA. The OECD Working Group of National Coordinators will consider these new and updated test guidelines at their 2010 meeting.

Endocrine Disruptor Testing

ICCVAM members and NICEATM staff are participating in the development of OECD test guidelines for detection of endocrine disruptors. Data from the LUMI-CELL® estrogen receptor assay validation study will be used to develop test method performance standards for stably-transfected transcriptional activation assays for the detection of estrogen receptor agonists. NICEATM-ICCVAM will submit the performance standards to OECD for consideration as part of a draft performance-based test guideline for this type of assay. See the preceding chapter (page 45) for more information about the LUMI-CELL® ER assay validation study.

NICEATM and ICCVAM also reviewed and commented on the following draft OECD documents:

- *Test Guideline 441: Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti) Androgenic Properties*, accepted by OECD in 2009 (OECD 2009g)
- *Guidance Document for the Weanling Hershberger Assay*
- *Test Guideline 455: Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay for the Detection of Estrogenic Agonist-Activity of Chemicals*, accepted by OECD in 2009 (OECD 2009h)

Dr. Raymond Tice, NICEATM Deputy Director (through February 2009) and ICCVAM representative from NIEHS, represented NICEATM and ICCVAM at meetings of the OECD Validation Management Group for Non-animal Testing. These were held in Brussels, Belgium in November 2008, and in Washington, DC, in November 2009. Dr. Tice reviewed results of Phases 1 and 2a of the LUMI-CELL® ER Transcriptional Activation Assay International Validation Study via teleconference at the 2008 meeting. He reviewed results of Phase 2b in person at the 2009 meeting.

Genetic Toxicity Testing

ICCVAM provided comments on cytotoxicity evaluation procedures and study design for a draft OECD Test Guideline 487 for the *in vitro* micronucleus test.

NICEATM and ICCVAM, along with CPSC, co-hosted an OECD expert meeting to consider revisions to existing test guidelines for the murine local lymph node assay that will reduce animal use for these tests.

ICCVAM members and NICEATM employees and contract support staff were among the participants, pictured here, at an OECD expert meeting on the murine local lymph node assay in October 2009.

Awards Received by ICCVAM Members and NICEATM Staff for Activities in Support of ICCVAM's Mission

NIH Director's Award

The members of the ICCVAM subcommittee that developed the *NICEATM-ICCVAM Five-Year Plan* received the 2008 NIH Director's Award. The award was given in recognition of their outstanding contributions in developing an innovative interagency strategic plan to promote research, development, translation, and validation of new safety assessment methods to support improved public health. The NIH Director's Award recognizes employees who exhibit superior performance or special efforts significantly beyond their regular duty requirements but directly related to fulfilling the NIH mission. The following individuals received this award:

- Alan Poland, M.D., National Cancer Institute, ICCVAM representative (Five-Year Plan Subcommittee Chair)
- Suzanne Fitzpatrick, Ph.D., DABT, U.S. Food and Drug Administration (FDA), ICCVAM representative
- David Hattan, Ph.D., FDA, ICCVAM representative
- Abigail C. Jacobs, Ph.D., FDA, ICCVAM representative
- Jodie Kulpa-Eddy, D.V.M., USDA, ICCVAM representative
- Deborah McCarley, NIEHS, NICEATM staff member
- Sheila Newton, Ph.D., NIEHS Office of Policy, Planning, and Evaluation
- Amy Rispin, Ph.D., EPA, ICCVAM representative
- Margaret Snyder, Ph.D., NIH Office of the Director, ICCVAM representative
- William Stokes, D.V.M., DACLAM, RADM, USPHS, NIEHS, NICEATM Director and ICCVAM representative
- Raymond R. Tice, Ph.D., NIEHS, NICEATM Deputy Director and ICCVAM representative
- Marilyn Wind, Ph.D., CPSC, ICCVAM Chair

James A. McCallam Award – Association of Military Surgeons

Dr. William Stokes received the 2008 James A. McCallam award, which is presented to a Doctor of Veterinary Medicine in recognition of outstanding accomplishments in the field of medicine and health. The award is named for Brigadier General James A. McCallam, a former Chief of the U.S. Army Veterinary Corps. General McCallam served in both World Wars and was the first veterinarian to hold the permanent rank of Brigadier General in the Regular Army.

United States and international organizations recognized ICCVAM members and NICEATM staff with five awards for their activities in support of ICCVAM's mission.

Dr. Andrew Rowan of the Humane Society of the United States (far right) and Dr. Leonard Sauers of Procter and Gamble (far left) present the 2008 North American Alternative Award to (from left) Dr. Robert Kavlock of the Environmental Protection Agency, Dr. James Inglese of the National Institutes of Health Chemical Genomics Center, and Dr. Raymond Tice of the National Toxicology Program, NICEATM Deputy Director (through February 2009) and ICCVAM representative from NIEHS.

Karl F. Meyer-James H. Steele Gold Head Cane Award – American Veterinary Medical Association

Dr. William Stokes received the 2008 Meyer-Steele Gold Head Cane award. This award, which is sponsored by the Hartz Mountain Corporation, recognizes achievements that have advanced human health through the practice of veterinary epidemiology and public health.

North American Alternative Award – Humane Society of the United States/Procter and Gamble Company

The North American Alternative Award was presented to the interagency high throughput screening initiative known as the Tox21 Program. Tox21 is under the leadership of Christopher Austin, M.D., of the National Human Genome Research Institute, Robert Kavlock, Ph.D., of the Environmental Protection Agency, and Raymond Tice, Ph.D., of the National Toxicology Program. Dr. Tice was the NICEATM Deputy Director through February 2009, and is an ICCVAM representative from NIEHS. The North American Alternative Awards are given for outstanding scientific contributions to the advancement of viable alternatives to animal testing.

Doerenkamp-Zbinden Foundation Poster Awards at the Seventh World Congress on Alternatives and Animal Use in the Life Sciences

The Doerenkamp-Zbinden Foundation (DZF) recognized NICEATM and ICCVAM with an award for a poster presented at the Seventh World Congress on Alternatives and Animal Use in the Life Sciences (WC7) in 2009. The poster, entitled "International Acceptance of *In Vitro* Alternative Ocular Safety Testing Methods: The Bovine Corneal Opacity and Permeability Test Method (Draft OECD TG 437)," described the international acceptance of the use of the BCOP test method for identification of ocular corrosives and severe irritants. The Doerenkamp-Zbinden Foundation awards recognized posters at WC7 that best supported the principles of the DZF, a Swiss foundation that awards prizes for exceptional achievements in animal protection in biomedical research and funds research into reducing the suffering of experimental animals. The poster was co-authored by ICCVAM members from the FDA, the CPSC, and the EPA, along with NICEATM staff and representatives from OECD, ECVAM, and JaCVAM.

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

NICEATM staff and ICCVAM members participated in numerous international workshops, conferences, and meetings in 2008 and 2009. Brief descriptions of selected events are provided below.

Please note that any conclusions and recommendations issued in the proceedings of the meetings outlined below are those of the meeting 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.

Spotlight on Ingredients – In Vitro *Alternatives Forum*

A forum entitled *Spotlight on Ingredients:* In Vitro *Alternatives* was held October 23–24, 2008, at the Chemical Heritage Foundation in Philadelphia, Pennsylvania. The forum was planned and cosponsored by the Alternatives Research and Development Foundation, the Institute for *In Vitro* Sciences, Inc., and the Chemical Heritage Foundation. Experts and representatives from major U.S. and European companies and trade groups convened for a briefing on state-of-the-art alternative testing methods, the policy and consumer demands behind them, and how to move forward with *in vitro* testing programs. Dr. William Stokes, Director of NICEATM, gave a presentation on "NICEATM and ICCVAM: Role in the Validation and Regulatory Acceptance of Alternative Safety Testing Methods and Recent Progress."

European Partnership for Animal Alternatives

The European Partnership for Animal Alternatives (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.

The Fourth Annual Conference of the EPAA took place in Brussels, Belgium, on November 3, 2008. Dr. Raymond Tice, NICEATM Deputy Director (through February 2009) and ICCVAM representative from NIEHS, spoke on "U.S. Research Programs and Screening Activities" and participated on a panel discussion of "Research Strategies to Implement the 3Rs Principles."

New Jersey Association for Biomedical Research

The New Jersey Association for Biomedical Research (NJABR) is dedicated to promoting the improvement of human and animal health through biomedical research. NJABR is a comprehensive resource center for students and educators, government, the media, the public, and New Jersey's research community. The NJABR includes members from academic institutions, industry, hospitals, voluntary health organizations, and affiliated trade and professional organizations.

Dr. Stokes was a keynote speaker at the NJABR's first Alternatives Forum on November 8, 2008. He also led a discussion section that considered the understanding of the concept of alternatives within the scientific community and addressed the status of alternatives in the United States.

Dr. Stokes also delivered a plenary presentation entitled "Defining, Refining, and Anticipating Humane Endpoints" at *IACUC 16: The Charge and the Challenge*, NJABR's annual interactive training seminar for Institutional Animal Care and Use Committee members. The seminar took place on November 9, 2008.

American Association for the Advancement of Science

The American Association for the Advancement of Science (AAAS), publisher of the journal *Science*, is an international nonprofit organization dedicated to advancing science around the world by serving as an educator, leader, spokesperson, and professional association. The 2009 AAAS Annual Meeting took place in Chicago, Illinois on February 12-16. Dr. William Stokes gave a presentation on "A Five-Year Plan for Advancing Alternative Methods."

7th World Congress on Alternatives and Animal Use in the Life Sciences

NICEATM and ICCVAM participated in the 7th World Congress on Alternatives and Animal Use in the Life Sciences (WC7) in Rome, Italy, on August 31–September 3, 2009. This biennial international conference included sessions on the development, validation, and evaluation of alternative test methods that might be used to reduce, refine, and/or replace the use of animals in regulatory testing strategies. The specific objective of WC7 was to emphasize how progress in the development of new technologies can support progress toward the reduction, refinement and replacement of experimental animals.

Dr. William Stokes and Dr. Raymond Tice served on the WC7 Scientific Organizing Committee, as did ICCVAM members Dr. Suzanne Fitzpatrick, Dr. Abigail Jacobs, and Dr. Marilyn Wind. Drs. Fitzpatrick, Jacobs, and Wind served on the Programme Review Panel along with former ICCVAM member Dr. Amy Rispin. Dr. Stokes cochaired scientific sessions on "Current and Evolving Concepts for the Validation of Safety Assessment Methods" and "Recent Progress and Future Directions in the Validation and Regulatory Acceptance of Alternative Test Methods that Reduce, Refine, and Replace Animal Use." Dr. Tice presented a plenary session lecture entitled "The U.S. Tox21 Community and the Future of Toxicology Testing." Dr. Wind chaired a session at which participating countries delivered status reports on ICATM. Nine NICEATM and ICCVAM scientists from four Federal agencies attended WC7, contributing to 10 platform and eight poster presentations. Titles and authors of these presentations are included in the list of NICEATM-ICCVAM publications in **Appendix C.**

Korean Society for Alternatives to Animal Experimentation–Inaugural Symposium for Korean Center for the Validation of Alternative Methods

The Korean Center for the Validation of Alternative Methods (KoCVAM) was established in South Korea as part of the National Institute of Food and Drug Safety (NIFDS) in the Korean Food and Drug Administration. Dr. Stokes delivered the keynote address at an international symposium convened to recognize the formal establishment of KoCVAM. The symposium was held in Seoul, Korea, on November 3, 2009, at Seoul National University. It was held in conjunction with the Sixth Congress of the Korean Society for Alternatives to Animal Experimentation (KSAAE). The KSAAE was founded in collaboration with industry, academia, and government to facilitate the research and development of alternative test methods in Korea.

Dr. Stokes' presentation was entitled "Validation and Regulatory Acceptance of Alternative Methods for Safety Testing: Recent Progress and Future Directions." He emphasized the need for high-quality scientific validation studies as a prerequisite for regulatory acceptance and use of proposed new safety test methods. He highlighted new technologies and scientific advances that are expected to support the future development of more predictive safety tests.

Trisociety International Conference on Dermatoallergy, Environmental and Occupational Dermatology, and Contact Dermatitis

The Trisociety International Conference of Dermatoallergy, Environmental/Occupational Dermatology, and Contact Dermatitis was held in Kyoto, Japan, on November 5-8, 2009. Dr. Stokes participated as an invited speaker at the conference's *International Workshop of Skin Safety Evaluation of Cosmetics and Chemicals*. His presentation was on the "The Local Lymph Node Assay, an Alternative Test Method for Allergic Contact Dermatitis. Update on the Validation and Regulatory Acceptance of New Versions and Applications."

22nd Annual Meeting of the Japanese Society for Alternatives to Animal Experiments

Dr. Stokes attended the 22nd Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, held at Osaka University, Osaka, Japan, on November 13, 2009. His presentation was entitled "Advancing Laboratory Animal Welfare and Public Health Science: The Role of Innovative Refinement, Reduction, and Replacement Strategies. He also participated in the *International Symposium Among Japan, Korea, and China in Alternatives to Animal Experimentation* as an expert on alternatives on November 14, 2009.

47th and 48th Annual Meetings of the Society of Toxicology

NICEATM and ICCVAM participated in the 2008 and 2009 annual meetings of the Society of Toxicology (SOT).

The 47th Annual SOT Meeting was held on March 16-20, 2008, in Seattle, WA. Twenty members of NICEATM and ICCVAM contributed to five poster presentations. Titles and authors of these presentations are included in the list of NICEATM-ICCVAM publications in **Appendix C.**

The 48th Annual SOT Meeting was held on March 15-19, 2009, in Baltimore, MD. Twelve members of NICEATM and ICCVAM contributed to four poster presentations. Titles and authors of these presentations are included in the list of NICEATM-ICCVAM publications in **Appendix C.**

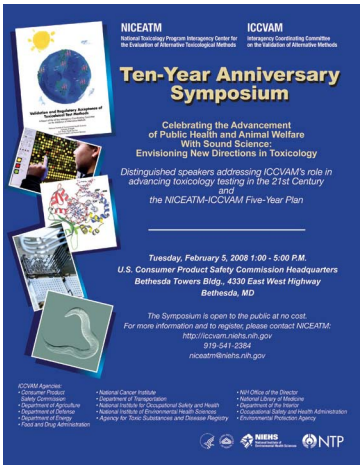
NICEATM staff and ICCVAM members participated in numerous international workshops, conferences, and meetings in 2008 and 2009.

NICEATM-ICCVAM Communications

During 2008-2009, NICEATM and ICCVAM presented a symposium to commemorate ICCVAM's tenth anniversary, added new pages to the NICEATM-ICCVAM website, and published 10 reports, 23 publications, and 16 notices in the ***Federal Register.***

Highlights of ICCVAM Activities

- NICEATM and ICCVAM organized a symposium in February 2008 to commemorate the tenth anniversary of the establishment of ICCVAM and to announce the release of the *NICEATM-ICCVAM Five-Year Plan*.
- New pages were added to the NICEATM-ICCVAM website to enable easy access to key information about NICEATM and ICCVAM activities and progress of NICEATM-ICCVAM projects.
- ICCVAM publications in 2008 and 2009 included test method evaluation reports, background review documents, workshop reports, peer review panel reports, and the *NICEATM-ICCVAM Five-Year Plan (2008-2012)*. NICEATM published 16 *Federal Register* notices, and ICCVAM members or NICEATM staff were authors of 23 manuscripts and abstracts describing NICEATM-ICCVAM activities.



Over one hundred people representing many of the ICCVAM stakeholder groups attended a symposium commemorating the tenth anniversary of the establishment of ICCVAM and announcing the release of the ***NICEATM-ICCVAM Five Year Plan****.*

ICCVAM Ten-Year Anniversary Symposium

To commemorate the tenth anniversary of ICCVAM's establishment and to announce the release of the *NICEATM-ICCVAM Five-Year Plan*, NICEATM and ICCVAM organized a symposium entitled *Celebrating the Advancement of Public Health and Animal Welfare With Sound Science: Envisioning New Directions in Toxicology.* The symposium was held in February 2008 at Consumer Product Safety Commission Headquarters in Bethesda, Maryland. More than 100 people attended. Attendees represented many of the ICCVAM stakeholder groups including Federal agencies, academia, industry, international organizations, and the animal welfare community. Distinguished speakers addressed ICCVAM's role in advancing toxicology testing in the 21st century. Representatives from ICCVAM stakeholder groups participated in a panel discussion with the theme "Test Method Research, Development, Translation and Validation: The Way Forward for ICCVAM and its Stakeholders."

More information about the symposium, including the agenda, speaker presentations, biographical information on the speakers and panelists, and the *NICEATM-ICCVAM Five-Year Plan,* can be found on the NICEATM-ICCVAM website.[27]

[27] Information about the Symposium and a link to the *NICEATM-ICCVAM Five-Year Plan* is available on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/meetings/10thAnnivSymp/10thAnnivSymp.htm.

NICEATM-ICCVAM Website

The NICEATM-ICCVAM website is a vital tool for effective communication with stakeholders. The site receives over 20,000 visits in a typical month, indicating a high level of public interest.

Recognizing that the Internet represents a primary source of information for ICCVAM stakeholders, NICEATM added new pages to ensure easy access to key information:

- The Alternative Test Method Project Milestones page (http://iccvam.niehs.nih.gov/methods/milestones.htm) provides a table summarizing the status of all ongoing and completed NICEATM-ICCVAM test method evaluations, as well as projects to which NICEATM, ICCVAM, and agency scientists are contributing. The summary table contains links to tables providing a timeline of project milestones. These tables in turn provide links to key web pages and documents.
- The News Updates page (http://iccvam.niehs.nih.gov/announcements/update.htm) is updated with summaries of key NICEATM-ICCVAM activities as they occur.
- The NICEATM-ICCVAM Five-Year Plan page (http://iccvam.niehs.nih.gov/docs/5yearplan.htm) is updated regularly with information about activities that support the *NICEATM-ICCVAM Five-Year Plan.*
- A table summarizing regulatory acceptance of alternative toxicological methods in the United States and internationally is available at http://iccvam.niehs.nih.gov/about/accept.htm. It provides an up-to-date chronological list of internationally-accepted alternative test methods, including information on OECD adoption. A similar list organized by test method area can be found at http://iccvam.niehs.nih.gov/about/accept-area.htm.
- Answers to "Frequently Asked Questions" about NICEATM and ICCVAM are available at http://iccvam.niehs.nih.gov/about/ni_QA.htm.

Other information available on the website includes:

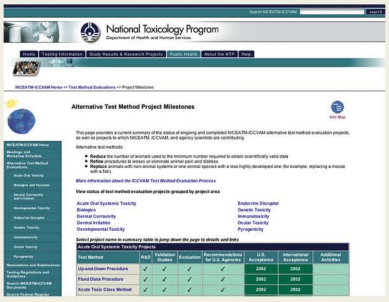
- Detailed information about NICEATM-ICCVAM test method evaluation activities
- Procedures for making nominations and submissions to ICCVAM
- Background information about NICEATM and ICCVAM
- Databases of NICEATM-ICCVAM documents
- Online forms that enable visitors to submit public comments to NICEATM and register for NICEATM meetings and workshops

Navigation menus on each page and a site map enable easy navigation throughout the website. Information is presented in a manner consistent with U.S. Federal Government Plain Language Guidelines.

Five-Year Plan Implementation: NICEATM-ICCVAM Communications

The NICEATM-ICCVAM website now includes the following resources:

- A list of Frequently Asked Questions, which provides a quick reference guide to broad issues related to the ICCVAM test method evaluation process.
- A table summarizing the status of all ongoing and completed NICEATM-ICCVAM test method evaluations, as well as projects to which NICEATM, ICCVAM, and agency scientists are contributing



Reports, Federal Register Notices, and Publications

NICEATM and ICCVAM published 10 reports, 16 *Federal Register* notices, and 23 abstracts and manuscripts during 2008 and 2009. NICEATM and ICCVAM activities were also reported in three NIEHS news releases and eight articles in the NIEHS *Environmental Factor* newsletter. A complete list of these publications is available in **Appendix C.**

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Appendix A: ICCVAM Agency Representatives 2008-2009

Below are listed individuals who served as designated representatives from ICCVAM member agencies in 2008 and 2009. Unless otherwise noted, individuals were serving as of January 2010.

❖Principal agency representative ◊Alternate agency representative

Agency for Toxic Substances and Disease Registry

❖Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Ed Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission

❖Marilyn L. Wind, Ph.D. (Chair)
◊Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

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., DACLAM
Harry Salem, Ph.D.

Department of Energy

❖Michael Kuperberg, Ph.D.
◊Marvin Stodolsky, Ph.D.

Department of the Interior

❖Barnett A. Rattner, Ph.D.
◊Sarah Gerould, Ph.D. (through 2/09)

Department of Transportation

❖George Cushmac, Ph.D.
◊Steve Hwang, Ph.D.

Environmental Protection Agency

Office of Pesticide Programs
❖John A. "Jack" Fowle III, Ph.D., DABT
◊Vicki Dellarco, Ph.D.
◊Tina Levine, Ph.D.
Amy Rispin, Ph.D. (through 1/09)
Deborah McCall
Christine Augustyniak, Ph.D.

Office of Science Coordination and Policy
Karen Hamernik, Ph.D. (through 6/09)

Office of Research and Development
Julian Preston, Ph.D. (through 6/09)
Suzanne McMaster, Ph.D. (though 1/09)
Stephanie Padilla, Ph.D. (through 6/09)

Office of Pollution Prevention and Toxics
Jerry Smrchek, Ph.D. (through 3/09)

Food and Drug Administration

Office of Science
❖Suzanne Fitzpatrick, Ph.D., DABT

Center for Drug Evaluation and Research
◊Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.

Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D. (through 12/09)
Vasant Malshet, Ph.D., DABT

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. (through 3/09)
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.

Office of Regulatory Affairs
Lawrence A. D'Hoostelaere, Ph.D.

National Cancer Institute

❖T. Kevin Howcroft, Ph.D.
Alan Poland, M.D. (through 11/08)
◊Chand Khanna, D.V.M., Ph.D.

National Institute of Environmental Health Sciences

❖William S. Stokes, D.V.M., DACLAM
◊Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

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

❖Pertti (Bert) Hakkinen, Ph.D.
◊Jeanne Goshorn, M.S.

Occupational Safety and Health Administration

❖Surender Ahir, Ph.D.

Appendix B: *ICCVAM Test Method Evaluation Process*

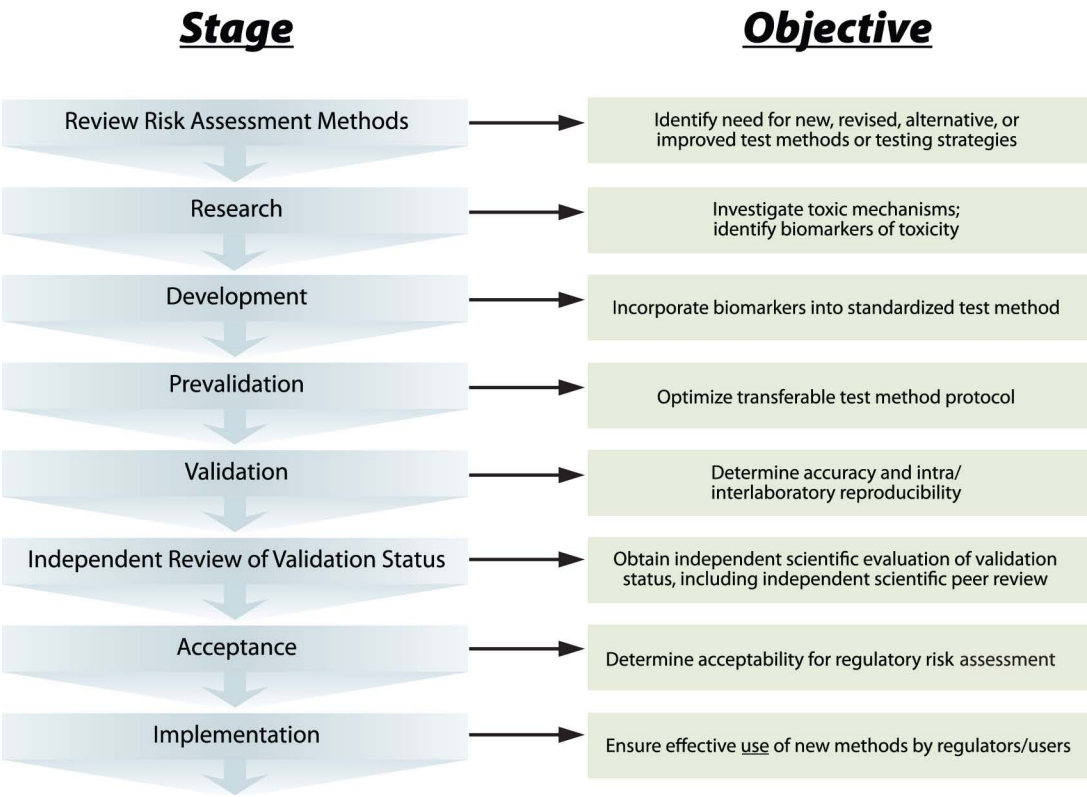
Alternative test methods are those that reduce, refine, or replace animal use in regulatory toxicity testing. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) evaluate the usefulness and limitations of new, revised, and alternative test methods, and promote regulatory acceptance of test methods found to be scientifically valid for their intended purposes. NICEATM and ICCVAM foster cooperation among Federal agencies, providing an efficient and effective mechanism for Federal review of test methods. They promote adoption of 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, while protecting human health, animal health, and the environment.

However, NICEATM and ICCVAM do not have authority to approve new, revised, or alternative testing regulations or guidelines. Nor can they require that a test method be used for a particular purpose. Only Federal agencies can approve new test methods. Only relevant agencies can determine whether and how data from new test methods can be accepted and used in their respective programs.

Proposed test methods advance from concept to regulatory acceptance in a number of stages (**Figure B-1**). ICCVAM coordinates interagency technical reviews of new, revised, and alternative test methods and provides a means by which issues relating to the validation, acceptance, and national and international harmonization of toxicological test methods may be resolved. ICCVAM places priority on evaluations of test methods that may (1) better predict adverse human, animal, or environmental effects and (2) reduce, refine, or replace animal use.

NICEATM and ICCVAM evaluate each proposed test method's validation status (i.e., the usefulness and limitation of the test method for a specific purpose) and conduct independent scientific peer reviews (**Figure B-2**). This appendix outlines the stages by which ICCVAM (1) considers and prioritizes nominations and submissions, (2) conducts test method evaluations, and (3) reports the results of its test method evaluations to Federal agencies and other interested parties.

Figure B-1: Development, Evaluation, and Acceptance of Test Methods



Definitions of Key Terms

Accuracy: the closeness of agreement between a test method result and an accepted reference value, or the test method's proportion of correct outcomes

Alternative test methods: methods that reduce, refine or replace animal use in product safety testing

Nomination: a proposal to ICCVAM to conduct activities related to the current validation status of test method(s), such as gathering information, conducting workshops and peer reviews, and performing validation studies

Performance standards: the basis for evaluating a proposed test method against a similar, previously validated test method

Reproducibility: the consistency of a test method's results in a single laboratory (intralaboratory) or in different laboratories (interlaboratory) using the same protocol

Repeatability: the success rate of a test method in successive experiments

Relevance: the extent to which a test method correctly predicts or measures the biological effect of interest

Reliability: the degree to which a test method produces consistent results within and among laboratories over time

Submission: a test method proposed to ICCVAM for review and evaluation with adequate validation studies completed and validated according to the *ICCVAM Guidelines*

Validation: the process in which laboratory studies establish the reliability and relevance of a test method for its intended application

ICCVAM Test Method Nomination and Submission

ICCVAM Guidelines. In 2003, ICCVAM published the *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods* (ICCVAM 2003). The document tells test method sponsors the information that ICCVAM needs, at each stage, to evaluate the validation status of new, revised, and alternative test methods. It includes an outline for organizing the necessary information and data in nominations and submissions. A *nomination* is a proposed test method for which a complete test method submission is not available. A *submission* is one for which adequate validation studies have been completed and adequate documentation of the scientific validity has been prepared according to the *ICCVAM Guidelines*.

ICCVAM established a process to consider test method nominations and submissions and prioritize them for review and evaluation. **Figure B-2** provides an overview of this process. Submissions should include all information requested in the *ICCVAM Guidelines*. Providing as much of the requested information as possible will expedite ICCVAM's consideration of a proposed test method. If requested information is unavailable or incomplete, the sponsor should explain the scientific approach(es) with which they plan to gather or generate the data.

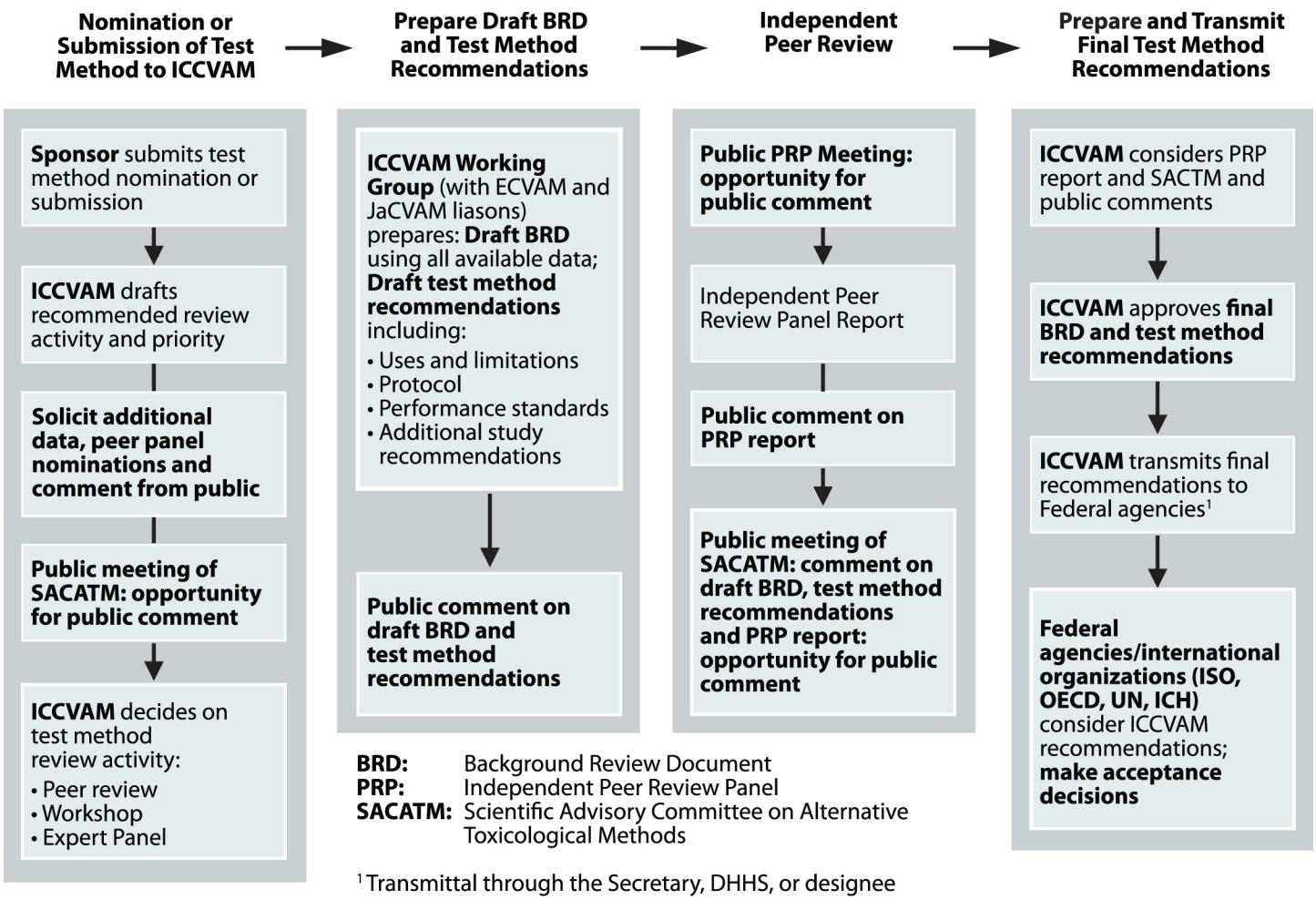
Preliminary Review and Evaluation. The Director of NICEATM tracks the status of test method nominations and submissions, updates ICCVAM, and arranges for NICEATM to conduct preliminary evaluations as resources permit. Preliminary evaluations of test method nominations and submissions summarize the following:

- 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 environmental health
- 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 gives ICCVAM the results of NICEATM's preliminary evaluations, along with recommendations for validation studies or further evaluations (e.g., workshops, expert panel meetings, independent peer review, or expedited reviews). ICCVAM then:

- Reviews the NICEATM preliminary evaluation report
- Determines whether the test method warrants further evaluation (e.g., applicable to one or more agencies, potential for widespread use)
- Develops draft test method recommendations for evaluation priority, validation studies, and further evaluations

Figure B-2. ICCVAM Test Method Evaluation Process



Public Comment. Throughout the process, NICEATM and ICCVAM invite public comments on test method nominations and submissions. They hold public meetings, manage electronic forums (e.g., ICCVAM e-mail lists and the NICEATM-ICCVAM website), and provide printed materials and publications. NICEATM-ICCVAM provides information to and requests comments from the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM).

Initiation of Evaluation Activity. ICCVAM considers comments from SACATM and the public, develops final recommendations for future activities, and prioritizes these activities. The Director of NICEATM estimates the resources needed to conduct the recommended evaluations and validation studies. The Director forwards these, along with recommendations from SACATM and ICCVAM, to the Associate Director of the National Toxicology Program (NTP). The NTP Associate Director considers the submitted material and forwards a recommendation to the Director of the National Institute of Environmental Health Sciences (NIEHS). Based on this information, the NIEHS Director finalizes priorities and allocates resources for approved activities.

The Director of NICEATM informs ICCVAM of the available funds from NIEHS, other ICCVAM agencies, or other stakeholders. These funds can be used to support the recommended activities (e.g., workshops, expert panel meetings, independent peer review, expedited reviews, and validation studies). When resources are available, NICEATM collaborates with ICCVAM and the appropriate working group to organize the recommended activity.

Development of ICCVAM Test Method Recommendations

Preparation of Background Review Document and Draft Test Method Recommendations. 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 (see **Figure B-2**). The working group collaborates with NICEATM to prepare a comprehensive draft background review document (BRD) on the test method. This draft BRD provides (1) the rationale and scientific basis for use of the test method, (2) the test method protocol, and (3) substances used to evaluate the test method and comparable *in vivo* reference data. The draft BRD includes information provided by the sponsor to support the nomination or submission. It may also include relevant data submitted by interested parties and information on use of the test method obtained from searches of the scientific literature. The draft BRD may analyze the accuracy and reliability of the test method and discuss animal welfare considerations and other parameters—such as time, cost, and infrastructure requirements—to be considered when using the test method. NICEATM and the ICCVAM working group consider the draft BRD while drafting recommendations on use of the test method.

Independent Scientific Peer Review. NICEATM posts the draft BRD and draft test method recommendations on the NICEATM-ICCVAM website, and a *Federal Register* notice is published to announce their availability for public review and comment. NICEATM then gathers an independent scientific peer review panel to review the documents. Members of this panel include research scientists, clinicians, test method developers, statisticians, and other professionals with relevant expertise. They are drawn from industry, academia, animal welfare organizations, and regulatory agencies (normally agencies other than those represented on ICCVAM). The peer review panel should have international representation and reflect the viewpoints of all interested parties when they consider the test methods.

The peer review panel meets in public session, and public comments are welcomed during the meeting. The panel publishes its conclusions and recommendations in an independent report shortly after the meeting. This document is also posted on the NICEATM-ICCVAM website, and a *Federal Register* notice is published to announce its availability.

Test Method Evaluation Report. ICCVAM considers the panel's review of the ICCVAM draft BRD and draft test method recommendations, as well as comments received from the public and SACATM, while preparing a test method evaluation report.

The test method evaluation report includes ICCVAM recommendations on the regulatory applicability of the method and its demonstrated usefulness and limitations for proposed hazard and safety assessments. Typically, the report will also recommend the following:

- *A standardized test method protocol*. ICCVAM develops the protocol from information gathered during the test method evaluation. This protocol specifies how to conduct the test method. It may include information about the purpose and applicability of the test method, study design, data evaluation, decision criteria, and study report preparation. The standardized protocol can generate consistent data to expand the test method's validation database.
- *Performance standards (if applicable):* Performance standards communicate how the new test method can be deemed to be sufficiently accurate and reliable for specific testing purposes (see below).
- *Future studies:* ICCVAM may identify and recommend additional research, development, and/or validation studies that can improve or broaden the test method's applicability.

Performance Standards. ICCVAM develops and recommends performance standards when evaluating proposed test methods. They communicate the basis by which new proprietary (i.e., copyrighted, trademarked, or registered) and nonproprietary test methods have been deemed sufficiently accurate and reliable for specific testing purposes. *Accuracy* is a measure of test method performance. It 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. *Reliability* is a measure of how well a test method produces the same results over time within the same laboratory (intralaboratory) and across different laboratories (interlaboratory).

Once a proposed test method has been accepted by regulatory agencies, performance standards can be used to evaluate the accuracy and reliability of other test methods that (1) are based on similar scientific principles and (2) measure or predict the same biological or toxic effect. During the test method evaluation process, NICEATM and the appropriate ICCVAM working group draft performance standards that take into account performance standards proposed by the test method sponsor, information provided in the test method submission, and other available data.

The NICEATM-ICCVAM peer review panel evaluates the proposed performance standards when it evaluates the validation status of the proposed test method. The proposed performance standards and the draft test method evaluation report are made available to the public for comment before and during the peer review panel meeting. With additional public comments, feedback from the peer review panel, and ICCVAM's endorsement, NICEATM and the appropriate ICCVAM working group finalize the recommended performance standards. Regulatory authorities can reference 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.

Regulatory Acceptance

Transmittal to Federal Agencies. ICCVAM submits finalized test method evaluation reports, with recommended performance standards, to U.S. Federal agencies represented on ICCVAM. NICEATM makes these documents available to the public on behalf of ICCVAM via the NICEATM-ICCVAM website. ICCVAM announces the availability of test method evaluation reports in the *Federal Register,* NTP newsletters, and NICEATM-ICCVAM e-mail lists.

The ICCVAM Authorization Act specifies that, within 180 days of transmittal, each ICCVAM member agency must review the ICCVAM test method recommendation and notify ICCVAM in writing of its findings.

Responses from Federal Agencies. The final step in the ICCVAM test method evaluation process is the receipt by NICEATM of the responses of the ICCVAM member agencies to the ICCVAM test method recommendation. Once an alternative test method has been accepted, ICCVAM works to promote the use of the test method by sponsoring and participating in training workshops and scientific meetings to reach interested stakeholders who may want to use or consider data from the test method.

Acceptance of Alternative Methods by Regulatory Agencies, 1998-2009

Method	ICCVAM Background Review Documents and Peer Review	U.S. Regulatory Acceptance/ Endorsement	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Murine local lymph node assay for skin sensitization	ICCVAM peer review and report; recommended in 1999	Accepted by U.S. in 1999; as OECD TG 429 in 2002	OECD TG 429 (2002) ISO (2002)	Via OECD
CORROSITEX® *in vitro* membrane barrier skin corrosivity test	ICCVAM peer review and report; recommended in 1999	Accepted by U.S. in 1999; as OECD TG 435 in 2006	OECD TG 435 (2006)	Via OECD
Up-and-down procedure for acute oral toxicity	ICCVAM peer review and report; recommended in 2001	Accepted by U.S. in 2003; as OECD TG 425 in 2001	OECD TG 425 (2001)	Via OECD
Fixed dose procedure for acute oral toxicity	ICCVAM working group contributed to test guideline development	OECD TG 420 accepted in 2001	OECD TG 420 (2001)	Via OECD
Acute toxic class method for acute oral toxicity	ICCVAM working group contributed to test guideline development	OECD TG 423 accepted in 2001	OECD TG 423 (2001)	Via OECD
EPISKIN™ *in vitro* human skin model skin corrosivity test	ICCVAM review and report; recommended in 2002	OECD TG 431 accepted in 2004	OECD TG 431 (2004)	67/548/EEC
EpiDerm™ *in vitro* human skin model skin corrosivity test	ICCVAM review and report; recommended in 2002	OECD TG 431 accepted in 2004	OECD TG 431 (2004)	67/548/EEC
SkinEthic™ *in vitro* human skin model skin corrosivity test	ICCVAM contributed to U.S. OECD test guideline review	OECD TG 431 accepted in 2004	OECD TG 431 (2004)	Via OECD
Rat TER *in vitro* skin corrosivity test	ICCVAM review and report; recommended in 2002	OECD TG 430 accepted in 2004	OECD TG 430 (2004)	67/548/EEC
3T3 NRU phototoxicity test for skin photo-irritation	ICCVAM contributed to U.S. OECD test guideline review	OECD TG 432 accepted in 2004	OECD TG 432 (2004)	67/548/EEC
3T3 NRU phototoxicity test: application to UV filter chemicals	ICCVAM contributed to U.S. OECD test guideline review	OECD TG 432 accepted in 2004	OECD TG 432 (2004)	Via OECD
In vitro dermal absorption methods	ICCVAM contributed to U.S. OECD test guideline review, expert consultation meetings	OECD TG 428 accepted in 2004	OECD TG 428 (2004)	Via OECD
Use of humane endpoints in animal testing of biological products	ICCVAM agency initiative	9 CFR 117.4e	NA	
Rabies vaccine, humane endpoints	ICCVAM agency initiative	9 CFR 117.4e	NA	
Uterotrophic bioassay in rodents: a short-term screening test for estrogenic properties	ICCVAM contributed to U.S. OECD test guideline review	OECD TG 440 accepted in 2007	OECD TG 440 (2007)	Via OECD
Bovine corneal opacity and permeability test method to identify severe eye irritants/ corrosives	ICCVAM review and report; recommended in 2007	Accepted in 2008	OECD TG 437 (2009)	Via OECD
Isolated chicken eye test method to identify severe eye irritants/ corrosives	ICCVAM review and report; recommended in 2007	Accepted in 2008	OECD TG 438 (2009)	Via OECD
Acute toxicity *in vitro* starting dose procedure, 3T3 cells	ICCVAM 2001 workshop report; ICCVAM 2006 peer review and report; recommended in 2008	Accepted in 2008	*OECD Guidance Document in review*	*OECD Guidance Document in review*
Acute toxicity *in vitro* starting dose procedure, NHK cells	ICCVAM 2001 workshop report; ICCVAM 2006 peer review and report; recommended in 2008	Accepted in 2008	*OECD Guidance Document in review*	*OECD Guidance Document in review*

Method	ICCVAM Background Review Documents and Peer Review	U.S. Regulatory Acceptance/ Endorsement	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
ELISA test for batch potency testing of erysipelas vaccines (refinement: antibody quantification)	ICCVAM coordinated agency consideration	Per 27 CFR 610.10; are reviewed on a case-by-case basis	NA	Published in European Pharmacopeia
Relevance of the target animal safety test for batch safety testing of vaccines for veterinary use	ICCVAM coordinated agency consideration	Per 9 CFR 113.4	NA	Published in European Pharmacopeia
ELISA test for batch potency testing of human tetanus vaccines (refinement: antibody quantification)	ICCVAM coordinated agency consideration	Per 27 CFR 610.10; are reviewed on a case-by-case basis	NA	Published in European Pharmacopeia
ToBI test for batch potency testing of human tetanus vaccines (refinement: antibody quantification)	ICCVAM coordinated agency consideration	Per 27 CFR 610.10; are reviewed on a case-by-case basis	NA	Published in European Pharmacopeia
Human whole blood/interleukin-1β *in vitro* pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted in 2009	NA	Published in European Pharmacopeia
Human whole blood/interleukin-1β *in vitro* pyrogen test: application of cryopreserved human whole blood	ICCVAM peer review and report; recommended in 2008	Accepted in 2009	NA	Published in European Pharmacopeia
Human whole blood/ interleukin-6 *in vitro* pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted in 2009	NA	Published in European Pharmacopeia
Human peripheral blood mononuclear cell/interleukin-6 *in vitro* pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted in 2009	NA	Published in European Pharmacopeia
Monocytoid cell line Mono Mac 6/interleukin-6 *in vitro* pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted in 2009	NA	Published in European Pharmacopeia
Inhalation toxicity – acute toxic class method	ICCVAM contributed to U.S. OECD test guideline review	OECD TG 436 accepted in 2009	OECD TG 436 (2009)	Via OECD
Hershberger bioassay in rats: a short-term screening assay for (anti) androgenic properties	ICCVAM contributed to U.S. OECD test guideline review	OECD TG 441 accepted in 2009	OECD TG 441 (2009)	Via OECD
Stably transfected human estrogen receptor-α mediated reporter gene assay using hER-HeLa-9903 cells to detect estrogenic agonist-activity of chemicals	ICCVAM contributed to U.S. OECD test guideline review, expert consultation meetings	OECD TG 455 accepted in 2009	OECD TG 455 (2009)	Via OECD
Totals	**31**	**31**	**18**	**27**

Abbreviations

CFR U.S. Code of Federal Regulations
ELISA Enzyme-linked immunosorbent assay
ICCVAM Interagency Coordinating Committee on the Validation of Alternative Methods
ISO International Organization for Standardization
NHK Normal human keratinocyte
NRU Neutral red uptake
OECD Organisation for Economic Co-operation and Development
TER Transcutaneous electrical resistance
TG Test guideline
ToBI Toxin binding inhibition
WG Working group

Appendix C: *NICEATM and ICCVAM Publications 2008-2009*

NICEATM and ICCVAM Reports

ICCVAM. 2009. Independent Scientific Peer Review Panel Report. Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches. Research Triangle Park, NC: National Institute of Environmental Health Sciences.
Available: http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf.

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Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf.

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ICCVAM. 2009. ICCVAM Test Method Evaluation Report. The Reduced Murine Local Lymph Node Assay: An Alternative Test Method Using Fewer Animals to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication 09-6439. Research Triangle Park, NC: National Institute of Environmental Health Sciences.
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ICCVAM. 2008. ICCVAM Background Review Document: Validation Status of Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products. NIH Publication 08-6391. Research Triangle Park, NC:National Institute of Environmental Health Sciences.
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Appendix D:
List of Acronyms and Abbreviations

AAAS	American Association for the Advancement of Science
ACD	Allergic contact dermatitis
AMCP	Antimicrobial cleaning product
BCOP	Bovine corneal opacity and permeability
BET	Bacterial endotoxin test
BPA	Bisphenol A
BRD	Background review document
BrdU	Bromodeoxyuridine
CFR	U.S. Code of Federal Regulations
CM	Cytosensor® Microphysiometer
CPSC	U.S. Consumer Product Safety Commission
DZF	Doerenkamp-Zbinden Foundation
ECVAM	European Centre for the Validation of Alternative Methods
ELISA	Enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
EPAA	European Partnership for Animal Alternatives
ER	Estrogen receptor
EU	European Union
FDA	U.S. Food and Drug Administration
FR	*Federal Register*
HET-CAM	Hen's egg test–chorioallantoic membrane
ICATM	International Cooperation on Alternative Test Methods
ICCR	International Cooperation on Cosmetics Regulation
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
IL	Interleukin
IRE	Isolated rabbit eye
ISO	International Organization for Standardization
JaCVAM	Japanese Center for the Validation of Alternative Methods
KoCVAM	Korean Center for the Validation of Alternative Methods
LLNA	Murine local lymph node assay
LLNA: BrdU-ELISA	Murine local lymph node assay using BrdU in an ELISA format
LLNA: BrdU-FC	Murine local lymph node assay using BrdU in a flow cytometry platform
LLNA:DA	Murine local lymph node assay measuring adenosine triphosphate content in lymph nodes
LVET	Low volume eye test
NHK	Normal human keratinocyte
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIFDS	National Institute of Food and Drug Safety (Korea)
NIOSH	National Institute for Occupational Safety and Health
NIH	National Institutes of Health
NJABR	New Jersey Association for Biomedical Research
NRU	Neutral red uptake
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
RDWG	ICCVAM Research and Development Working Group

rLLNA	Reduced murine local lymph node assay
RPT	Rabbit pyrogen test
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SOT	Society of Toxicology
TG	Test guideline
U.S.C.	United States Code
USDA	United States Department of Agriculture
WC7	Seventh World Congress on Alternatives and Animal Use in the Life Sciences

Appendix E: *The ICCVAM Authorization Act*

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.

Appendix F:
Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

This Appendix lists all SACATM members during 2008 and 2009; ending dates of appointments are indicated.

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

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

June A. Bradlaw, Ph.D.
Science Advisor
International Foundation for Ethical Research
Rockville, MD
Appointment ended: 6/30/2008

Karen K. Brown, Ph.D.
President, Pair O'Docs Enterprises
Parkville, MO
Appointment ends: 06/30/2011

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

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

George B. Corcoran, Ph.D., ATS
Chairman and Professor, Department of Pharmaceutical Sciences
Eugene Applebaum College of Pharmacy and Health Sciences
Wayne State University
Detroit, MI
Appointment ends: 06/30/2011

Mary Jane Cunningham, Ph.D.
OMICS Program Manager
Integrated Laboratory Systems, Inc.
Research Triangle Park, NC
Appointment ended: 6/30/2008

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

Helen E. Diggs, D.V.M.
Associate Dean Hospital Programs
Director, Veterinary Teaching Hospital
College of Veterinary Medicine
Oregon State University
Corvallis, OR
Appointment ends: 6/30/2010

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

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

Donald A. Fox, Ph.D.
Professor, Department of Pharmacological & Pharmaceutical Sciences
College of Optometry, University of Houston
Houston, TX
Appointment ended: 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

Steven R. Hansen, D.V.M., M.S., M.B.A., DABT, ABVT
ASPCA Poison Control Center
Urbana, IL
Appointment ends: 6/30/2012

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

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

Sharon Meyer, Ph.D.
Professor, Department of Toxicology
University of Louisiana at Monroe
Monroe, LA
Appointment ends: 6/30/2011

Annie (Peiyong) Qu, Ph.D.
Department of Statistics
University of Illinois-Champaign
Champaign, IL
Appointment ends: 6/30/2010

Gary Wnorowski, M.B.A., LAT
President, Eurofins/Product Safety Laboratories
Dayton, NJ
Appointment ended: 6/30/2011

SACATM Meetings

- June 18-19, 2008, Research Triangle Park, NC; announced in *Federal Register* notice 73 FR 25754.
- June 25-26, 2009, Arlington, VA; announced in *Federal Register* notice 74 FR 19562.

Appendix G:
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*

Official Title (caption):

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.

ICCVAM is administered by, and receives scientific support from, the 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., DACLAM – Director and Project Officer

Deborah McCarley – Special Assistant and Assistant Project Officer

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

David Allen, Ph.D. – Principal Investigator

Steven Morefield, M.D. – Project Manager

Thomas Burns, M.S.

Patricia Ceger, M.S.

Frank Deal, M.S.

Nelson Johnson

Brett Jones, Ph.D.

Linda Litchfield

Elizabeth Lipscomb, Ph.D

Anna Lee Mosley

Michael Paris

Eleni Salicru, Ph.D.

Catherine Sprankle

Frank Stack

Judy Strickland, Ph.D., DABT

James Truax, M.A.

Linda Wilson

NICEATM-ICCVAM reports, *Federal Register* notices, and other information about NICEATM-ICCVAM test method evaluations are available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/. Hard copies of NICEATM-ICCVAM reports are also available on request from NICEATM:

NICEATM
National Institute of Environmental Health Sciences
P.O. Box 12233, MD K2-16
Research Triangle Park, NC 27709 USA
Telephone: 919-541-2384 Fax: 919-541-0947
E-mail: niceatm@niehs.nih.gov

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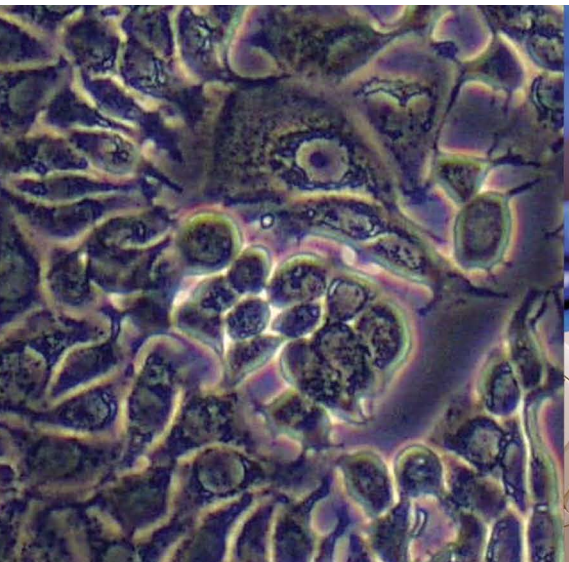

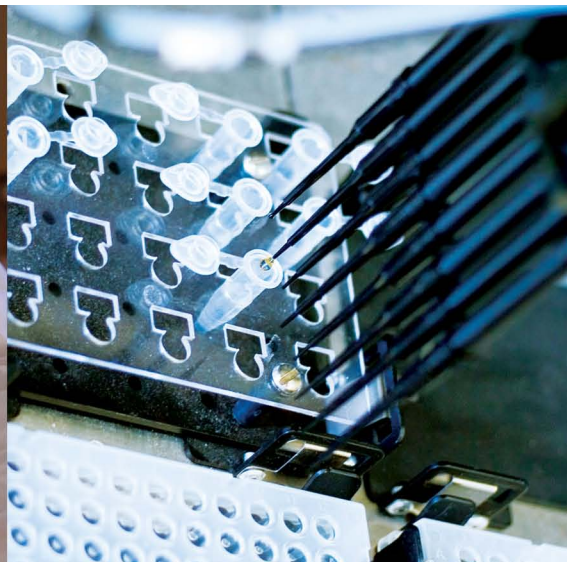

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

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National Institutes of Health

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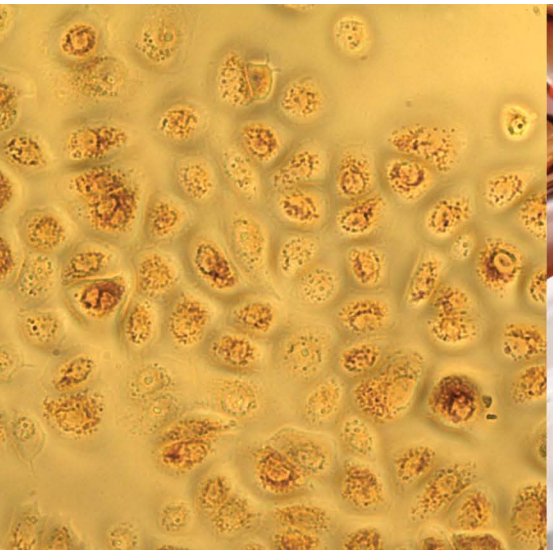
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U.S. Department of Health and Human Services