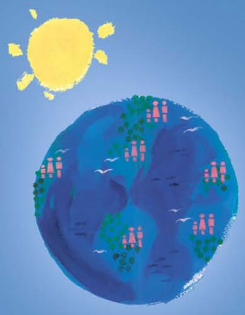
National Toxicology Program
U.S. Department of Health and Human Services



**Interagency Coordinating Committee on the Validation of Alternative Methods**

# Biennial Progress Report 2010-2011

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. 12-7873

# 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

The ICCVAM Authorization Act of 2000 (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 also promotes the scientific validation and regulatory acceptance of toxicological and safety testing methods that more accurately assess the safety or hazards of chemicals and products and that reduce, refine (enhance animal well-being and lessen or avoid pain and distress), or replace animal use. NICEATM administers ICCVAM; provides scientific and operational support for ICCVAM-related activities; and conducts independent validation studies to assess the usefulness and limitations of new, revised, and alternative test methods and strategies. 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).

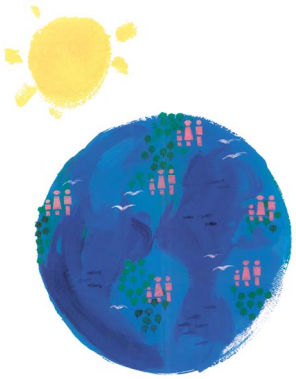
ICCVAM is an interagency committee composed of representatives from the following 15 U.S. Federal regulatory and research member agencies that require, use, generate, or disseminate toxicological and safety testing information:*

- *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 for Occupational Safety and Health*
  - *Food and Drug Administration*
  - *National Institutes of Health*
    - *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*

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

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

**Interagency Coordinating Committee on the Validation of Alternative Methods**



# Biennial Progress Report 2010–2011

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

National Toxicology Program
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Research Triangle Park, NC 27709

NIH Publication No. 12-7873
U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences
U.S. Public Health Service

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

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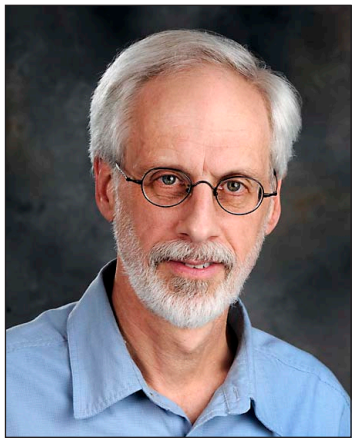
# The ICCVAM Member Agencies

The ICCVAM Authorization Act (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 2010 and 2011 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 for Occupational Safety and Health*
  - *Food and Drug Administration*
  - *National Institutes of Health*
    - *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*

NIH...Turning Discovery Into Health

NIEHS National Institute of Environmental Health Sciences



## A Message from NIEHS and NTP

The mission of the National Institute of Environmental Health Sciences (NIEHS) is to discover how the environment affects people in order to promote healthier lives. Our vision is to provide global leadership for innovative research that improves public health by preventing disability and disease from our environment. 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 and vision by advancing improved safety assessment tools to protect, promote, and advance the health of people, animals, and the environment.

The U.S. government's National Prevention Council adopted a National Prevention Strategy in 2011 that seeks to promote health and wellness and to empower people to make healthier choices. Having accurate safety testing information about chemicals and other substances that might cause injury or disease is key to injury prevention and good health. Innovative new safety testing methods are helping to provide improved information to support these prevention efforts.

This biennial report documents the significant progress and contributions that NICEATM and ICCVAM have made during the past two years in achieving the regulatory acceptance and implementation of scientifically valid new safety testing methods. Their careful evaluations have been essential to identify whether and to what extent these new test methods will continue to protect and advance the health of people, animals, and the environment compared to existing test methods and approaches. Each of the new methods also supports improved animal welfare and uses fewer or no animals.

We would like to note the growing collaborations between NICEATM and ICCVAM and their international partners. Last year we signed an updated Memorandum of Cooperation that expanded the International Cooperation on Alternative Test Methods (ICATM) to include the Republic of Korea, in addition to Canada, the European Union, and Japan. During its first three years, this partnership has demonstrated its value in achieving faster and more efficient worldwide acceptance of several new safety testing methods.

We are pleased to acknowledge NICEATM and ICCVAM for their accomplishments during the past two years and look forward to continued progress.

Linda S. Birnbaum, PhD, DABT, ATS
Director, NIEHS and NTP

John R. Bucher, PhD, DABT
Director, Division of the NTP, NIEHS
Associate Director, NTP

# A Message from NICEATM and ICCVAM

On behalf of 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) we are pleased to present this ICCVAM Biennial Progress Report for the 2010–2011 reporting period. The report describes the progress of NICEATM and ICCVAM in advancing innovative new safety testing methods and strategies that will protect and improve the health of people, animals, and the environment, and that will also reduce, refine (enhance animal well-being and lessen or avoid pain and distress), and replace animal use when scientifically feasible.

During the past two years, NICEATM, ICCVAM, and ICCVAM agencies have contributed to the national and international endorsement and adoption of 14 new and updated alternative methods. Since the establishment of ICCVAM, over 50 alternative methods have been adopted.

NICEATM and ICCVAM continue to foster stronger collaborations with our international partners. In 2009, we joined together with national validation organizations in the European Union, Canada, and Japan to establish the International Cooperation on Alternative Test Methods (ICATM). In 2011, this agreement was expanded to include the Republic of Korea. ICATM provides a framework for NICEATM and ICCVAM to work cooperatively with these organizations to accelerate the adoption of new harmonized test methods.

We gratefully acknowledge the contributions of the ICCVAM representatives and ICCVAM interagency working group members from the 15 ICCVAM member agencies. We also acknowledge the contributions from NICEATM and its support contract staff, members of the Scientific Advisory Committee on Alternative Toxicological Methods, our international experts and peer review panel members, and our many other stakeholders. Their collective commitment to high-quality science and animal welfare was instrumental to the progress made during 2010 and 2011.

We especially recognize the sustained outstanding leadership and contributions of Dr. Marilyn Wind during her 17 years of service to ICCVAM as the principal ICCVAM representative from the Consumer Product Safety Commission. Marilyn began her service on the original *ad hoc* ICCVAM in 1994, and served as Vice-Chair from 2002 to 2006, and Chair from 2007 until her retirement in July 2010.

We also recognize the service and contributions of Dr. George Cushmac of the Department of Transportation, who retired in 2010 after serving as the principal DOT representative since 1994, and Dr. Devaraya "Jag" Jagannath, who retired after serving as the Food and Drug Administration's Center for Veterinary Medicine representative since 2001.

The activities summarized in this report exemplify NICEATM and ICCVAM's continued commitment to gaining regulatory acceptance of scientifically valid new and updated test methods that will continue to support the health of people, animals, and the environment while improving animal welfare and reducing animal use. We look forward to continued future progress.

Jodie Kulpa-Eddy, DVM
Chair, ICCVAM
Animal and Plant Health Inspection Service
U.S. Department of Agricuture

Rear Admiral William S. Stokes, DVM, DACLAM
Assistant Surgeon General, U.S. Public Heath Service
Director, NICEATM
Executive Director, ICCVAM
National Institute of Environmental Health Sciences

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established in 1997 to conduct interagency technical evaluations of new safety testing methods, including alternative testing methods that will reduce, refine (enhance animal well-being and lessen or avoid pain and distress), and replace the use of animals. ICCVAM was also established to coordinate cross-agency activities relating to development, validation, acceptance, and national and international harmonization of new, modified, and alternative toxicological test methods.

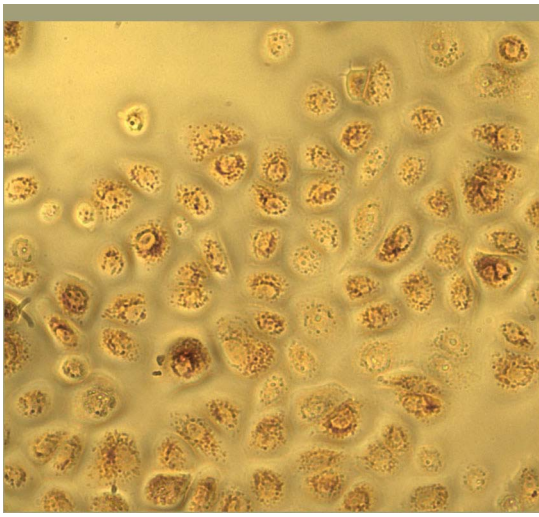
The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), ICCVAM, and the ICCVAM member agencies have contributed to the evaluation of 50 alternative methods that have been approved or endorsed by Federal regulatory agencies and international test guideline organizations. Of these, 33 are *in vitro* methods that reduce or replace animal use. The other 17 are *in vivo* methods that significantly reduce the number of animals used or significantly improve animal welfare by minimizing or avoiding potential pain and distress.

This report describes test method evaluations and other activities that ICCVAM conducted in 2010 and 2011 in conjunction with NICEATM. Selected highlights follow.

## Eye Safety Testing

- In 2010, ICCVAM recommended alternative methods and strategies to reduce animal use and to minimize or avoid unrelieved pain and distress during eye safety testing. Federal agencies accepted or endorsed ICCVAM recommendations for the following alternative methods in 2011:
  - Pain management procedures that should always be used to avoid or minimize unrelieved pain and distress when *in vitro* methods do not provide sufficient eye safety information and it is necessary to use animals to meet regulatory safety testing requirements. These procedures include the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints.
  - An *in vitro* Cytosensor microphysiometer (CM) test method that can be used as a screening test to identify some types of substances that may cause permanent or severe eye injuries and to determine if some types of substances will not cause sufficient injury to require hazard labeling for eye irritation. *The CM test method is the first accepted* in vitro *test method that can be used instead of animals to identify substances that do not require eye hazard labeling.*
  - Reports on the current validation status of four *in vitro* test methods for identifying substances with the potential to cause nonsevere ocular injuries, and recommended studies to further characterize their usefulness and limitations.
  - Reports on the current validation status and recommended additional studies for a non-animal *in vitro* testing strategy proposed to assess the eye irritation potential of antimicrobial cleaning products using the bovine corneal opacity and permeability (BCOP), CM, and EpiOcular™ (MatTek) test methods. The recommended studies will provide data necessary to support evaluation of the usefulness and limitations of the proposed testing strategy.
  - A report on the validation status of the low volume rabbit eye test, and recommendations that it should not be used for prospective *in vivo* ocular safety testing due to performance issues.
- NICEATM and ICCVAM developed draft eye injury hazard classification criteria to support consumer product safety testing with 3 animals rather than the current 6 to 18 animals. The recommended classification criteria provide the same or greater level of eye injury hazard labeling as current requirements, while using 50% to 83% fewer animals.

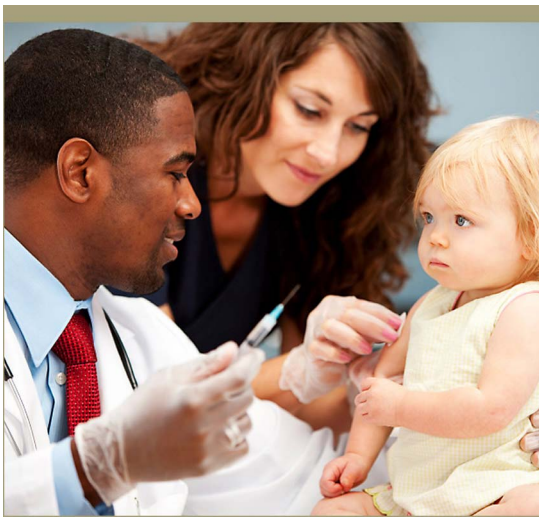
- NICEATM and ICCVAM initiated collaborations with the Japanese Center for the Validation of Alternative Methods to review the validation status of a short time exposure model that uses cultured corneal cells to rapidly identify whether substances may pose an eye injury hazard.
- NICEATM and ICCVAM prepared a guidance document on histopathology from *in vitro* and *in vivo* models used for eye injury hazard testing. Collection of histopathology data will be used to determine whether this information can increase the accuracy of some *in vitro* test systems such as the BCOP, isolated chicken eye, and isolated rabbit eye. The guidance document was formally adopted internationally by the Organisation for Economic Co-operation and Development (OECD) in 2011.



## Acute Systemic Toxicity Testing

- NICEATM and ICCVAM prepared a guidance document describing how to use two ICCVAM-recommended *in vitro* test methods to estimate starting doses for acute oral systemic toxicity tests. The tests can reduce animal use per test by up to 50%. The OECD formally adopted this international guidance document in 2010.
- NICEATM and members of the ICCVAM Interagency Acute Toxicity Working Group participated on the validation management team for an international study to determine whether two types of cultured liver cells reliably predict drug metabolism and associated toxicity.
- NICEATM initiated development of an acute dermal systemic toxicity up-and-down procedure that is expected to reduce the number of animals needed to determine if substances can be poisonous when they come in contact with the skin.

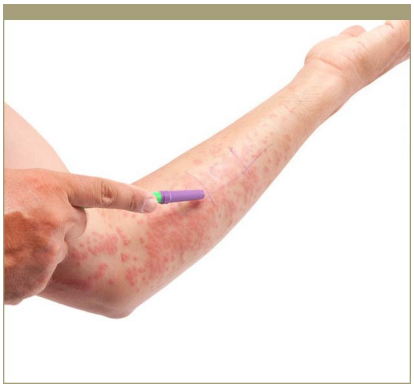
## Skin Safety Testing



- NICEATM and ICCVAM developed and submitted proposed revisions to two OECD test guidelines for *in vitro* test methods that can identify substances with the potential to cause skin burns. The revisions provide performance standards that can be used to validate similar test methods that may be more accurate, faster to perform, and less expensive.
- NICEATM completed a study to determine if revised procedures for reconstructed human skin models could increase the accuracy of the test methods for identifying whether chemicals can cause skin injuries. The results characterize limitations of the *in vitro* test methods that will need to be addressed by other approaches or models in a non-animal integrated testing strategy.

## Biologics Testing

- In September 2010, NICEATM, ICCVAM, and their partners in the International Cooperation on Alternative Test Methods (ICATM) held an international workshop on alternative methods that can reduce, refine, and replace animals for vaccine potency and safety testing. The workshop reviewed the state of the science of alternative methods that are currently

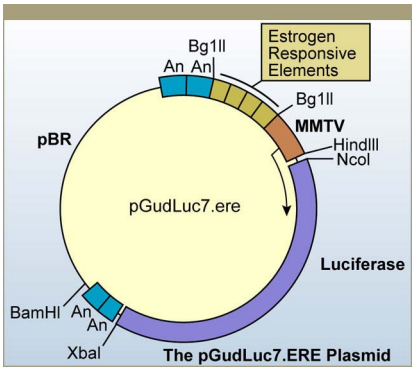


available for this purpose and developed recommendations for priority research needed to further advance alternative methods. Proceedings from the workshop were published in December 2011 (Kulpa-Eddy et al. 2011).

- NICEATM, ICCVAM, and their international partners convened a workshop on alternative methods for rabies vaccine potency testing in October 2011. Participants reviewed the available methods and approaches to reduce, refine, and replace animals used in rabies vaccine potency testing and developed an implementation strategy to achieve global acceptance and use of these alternatives.

## Immunotoxicity Testing: Allergic Contact Dermatitis

- ICCVAM recommended several new versions and applications of the murine local lymph node assay (LLNA) that will further reduce animal use and expand the applicability of the LLNA for assessing the allergic contact dermatitis hazard potential of chemicals and products. Federal agencies accepted or endorsed the following ICCVAM recommendations in 2010 and 2011:
  - An updated LLNA protocol that achieves a 20% reduction in the number of required animals
  - Routine use of the reduced LLNA, when dose–response information is not required, to determine the allergic contact dermatitis hazard potential of chemicals and products, enabling a 40% reduction in animal use for each test
  - Performance standards for the LLNA, which will enable more rapid and efficient evaluation of the validity of new versions that are mechanistically and functionally similar to the LLNA
  - Two new "green" versions of the LLNA that do not require radioactive reagents and will allow use of the LLNA in nearly all laboratories worldwide




- NICEATM and ICCVAM forwarded proposals to update the OECD test guideline for the LLNA and to create two new test guidelines for the nonradiolabeled versions of the LLNA. The OECD adopted the test guidelines in 2010, resulting in worldwide acceptance of these important methods.
- ICCVAM forwarded recommendations to Federal agencies that the LLNA may be used as a screening test to categorize substances as strong skin sensitizers. Agency acceptance responses will be received in early 2012.
- NICEATM initiated an evaluation of multiple *in vitro* methods used in integrated testing strategies to reduce, refine, and replace animal use for identification of substances that may cause allergic contact dermatitis.

## Endocrine Disruptor Testing

- ICCVAM completed an evaluation of the *in vitro* BG1Luc ER TA test method for its use as an initial screen to identify substances with the potential to induce or inhibit activation of the estrogen receptor. A draft international test guideline and performance standards were forwarded to the OECD, which initiated international review.

- NICEATM completed its coordination of an international validation study to evaluate an *in vitro* test method that measures proliferation of cultured cells to identify substances with the potential to induce or inhibit activation of the estrogen receptor.

## Pyrogen Testing

- The ICCVAM Interagency Pyrogenicity Working Group reviewed a nomination for the monocyte activation test, one of five *in vitro* pyrogen test methods previously reviewed by ICCVAM, to evaluate its usefulness to detect non-endotoxin pyrogens. ICCVAM considered this method of sufficient interest and applicability to warrant further evaluation.

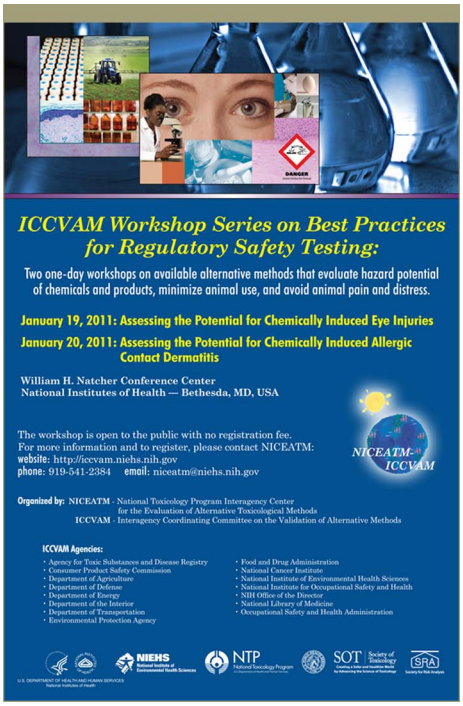
Photo courtesy of the NIH Center for Translational Therapeutics

## Genetic Toxicity Testing

- The ICCVAM Interagency Genetic Toxicity Working Group commented on validation study reports for three cell transformation assays conducted by the European Centre for the Validation of Alternative Methods. These cell transformation assays use cultured mouse or hamster cells to detect genotoxic and nongenotoxic carcinogens. These test methods are intended to reduce the number of animals used to detect substances that may cause cancer.

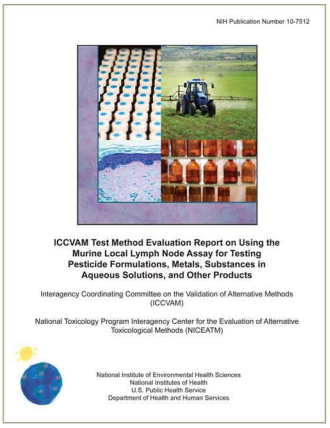
## Research and Development Activities Supporting Alternative Methods Development

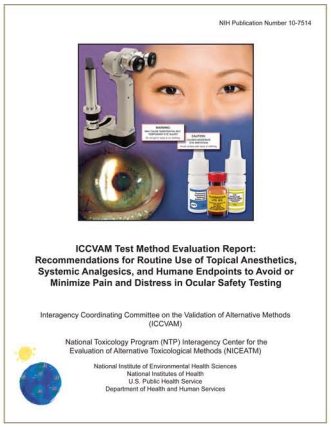
- NICEATM and ICCVAM agencies initiated collaborations to speed the translation of research advances and new technologies into scientifically valid safety testing methods that can further reduce, refine, and replace animal use. Agency initiatives and collaborations include Tox21, ToxCast™ (U.S. Environmental Protection Agency), and the National Institutes of Health–Food and Drug Administration–Defense Advanced Research Projects Agency Regulatory Science Initiative.

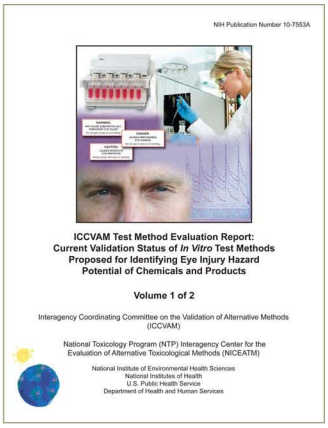



## ICCVAM Outreach and Collaborative Activities

- NICEATM and ICCVAM convened two workshops on Best Practices for Regulatory Safety Testing in January 2011. Participants learned about how ICCVAM-recommended alternative methods can be used to determine whether chemicals and products may cause eye injuries or allergic contact dermatitis.
- The National Institute of Environmental Health Sciences, on behalf of NICEATM and ICCVAM, signed an international agreement in March 2011 to add the Republic of Korea to ICATM, which was originally established in 2009 by the United States, the European Union, Japan, and Canada. The expanded international agreement is expected to further reduce, refine, and replace animal use in toxicity testing worldwide.


- NICEATM and ICCVAM collaborated with international partners on validation studies for test methods to identify substances with the potential to cause eye injuries, acute poisoning, allergic contact dermatitis, disturbances to the endocrine system, and DNA damage.
- NICEATM and ICCVAM participated in annual public meetings of the Scientific Advisory Committee on Alternative Toxicological Methods, providing comprehensive reports on activities and progress.
- NICEATM and ICCVAM prepared, commented on, or otherwise contributed to the development of 18 new test guidelines or proposals for new test guidelines, revisions of existing test guidelines, and guidance documents for alternative test methods considered by the OECD.
- NICEATM and ICCVAM scientists delivered presentations at the 2010 and 2011 annual meetings of the Society of Toxicology and at the 8th World Congress on Alternatives and Animal Use in the Life Sciences in 2011. NICEATM and ICCVAM scientists also attended and made presentations at five additional international meetings and conferences.
- Publications in 2010 and 2011 included test method evaluation reports, background review documents, workshop reports, and peer review panel reports. NICEATM published 17 *Federal Register* notices in 2010 and 2011. NICEATM and ICCVAM scientists published 21 manuscripts in peer-reviewed journals and presented 28 posters and platform presentations describing NICEATM–ICCVAM activities.

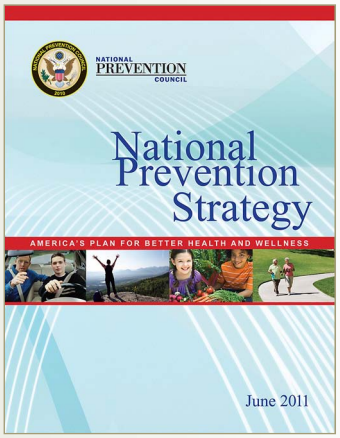
## DEVELOPMENT OF A PROCESS FOR EVALUATION OF ALTERNATIVE TEST METHODS

U.S. regulatory agencies are charged with protecting human and animal health and the environment. As part of this mission, agencies must determine the possible hazards presented by substances such as pesticides, consumer products, and workplace chemicals. Safety 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 those that *reduce* the number of animals required for a specific test procedure, *refine* animal use to enhance animal well-being and lessen or avoid pain and distress, or *replace* animal use with non-animal test systems or lower species. Collectively, these methods are referred to as the 3Rs of alternatives.

In September 1994, the Director of the National Institute of Environmental Health Sciences (NIEHS) created an *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to respond to directives in the National Institutes of Health (NIH) Revitalization Act of 1993 (42 U.S.C. 285*l*-1 and 42 U.S.C. 283*e*).[1] This Act required NIEHS to establish criteria for the validation and regulatory acceptance of alternative 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 present on ICCVAM today.

[1] The excerpts of the NIH Revitalization Act relevant to the establishment of NICEATM and ICCVAM (42 U.S.C. 285*l*-1 and 42 U.S.C. 283*e*) are included in this report as **Appendix G**. This information can also be found on the NICEATM–ICCVAM website at http://iccvam.niehs.nih.gov/docs/about_docs/pl103_43.pdf.

## CHEMICAL SAFETY TESTING PREVENTS ILLNESS AND INJURY




Chemicals make our lives more comfortable, but many of the chemicals that improve the quality of our lives can also cause illness or injury if handled inappropriately. Testing substances such as pesticides, consumer products, and workplace chemicals to evaluate their safety or toxicity provides information about possible hazards. This information enables people to make informed decisions about using, storing, and disposing of these substances in ways that protect themselves and the environment.

In June 2011, the Office of the Surgeon General issued ***The National Prevention Strategy: America's Plan for Better Health and Wellness.*** Developed by the leaders of 18 Federal government agencies and offices concerned with public health and safety, this strategy "aims to guide our nation in the most effective and achievable means for improving health and well-being" (National Prevention Council 2011). The National Prevention Strategy recognizes that good health comes not just from receiving quality medical care but also from clean air and water, safe outdoor spaces for physical activity, safe worksites, healthy foods, violence-free environments, and healthy homes.

Obtaining accurate information about whether substances might cause injury or disease supports two of the Strategic Directions of the National Prevention Strategy. Information about possible health hazards presented by chemical products ***empowers people*** to make healthier choices. Information about how a chemical product could affect the environment encourages people to handle these substances properly to maintain ***healthy and safe community environments***. Federal agencies and offices represented on the National Prevention Council include entities that are both responsible for regulating the labeling, shipping, handling, and disposal of potentially hazardous substances and engaged in research to develop testing methods that will provide better information about potentially hazardous substances.

In 1997, the *ad hoc* ICCVAM committee published its final report, *Validation and Regulatory Acceptance of Toxicological Test Methods* (ICCVAM 1997). The same year, NIEHS, with the cooperation and agreement of the other 14 agencies, established a standing ICCVAM committee to (1) implement a process to evaluate new test methods of agency interest and (2) coordinate agency interactions related to the development, validation, acceptance, and national and international harmonization of toxicological test methods.

## ICCVAM AGENCIES

The ICCVAM Authorization Act defines the composition of ICCVAM as the heads, or their designees, of the 15 U.S. Federal agencies indicated by italics in the list below:

- *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 for Occupational Safety and Health*
  - *Food and Drug Administration*
  - *National Institutes of Health*
    - *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*

## ESTABLISHMENT OF NICEATM AND ICCVAM

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) is located within the Division of the NTP at NIEHS. NIEHS established NICEATM in 1997 to:

- Administer ICCVAM and its scientific advisory committee
- Coordinate and provide technical and scientific support 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 high-priority alternative test methods that may reduce, refine, or replace animal use for regulatory safety testing

The ICCVAM Authorization Act of 2000[2] 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 safety testing methods that protect human and animal health and the environment while reducing, refining, and 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, and replace the use of animals in testing, where feasible

[2] The ICCVAM Authorization Act (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 located within the Division of the National Toxicology Program at the National Institute of Environmental Health Sciences in Research Triangle Park, NC

## 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
- 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 them available to the public

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 evaluations. 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 2003)
- Assessing the completeness of background review documents for test methods submitted for ICCVAM evaluation and preparing draft background review documents where necessary
- Determining whether and to what extent new, revised, and alternative test methods proposed for ICCVAM evaluation are applicable to regulatory safety testing
- Assembling and distributing information about current best practices for the humane care and use of animals in toxicological research and testing

NICEATM and ICCVAM also carry out activities required by the NIH Revitalization Act of 1993 (see **Appendix G**). This Act directed NIEHS to develop and validate improved testing methods for acute and chronic toxicity, including methods to reduce, refine, or replace animal use. To address this mandate, NICEATM conducts and coordinates international test method validation studies as resources allow. These studies are designed to evaluate the usefulness and limitations of alternative test methods that reduce, refine, or replace animal use for toxicity testing and that may improve safety assessments for people, animals, and the environment.

## WHY DO SAFETY TESTING METHODS REQUIRE VALIDATION AND EVALUATION?

New regulatory safety testing methods must protect human health, animal health, and the environment as well as or better than currently accepted methods. The validation process generates data to characterize the usefulness and limitations of alternative test methods for specific safety testing applications. This information is then used to determine if use of an alternative test method is as protective as currently accepted methods and strategies.

During the test method validation process, the test method is 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 includes its *accuracy*, the proportion of test method results that agree with an accepted reference value. Test methods must be reliable and relevant enough that their results can be used in regulatory safety testing to adequately predict the effect of a tested substance on human health, animal health, and the environment.

ICCVAM evaluation of 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 method. Data generated using the new test method are compared to those from the currently approved test method(s) and to any relevant human toxicity data. If the available data from the new test method are insufficient, validation studies must generate data to specifically evaluate 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 facilitate development, validation, and regulatory acceptance of new and revised regulatory test methods that reduce, refine, and replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment.**

### 1997

**1997 *AD HOC* ICCVAM COMMITTEE** (established by NIEHS in response to the NIH Revitalization Act of 1993) issues its final report recommending validation and acceptance criteria for toxicological test methods; NIEHS establishes a standing ICCVAM committee and the Advisory Committee on Alternative Toxicological Methods.

### 2002

**2002 ICCVAM ISSUES RECOMMENDATIONS** on use of the up-and-down procedure (UDP) for assessing acute oral systemic toxicity and on use of three *in vitro* test methods that identify substances that are corrosive to the skin; NICEATM initiates a validation study of two *in vitro* test methods to set starting doses for acute oral toxicity tests; NTP establishes the the Scientific Advisory Committee on Alternative Toxicological Methods.

### 2007

**2007 ICCVAM RECOMMENDS** *in vitro* test methods to set starting doses for acute oral toxicity tests; NICEATM–ICCVAM launches improved website; the Consumer Product Safety Commission nominates new versions and applications of the LLNA for evaluation.

### 2008

**2008 ICCVAM RELEASES THE 2008–2012 FIVE-YEAR PLAN** at its Ten-Year Anniversary Symposium; ICCVAM recommends five *in vitro* methods for identification of pyrogens; ICCVAM cosponsors international workshop on *in vitro* approaches and humane endpoints in acute toxicity testing.

## 1999

**1999 ICCVAM ISSUES RECOMMENDATIONS** on use of the murine local lymph node assay (LLNA) to identify substances with the potential to cause allergic contact dermatitis and on use of Corrositex® (InVitro International) to identify substances that are corrosive to the skin.

## 2000

**2000 ICCVAM AUTHORIZATION ACT** establishes ICCVAM as a permanent interagency committee under NICEATM; ICCVAM sponsors a workshop on the use of *in vitro* methods in acute systemic toxicity testing.

## 2001

**2001 ICCVAM PUBLISHES A REPORT** on the *in vitro* methods workshop presented in 2000, a guidance document on using *in vitro* methods to set starting doses for acute systemic toxicity testing, and ICCVAM's first progress report.

## 2003

**2003 ICCVAM PUBLISHES RESULTS** of its evaluation of the validation status of *in vitro* methods to identify potential endocrine disruptors, recommendations on procedures for future validation studies, and revised guidelines for nominations and submissions; the Environmental Protection Agency nominates eye safety test methods for evaluation.

## 2004

**2004 ICCVAM HOLDS A STRATEGIC PLANNING MEETING** and develops a *Mission, Vision and Strategic Priorities* document (ICCVAM 2004a); ICCVAM publishes performance standards for skin corrosion test methods; test method developers nominate two *in vitro* test methods to identify potential endocrine disruptors for NICEATM-sponsored validation studies.

## 2006

**2006 ICCVAM ISSUES RECOMMENDATIONS** on use of the first *in vitro* test methods to identify ocular corrosives and severe irritants; NICEATM initiates validation study of an *in vitro* test method to identify potential endocrine disruptors; NICEATM and ICCVAM convene a workshop on alternative botulinum toxin testing methods; NICEATM and ICCVAM submit test guidelines based on Corrositex and the UDP that are adopted by the Organisation for Economic Co-operation and Development (OECD).

## 2009

**2009 THE U.S., CANADA, EUROPEAN UNION, AND JAPAN SIGN** a Memorandum of Cooperation establishing the International Cooperation on Alternative Test Methods; ICCVAM recommends the reduced LLNA test method for assessment of allergic contact dermatitis hazards; ICCVAM publishes LLNA performance standards; the OECD adopts the first two *in vitro* ocular test guidelines, which were developed and submitted by NICEATM and ICCVAM.

## 2010

**2010 ICCVAM RECOMMENDS** the first two "green" nonradioactive versions of the LLNA and an expanded LLNA applicability domain; ICCVAM recommends additional *in vitro* eye safety testing methods and strategies, including the first *in vitro* test method used for substances not labeled as irritants; ICCVAM recommends routine use of topical anesthetics, systemic analgesics, and humane endpoints for *in vivo* eye safety testing; ICCVAM cosponsors the first international workshop on alternative methods to reduce, refine, and replace animal use for human and veterinary vaccine potency and safety testing; ICCVAM develops test guidelines on use of new versions and applications of the LLNA that are adopted by the OECD.

## 2011

**2011 NICEATM AND ICCVAM CONVENE WORKSHOPS** on best practices for regulatory safety testing; ICCVAM issues recommendations on use of the LLNA for potency categorization; NICEATM and ICCVAM organize an international workshop on alternative methods for human and veterinary rabies vaccine testing; ICCVAM develops final recommendations on *in vitro* BG1 Luc ER TA test method to detect potential endocrine disruptors.

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

## 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, NICEATM, and ICCVAM about federally mandated ICCVAM functions and ICCVAM activities.

The ICCVAM Authorization Act states that SACATM must 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
- Additional representatives selected from among the following:
  - Academic institutions
  - State government agencies
  - An international regulatory body
  - Any corporation developing or marketing new or revised or alternative test methodologies, including contract laboratories

The SACATM charter, related *Federal Register* notices, past meeting materials, and future meeting announcements may be found on the NTP website (http://ntp.niehs.nih.gov/go/167).

The SACATM charter directs SACATM to meet at least once each fiscal year. 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.

SACATM held meetings in June 2010 and June 2011. Summaries of the 2010 and 2011 meetings are provided on pages 59 and 60. A list of SACATM members during 2010 and 2011 can be found in **Appendix F.**

## NICEATM–ICCVAM FIVE-YEAR PLANS

In 2008, 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 2008a). The *NICEATM–ICCVAM Five-Year Plan* described goals and objectives that support ICCVAM's purposes as outlined in the ICCVAM Authorization Act. It also outlined 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* (ICCVAM 2004a).

The *NICEATM–ICCVAM Five-Year Plan (2008–2012)* 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. In June 2009, the subcommittee released an Implementation Plan. This working document describes how the strategies outlined in the *NICEATM–ICCVAM Five-Year Plan* are being implemented and how the four key challenges are being addressed. It includes goals, specific objectives, planned activities, and progress toward the goals and objectives outlined in the *NICEATM–ICCVAM Five-Year Plan*.

In 2011, NICEATM began to develop a five-year plan for 2013 through 2017. A request for information relevant to the development of this five-year plan was sent to ICCVAM agencies in November 2011. This request asked agencies for information on high-priority areas for alternative test methods activities and planned research, development, and validation activities relevant to the ICCVAM mission. A *Federal Register* notice was published in November 2011 requesting comments from the public relevant to the development of the plan.

NICEATM and ICCVAM will continue to develop the 2013–2017 five-year plan through 2012, with multiple opportunities for public comments. Public release of the 2013–2017 five-year plan is scheduled for December 2012.

## 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 methods, such as gathering additional information, conducting workshops, or 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 enhance animal well-being and lessen or avoid pain or distress in animals

**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 a non-animal system or one animal species with a phylogenetically lower one

**Submission:** a proposal to ICCVAM for review and evaluation of a test method that has had validation studies completed according to the *ICCVAM Guidelines* (ICCVAM 2003)


## FIVE-YEAR PLAN IMPLEMENTATION

Descriptions of NICEATM–ICCVAM activities will note how these activities relate to the goals and objectives of the *NICEATM–ICCVAM Five-Year Plan (2008–2012)* and the Implementation Plan.

## REGULATORY ACCEPTANCE OF TEST METHODS EVALUATED BY ICCVAM

Test methods that can accurately identify 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 systemic toxicity (risk of poisoning when absorbed through the skin)
- Eye injuries, temporary and permanent
- Skin injuries, temporary and permanent
- Dermal sensitization (production of allergic contact dermatitis upon repeated skin exposure)

ICCVAM's goal in fostering and evaluating new test methods is to translate research advances and new technologies into scientifically valid safety testing methods that can be used to meet regulatory testing requirements. Since its establishment, ICCVAM and its member agencies have contributed to the evaluation of 50 alternative test methods endorsed by Federal regulatory agencies (see table in **Appendix B** for list). NICEATM and ICCVAM coordinated comprehensive technical evaluations of 21 of these methods. Of the alternative methods accepted by Federal agencies, 33 are *in vitro* methods that either replace animal use or reduce the number of animals required for testing. The remaining 17 are either modifications of existing *in vivo* test methods or new alternative *in vivo* test methods that reduce animal use or animal pain and distress.

The 50 alternative methods endorsed by Federal regulatory agencies provide alternatives for five of the six most commonly conducted types of testing: temporary or permanent skin or eye injuries, dermal sensitization, or poisoning when swallowed or inhaled. ICCVAM is currently developing recommendations on an up-and-down procedure to reduce animal use for the sixth test method area, acute dermal systemic toxicity testing.

ICCVAM test method evaluation activities are prioritized based on consideration of one or more of the following:

- 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 (enhancing animal well-being and lessening or avoiding pain and distress), or replacing animals used in testing
- The potential for an alternative test method to apply to testing required by multiple agencies

This chapter provides an update on ICCVAM test method evaluations and related activities during 2010 and 2011. The four highest priorities are eye safety testing, acute toxicity testing, skin safety testing, and biologics testing. Other priorities are testing to identify allergic contact dermatitis hazards, endocrine disruptor testing, pyrogen testing, and genetic toxicity 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 to assess their potential to cause eye injuries. 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 in order to prevent exposures that may result in injuries.

Multiple regulatory agencies require ocular (eye) safety testing, which is one of the four most commonly required product safety tests. Despite progress in developing alternative test methods that do not use live animals, a large proportion of eye safety tests still use the rabbit eye test (Draize et al. 1944). This test is performed 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. Eye safety testing can involve large numbers of animals, and animals used in tests to identify eye hazards can experience significant pain and distress when test articles are applied to the eye and when eye injuries occur. Therefore, development of alternative *in vitro* test methods and approaches to the rabbit eye test is a high priority for NICEATM–ICCVAM.

## HIGHLIGHTS OF ICCVAM ACTIVITIES

- In 2010, ICCVAM recommended alternative methods and strategies to reduce animal use and to miminize or avoid unrelieved pain and distress during eye safety testing. Federal agencies accepted or endorsed ICCVAM recommendations for the following alternative methods in 2011:
  - Pain management procedures that should always be used to avoid or minimize unrelieved pain and distress when *in vitro* methods do not provide sufficient eye safety information and it is necessary to use animals to meet regulatory safety testing requirements. These procedures include the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints.
  - An *in vitro* Cytosensor microphysiometer (CM) test method that can be used as a screening test to identify some types of substances that may cause permanent or severe eye injuries and to determine if some types of substances will not cause sufficient injury to require hazard labeling for eye irritation. *The CM test method is the first accepted* in vitro *test method that can be used instead of animals to identify substances that do not require eye hazard labeling.*
  - Reports on the current validation status of four *in vitro* test methods for identifying substances with the potential to cause nonsevere eye injuries, and recommended studies to further characterize their usefulness and limitations.

## DEFINITIONS OF KEY TERMS

**Antimicrobial cleaning product (AMCP):** a commercially available 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:** a predetermined criterion used to evaluate whether a study should be discontinued early to stop or minimize test animal pain or distress

**Ocular corrosive:** a substance that causes permanent tissue damage in the eye following application

**Ocular irritant:** a substance that causes temporary tissue damage in the eye. Damage from a *severe* irritant is still evident 21 days after application or causes 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


  - Reports on the current validation status and recommended additional studies for a non-animal *in vitro* testing strategy proposed to assess the eye irritation potential of antimicrobial cleaning products using the bovine corneal opacity and permeability (BCOP), CM, and EpiOcular™ (MatTek) test methods. The recommended studies will provide data necessary to support evaluation of the usefulness and limitations of the proposed testing strategy.
  - A report on the validation status of the low volume rabbit eye test, and recommendations that it should not be used for prospective *in vivo* eye safety testing due to performance issues.

- NICEATM and ICCVAM developed draft eye injury hazard classification criteria to support consumer product safety testing with 3 animals rather than the current 6 to 18 animals. The recommended classification criteria provide the same or greater level of eye injury hazard labeling as current requirements, while using 50% to 83% fewer animals.
- NICEATM and ICCVAM initiated collaborations with the Japanese Center for the Validation of Alternative Methods to review the validation status of a short time exposure model that uses cultured corneal cells to rapidly identify whether substances may pose an eye injury hazard.
- NICEATM and ICCVAM prepared a guidance document on histopathology from *in vitro* and *in vivo* models used for eye injury hazard testing. Collection of histopathology data will be used to determine whether this information can increase the accuracy of some *in vitro* test systems such as the BCOP, isolated chicken eye, and isolated rabbit eye. The guidance document was formally adopted internationally by the Organisation for Economic Co-operation and Development (OECD) in 2011.

## Guidance Documents for Use of *In Vitro* Test Methods to Identify Ocular Corrosives and Severe Irritants

In 2006, ICCVAM recommended that the BCOP and the isolated chicken eye (ICE) test methods could be used to screen for ocular corrosives and severe irritants in appropriate circumstances and with specific limitations. If either of these alternative test methods yields a positive response, the product can be labeled as an ocular corrosive or severe irritant and no live animal testing is required.

ICCVAM's recommendations formed the basis for test guidelines adopted by the Organisation for Economic Co-operation and Development (OECD) in 2009. The BCOP and ICE test methods can now be used worldwide to identify substances as ocular corrosives or 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 pain and distress caused by animal testing.

NICEATM and the ICCVAM Interagency Ocular Toxicity Working Group prepared and submitted a guidance document for use with Test Guidelines 437 and 438. This document, which was formally adopted internationally by the OECD in 2011 (OECD 2011a), (1) promotes histopathology evaluation as an additional endpoint for eye safety testing and (2) provides specific guidance on expanding the data available for the BCOP and ICE test methods to optimize their use for identifying all hazard categories. The document includes detailed protocols that describe the routine collection of tissues for histopathology evaluation and recommends decision criteria for the BCOP and ICE test methods to identify moderate and mild irritants and substances not labeled as eye irritants. Both of these practices support the development of an expanded database of reference data that may result in broader use of the BCOP and ICE test methods.

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

In the *ICCVAM Test Method Evaluation Report: Recommendations for Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing* (published in September 2010),[1] ICCVAM recommended pain management procedures that should always be used to avoid or minimize pain and distress when it is necessary to conduct the rabbit eye test for regulatory safety purposes. The recommended procedures, including use of topical anesthetics, are commonly used in human eye surgery procedures. The test method evaluation report includes procedures detailing specific pain-relieving drugs and a schedule of administration to effectively avoid or minimize pain and distress. The report includes ICCVAM's recommendation that rabbits be routinely evaluated for clinical signs of pain and distress. Examples of humane endpoints that should be used to end a study early and thereby avoid or minimize pain and distress experienced by test animals are also provided.

ICCVAM forwarded these recommendations to U.S. Federal agencies in 2010, and the recommendations were endorsed in 2011. Adoption and use of these recommendations will result in the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints whenever the rabbit eye test is conducted for regulatory safety testing, unless pain response monitoring is required (for example, when testing tolerability of topical medicines for the eye). Use of these modified procedures for *in vivo* eye safety testing will substantially reduce or eliminate animal pain and distress.

The ICCVAM Interagency Ocular Toxicity Working Group updated OECD Test Guideline 405: Acute Eye Irritation/Corrosion (OECD 2002) to include these recommendations to incorporate procedures for routine use of topical anesthetics, systemic analgesics, and humane endpoints in *in vivo* ocular safety testing. An OECD expert working group is currently considering these revisions to Test Guideline 405.

[3] Documents mentioned in this chapter that were published during 2010 and 2011 are listed in **Appendix C**. All ICCVAM documents are available electronically on the NICEATM–ICCVAM website (see **Appendix C** for locations of specific reports or website pages). They are also available in hard copy from NICEATM (see page 97 for contact information).

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

In September 2010, ICCVAM published 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.* In this report, ICCVAM made further recommendations to Federal agencies on the use of *in vitro* test methods that identify substances with the potential to cause eye injuries without the use of live animals:

- Bovine corneal opacity and permeability
- Cytosensor microphysiometer
- Hen's egg test–chorioallantoic membrane
- Isolated chicken eye
- Isolated rabbit eye

ICCVAM recommended that the Cytosensor microphysiometer (CM) test method can be used as a screening test to identify certain types of water-soluble substances that may cause permanent or severe eye injuries. Substances that are appropriate for testing using the CM test method and that yield positive results can be classified as having the potential to cause severe or permanent eye injuries without additional testing using animals. However, a substance that tests negative with the CM test method must be tested using another test method capable of distinguishing between different hazard classifications of substances that can cause reversible and nonsevere eye injuries.

ICCVAM also recommended that the CM test method can be used to test certain substances (water-soluble surfactants and surfactant-containing formulations such as cosmetics and personal care products) to determine if they do not have sufficient potential to cause eye injuries and therefore do not require eye hazard labeling. The CM test method is the first *in vitro* test method available in the United States for this purpose and is now under consideration for international adoption as an OECD test guideline.

ICCVAM concluded that studies are needed to further characterize the usefulness and limitations of the other four test methods before they can be used in regulatory safety testing to classify substances as having the potential to cause nonsevere eye injuries or as not requiring hazard labeling for eye irritation. The 2010 report on the ICCVAM evaluation of these test methods includes recommendations for these future studies and also includes recommended test method protocols. ICCVAM forwarded these recommendations to U.S. Federal agencies in 2010, and the recommendations were endorsed in 2011.

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

In response to a request by the U.S. Environmental Protection Agency (EPA), NICEATM and ICCVAM evaluated a non-animal testing strategy to assess the potential of antimicrobial cleaning products (AMCPs) to cause eye irritation. This proposed testing strategy utilized the BCOP, CM, and EpiOcular test methods to determine the EPA hazard category and labeling requirements for these substances.

ICCVAM evaluated the proposed strategy and recommended further studies to characterize its usefulness and limitations. The results from these studies will provide data necessary to support evaluation of the usefulness and limitations of the proposed testing strategy. ICCVAM provided 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 Cleaning Products* (published in September 2010). ICCVAM forwarded these recommendations to U.S. Federal agencies in 2010, and the recommendations were endorsed in 2011.

*In vitro* test methods for identifying substances with the potential to cause eye injuries were a topic of the NICEATM-ICCVAM workshop on "Best Practices for Regulatory Safety Testing: Assessing the Potential for Chemically Induced Eye Injuries," held in January 2011. In this photo, NICEATM Director Dr. William Stokes, ICCVAM Chair Dr. Jodie Kulpa-Eddy, and ICCVAM Interagency Ocular Toxicity Working Group Chair Dr. Jill Merrill consider a question from a workshop participant.

## 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. Based on the review, ICCVAM does not consider the LVET a valid replacement for the rabbit eye test and, therefore, does not recommend it for prospective *in vivo* eye safety testing. If animals must be used for eye safety testing, ICCVAM recommends using the rabbit eye test with topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize animal pain and distress.

ICCVAM forwarded these recommendations to U.S. Federal agencies in 2010, and the recommendations were endorsed in 2011. They are presented in the *ICCVAM Test Method Evaluation Report: Recommendation to Discontinue Use of the Low Volume Eye Test for Ocular Safety Testing* (published in September 2010).

## Eye Injury Hazard Classification Criteria to Support Consumer Product Safety Testing

Current procedures used to determine appropriate eye hazard classification for consumer products require 6 animals per test and may require up to three sequential tests for each substance, thereby requiring 6, 12, or 18 animals to reach a hazard decision. The requirement for second and third sequential tests is based on the number of positive responses in the previous test.

Results from testing consistent with the OECD test guideline for eye irritation/corrosion, which specifies 3 animals, can also be submitted to U.S. agencies. However, current U.S. regulations for eye hazard classification of consumer products do not provide criteria for using results from a 3-animal test.

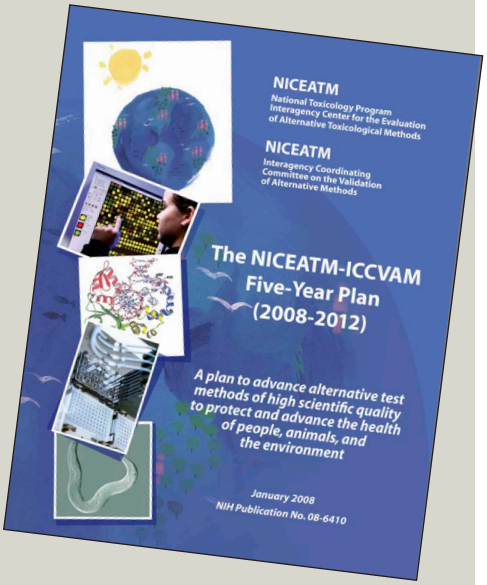
Therefore, NICEATM and ICCVAM analyzed the results from 3-animal tests to determine classification criteria that would provide labeling equivalent to that provided by current testing approaches. The analysis showed that using a classification criterion of one or more positive animals in a 3-animal test for the identification of eye hazards will provide a level of eye hazard classification the same as or greater than that of current testing approaches, while using 50% to 83% fewer animals.

A manuscript describing the NICEATM analysis was published in *Regulatory Toxicology and Pharmacology* (Haseman et al. 2011). Draft ICCVAM recommendations based on the results of this analysis were made available for comment to the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) at their 2010 and 2011 meetings and to the public via a *Federal Register* notice published in August 2011 (76 FR 50220). ICCVAM is considering public and SACATM comments during development of final recommendations, which will be forwarded to U.S. Federal agencies in 2012.

### Evaluation of the Short Time Exposure Test

The short time exposure test is an *in vitro* test method that rapidly identifies substances with the potential to cause eye injuries by assessing cytotoxicity (cell damage or death) in cultured rabbit corneal cells. Validation studies have been conducted in Japan to evaluate the performance of the short time exposure test using substances that represent a variety of chemical classes. ICCVAM initiated collaborations with the Japanese Center for the Validation of Alternative Methods to review the validation status of the short time exposure test in accordance with the International Cooperation on Alternative Test Methods framework.

## FIVE-YEAR PLAN IMPLEMENTATION: EYE SAFETY TESTING




- ICCVAM made recommendations to Federal agencies on the use of *in vitro* test methods proposed for identifying eye injury hazard potential of chemicals and products:
  - Bovine corneal opacity and permeability
  - Cytosensor microphysiometer
  - Hen's egg test—chorioallantoic membrane
  - Isolated chicken eye
  - Isolated rabbit eye
- ICCVAM made recommendations on an *in vitro* testing strategy to determine the ocular irritation potential of antimicrobial cleaning product formulations.
- ICCVAM made recommendations to Federal agencies on the use of topical anesthetics and systemic analgesics for reducing pain and distress during *in vivo* eye safety testing.
- ICCVAM proposed the evaluation of ocular histopathology for its potential to improve test method predictivity. As a result of this ICCVAM proposal, in 2011 the OECD adopted a guidance document for use with the test guidelines for the bovine corneal opacity and permeability and isolated chicken eye test methods.

## PUBLIC HEALTH AND ANIMAL WELFARE PERSPECTIVE

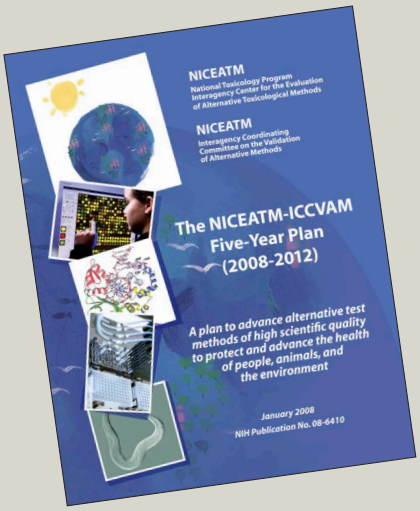
Death rates from accidental poisoning have more than tripled over the past 20 years. In the United States, accidental poisonings are now second only to motor vehicle crashes as the leading cause of accidental deaths (Murphy et al. 2012). Every day, more than 80 people in the United States die from accidental poisonings, and nearly 2000 are treated in emergency rooms (Centers for Disease Control 2011). More than half of these are children (Bronstein et al. 2011). 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. Potential poisons must be accurately identified in order to adequately protect human and animal health and to determine the appropriate use of child-resistant packaging.

Acute 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 acute systemic toxicity testing.

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, reduce the number of animals required for acute oral systemic toxicity testing (identification of substances that are poisonous when swallowed). However, identification of methods to further reduce animal use in acute systemic toxicity testing remains a top priority for NICEATM and ICCVAM.

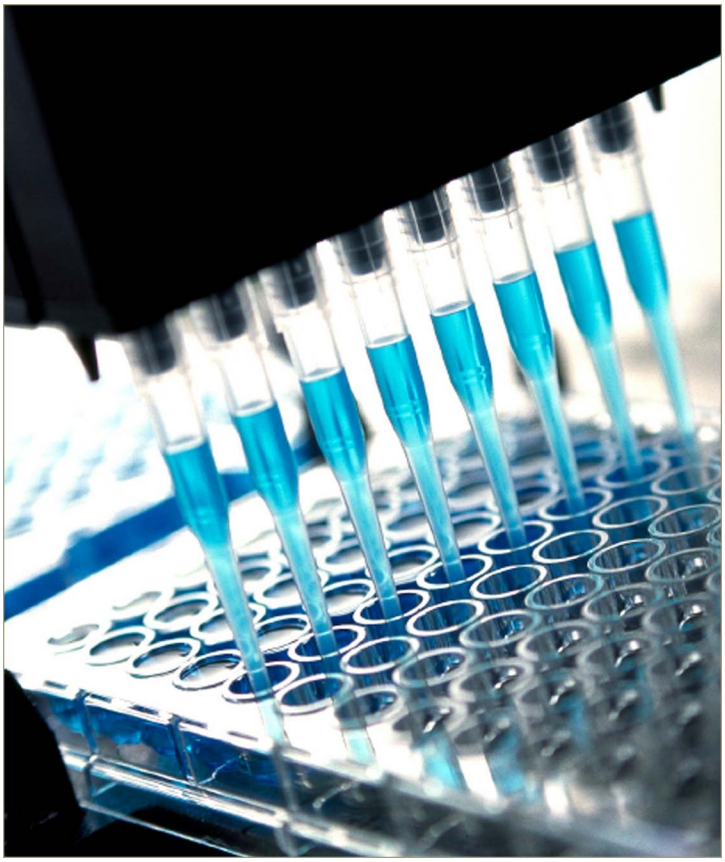
## HIGHLIGHTS OF ICCVAM ACTIVITIES

- NICEATM and ICCVAM prepared a guidance document describing how to use two ICCVAM-recommended *in vitro* test methods to estimate starting doses for acute oral systemic toxicity tests. The tests can reduce animal use per test by up to 50%. The OECD formally adopted this international guidance document in 2010.
- NICEATM and members of the ICCVAM Interagency Acute Toxicity Working Group participated on the validation management team for an international study to determine whether two types of cultured liver cells can reliably predict drug metabolism and associated toxicity.
- NICEATM initiated development of an acute dermal systemic toxicity up-and-down procedure that is expected to reduce the number of animals needed to determine whether substances can be poisonous when they come in contact with the skin.




## FIVE-YEAR PLAN IMPLEMENTATION: ACUTE SYSTEMIC TOXICITY TESTING

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.



### International Acceptance 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 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.

In 2008, ICCVAM sent recommendations to Federal agencies on the use of two *in vitro* 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, potentially reducing the number of animals required for this type of testing.

NICEATM and ICCVAM prepared and submitted a guidance document that describes use of these two test methods for estimating starting doses for acute oral systemic toxicity tests. Use of the *in vitro* test methods can reduce animal use in the acute oral systemic toxicity tests by up to 50% per test. The OECD published the guidance document submitted by NICEATM and ICCVAM in July 2010 (OECD 2010a). Acceptance by OECD member countries will facilitate worldwide use of these test methods to set starting doses for acute oral systemic toxicity tests, further reducing the number of animals required for this testing.

### Validation Study of an *In Vitro* Enzyme Induction Assay to Assess Acute Toxicity

NICEATM and the ICCVAM Interagency Acute Toxicity Working Group are participating on the validation management team for an international study sponsored by the European Centre for the Validation of Alternative Methods (ECVAM). The goal of this validation study is to determine whether two types of cultured liver cells (HepaRG cells and cryopreserved human hepatocytes) reliably predict drug metabolism and associated toxicity. This validation study includes exposing the two cell types to compounds that may induce cytochrome p450 enzymes and then measuring the metabolites produced. The goal of the study is to validate an *in vitro* model for assessing the metabolism and toxicity of drugs. However, the study may lead to development of a novel *in vitro* platform for assessing the metabolism and toxicity of other potentially poisonous substances.

The validation study is ongoing. Test results from a limited number of compounds demonstrate that the current protocol generates reproducible enzyme induction among several laboratories. Additional substances are being tested, and the study is scheduled to be completed in December 2012.

### Development of an Up-and-Down Procedure for Acute Dermal Systemic Toxicity

Acute dermal systemic toxicity testing identifies substances that are poisonous when absorbed through the skin. NICEATM is using acute dermal systemic toxicity reference data to develop a dermal up-and-down test. This test is expected to reduce the use of animals in acute dermal systemic toxicity tests. An independent peer review of the NICEATM analysis is planned for 2013.

## 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:** a condition under which cells are killed or harmed through interference with the structures or processes essential for cell survival, proliferation, and/or function

**Cytochrome P450 enzymes:** a group of enzymes in the liver that metabolize (alter the structure of) drugs and other molecules

**Cytotoxicity:** a condition under which cells are killed or harmed

**Enzyme induction:** a process in which the production of an enzyme is initiated or increased

**Enzyme substrate:** a chemical upon which an enzyme acts to catalyze a chemical reaction that will alter the structure of the chemical

**Hepatocytes:** the primary cells of the liver that carry out various activities, such as metabolism and the synthesis of proteins and other substances

**Metabolism:** the sum of the processes by which a particular substance is handled in a living organism, such as assimilation and incorporation or detoxification and excretion

**Metabolite:** the chemical produced after the metabolism of a substance

**Up-and-down procedure:** an acute systemic toxicity test method that involves sequentially testing single animals. The dose to an animal is increased if the previous animal lives and is decreased if the previous animal dies.

## PUBLIC HEALTH AND ANIMAL WELFARE PERSPECTIVE

Injuries to the skin, also called dermal injuries, fall into two categories. Skin *corrosion* is *irreversible* destruction of skin resulting in permanent damage that occurs when contact with a substance kills or destroys skin cells and in effect produces a chemical burn. Skin *irritation* is *reversible* damage resulting in redness or swelling that occurs when a chemical injures skin cells.

Every year, thousands of workers experience injuries from chemical burns, many of which result in lost workdays (Bureau of Labor Statistics 2010a). Chemical products used in the home, such as bleach, swimming pool chemicals, drain cleaners, and other cleaning products, can also cause chemical burns. Chemical burns can be difficult to treat, and long-term complications from these injuries are common (Palao et al. 2010). To protect workers and consumers, regulatory agencies test substances to determine whether they may present skin injury 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.

Substances with the potential to cause skin injuries are typically identified by applying the test substance to the skin of a laboratory animal. As an alternative, ICCVAM 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 corrosives without animal testing. Similar test methods to identify skin irritants can further reduce the number of animals needed.

## HIGHLIGHTS OF ICCVAM ACTIVITIES

- NICEATM and ICCVAM developed and submitted proposed revisions to two OECD test guidelines for *in vitro* test methods that can identify substances with the potential to cause skin burns. The revisions provide performance standards that can be used to validate similar test methods that may be more accurate, faster to perform, and less expensive.
- NICEATM completed a study to determine if revised procedures for reconstructed human skin models could increase the accuracy of the test methods for identifying whether chemicals can cause skin injuries. The results characterize limitations of the *in vitro* test methods that will need to be addressed by other approaches or models in a non-animal integrated testing strategy.


## FIVE-YEAR PLAN IMPLEMENTATION: SKIN 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 for identification of substances with the potential to cause skin injuries.

## Revisions to OECD Test Guidelines

NICEATM and ICCVAM developed and submitted proposed revisions to OECD test guidelines for methods that can identify substances with the potential to cause damage to the skin without using live animals.

- NICEATM and ICCVAM submitted revisions to OECD Test Guideline 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method. The ICCVAM Interagency Dermal Corrosivity and Irritation Working Group commented on the draft revised test guideline. The OECD adopted the revised test guideline in 2010 (OECD 2010b).
- NICEATM and ICCVAM submitted revisions to OECD Test Guidelines 430 and 431, which describe methods to identify substances with the potential to cause permanent damage to the skin without using live animals. These revisions incorporate features of ICCVAM performance standards for *in vitro* test methods for skin corrosion (ICCVAM 2004b) that were developed after the OECD test guidelines were initially published in 2002. The OECD Dermal Expert Consultation Group will meet in January 2012 to review the revisions to the test guidelines and is scheduled to discuss final revisions in September 2012.

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

Any testing strategy for substances with the potential to cause skin injuries 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* test methods evaluated by ICCVAM to identify substances with the potential to cause these types of severe skin injuries 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 their potential to cause skin 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 recently completed a study to determine the extent to which revised procedures could increase the accuracy of *in vitro* test methods. 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. NICEATM is currently analyzing the results of this study.

## DEFINITIONS OF KEY TERMS

**Performance standards:** criteria, 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

**Weight-of-evidence approach:** an approach that considers a collection of characteristics and test results as the basis for a conclusion that may not be evident from the individual data

## 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 (FDA) 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, and the animals used may experience significant pain and distress during testing. Therefore, identification of methods that would reduce or eliminate the need for animal testing for biologics is a high priority for NICEATM and ICCVAM.

## HIGHLIGHTS OF ICCVAM ACTIVITIES

- In September 2010, NICEATM, ICCVAM, and their partners in the International Cooperation on Alternative Test Methods (ICATM) held an international workshop on alternative methods that can reduce, refine, and replace animals for vaccine potency and safety testing. The workshop reviewed the state of the science of alternative methods that are currently available for this purpose and developed recommendations for priority research needed to further advance alternative methods. Proceedings from the workshop were published in December 2011 (Kulpa-Eddy et al. 2011).
- NICEATM, ICCVAM, and their international partners convened a workshop on alternative methods for rabies vaccine potency testing in October 2011. Participants reviewed the available methods and approaches to reduce, refine, and replace animals used in rabies vaccine potency testing and developed an implementation strategy to achieve global acceptance and use of these alternatives.

### 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 to public health. 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.

NICEATM, ICCVAM, the European Centre for the Validation of Alternative Methods (ECVAM), the Japanese Center for the Validation of Alternative Methods (JaCVAM), and Health Canada organized an international workshop in September 2010 to address these issues. The workshop was attended by nearly 200 scientists representing relevant stakeholder organizations from 13 countries.

Workshop participants identified knowledge and data gaps that need to be addressed in order to develop methods that can further reduce, refine, and replace the use of animals in vaccine testing. Participants also identified and prioritized research, development, and validation activities needed to address these knowledge and data gaps, including the application of new science and technology to develop improved methods. They agreed that vaccines that use the largest number of animals and that are associated with the greatest pain and distress should be given the highest priority for development and validation of alternative test methods. Participants also emphasized the need to find ways to avoid or minimize testing with live viruses and bacteria that are hazardous to workers. Ways to promote the increased use of accepted methods were also discussed. Implementation of the workshop recommendations is expected to advance the availability and use of alternative methods for vaccine potency and safety testing while ensuring continued protection of human and animal health.


Complete proceedings of the workshop, including manuscripts from speakers and breakout group sessions, were published in 2011 as a dedicated issue of *Procedia in Vaccinology* (Kulpa-Eddy et al. 2011; see **Appendix C** for a listing of articles with NICEATM–ICCVAM authors). ICCVAM will continue to use the conclusions and recommendations from the workshop to prioritize future research, development, and validation activities for alternative test methods that reduce, refine, and replace the use of animals in vaccine potency and safety testing.

## International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing

Rabies is a deadly disease that kills over 70,000 people worldwide each year, and rabies vaccines are the most important resource available for prevention of rabies infections. However, the current method to evaluate the effectiveness of human and veterinary rabies vaccines involves vaccinating animals and then challenging them with the rabies virus. This approach requires large numbers of laboratory animals and causes significant pain and distress. New methods and approaches are sought that (1) are more humane and use fewer or no animals; (2) are faster, more accurate, and less expensive; and (3) do not require handling of live rabies virus and thus are safer for laboratory workers.

The September 2010 NICEATM–ICCVAM vaccine workshop identified rabies vaccines as one of the three highest priorities for future research, development, and validation of alternative test methods that could further reduce, refine, and ultimately replace animal use for potency and safety testing. One of the highest priority implementation activities was organization of an international workshop on alternative methods for rabies vaccine potency testing. In October 2011, NICEATM, ICCVAM, and their international partners convened a workshop on alternative methods for rabies vaccine potency testing.

The October 2011 workshop focused on three areas: reduction and refinement opportunities for the *in vivo* mouse challenge test; validation status, data gaps, and implementation strategies for serological antibody quantification methods; and validation status, data gaps, and implementation strategies for *in vitro* antigen quantification methods. Some of the key preliminary recommendations from the workshop include:

- The ultimate goal is replacement of the *in vivo* mouse challenge test for potency. Until that can be achieved, refinements such as the use of anesthetics, analgesics, and humane endpoints are recommended. Efforts should also be made to reduce animal use by eliminating repeat potency testing for human rabies vaccines and encouraging manufacturers to test the potency of multiple vaccine batches simultaneously.
- A serological method is available for veterinary vaccine use that is sufficiently standardized to provide the framework to replace the *in vivo* mouse challenge test. This method, the serum neutralization test, has undergone interlaboratory

validation (Kramer et al. 2010) and is expected to be approved for publication in European Pharmacopoeia Monograph 0451 (EDQM 2011). Manufacturers of veterinary rabies vaccines, in collaboration with regulatory authorities, should initiate product-specific validation of this test. While the serum neutralization test still requires animal use for vaccination, it uses fewer animals and eliminates animal pain and distress associated with rabies challenge.

- Human rabies vaccines tend to be simpler products than veterinary rabies vaccines because they are typically monovalent and non-adjuvanted. Because of this, it may be possible to directly replace the *in vivo* mouse challenge test with a non-animal *in vitro* antigen quantification method for potency testing of human vaccines. Workshop participants made specific recommendations on the identification of critical reagents and on the validation of the test method.


Final speaker presentations and a summary of preliminary recommendations from the workshop are available on the NICEATM–ICCVAM website at http://iccvam.niehs.nih.gov/meetings/RabiesVaccWksp-2011/RabiesVaccWksp.htm. The final report from the workshop, including final recommendations, will be published in 2012 in *Biologicals*.

## Alternative Methods to Assess the Potency of Vaccines Used to Prevent Diseases Caused by *Leptospira* and *Clostridium* Species

The genera *Leptospira* and *Clostridium* include a number of bacterial species that can cause serious or fatal diseases in livestock, pets, and humans. For example, *Clostridium tetani,* a bacterium found in soil and in the gastrointestinal tracts of animals, causes tetanus, which can be fatal to animals and humans. Vaccination against these bacteria is important for prevention of these diseases.

Production of *Leptospira* and *Clostridium* vaccines requires testing of each lot of vaccine to ensure potency. This testing uses large numbers of animals, many of which experience unrelieved pain and distress. Serological antibody quantification methods (which eliminate animal pain and distress) and *in vitro* antigen quantification methods (which do not use animals) have been developed for potency testing of some *Leptospira* species serovars. The U.S. Department of Agriculture has developed an *in vitro* enzyme-linked immunosorbent assay (ELISA) to compare the relative potency of specific vaccines to a qualified reference standard for a number of *Leptospira interrogans* serovars. Additionally, serological tests were developed and validated for other *Leptospira* species such as *L. hardjo* and are now included in the European Pharmacopoeia monographs for vaccine testing.

Similarly, for some *Clostridium* species vaccines, serological and *in vitro* methods were developed to replace current tests using rabbits and mice. The possibility of using alternative methods to test *Clostridium* species vaccine potency is now supported by general guidance published for several *Clostridium* species. Global implementation is still required for many serological methods that are now only used on a regional basis.


## FIVE-YEAR PLAN IMPLEMENTATION: BIOLOGICS TESTING

- NICEATM and ICCVAM convened a workshop in September 2010 to evaluate alternative methods and testing strategies for vaccine potency and safety testing.
- Acting on a recommendation of the September 2010 workshop, NICEATM and ICCVAM convened a workshop in October 2011 to develop an implementation strategy to achieve global acceptance and use of alternative methods for rabies vaccine potency testing. Additional workshops to address recommendations from the September 2010 workshop are planned for 2012 and 2013.

Acting on high-priority recommendations from the September 2010 vaccine workshop, NICEATM, ICCVAM, and their international partners began planning for future workshops on alternative methods to assess the potency and safety of vaccines used to prevent diseases caused by *Leptospira* and *Clostridium* species. A workshop on *Leptospira* vaccines will be held September 19–21, 2012; and a workshop on *Clostridium* vaccines is planned for 2013. As with the October 2011 rabies vaccine workshop, key goals of these workshops will be to review available methods and to define efforts necessary to achieve global acceptance and implementation.

## Alternative Methods for Detection and Quantification of Botulinum Neurotoxin

NICEATM and ICCVAM, along with international counterparts, held a workshop in November 2006 to evaluate the state of the science of potential alternatives to the mouse $LD_{50}$ assay for botulinum neurotoxin potency testing (ICCVAM 2008b). Since then, significant progress has been made in the availability of alternative methods for this purpose, including the June 2011 approval by the FDA of an *in vitro* cell-based method for potency and stability testing of formulations of botulinum neurotoxin for therapeutic purposes.

To provide an update on advancements in this area, NICEATM, ICCVAM, and their ICATM partners will convene a workshop in 2012 to review the current status of development of alternative botulinum neurotoxin test methods. The workshop will bring together scientific experts from government, industry, and academia to review these methods and to define efforts necessary to achieve U.S. regulatory acceptance and implementation. These discussions are intended to establish a roadmap for progress toward acceptance and use of methods that reduce, refine, and replace the use of animals for botulinum neurotoxin testing required by U.S. government agencies.

In May 2011, BioSentinel Pharmaceuticals nominated a suite of test methods for detection and quantification of botulinum neurotoxin (BoTest™, BoTest™ Matrix, and BoCell™) to ICCVAM for consideration for interlaboratory validation studies. After reviewing the nomination and public and SACATM comments received on the nomination, ICCVAM and the ICCVAM Interagency Biologics Working Group concluded that these and any other *in vitro* test methods for detecting and quantifying botulinum neurotoxin should have a high priority for further discussion to determine what information is needed to adequately characterize their usefulness and limitations. Studies considered necessary to adequately characterize the validation status of specific test methods for regulatory testing purposes will be prioritized based on likelihood of success.

ICCVAM will establish a botulinum-focused interagency working group to identify knowledge gaps, studies needed to address these gaps and their relative priority, and any other relevant issues.

## DEFINITIONS OF KEY TERMS

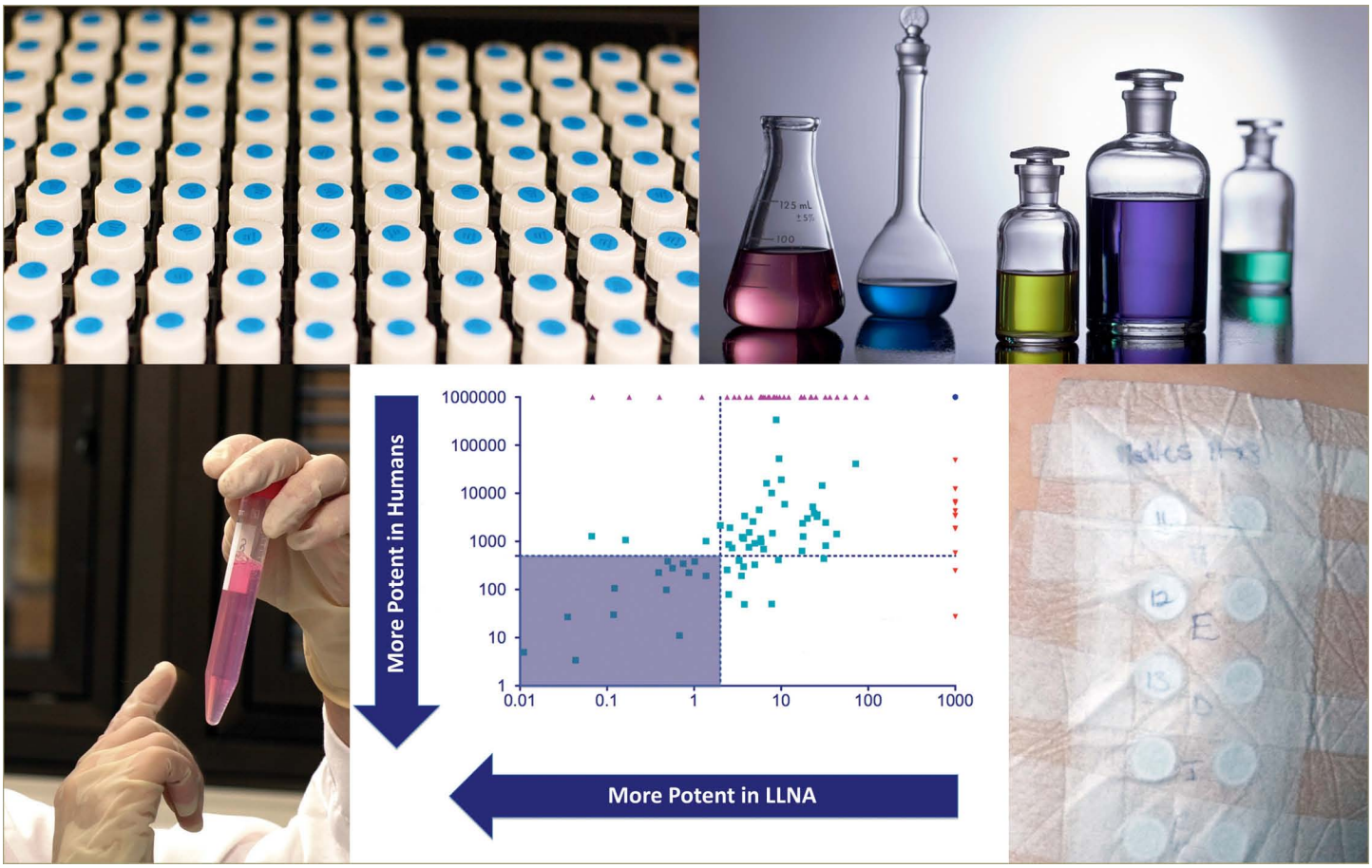
**Antigen quantification method:** a potency test that measures the amount of antigen in a vaccine to determine if there is a sufficient amount to induce a protective immune response in animals

**Challenge test:** a potency test requiring the vaccination of animals followed by infection with a virulent pathogen to assess the protection afforded by a specific vaccine

**Product-specific validation:** a process by which a test method is demonstrated to consistently confirm that the product being tested meets particular specifications

**Serological antibody quantification method:** a vaccine potency test in which animals are immunized and the amount (titer) of a specific immunoglobulin (antibody) produced in response is measured from blood serum samples

**Serovar:** a group of closely related microorganisms distinguished by a characteristic set of antigens, e.g., *Leptospira interrogans* serovars: *pomona, canicola, icterohaemorrhagiae*




## PUBLIC HEALTH AND ANIMAL WELFARE PERSPECTIVE

Occupational skin diseases, including allergic contact dermatitis, are the most common type of occupational disease, with estimated annual costs exceeding $1 billion (NIOSH 2010).

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 prognosis for ACD is poor, it is important to prevent exposure to potential sensitizers whenever they are handled.

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 chemicals such as formaldehyde, formulations such as pesticides, and 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 pain and distress to the test animal.

Based on a 1998 evaluation (ICCVAM 1999), ICCVAM recommended the murine local lymph node assay (LLNA), a test method that virtually eliminates pain and distress experienced by the test animal, as a valid alternative to these guinea pig tests for most testing situations. More recently, ICCVAM evaluated new applications and versions of the LLNA that should promote more widespread use.

## HIGHLIGHTS OF ICCVAM ACTIVITIES

- ICCVAM recommended several new versions and applications of the murine local lymph node assay (LLNA) that will further reduce animal use and expand the applicability of the LLNA for assessing the ACD hazard potential of chemicals and products. Federal agencies accepted or endorsed the following ICCVAM recommendations in 2010 and 2011:
  - An updated LLNA protocol that achieves a 20% reduction in the number of required animals
  - Routine use of the reduced LLNA, when dose–response information is not required, to determine the ACD hazard potential of chemicals and products, enabling a 40% reduction in animal use for each test
  - Performance standards for the LLNA, which enable more rapid and efficient evaluation of the validity of new versions that are mechanistically and functionally similar to the LLNA
  - Two new "green" versions of the LLNA that do not require radioactive reagents and will allow use of the LLNA in nearly all laboratories worldwide
- NICEATM and ICCVAM forwarded proposals to update the OECD test guideline for the LLNA and to create two new test guidelines for the nonradiolabeled versions of the LLNA. The OECD adopted the test guidelines in 2010, resulting in worldwide acceptance of these important methods.
- ICCVAM forwarded recommendations to Federal agencies that the LLNA may be used as a screening test to categorize substances as strong skin sensitizers. Agency acceptance responses will be received in early 2012.
- NICEATM initiated an evaluation of multiple *in vitro* methods used in integrated testing strategies to reduce, refine, and replace animal use for identification of substances that may cause ACD.

### Background on the LLNA Evaluation

Traditional test methods for skin sensitization evaluate allergic responses in guinea pigs. In contrast, the LLNA assesses skin sensitization potential by using incorporation of radioisotopes ($^{3}$H-methyl thymidine or $^{125}$I-iododeoxyuridine) to detect 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 is 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 the potential for sensitizing substances to cause test animal pain and distress, and (3) providing information about the relationship between the dose of a test substance and the resulting effect. 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 (EPA 2003; ISO 2002; OECD 2010c).

## DEFINITIONS OF KEY TERMS

**Allergic contact dermatitis (ACD):** an allergic reaction that results from repeated direct 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

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

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

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

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

**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 amount of a chemical that produces a skin sensitization reaction

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

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 "green" versions of the traditional LLNA that do not require the use of radioactive reagents:
  - A version of the LLNA that measures incorporation of the nucleotide analog bromodeoxyuridine (BrdU) using an enzyme-linked immunosorbent assay (ELISA) 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)
  - A version of the LLNA that measures adenosine triphosphate content in the draining lymph nodes of the ears (LLNA: DA)
- The reduced LLNA (rLLNA; also referred to as the LLNA limit dose procedure), a procedure in which a substance is tested at a single high dose
- Use of the LLNA to test formulations, metals, and aqueous solutions
- Use of the LLNA to categorize allergic contact dermatitis potency for hazard classification

Test methods for identifying potential chemical sensitizers were the focus of the NICEATM–ICCVAM workshop on "Best Practices for Regulatory Safety Testing: Assessing the Potential for Chemically Induced Allergic Contact Dermatitis," held in January 2011. In this photo, ICCVAM Interagency Immunotoxicity Working Group Co-Chair Dr. Joanna Matheson makes a point during her "Review of Alternative Test Methods and Integrated Strategies for ACD Hazard Assessments."

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.

## Evaluation of the Reduced LLNA

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

ICCVAM concluded that the rLLNA, when conducted according to the updated ICCVAM-recommended LLNA protocol, can distinguish between skin sensitizers and nonsensitizers. In light of the reduction in animal use possible by using the rLLNA, ICCVAM recommended that the rLLNA should be used routinely to determine the ACD hazard potential of chemicals and products, unless a substance is expected to produce positive results and dose–response information is needed. The ICCVAM recommendations for the rLLNA were included in an ICCVAM test method evaluation report that was forwarded to U.S. Federal agencies in September 2009 (ICCVAM 2009a). ICCVAM agencies endorsed the ICCVAM recommendations in 2010.

## Development of LLNA Performance Standards

Before a new test method is accepted for regulatory applications, validation studies are performed to 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 can achieve these objectives for a specific testing purpose. The performance standards can 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.

Performance standards for the LLNA were not developed as part of the original ICCVAM evaluation of the LLNA. Therefore, NICEATM and ICCVAM developed LLNA performance standards in conjunction with the test method evaluation conducted in response to the 2007 CPSC nomination. ICCVAM recommended the performance standards to U.S. Federal agencies in 2009 (ICCVAM 2009b), and the agencies endorsed them in 2010. These performance standards can be used to rapidly and efficiently determine the validity of nonradiolabeled and other modified versions of the LLNA that are mechanistically and functionally similar to the LLNA.


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 an additional 40%. The OECD adopted the updated guideline in 2010 (OECD 2010c), resulting in worldwide acceptance of these important revisions.

## 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 NICEATM and ICCVAM to 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.

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* (published in June 2010), ICCVAM recommended that the LLNA may be used to test any chemical or product for ACD hazard potential, including pesticide formulations and other products, metals (with the exception of nickel), substances tested in aqueous solutions, and other products and substances unless the substance has properties associated with it that may interfere with the ability of the LLNA to detect sensitizing substances. These recommendations were endorsed by Federal agencies in 2011.

## 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. The CPSC nomination requested that NICEATM and ICCVAM also evaluate modified LLNA test methods that do not require the use of radioactive reagents. Acceptance of such methods would allow use of the LLNA in nearly all laboratories worldwide, 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.

## FIVE-YEAR PLAN IMPLEMENTATION: IMMUNOTOXICITY TESTING


- ICCVAM evaluated the possible expansion of the scope of substances for which the LLNA may be used and whether it can be used as a stand-alone method to categorize the potency of potential sensitizers.
- ICCVAM evaluated modifications to the LLNA that may further reduce the number of animals used or eliminate the need to use radioactive reagents:
  - Evaluation of the reduced LLNA
  - Evaluation of three modified nonradiolabeled versions of the LLNA
  - Development of an updated LLNA 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 sensitizers.

ICCVAM recommended the use of two modified nonradiolabeled "green" versions of the LLNA, the LLNA: DA and the LLNA: BrdU-ELISA, to identify substances as potential skin sensitizers or nonsensitizers, with certain limitations. ICCVAM recommendations on the usefulness and limitations of these test methods, protocols for each test method, and future studies are included in two reports published in June 2010 (see **Appendix C** for report titles). The database for the LLNA: BrdU-FC method was inadequate for the development of final ICCVAM recommendations for test method uses and limitations.

Based on these recommendations, NICEATM and ICCVAM submitted to the OECD two new draft test guidelines to facilitate the international acceptance of the LLNA: DA and the LLNA: BrdU-ELISA. These test guidelines were formally adopted by the OECD in 2010 as Test Guidelines 442A (OECD 2010d) and 442B (OECD 2010e), respectively.

### Use of the LLNA to Categorize Skin Sensitization Potency

ICCVAM's 1998 evaluation of the LLNA supported its use for classifying substances only as potential sensitizers or nonsensitizers. In the 2007 nomination, the CPSC asked NICEATM and ICCVAM to assess the usefulness and limitations of the LLNA as a stand-alone test method to classify sensitizers according to the relative amount of a sensitizer that produces skin sensitization. Under current regulations, the CPSC requires only products that are considered to be strong skin sensitizers to carry hazard labeling warning of ACD hazard potential. Strong sensitizers are those substances considered to have a significant potential for causing hypersensitivity.

ICCVAM concluded that the LLNA, using specific classification criteria, can be used as a screening test to categorize substances as strong sensitizers. However, because almost half of the known strong human skin sensitizers did not meet the criteria for classification of chemicals as strong sensitizers, the LLNA cannot be considered a stand-alone test method to determine skin sensitization potency categories. Additional information is required to categorize a substance as something other than a strong sensitizer.

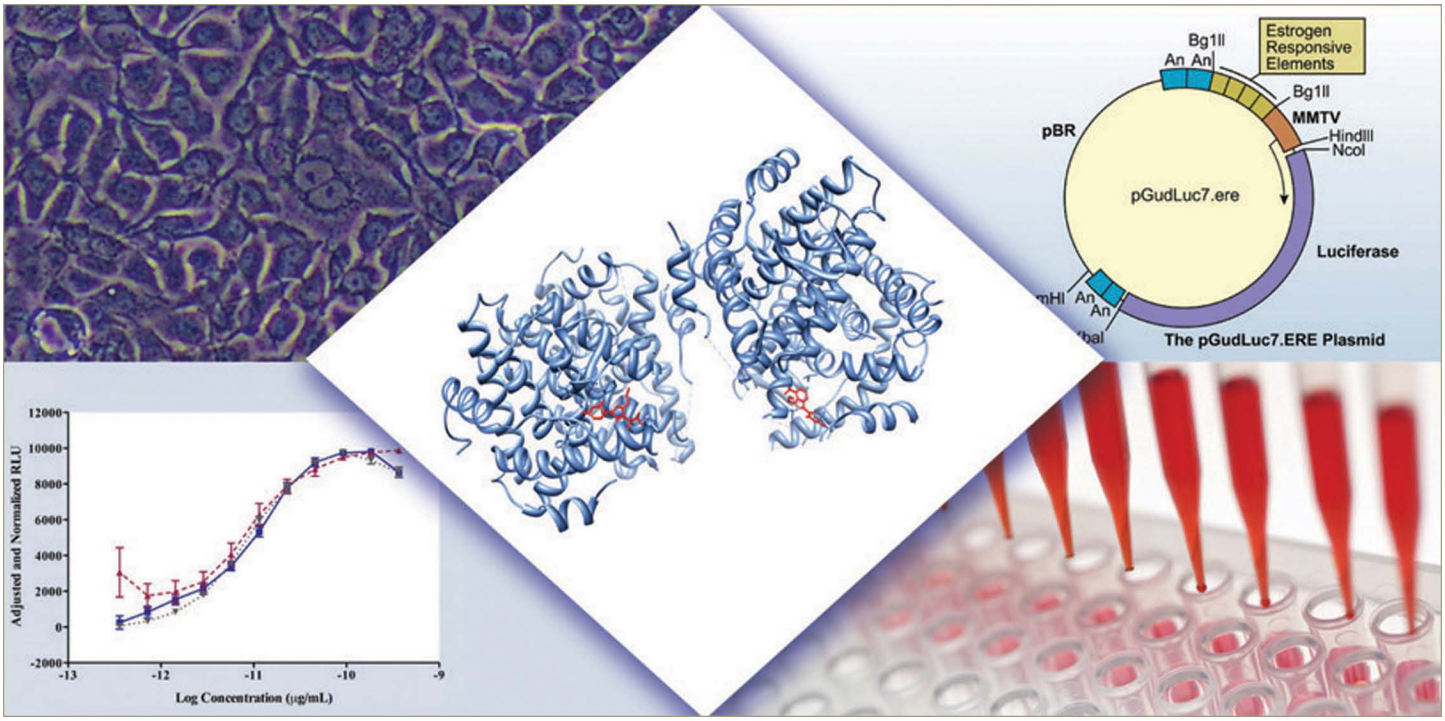
ICCVAM recommendations on the use of the LLNA for potency categorization are included in the *ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans* (published in June 2011). Responses to the ICCVAM recommendations are due from Federal agencies in early 2012.

## Evaluation of *In Vitro* Test Methods to Identify Potential Sensitizers

NICEATM is evaluating the use of *in vitro* test methods to assess ACD hazards.

A validation study of a direct peptide reactivity assay (also known as DPRA) 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 test methods could be used in combination with existing data from *in vivo* tests, physical and chemical properties (such as chemical class, molecular weight, melting point, and boiling point), and other information to make regulatory decisions about potential sensitizers. NICEATM and ICCVAM scientists are serving as liaisons to the ECVAM-led study management team, which also includes JaCVAM liaisons.

NICEATM initiated an evaluation into the use of multiple *in vitro* test methods in combination with information about physical and chemical properties of potential sensitizers in integrated testing strategies to reduce, refine, and replace animal use for identification of substances that may cause ACD. NICEATM and ICCVAM will also propose *in vitro* test methods that can be used with high throughput screening platforms to increase the accuracy of *in vitro* predictions of whether substances may cause ACD. NICEATM will evaluate the high throughput screening results to identify the most useful test methods.




## 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, causing adverse health effects by interfering with normal hormone function. 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 classify a variety of substances as endocrine disruptors. For example, polycarbonate plastic, widely used to make plastic bottles, contains a chemical called bisphenol A, a known endocrine disruptor.

In response to early findings, Congress passed the Food Quality Protection Act (7 U.S.C. 136) in 1996. This law directs the EPA to screen pesticides and environmental contaminants for their potential to affect the endocrine systems of humans and wildlife. The EPA 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 evaluated the validation status of and conducted validation studies of *in vitro* test methods to identify potential endocrine disruptors.

## HIGHLIGHTS OF ICCVAM ACTIVITIES

- ICCVAM completed an evaluation of the *in vitro* BG1Luc estrogen receptor (ER) transactivation (TA) test method for its use as an initial screen to identify substances with the potential to induce or inhibit activation of the estrogen receptor. A draft international test guideline and performance standards were forwarded to the OECD, which initiated international review.
- NICEATM completed its coordination of an international validation study to evaluate an *in vitro* test method that measures proliferation of cultured cells to identify substances with the potential to induce or inhibit activation of the estrogen receptor.

## Validation Study of the BG1Luc ER TA Test Method for Estrogenic and Anti-Estrogenic Activity

The BG1Luc ER TA test method, also known as the LUMI-CELL® (XDS) ER test method, uses an immortalized genetically modified cell line (BG1Luc4E2). The BG1Luc4E2 cells express firefly luciferase, which causes them to glow in response to estrogen and estrogen-like substances. The test method measures the light emitted when the cells are exposed to a test substance. Xenobiotic Detection Systems, Inc. (XDS), developed the LUMI-CELL ER test method with the support of a National Institute of Environmental Health Sciences Small Business Innovation Research grant.

NICEATM coordinated an international validation study of the BG1Luc ER TA test method at laboratories in Europe, the United States, and Japan. The study was completed in 2010. An independent scientific peer review panel evaluated the results of the study at a public meeting in March 2011. The peer review panel agreed with ICCVAM that the BG1Luc ER TA test method can be used as a screening test to identify substances with *in vitro* ER agonist and/or antagonist activity. ICCVAM considered the comments of the peer review panel, as well as comments from the public and SACATM, as it developed final recommendations on the BG1Luc ER TA test method.

ICCVAM recommended that the accuracy and reliability of the BG1Luc ER TA test method support its use as a screening test to identify substances that can induce or inhibit human ER activity *in vitro*. ICCVAM concluded that the accuracy of this assay is at least equivalent to the only ER TA test method currently in a U.S. regulatory test guideline, EPA OPPTS 890.1300 (EPA 2009). In addition, the BG1Luc ER TA test method was found to offer several advantages over the existing ER TA method, including (1) validation for use over a wider concentration range of test substances, (2) potential to detect a wider range of ER-active substances, (3) ability to identify both substances that induce and inhibit the estrogen receptor, and (4) availability of the cell line used for the test from more than one source.

The ICCVAM recommendations are included in *The LUMI-CELL® ER (BG1Luc ER TA) Test Method: An* In Vitro *Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals.* The report was published in late 2011, and ICCVAM recommendations will be transmitted to Federal agencies in early 2012.

The ICCVAM recommendations on the use of the BG1Luc ER TA test method also form the basis for a new test guideline currently being considered by the OECD Test Guidelines Programme. The draft test guideline will be considered by the National Coordinators of the Test Guidelines Programme in early 2012. NICEATM is also facilitating the development a performance-based test guideline for ER TA test methods to be submitted to the OECD. This test guideline will facilitate the timely adoption of functionally and mechanistically similar test methods.

The BG1Luc ER TA test method was adapted to a high throughput format using 1536-well plates by the National Institutes of Health (NIH) Center

## DEFINITIONS OF KEY TERMS

**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

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

**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). It can be used in the laboratory to detect chemical reactions.

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

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

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


for Translational Therapeutics (NCTT; formerly the NIH Chemical Genomics Center). Preliminary results are promising, and it is expected that this method will be incorporated into the Tox21 screening paradigm in 2012.

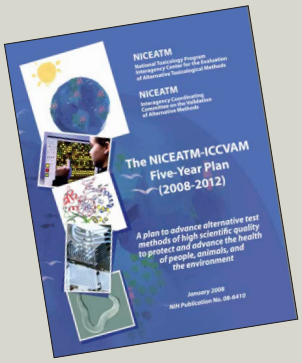
### Validation of CertiChem® MCF-7 Cell Proliferation Assay

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.

NICEATM coordinated an international interlaboratory validation study of the CertiChem MCF-7 assay, which was conducted by laboratories in the United States, Japan, and South Korea. Results from the study, which was completed in 2011, are currently being evaluated to determine the usefulness and limitations of the test method.

### Other Activities Supporting Development of *In Vitro* Test Methods to Identify Potential Endocrine Disruptors

NICEATM provided advice to Thermo Fisher Scientific on a validation study design to evaluate their Endocrine Profiler system, an *in vitro* test method that uses human cells to identify potentially endocrine-disrupting substances. Once the validation study is complete, the results will be reviewed by NICEATM with the potential for evaluation by an independent peer review panel and subsequent ICCVAM recommendations.




## FIVE-YEAR PLAN IMPLEMENTATION: IDENTIFICATION OF POTENTIAL ENDOCRINE DISRUPTORS

NICEATM completed an international study with the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods to evaluate the usefulness and limitations of the BG1Luc ER TA *in vitro* test method, a test method to identify estrogen-like chemicals that act like estrogens or anti-estrogens.

## PUBLIC HEALTH AND ANIMAL WELFARE PERSPECTIVE

Pyrogens cause inflammation and fever when introduced into the body via injectable drugs or implanted medical devices. Sources of pyrogenic material 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 FDA require that medical devices and pharmaceutical products intended for administration by injection be tested for and found to be free of pyrogen contamination before use in humans and animals.

The two most commonly used pyrogen tests, the rabbit pyrogen test (RPT) and the bacterial endotoxin test (BET), require the use of animals. The RPT measures the rise in temperature of rabbits after intravenous injection of a test solution. The BET is an *in vitro* test method that measures coagulation of hemolymph extract from the horseshoe crab after exposure to endotoxin. The BET was accepted by the FDA in the 1980s and has substantially decreased rabbit use in the United States for pyrogen testing. Other *in vitro* methods are needed, however, for cases in which use of the BET is not appropriate. Declining populations of horseshoe crabs are also raising concerns about the use of hemolymph extract for the BET (Wheeler 2011).

ICCVAM made recommendations in 2009 on the use of five *in vitro* test methods to assess the potential pyrogenicity of pharmaceuticals and other products. Use of these tests may reduce the number of animals required for pyrogen testing. ICCVAM also recommended future studies that could expand the applicability of these methods to detect a wider range of pyrogens.

## HIGHLIGHTS OF ICCVAM ACTIVITIES

- The ICCVAM Interagency Pyrogenicity Working Group reviewed a nomination for the monocyte activation test, one of five *in vitro* pyrogen test methods previously reviewed by ICCVAM, to evaluate its usefulness to detect non-endotoxin pyrogens. ICCVAM considered this method of sufficient interest and applicability to warrant further evaluation.

## DEFINITIONS OF KEY TERMS

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

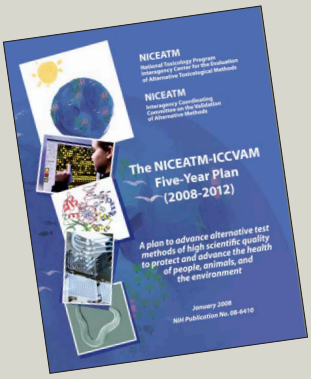
**Gram-negative:** a type of bacteria that can react with the immune system, causing inflammation and infection

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

**Monocytes:** white blood cells that release fever-producing substances

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

**Pyrogen:** a substance that can cause fever




## FIVE-YEAR PLAN IMPLEMENTATION: PYROGEN TESTING

NICEATM reviewed a nomination of the monocyte activation test, one of five *in vitro* pyrogen test methods on which ICCVAM made recommendations for future studies in a 2008 test method evaluation report (ICCVAM 2008c). The current nomination of the monocyte activation test addressed the ICCVAM recommendations. ICCVAM concluded that the nomination was of sufficient interest and applicability to warrant further discussion, and the ICCVAM Interagency Pyrogenicity Working Group will continue to consider and evaluate the topic of pyrogen testing and make recommendations to ICCVAM.

### ICCVAM Nomination Prioritization and Activities

In 2009, all applicable Federal agencies, including the FDA, endorsed ICCVAM recommendations on the use of five *in vitro* test methods to assess the potential pyrogenicity of pharmaceuticals and other products. These test methods may now be used instead of animal tests to detect Gram-negative endotoxin in human parenteral drugs on a case-by-case basis, subject to product-specific validation to demonstrate equivalence to the RPT. These methods should now be considered before *in vivo* pyrogen testing and used when appropriate for specific testing situations. They may reduce the number of animals required for pyrogen testing. ICCVAM recognized that these test methods could be applicable for detection of a wider range of pyrogens (specifically non-endotoxin pyrogens) and made recommendations for future studies that could expand their applicability.

In 2011, one of the *in vitro* tests recommended by ICCVAM, the monocyte activation test, was nominated to ICCVAM for further evaluation in order to expand its applicability domain to non-endotoxin pyrogens. The ICCVAM Interagency Pyrogenicity Working Group reviewed the nomination, NICEATM's preliminary evaluation, the ICCVAM test method evaluation report (ICCVAM 2008c), and the public and SACATM comments received on the nomination. The working group concluded that the nomination was of sufficient interest and applicability to warrant further discussion.

## PUBLIC HEALTH AND ANIMAL WELFARE PERSPECTIVE

Genetic toxicity refers to damage to the DNA and 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.

The EPA, FDA, and CPSC 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 collaborate with international partners to evaluate genetic toxicity test methods that can reduce, refine, and replace the use of animals for this purpose.

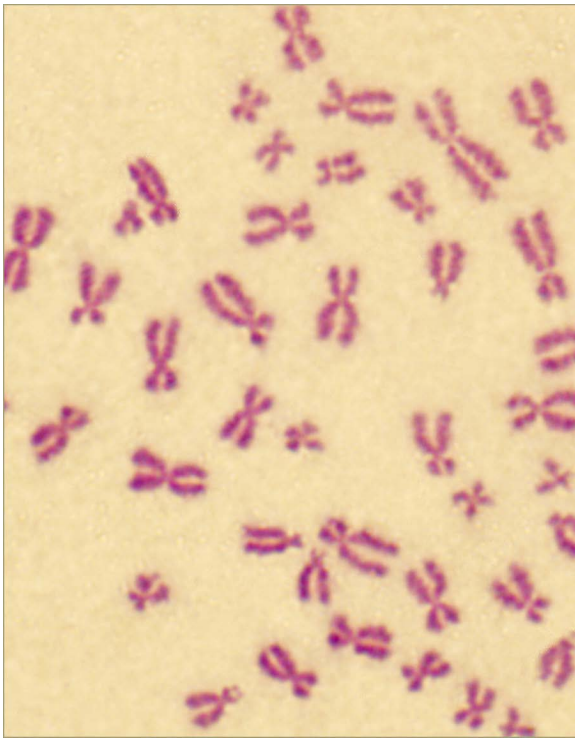
## HIGHLIGHTS OF ICCVAM ACTIVITIES

- The ICCVAM Interagency Genetic Toxicity Working Group commented on validation study reports for three cell transformation assays conducted by the European Centre for the Validation of Alternative Methods. These cell transformation assays use cultured mouse or hamster cells to detect genotoxic and nongenotoxic carcinogens. These test methods are intended to reduce the number of animals used to detect substances that may cause cancer.

### ICCVAM Involvement in Genetic Toxicity Validation Studies

ECVAM conducted studies on three cell transformation assays that use mouse or hamster cells to detect genotoxic and nongenotoxic carcinogens. These test methods are intended to reduce the number of animals used to detect substances that may cause cancer.

The ICCVAM Interagency Genetic Toxicity Working Group provided technical comments on the ECVAM Validation Management Team's conclusions from these studies.



Courtesy of Smith College Center for Microscopy and Imaging

## DEFINITIONS OF KEY TERMS

**Cell transformation:** the induction in cultured cells of certain alterations that are characteristic of tumor cells. Cell transformation assays assess the extent to which these alterations occur as the result of treatment with a specific chemical or mixture.

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


Photo courtesy of the NIH Center for Translational Therapeutics

## 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 achieve this goal, NICEATM and ICCVAM are working to identify research activities relevant to the development of such methods, including new technologies that can be expected to support future test method development. Effective translation of technological advances into new test methods will allow better protection of public health while addressing animal use and welfare concerns.

## HIGHLIGHTS OF ICCVAM ACTIVITIES

- NICEATM and ICCVAM agencies initiated collaborations to speed the translation of research advances and new technologies into scientifically valid safety testing methods that can further reduce, refine, and replace animal use. Agency initiatives and collaborations include Tox21, ToxCast™ (U.S. Environmental Protection Agency), and the NIH–FDA–Defense Advanced Research Projects Agency (DARPA) Regulatory Science Initiative.

### Development of New Technologies for Safety Testing

In recent years, dramatic technological advances in molecular biology and computer science have created an opportunity to use *in vitro* biochemical- and cell-based assays and non-mammalian animal models for toxicological testing. 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 (National Research Council 2007).

In support of this new approach to toxicity testing, the U.S. National Toxicology Program has partnered with the EPA, the NCTT, and the FDA to create the Tox21 community. The goal of this project is to use high throughput laboratory technologies and computational methods to define the toxicological characteristics of many compounds. This will ultimately allow definition of the molecular basis of toxicological effects, enabling the use of *in vitro* technologies to better predict the biological effects of as-yet uncharacterized chemicals and chemical mixtures.

NICEATM and ICCVAM are closely monitoring the progress of this collaboration. Promising methods and approaches will be reviewed by ICCVAM, which will then forward recommendations on their appropriate use to Federal agencies.

In moving toward the Tox21 goal of pathway-based *in vitro* screens, the ability of test methods to be run on a high throughput screening platform will be an important aspect of their utility. The BG1Luc ER TA test method, which was

recommended by ICCVAM for screening substances for *in vitro* ER agonist and antagonist activities, has been adapted to a high throughput format using 1536-well plates by the NCTT. Preliminary results are promising, and it is expected that this method will be incorporated into the Tox21 screening paradigm in 2012.

### Research and Development Activities Within ICCVAM Member Agencies

The majority of ICCVAM member agencies support research activities. Many of these research activities relate to the development, translation, validation, and regulatory acceptance of new and alternative test methods that can reduce, refine, and replace the use of animals in testing. NICEATM and ICCVAM maintain information on ongoing research within ICCVAM member agencies relevant to the ICCVAM mission and the *NICEATM–ICCVAM Five-Year Plan (2008–2012*), and provide information on these activities to stakeholders via the NICEATM–ICCVAM website. Notable examples of ICCVAM member agency research activities and initiatives include:

- The FDA, the NIH, and DARPA are partners in the Regulatory Science Program. The goal of this partnership is to accelerate development and use of new tools, standards, and approaches to more effectively evaluate product safety, efficacy, and quality. One of the early grants awarded via this partnership will support development of an *in vitro* test battery, known as the replacement ocular battery or "ROBatt," for assessment of the potential for test substances to cause eye injury. Another early grant will support development of a heart–lung model to test the safety and efficacy of drugs.
- The EPA is developing a suite of computational tools, known as ToxCast, to prioritize chemicals for toxicology evaluation. Preliminary phases of ToxCast are underway. If they are successful, the project will move to an implementation phase in which data on chemicals in need of toxicological evaluation will be analyzed to develop recommendations for testing priorities.
- The National Institute of Environmental Health Sciences, through a Small Business Innovation Research grant, is supporting the development of an imaging system to evaluate changes in zebrafish embryos exposed to test substances.
- The National Institute of Allergy and Infectious Diseases is using nematodes (roundworms) and fruit flies in a high throughput strategy for screening new antibiotics. Drugs that are toxic to the test organisms are eliminated from further development, increasing efficiency of the drug development process.
- The U.S. Department of Agriculture has completed validation of an *in vitro* test to assess potency of vaccines used to prevent leptospirosis, a disease against which pets and livestock are commonly vaccinated.

NICEATM's complete list of ICCVAM member agency research and development activities is available on the NICEATM–ICCVAM website at http://iccvam.niehs.nih.gov/docs/5yearplan.htm.

NICEATM periodically informs stakeholder organizations, via the ICCVAM-all email list, about relevant funding opportunities available from ICCVAM member agencies. Recent announcements have highlighted funding available from NIH institutes and centers, the Department of Defense, the National Science Foundation, and the EPA. Information about the ICCVAM-all e-mail list is available on the NICEATM–ICCVAM website at http://iccvam.niehs.nih.gov/contact/ni_list.htm.

## FIVE-YEAR PLAN IMPLEMENTATION: RESEARCH AND DEVELOPMENT ACTIVITIES SUPPORTING ALTERNATIVE METHODS DEVELOPMENT

The BG1Luc ER TA test method, for which a NICEATM-coordinated validation study was completed, is now being adapted to a high throughput platform. It is expected that this method will be incorporated into the Tox21 screening paradigm in 2012.

# HIGHLIGHTS OF ICCVAM ACTIVITIES

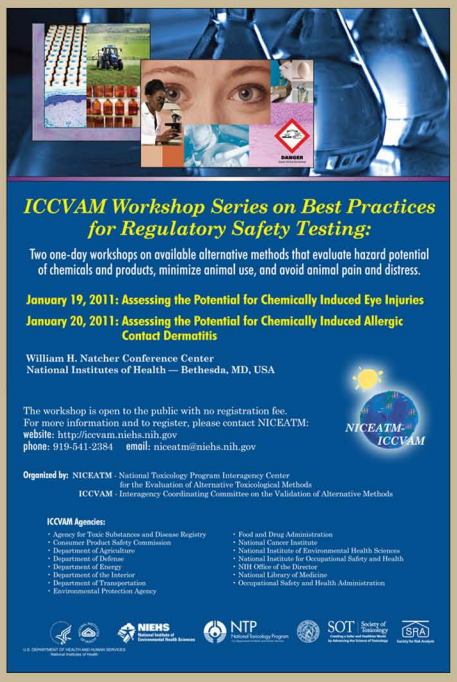
- NICEATM and ICCVAM convened two workshops on Best Practices for Regulatory Safety Testing in January 2011. Participants learned about how ICCVAM-recommended alternative methods can be used to determine whether chemicals and products may cause eye injuries or allergic contact dermatitis.
- The National Institute of Environmental Health Sciences (NIEHS), on behalf of NICEATM and ICCVAM, signed an international agreement in March 2011 to add the Republic of Korea to the International Cooperation on Alternative Test Methods, which was originally established in 2009 by the United States, the European Union, Japan, and Canada. The expanded international agreement is expected to further reduce, refine, and replace animal use in toxicity testing worldwide.
- NICEATM and ICCVAM collaborated with international partners on validation studies for test methods to identify substances with the potential to cause eye injuries, acute poisoning, allergic contact dermatitis, disturbances to the endocrine system, and DNA damage.
- NICEATM and ICCVAM participated in annual public meetings of the Scientific Advisory Committee on Alternative Toxicological Methods, providing comprehensive reports on activities and progress.
- NICEATM and ICCVAM prepared, commented on, or otherwise contributed to the development of 18 new test guidelines or proposals for new test guidelines, revisions of existing test guidelines, and guidance documents for alternative test methods considered by the Organisation for Economic Co-operation and Development.
- NICEATM and ICCVAM scientists delivered presentations at the 2010 and 2011 annual meetings of the Society of Toxicology and at the 8th World Congress on Alternatives and Animal Use in the Life Sciences in 2011. NICEATM and ICCVAM scientists also attended and made presentations at five additional international meetings and conferences.
- Publications in 2010 and 2011 included test method evaluation reports, background review documents, workshop reports, and peer review panel reports. NICEATM published 17 *Federal Register* notices in 2010 and 2011. NICEATM and ICCVAM scientists published 21 manuscripts in peer-reviewed journals and presented 28 posters and platform presentations describing NICEATM–ICCVAM activities.

## Workshops on Best Practices for Regulatory Safety Testing

Chemically induced eye injuries and allergic contact dermatitis both have a significant impact on public health. For example, household cleaners and other chemical products are the leading cause of consumer product-related eye injuries in children under age 10 (McGwin et al. 2006a, 2006b; Moren Cross et al. 2008). Skin diseases, including allergic skin reactions, comprise the largest category of occupational illness (Bureau of Labor Statistics 2010b), and allergic contact dermatitis accounts for an estimated 7 million health care visits each year in the United States (Asthma and Allergy Foundation of America).

NICEATM and ICCVAM organized two workshops that reviewed available alternative test methods to protect consumers and workers from these hazards. The workshops, the first in a planned series on Best Practices for Regulatory Safety Testing, convened in January 2011. The workshops presented information and case studies about considering and using ICCVAM-recommended alternative methods to determine if chemicals and products may cause eye injuries and allergic contact dermatitis.

Presenters at the workshop on "Best Practices for Regulatory Safety Testing: Assessing the Potential for Chemically Induced Eye Injuries" included (from left) NICEATM Director Dr. William Stokes, Mr. Hans Raabe (Institute for In Vitro Sciences), Dr. Elizabeth Lipscomb (ILS/NICEATM), Dr. Rodger Curren (Institute for In Vitro Sciences), ICCVAM Chair Dr. Jodie Kulpa-Eddy, Dr. David Allen (ILS/NICEATM), and ICCVAM Interagency Ocular Toxicity Working Group Chair Dr. Jill Merrill.




Presenters at the workshop on "Best Practices for Regulatory Safety Testing: Assessing the Potential for Chemically Induced Allergic Contact Dermatitis" included (from left) NICEATM Director Dr. William Stokes, Dr. Darrell Boverhof (The Dow Chemical Company), Dr. Michael Woolhiser (The Dow Chemical Company), ICCVAM Interagency Immunotoxicity Working Group Co-Chairs Dr. Joanna Matheson and Dr. Abby Jacobs, ICCVAM Chair Dr. Jodie Kulpa-Eddy, Dr. David Allen (ILS/NICEATM), Dr. Judy Strickland (ILS/NICEATM), Dr. Hitoshi Sakaguchi (Kao Corporation), and Dr. Eleni Salicru (ILS/NICEATM).

More than 70 scientists from industry, academia, and Federal research and regulatory agencies gathered at the National Institutes of Health campus in Bethesda, Maryland, for each workshop. The workshops were also webcast, enabling nearly 100 additional viewers to attend each workshop remotely. Both workshops were cosponsored by the Society of Toxicology and the Society for Risk Analysis.

The workshop on Assessing the Potential for Chemically Induced Eye Injuries, which took place on January 19, 2011, included discussion of three test methods that allow for hazard classification of certain types of substances without using animals. ICCVAM recommendations for routine pain management procedures were also reviewed. These procedures will virtually eliminate pain and distress whenever it is necessary to use rabbits for eye safety testing required by Federal regulatory agencies. (See pages 21–26 in **Chapter 2** for descriptions of relevant test method evaluation activities.)

The January 20, 2011, workshop on Assessing the Potential for Chemically Induced Allergic Contact Dermatitis primarily focused on recent ICCVAM recommendations on new versions and applications of the murine local lymph node assay (LLNA). The LLNA allows identification of substances with the potential to cause allergic contact dermatitis in humans using fewer animals than traditional methods. The LLNA eliminates the pain and distress experienced by animals with these methods. Nonradioactive versions of the LLNA recommended by ICCVAM will allow more institutions to take advantage of the animal welfare benefits provided by the LLNA. (See pages 36–41 in **Chapter 2** for descriptions of relevant test method evaluation activities.)

Each workshop featured several case studies to allow regulators and stakeholders to gain experience in selecting appropriate test methods and interpreting results from actual studies. Each workshop also included roundtable discussions with regulatory agencies and concluded with presentations on promising *in vitro* and *in chemico* methods in the validation pipeline. New methods were also the focus of many of the 34 poster presentations available for viewing throughout both workshops.

Materials from both workshops, including presentations, poster session abstracts, links to ICCVAM-recommended protocols, and links to the archived webcasts, are available on the NICEATM–ICCVAM website at http://iccvam.niehs.nih.gov/meetings/Implement-2011/ImplmtnWksp.htm.


*NIEHS joined international counterparts in signing an updated International Cooperation on Alternative Test Methods agreement in March 2011.*

## Expansion of the International Cooperation on Alternative Test Methods

NIEHS, on behalf on NICEATM and ICCVAM, joined international counterparts in signing an agreement that will expand international efforts to reduce the number of animals required for chemical safety testing. The agreement brought the Republic of Korea into the International Cooperation on Alternative Test Methods (ICATM). The Republic of Korea participates in ICATM via the Korean Center for the Validation of Alternative Methods (KoCVAM).

ICATM represents an effort to promote international cooperation that should permit more rapid acceptance of new safety testing methods for chemicals and products. The updated ICATM agreement was signed at a ceremony March 8, 2011, during the 50th Annual Meeting of the Society of Toxicology. In addition to the United States and the Republic of Korea, representatives of the European Commission and the governments of Canada and Japan signed the updated agreement.

### *Background on ICATM*

In April 2009, representatives from four international agencies involved in the evaluation of alternative toxicological methods signed a Memorandum of Cooperation establishing ICATM. The agreement promotes enhanced international cooperation and coordination on the scientific validation of non- and reduced-animal toxicity testing methods. National and international regulatory agencies more rapidly accept toxicity testing methods that are shown to be reproducible based on strong scientific information and that can accurately identify product-related health hazards.

ICATM was established in response to a recommendation by the International Cooperation on Cosmetic Regulation, a group of cosmetic regulatory authorities from the United States, Japan, the European Union, and Canada. At its first meeting in September 2007, the International Cooperation on Cosmetic Regulation 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 interaction among scientific experts from the regulatory bodies within the participating countries. In response, NICEATM–ICCVAM, the European Centre for the Validation of Alternative Methods (ECVAM), the Japanese Center for the Validation of Alternative Methods (JaCVAM), and Health Canada developed a framework to ensure collaboration on this issue.

## 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 activities 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.

**Health Canada's** Environmental Health Science and Research Bureau 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 ICATM Framework*

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

- To establish international cooperation in the critical areas of validation studies, independent peer review, and development of harmonized 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 (available on the FDA website at http://www.fda.gov/InternationalPrograms/HarmonizationInitiatives/ucm114518.htm) has been endorsed by ICCVAM and adopted by the International Cooperation on Cosmetic Regulation.

NICEATM and ICCVAM scientists attended nine ICATM coordination meetings in 2010 and 2011.

## COLLABORATIONS WITH INTERNATIONAL VALIDATION ORGANIZATIONS

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

- NICEATM and ICCVAM collaborated with ECVAM, JaCVAM, and KoCVAM in conducting international validation studies. (See following page for list and refer to previous chapter for details.)
- The Head of ECVAM, the Directors of JaCVAM and KoCVAM, and a representative from Health Canada continued to participate in meetings of the Scientific Advisory Committee on Alternative Toxicological Methods as nonvoting liaison members.
- The Chair of ICCVAM and the Director of NICEATM continued to participate in ECVAM Scientific Advisory Committee meetings as official observers. Meetings were held in April and October 2010, and in February and October 2011. The Director of NICEATM also attended a meeting of the JaCVAM advisory council in November 2011.
- ECVAM and JaCVAM had liaisons to each ICCVAM interagency working group that was active during the reporting period, and Health Canada had liaison members to several working groups. Liaisons from the international validation organizations provided comments on behalf of those organizations during the development of ICCVAM recommendations on new versions and applications of the LLNA and *in vitro* test methods for detection of potential endocrine disruptors.

Dr. Marilyn Wind, former Chair of ICCVAM, was an active participant in ICCVAM's international interactions. She is pictured here with NICEATM Director Dr. William Stokes as a ceremony honoring her service at her retirement in July 2010.

- ICCVAM requested nominations of experts from the ICATM partner organizations for the independent scientific peer review panel on the BG1Luc ER TA *in vitro* test method for detection of potential endocrine disruptors.
- ICCVAM nominated experts to participate on ECVAM Scientific Advisory Committee peer reviews for the following test methods:
  - 3T3 neutral red uptake assay for the identification of chemicals that are not classified for acute oral toxicity
  - KeratinoSens (Givaudan SH) assay for identification of sensitizers
- ICATM partner organizations cosponsored the September 2010 International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing, and the October 2011 International Workshop on Alternative Methods for Rabies Vaccine Potency Testing.
- NICEATM Director Dr. William Stokes and ICCVAM Chair Dr. Jodie Kulpa-Eddy participated as observers on the ECVAM Scientific Advisory Committee peer reviews of studies on three cell transformation assays that use mammalian cells to detect genotoxic and nongenotoxic carcinogens.
- NICEATM and ICCVAM scientists served on the advisory board and attended meetings for the ACuteTox project, which concluded in 2010. The goal of ACuteTox, a 5-year project funded by the European Union, was to develop an *in vitro* test strategy sufficiently robust and powerful to completely replace *in vivo* testing of acute toxicity of chemicals.

## INTERNATIONAL COOPERATION ON VALIDATION STUDIES

### Eye Safety Testing

Evaluation of the *in vitro* EpiOcular™ (MatTek) and SkinEthic™ HCE (L'Oreal) test methods to discriminate between irritants and substances that do not cause classifiable ocular irritation

- NICEATM Director Dr. William Stokes and ICCVAM Interagency Ocular Toxicity Working Group Chair Dr. Jill Merrill served as liaison members to the ECVAM-led management team for the ongoing validation study for these two methods.
- NICEATM and ICCVAM provided comments on study design, chemical selection, and test method performance criteria.

### Acute Systemic Toxicity Testing

International validation study in the field of toxicokinetics and metabolism: human cryopreserved HepaRG and cryopreserved hepatocytes CYP induction test methods

- NICEATM and ICCVAM scientists are participating in the validation management team (VMT) of this ECVAM-sponsored study, providing comments on study designs, chemical selection, laboratory standard operating procedures, and study reports.
- NICEATM and ICCVAM VMT members attended six team meetings in 2010 and 2011.

### Immunotoxicity Testing: Allergic Contact Dermatitis

Evaluation of *in vitro* tests for assessing skin sensitization potential of chemicals

- NICEATM and ICCVAM scientists, along with JaCVAM associates, are serving on the VMT for this ECVAM-led study, providing comments on study designs, chemical selection, and laboratory standard operating procedures.
- NICEATM and ICCVAM VMT members attended six team meetings in 2010 and 2011.

### Endocrine Disruptor Testing

Validation studies of test methods to identify potential disruptors of estrogen receptor activity

- NICEATM coordinated the international validation study for the CertiChem® MCF-7 cell proliferation test method, which included participating laboratories in the United States, South Korea, and Japan.
- NICEATM also coordinated the international validation study for the BG1Luc estrogen receptor transactivation test method (also known as the LUMI-CELL® ER test method). This validation study, which included participating laboratories in the United States, Europe, and Japan, was completed in 2010 and was evaluated by an ICCVAM-sponsored international peer review panel in 2011.

### Genetic Toxicity Testing

Validation studies of three cell transformation assays that use mammalian cells to detect genotoxic and nongenotoxic carcinogens

- The ICCVAM Interagency Genetic Toxicity Working Group provided technical comments on the ECVAM VMT conclusions.

*(See **Chapter 2** for details on these activities.)*

# ICCVAM CONTRIBUTIONS TO OECD TEST GUIDELINES AND GUIDANCE DOCUMENTS

During 2010 and 2011, ICCVAM participated in the development and national review of guidelines for the testing of chemicals issued by the Organisation for Economic Co-operation and Development (OECD). OECD test guidelines represent internationally agreed-upon testing methods that can be used by government, industry, and independent laboratories in the 34 OECD member countries to determine the safety of chemicals and chemical preparations. Adopted OECD test guidelines may be found on the OECD iLibrary website at http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

## Eye Safety Testing

The ICCVAM Interagency Ocular Toxicity Working Group worked with NICEATM to prepare a guidance document for use with the *in vitro* test methods described in OECD Test Guidelines 437 and 438. The guidance document describes procedures for processing tissues for histopathology from *in vitro* and *in vivo* models used for eye injury hazard testing. Data suggest that histopathology information may increase the accuracy of some *in vitro* test systems, and the guidance document will be useful in collecting the data needed to determine the usefulness of histopathology data. The OECD formally accepted this guidance document in 2011 (OECD 2011a).

NICEATM and ICCVAM also submitted a revision to Test Guideline 405: Acute Eye Irritation/Corrosion (OECD 2002). The revision incorporates the routine use of topical anesthetics, systemic analgesics, and humane endpoints in the rabbit eye test.

NICEATM Director Dr. William Stokes and ICCVAM Interagency Ocular Toxicity Working Group Chair Dr. Jill Merrill represented NICEATM and ICCVAM at the OECD Expert Meeting on Eye Irritation/Corrosion held in Ispra, Italy, in September 2011. Dr. Stokes presented a summary of the revision to Test Guideline 405, and Dr. Merrill presented a rationale for proposed revisions to the positive control in Test Guideline 437 for the bovine corneal opacity and permeability test method.

NICEATM and ICCVAM also reviewed and commented on the following draft OECD documents or revisions to OECD test guidelines submitted to the OECD by other national validation organizations:

- Draft test guideline for use of the Cytosensor microphysiometer test method for eye safety testing (submitted by ECVAM)
- Draft test guideline for use of the fluorescein leakage test method for eye safety testing (submitted by ECVAM)
- Draft Standard Project Submission Form for use of the short time exposure test method for eye safety testing (submitted by JaCVAM)
- Revisions to Test Guideline 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants (OECD 2009) (submitted by ECVAM)

Dr. Stokes and Dr. Merrill participated in discussions relevant to each of these documents at the September 2011 OECD Expert Meeting on Eye Irritation/Corrosion.

## Acute Systemic Toxicity Testing

The ICCVAM Interagency Acute Toxicity Working Group worked with NICEATM to prepare a Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests. The procedure described in the guidance document can reduce animal use per test by up to 50%. The OECD published the guidance document, officially designated as Guidance Document 129, in 2010 (OECD 2010a).

The Acute Toxicity Working Group also provided comments and recommendations on the following OECD documents:

- Test Guideline 223: Avian Acute Oral Toxicity Test, adopted by the OECD in July 2010 (OECD 2010f)
- Draft Guidance Document for the Threshold Approach for Acute Fish Toxicity Testing

## ABOUT THE OECD TEST GUIDELINES PROGRAMME

The OECD Guidelines for the Testing of Chemicals are a collection of test methods that have been endorsed by representatives of the 34 member countries of the Organisation for Economic Co-operation and Development (OECD). 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." (OECD 1997)

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.

### Skin Safety Testing

NICEATM and ICCVAM submitted revisions to OECD Test Guideline 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method. The ICCVAM Interagency Dermal Corrosivity and Irritation Working Group provided comments on the draft revised test guideline. The revised test guideline was adopted by the OECD in 2010 (OECD 2010b).

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

- Test Guideline 430: *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test (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 to identify substances that can cause skin burns (ICCVAM 2004b). The ICCVAM performance standards were developed after the OECD test guidelines were initially published. The OECD Dermal Expert Consultation Group will meet in January 2012 to review the revisions to the test guidelines.

### Immunotoxicity Testing: Allergic Contact Dermatitis

After ICCVAM evaluated new versions and applications of the LLNA, NICEATM and the ICCVAM Interagency Immunotoxicity Working Group updated OECD Test Guideline 429 for the LLNA to incorporate the ICCVAM-recommended updated protocol, the reduced LLNA procedure, internationally harmonized performance standards, and the use of the LLNA for testing a broader range of substances. NICEATM and ICCVAM, in collaboration with JaCVAM, also submitted draft test guidelines to the OECD for the LLNA: DA and the LLNA: BrdU ELISA.

NICEATM scientists and ICCVAM members served on the OECD expert working group on the LLNA, which first met in 2009 and worked until February 2010. This group evaluated the updated Test Guideline 429 and the draft test guidelines for the LLNA: DA and the LLNA: BrdU-ELISA.

The OECD adopted the updated Test Guideline 429 in 2010 (OECD 2010c). The OECD also adopted two new test guidelines:

- Test Guideline 442A: Skin Sensitization: Local Lymph Node Assay: DA (OECD 2010d)
- Test Guideline 442B: Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA (OECD 2010e)

Adoption of these test guidelines describing "green" versions of the LLNA that do not require radioactive reagents will allow use of the LLNA in nearly all laboratories worldwide.

### Endocrine Disruptor Testing

NICEATM and ICCVAM developed a draft proposal for a new OECD test guideline for the BG1Luc estrogen receptor transactivation (BG1Luc ER TA) test method for identifying estrogen receptor agonists and antagonists. The draft test guideline is currently being reviewed by OECD member countries and will be considered for approval at the April 2012 meeting of the OECD National Coordinators. A draft performance-based test guideline incorporating the BG1Luc ER TA test method is currently under review by an OECD working group.

OECD work on endocrine disruptors is managed by the Task Force on Endocrine Disrupters Testing and Assessment, which oversees three validation management groups that focus on different aspects of endocrine disruptor testing. Dr. Warren Casey, Deputy Director of NICEATM and ICCVAM representative for NIEHS, is a member of the OECD Validation Management Group for Non-Animal Testing.

Dr. Casey represented NICEATM and ICCVAM at the meetings of the validation management group in Fall 2010 and 2011. Dr. Jack Fowle, principal ICCVAM representative from the U.S. Environmental Protection Agency (EPA), also attended the Fall 2010 meeting.

### Genetic Toxicity Testing

Members of the ICCVAM Interagency Genetic Toxicity Working Group provided comments on draft Test Guideline 488 for transgenic rodent *in vivo* gene mutation assays. Test Guideline 488 was adopted in 2011 (OECD 2011b).

## MEETINGS OF THE SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) is an independent committee of ICCVAM stakeholders that advises the NIEHS Director, NICEATM, and ICCVAM about ICCVAM activities. SACATM held two meetings in 2010 and 2011:

- June 17–18, 2010, Research Triangle Park, North Carolina (announced in 75 FR 26757)
- June 16–17, 2011, Arlington, Virginia (announced in 76 FR 23323)

SACATM members are shown with international liaison members, ICCVAM members, and NIEHS staff in this photo from the 2010 SACATM meeting at the Environmental Protection Agency facility in Research Triangle Park, NC.

At the 2010 meeting, NICEATM Director Dr. William Stokes presented an update on NICEATM–ICCVAM activities. ICCVAM members Dr. Donna Mendrick of the FDA National Center for Toxicological Research and Drs. Raymond Tice and Warren Casey of the NIEHS/National Toxicology Program (NTP) presented updates on current research activities, including high throughput screening, validation of endocrine disruptor test methods, new model systems, approaches to identify biomarkers of disease and toxicity, and bioimaging. ICCVAM Chair Dr. Jodie Kulpa-Eddy of the U.S. Department of Agriculture (USDA) and ICCVAM member Dr. Richard McFarland of the FDA gave presentations on alternative methods for vaccine testing and the planned September 2010 NICEATM–ICCVAM workshop on alternative methods for vaccine potency and safety testing. SACATM discussed a number of other important issues including the validation of alternative methods for assessing chemically induced eye injuries and assessment of acute and chronic pain in laboratory animals.

At their 2011 meeting, SACATM voted unanimously to give a high priority to validation efforts of botulinum *in vitro* test methods and a high priority to further discussions of an *in vitro* pyrogen test. SACATM also agreed with the conclusions of the May 2011 peer review panel report on the evaluation of an *in vitro* estrogen receptor transactivation test method for endocrine disruptor chemical screening. Dr. Stokes presented an update on NICEATM–ICCVAM activities, and ICCVAM member Dr. Margaret Snyder from the National Institutes of Health joined representatives of other ICCVAM agencies to provide updates on the NIH–FDA Regulatory Science Initiative and Small Business Innovation Research grants. ICCVAM interagency working group chairs Dr. Jill Merrill of the FDA and Dr. Joanna Matheson of the Consumer Product Safety Commission reported on the January 2011 workshops on Best Practices for Regulatory Safety Testing. Drs. Kulpa-Eddy and McFarland provided a summary of the September 2010 NICEATM–ICCVAM workshop on alternative methods for vaccine potency and safety testing.

At both meetings, liaisons from the European Centre for the Validation of Alternative Methods, the Korean Center for the Validation of Alternative Methods, the Japanese Center for the Validation of Alternative Methods, and Health Canada presented updates on the activities of their groups.

A list of SACATM members during 2010 and 2011 can be found in **Appendix F**.

NICEATM Deputy Director Warren Casey was presented with the NIH Individual Merit Award by NIEHS and NTP Director Dr. Linda Birnbaum.

## AWARDS RECEIVED BY NICEATM AND ICCVAM FOR ACTIVITIES IN SUPPORT OF ICCVAM'S MISSION

### National Institutes of Health Individual Merit Award

Dr. Warren Casey, Deputy Director of NICEATM and ICCVAM representative for NIEHS, received a 2011 National Institutes of Health Individual Merit Award for excellent performance in leading the NTP's international validation and interagency evaluation of new testing methods to support the federal government's endocrine disruptor chemical screening program.

# ICCVAM PARTICIPATION IN NATIONAL AND INTERNATIONAL WORKSHOPS, CONFERENCES, AND MEETINGS

NICEATM and ICCVAM scientists participated in numerous international workshops, conferences, and meetings in 2010 and 2011. Brief descriptions of selected events follow.

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

## 21st Century Validation Strategies for 21st Century Tools

The 2007 National Research Council report *Toxicity Testing in the 21st Century: A Vision and a Strategy* (National Research Council 2007) outlined a new approach to toxicity testing. The Johns Hopkins University Center for Alternatives to Animal Testing organized a workshop to address the scientific challenges presented by this vision of the future of toxicity testing. The workshop took place on July 13 and 14, 2010, in Baltimore, Maryland.

Dr. Jack Fowle, principal ICCVAM representative from the EPA, participated on the "Integrated Testing Strategies" panel, and NICEATM Director Dr. William Stokes took part on the "Evidence-Based Toxicology" panel.

## *In Vitro* Alternatives Forum

The emergence of new national and international programs mandating increased toxicological safety testing of products and ingredients is a major driving force for the development of a new toxicology. The Institute for In Vitro Sciences organized an "*In Vitro* Alternatives Forum" in October 2010 to provide an opportunity for scientists to learn about upcoming toxicity testing challenges and the current activities designed to meet them.

Dr. Stokes attended the conference and spoke on "ICCVAM Progress in Advancing Alternative Safety Testing Methods for Assessing Allergic Contact Dermatitis." Dr. Tina Levine, ICCVAM representative from the EPA, provided an "Update on EPA Activities on Animal Alternatives."

## Potency Testing of Veterinary Vaccines for Animals: The Way From *In Vivo* to *In Vitro*

On December 1–3, 2010, the Paul Ehrlich Institute sponsored and hosted this meeting, which gathered experts from around the world to explore ways to reduce, refine, and eventually replace animal use for vaccine testing. The Paul Ehrlich Institute, located in Langen, Germany, is an agency of the German Federal Ministry of Health. It promotes the quality, efficacy, and safety of biological medicinal products.

Dr. Stokes served on the scientific committee for the Langen meeting. He also gave a presentation summarizing the conclusions reached by the participants at the September 2010 NICEATM-sponsored "International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions." The recommendations from the NICEATM workshop included priorities for future research and development efforts needed to advance alternatives for vaccine potency and safety testing.

Participants at the Paul Ehrlich Institute workshop included Dr. Robin Levis (FDA Center for Biologics Evaluation and Research), NICEATM Director Dr. William Stokes, Dr. Donna Gatewood (USDA Center for Veterinary Biologics), Dr. Juan Arciniega (FDA Center for Biologics Evaluation and Research), Jean-Marc Spieser (European Directorate for the Quality of Medicines and HealthCare), ICCVAM Chair Dr. Jodie Kulpa-Eddy, and Dr. Carmen Jungback (Paul Ehrlich Institute).

ICCVAM Chair Dr. Jodie Kulpa-Eddy of the USDA spoke on "Successful Development and Validation of an *In Vitro* Replacement Assay for *Leptospira* Potency Tests." Another ICCVAM-affiliated presenter at the Langen meeting was Dr. Juan Arciniega of the FDA's Center for Biologics Evaluation and Research, a member of the ICCVAM Interagency Biologics Working Group. Dr. Arciniega spoke on "Potential Application of the Consistency Approach for Vaccine Potency Testing."


NICEATM and ICCVAM presenters at WC8 included NICEATM Deputy Director Dr. Warren Casey, NICEATM Director Dr. William Stokes, Dr. David Allen (ILS/NICEATM), and Dr. Richard McFarland (FDA Center for Biologics Evaluation and Research).

## 8th World Congress on Alternatives and Animal Use in the Life Sciences

NICEATM and ICCVAM participated in the 8th World Congress on Alternatives and Animal Use in the Life Sciences (WC8) in Montreal, Canada, on August 21–25, 2011. The World Congress meetings support progress in the life sciences and application of the ethical principles of animal use embodied in the "three Rs" (reduction, refinement, and replacement of animal use). The specific goals of WC8 were to bridge the distance between science and policy and to identify opportunities for collaboration.

Dr. Stokes served on the Scientific Program Committee for WC8 along with two ICCVAM representatives from the FDA, Dr. Suzanne Fitzpatrick and Dr. Richard McFarland. Dr. Stokes also co-chaired three sessions at WC8. One session presented reports on the 2010 NICEATM–ICCVAM vaccine workshop, a second provided opportunities for updates from the organizations participating in ICATM, and a third focused on validation of alternative methods. At the third session, Dr. Stokes spoke on "Validation of the 21st Century Toxicology Toolbox: Challenges, Opportunities, and the Way Forward."

Dr. Warren Casey, Deputy Director of NICEATM, co-chaired two sessions at the conference. At a session on "Validation and Three Rs Strategies for Assessment of Endocrine-Active Substances," he presented an update on the NICEATM-sponsored validation study of the BG1Luc ER TA test method. He also co-chaired the session "Update on New *In Vitro* Models for Detection and Potency Assessment of Botulinum Neurotoxin."

Nine poster presentations by NICEATM and ICCVAM at WC8 highlighted activities related to testing for potential allergic contact dermatitis and eye safety hazards and vaccine potency and safety testing. ICCVAM member Dr. Rajendra Chhabra, director of toxicology training and coordination at NTP, presented a poster on environmental enrichment of animals in NTP studies. Titles and authors of all presentations are included in the list of NICEATM–ICCVAM publications in **Appendix C**.

## Animal Models and Their Value in Predicting Drug Efficacy and Toxicity

A conference on "Animal Models and Their Value in Predicting Drug Efficacy and Toxicity" was held September 15 and 16, 2011, at The New York Academy of Sciences. The meeting provided participants an opportunity to examine the traditional role of animal models in drug discovery; the strengths and weaknesses of these animal models; and ways to reduce, refine, and replace animal models in biomedical research. The conference was organized by The Global Medical Excellence Cluster and The New York Academy of Sciences in collaboration with Imperial College London and King's College London. NIEHS, the National Center for Research Resources, and the National Institute of Diabetes and Digestive and Kidney Diseases provided support for the conference.

Dr. Stokes spoke on "Best Practices for the Use of Animals in Toxicological Research and Testing." The presentation focused on advances in science and technology that are being applied to develop new testing methods and strategies that can reduce, refine, and in some cases replace animal use.

### Japanese Society for Alternatives to Animal Experiments

The 24th annual meeting of the Japanese Society for Alternatives to Animal Experiments (JSAAE) was held on November 10 and 11, 2011, in Sendai, Japan. JSAAE is an academic research organization that promotes research, development, education, and studies related to animal welfare and alternatives to animal experiments.

Dr. Stokes attended the JSAAE conference and provided an update on recent and planned NICEATM–ICCVAM activities. His presentation summarized NICEATM–ICCVAM's contributions toward reducing, refining, and replacing animal use for safety testing. He noted U.S. interagency efforts to accelerate development and use of more efficient safety testing approaches with the potential to better protect human health.

### 49th and 50th Annual Meetings of the Society of Toxicology

NICEATM and ICCVAM participated in the 2010 and 2011 annual meetings of the Society of Toxicology (SOT).

The 49th Annual SOT Meeting was held on March 7–11, 2010, in Salt Lake City, Utah. Thirteen NICEATM scientists and 20 members of ICCVAM and ICCVAM interagency working groups contributed to eight poster presentations. Details of poster presentations are included in the list of NICEATM–ICCVAM publications in **Appendix C.**

The 50th Annual SOT Meeting was held on March 6–10, 2011, in Washington, DC. Dr. Stokes and retired ICCVAM Chair Dr. Marilyn Wind chaired an informational session entitled "The International Cooperation on Alternative Test Methods (ICATM): Translating Science to Provide Improved Public Health Safety Assessment Tools." They gave presentations about ICATM and about NICEATM–ICCVAM's contributions to ICATM activities. In addition, 10 NICEATM scientists and 16 members of ICCVAM and ICCVAM interagency working groups contributed to eight poster presentations. Details of platform and poster presentations are included in the list of NICEATM–ICCVAM publications in **Appendix C**.


## REPORTS, *FEDERAL REGISTER* NOTICES, AND PUBLICATIONS

NICEATM and ICCVAM published 10 reports, 17 *Federal Register* notices, and 48 abstracts and manuscripts during 2010 and 2011. NICEATM and ICCVAM activities were also reported in 17 articles in the NIEHS *Environmental Factor* newsletter. A complete list of these publications is available in **Appendix C.**

American Academy of Ophthalmology. 2009. Eye Injuries at Home [Internet].
Available: http://www.geteyesmart.org/eyesmart/injuries/home.cfm [accessed 02 February 2012].

Asthma and Allergy Foundation of America. Allergy Facts and Figures [Internet].
Available: http://www.aafa.org/display.cfm?id=9&sub=30 [accessed 02 February 2012].

Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Dart RC. 2011. 2010 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS); 28th Annual Report. Clin Toxicol 49: 910–941.

Bureau of Labor Statistics. 2010a. Number, incidence rate, and median days away from work for nonfatal occupational injuries and illnesses involving days away from work by selected injury or illness characteristics and private industry, state government, and local government, 2010 [Internet].
Available: http://www.bls.gov/news.release/osh2.t05.htm [accessed 10 February 2012].

Bureau of Labor Statistics. 2010b. Injuries, Illnesses, and Fatalities [Internet].
Available: http://www.bls.gov/iif/oshwc/osh/os/charts2009/charts.htm#Figure3 [accessed 02 February 2012].

Centers for Disease Control. 2011. Poisoning in the United States: Fact Sheet [Internet].
Available: http://www.cdc.gov/HomeandRecreationalSafety/Poisoning/poisoning-factsheet.htm [accessed 01 February 2012].

Draize J, Woodard G, Calvery H. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 82: 377–390.

EDQM. 2011. Monograph 01/2010:0451. Rabies Vaccine (Inactivated) for Veterinary Use. In: European Pharmacopoeia. 7th ed. Strasbourg, France:European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM).

EPA. 2003. Health Effects Test Guidelines OPPTS 870.2600 Skin Sensitization. EPA 712 C 03-197. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2009. Health Effects Test Guidelines OPPTS 890.1300 Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa- 9903)). EPA 740 C 09-006. Washington, DC:U.S. Environmental Protection Agency.

Food Quality Protection Act of 1996. 7 U.S.C. 136, Public Law 104–170.

Haseman JK, Allen DG, Lipscomb EA, Truax JF, Stokes WS. 2011. Using fewer animals to identify chemical eye hazards: revised criteria necessary to maintain equivalent hazard classification. Regul Toxicol Pharmacol 61(1): 98–104.

Hutchings CV, Shum KW, Gawkrodger DJ. 2001. Occupational contact dermatitis has an appreciable impact on quality of life. Contact Dermatitis 45: 17–20.

ICCVAM. 1997. Validation and Regulatory Acceptance of Toxicological Test Methods. A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf.

ICCVAM. 1999. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. Results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods. NIH Publication No. 99-4494. Research Triangle Park, NC:National Institute of Environmental Health Sciences.
Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf.

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. Research Triangle Park, NC:National Institute of Environmental Health Sciences.
Available: http://iccvam.niehs.nih.gov/SuppDocs/SubGuidelines/SD_subg034508.pdf.

ICCVAM. 2004a. ICCVAM Mission, Vision and Strategic Priorities [Internet]. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/about_docs/MisVisStrat.pdf.

ICCVAM. 2004b. Recommended Performance Standards for *In Vitro* Test Methods for Skin Corrosion. NIH Publication No. 04-4510. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/dermal_docs/ps/ps044510.pdf.

ICCVAM. 2008a. 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. NIH Publication No. 08-6410. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/5yearplan.htm.

ICCVAM. 2008b. Report on the NICEATM–ICCVAM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce or Replace the Mouse $LD_{50}$ Assay for Botulinum Toxin Testing. NIH Publication No. 08-6416. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/biologics-docs/BoNTwkshprept.pdf.

ICCVAM. 2008c. ICCVAM Test Method Evaluation Report: Validation Status of Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products. NIH Publication No. 08-6392. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/pyrogen/pyr_tmer.htm.

ICCVAM. 2009a. 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 No. 09-6439. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-LD/TMER.htm.

ICCVAM. 2009b. Recommended Performance Standards: Murine Local Lymph Node Assay. NIH Publication No. 09-7357. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm.

ICCVAM Authorization Act of 2000. 42 U.S.C. 285*l*-3, Public Law 106-545.

ISO (International Organization for Standardization). 2002. Biological evaluation of medical devices — Part 10: Tests for irritation and delayed-type hypersensitivity (ISO 10993-10). Geneva:International Organization for Standardization.

Kramer B, Bruckner L, Daas A, Milne C. 2010. Collaborative study for validation of a serological potency assay for rabies vaccine (inactivated) for veterinary use. Pharmeuropa Bio Sci Notes 2: 37–55.

Kulpa-Eddy J, McFarland R, Stokes WS, eds. 2011. International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions. Procedia Vaccinol 5. 266 p.

McGwin G Jr, Hall TA, Seale J, Xie A, Owsley C. 2006a. Consumer product-related eye injury in the United States, 1998-2002. J Safety Res 37(5): 501–506.

McGwin G Jr, Hall TA, Xie A, Owsley C. 2006b. Trends in eye injury in the United States, 1992-2001. Invest Ophthalmol Vis Sci 47(2): 521–527.

Moren Cross J, Griffin R, Owsley C, McGwin G Jr. 2008. Pediatric eye injuries related to consumer products in the United States, 1997-2006. J AAPOS 12(6): 626–628.

Murphy SL, Xu JQ, Kochenek KD. 2012. Deaths: Preliminary Data for 2010. National Vital Statistics Reports: Volume 60, Number 4. Hyattsville, MD:National Center for Health Statistics.

National Prevention Council. 2011. National Prevention Strategy. Washington, DC:Office of the Surgeon General, U.S. Department of Health and Human Services. Available: http://www.healthcare.gov/prevention/nphpphc/strategy/index.html.

National Research Council. 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC:National Academies Press.

NIOSH (National Institute for Occupational Safety and Health). 2010. Workplace Safety and Health Topics: Skin Exposures & Effects [Internet]. Available: http://cdc.gov/niosh/topics/skin [accessed 02 February 2012].

OECD. 1997. Decision of the Council concerning the Mutual Acceptance of Data in the Assessment of Chemicals. C(97)186/FINAL. Available: http://acts.oecd.org/Instruments/ShowInstrumentView.aspx?InstrumentID=263&InstrumentPID=263&Lang=en&Book=False.

OECD. 2002. Test No. 405: Acute Eye Irritation/Corrosion. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

OECD. 2004a. Test No. 430: *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test (TER) [adopted 13 April 2004]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

OECD. 2004b. Test No. 431: *In Vitro* Skin Corrosion: Human Skin Model Test [adopted 13 April 2004]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

OECD. 2009. Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants [adopted 7 September 2009]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

OECD. 2010a. Guidance Document 129: Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests [adopted 20 July 2010]. Paris:OECD Publishing.

OECD. 2010b. Test No. 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

OECD. 2010c. Test No. 429: Skin Sensitisation: Local Lymph Node Assay [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

OECD. 2010d. Test No. 442A: Skin Sensitization: Local Lymph Node Assay: DA [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

OECD. 2010e. Test No. 442B: Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

OECD. 2010f. Test No. 223: Avian Acute Oral Toxicity Test [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems. Paris:OECD Publishing.

OECD. 2011a. Guidance Document 160: The Bovine Corneal Opacity and Permeability (BCOP) and Isolated Chicken Eye (ICE) Test Methods: Collection of Tissues for Histopathological Evaluation and Collection of Data on Non-severe Irritants [adopted 25 October 2011]. Paris:OECD Publishing.

OECD. 2011b. Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays [adopted 28 July 2011]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing.

Palao R, Monge I, Ruiz M, Barret JP. 2010. Chemical burns: pathophysiology and treatment. Burns 36(3): 295–304.

Skoet R, Zachariae R, Agner T. 2003. Contact dermatitis and quality of life: a structured review of the literature. Br J Dermatol 149: 452–456.

Wheeler T. June 7, 2011. Horseshoe crab ban pushed to save dwindling shorebirds [Internet]. Baltimore Sun (Baltimore, MD). Available: http://weblogs.baltimoresun.com/features/green/2011/06/horseshoe_crab_ban_pushed_to_s.html [accessed 22 May 2012].

The individuals listed below served as designated representatives from ICCVAM member agencies in 2010 and 2011. Unless otherwise noted, individuals were serving as of January 2012.

❖ Principal agency representative ▲ Alternate agency representative

**Agency for Toxic Substances and Disease Registry**

❖ Moiz Mumtaz, PhD
Bruce Fowler, PhD (through June 2011)
Ed Murray, PhD
Eric Sampson, PhD

**Consumer Product Safety Commission**

❖ Marilyn L. Wind, PhD (Chair through July 2010)
▲ Kristina Hatlelid, PhD
❖ Joanna Matheson, PhD (Vice-Chair from January 2011)

**Department of Agriculture**

❖ Jodie Kulpa-Eddy, DVM (Chair from January 2011; Vice-Chair through December 2010)
▲ Elizabeth Goldentyer, DVM

**Department of Defense**

❖ Robert E. Foster, PhD (through August 2010)
▲ Patty Decot
Peter J. Schultheiss, DVM, DACLAM (through August 2010)
Harry Salem, PhD (through August 2010)
David Honey, PhD (through September 2011)
Terry Besch, DVM, DACLAM

**Department of Energy**

❖ Michael Kuperberg, PhD
▲ Marvin Stodolsky, PhD (through March 2011)

**Department of the Interior**

❖ Barnett A. Rattner, PhD

**Department of Transportation**

❖ George Cushmac, PhD (through December 2010)
▲ Steve Hwang, PhD

**Environmental Protection Agency**

***Office of Pesticide Programs***

❖ Jack Fowle, PhD, DABT (through December 2011)
▲ Vicki Dellarco, PhD
▲ Tina Levine, PhD (through September 2011)
Deborah McCall (through September 2011)
Christine Augustyniak, PhD (through September 2011)
Anna Lowit, PhD

**Food and Drug Administration**

***Office of the Chief Scientist/ Office of the Commissioner***

❖ Suzanne Fitzpatrick, PhD, DABT

***Center for Drug Evaluation and Research***

▲ Abigail C. Jacobs, PhD
Paul C. Brown, PhD

***Center for Devices and Radiological Health***

Vasant Malshet, PhD, DABT

***Center for Biologics Evaluation and Research***

Richard McFarland, PhD, MD
Ying Huang, PhD

***Center for Food Safety and Nutrition***

David G. Hattan, PhD
Neil Wilcox, PhD (through May 2011)
Diego Rua, PhD

***Center for Veterinary Medicine***

Devaraya Jagannath, PhD (through May 2011)
M. Cecilia Aguila, DVM
Li You, PhD

***National Center for Toxicological Research***

Paul Howard, PhD
Donna Mendrick, PhD

***Office of Regulatory Affairs***

Lawrence A. D'Hoostelaere, PhD (through March 2011)

**National Cancer Institute**

❖ T. Kevin Howcroft, PhD
▲ Chand Khanna, DVM, PhD

**National Institute of Environmental Health Sciences**

❖ William S. Stokes, DVM, DACLAM
Raymond R. Tice, PhD (through December 2010)
▲ Warren Casey, PhD, DABT
Rajendra S. Chhabra, PhD, DABT
Jerrold J. Heindel, PhD

**National Institute for Occupational Safety and Health**

❖ Paul Nicolaysen, VMD
▲ K. Murali Rao, MD, PhD (through October 2010)

**National Institutes of Health**

❖ Margaret D. Snyder, PhD

**National Library of Medicine**

❖ Pertti (Bert) Hakkinen, PhD
▲ Jeanne Goshorn, MS

**Occupational Safety and Health Administration**

❖ Surender Ahir, PhD

# APPENDIX B: ICCVAM TEST METHOD EVALUATION PROCESS

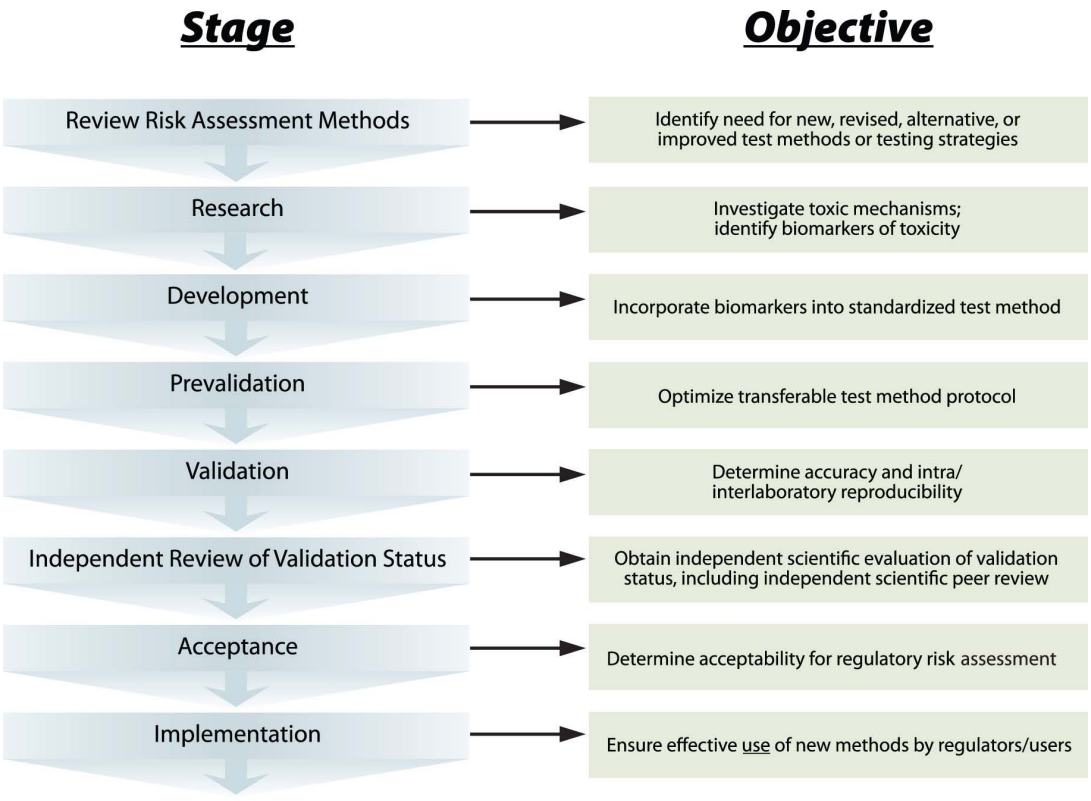
*Alternative test methods* reduce, refine (enhance animal well-being and lessen or avoid animal pain and distress), or replace animal use in regulatory toxicity testing. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency 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 to protect human health, animal health, and the environment while reducing, refining, and replacing the use of animals in testing where scientifically feasible.

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 limitations 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**

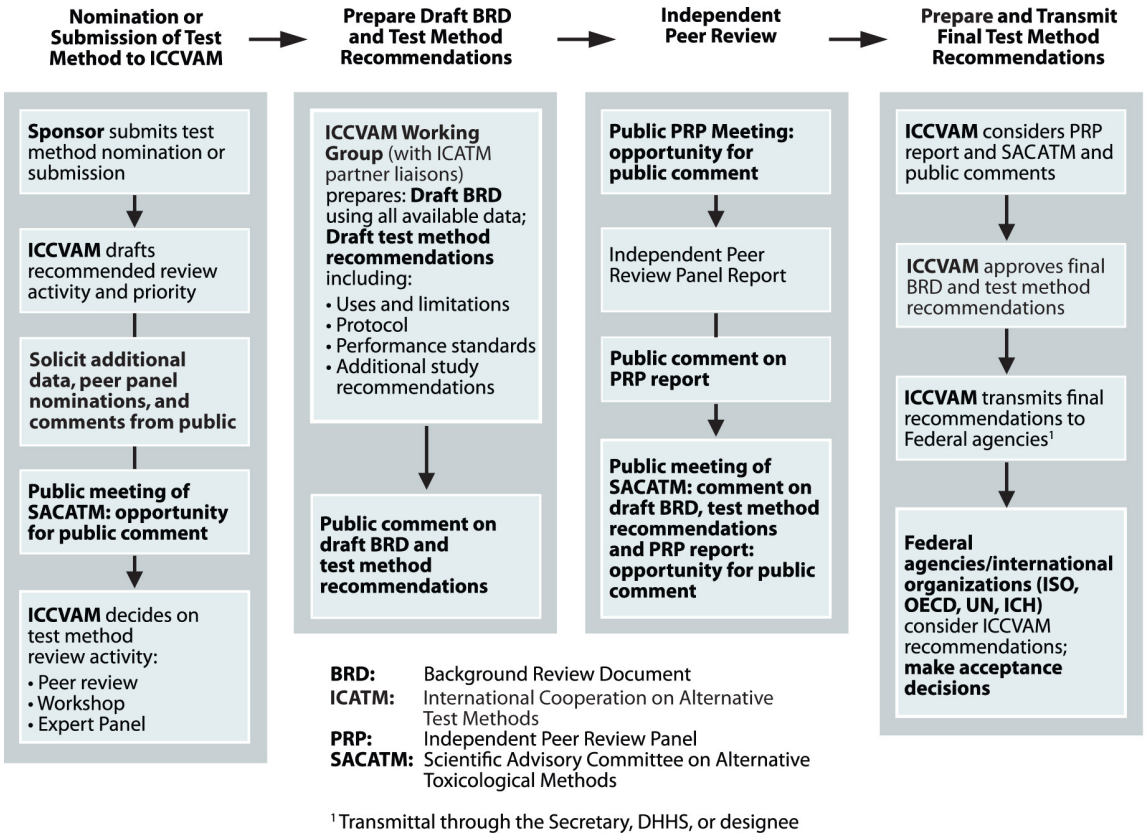


## ICCVAM Test Method Nomination and Submission

**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 *submission* consists of a proposal to ICCVAM for consideration of a test method for which adequate validation studies have been completed and the scientific validity has been adequately documented according to the *ICCVAM Guidelines*.
- A *nomination* consists of a proposal to ICCVAM for review and evaluation for which a complete test method submission is not available.

**Figure B-2. ICCVAM Test Method Evaluation Process**




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*. Receiving 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 reduce, refine, or replace animal use, compared to test methods currently 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, such as reduced cost and performance time, compared to current methods
- The completeness of the nomination or submission with regard to ICCVAM test method submission guidelines (ICCVAM 2003)

The Director of NICEATM gives ICCVAM the results of NICEATM's preliminary evaluations, along with recommendations for validation studies or further evaluations (such as 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 (that is, whether the test method is applicable to one or more agencies or has potential for widespread use)
- Develops draft recommendations for evaluation priority, validation studies, and further evaluations

**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 also provides information to and requests comments from the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM).

## 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:** criteria, based on a validated test method, that provide a basis for comparing a similar proposed test method

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

**Submission:** a proposal to ICCVAM for consideration of a test method that has had adequate validation completed and validated according to the *ICCVAM Guidelines*

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

**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. 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 submission 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 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 interagency 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 include 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 welcome 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 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 use of a standardized protocol will generate consistent data and increase the data available for evaluation of additional testing applications.

*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. Performance standards communicate the basis by which new proprietary 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 (1) how closely a test method's results agree with accepted reference values and (2) 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 interagency working group draft performance standards that take into account performance standards that may have been proposed by the test method sponsor, information provided in the test method submission, and other available data.

During its evaluation of the validation status of the proposed test method, the NICEATM–ICCVAM peer review panel also evaluates the proposed performance standards. The proposed performance standards are made available to the public for comment before and during the peer review panel meeting as part of the draft test method evaluation report. With additional public comments, feedback from the peer review panel, and ICCVAM's endorsement, NICEATM and the appropriate ICCVAM interagency 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 the NICEATM–ICCVAM e-mail list.

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 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.

**Regulatory Acceptance of Alternative Test Methods, 1998–2011**

| No. | Method | ICCVAM and ICCVAM Agency Contributions | U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance | OECD/Other Adoption | EU Regulatory Acceptance/ Endorsement |
|---|---|---|---|---|---|
| 1 | Murine local lymph node assay (LLNA) for skin sensitization | ICCVAM peer review and report; recommended in 1999 | Accepted by U.S. agencies in 1999; EPA OPPTS 870.2600 (2003) and FDA Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs (2002) | OECD TG 429 (2002); ISO (2002) | Via OECD |
| 2 | Corrositex® *in vitro* membrane barrier skin corrosivity test | ICCVAM peer review and report; recommended in 1999 | Accepted by U.S. agencies in 1999; 49 CFR 173.137 | OECD TG 435 (2006) | Via OECD |
| 3 | Up-and-down procedure for acute oral toxicity | ICCVAM peer review and report; recommended in 2001 | Accepted by U.S. agencies in 2003; EPA OPPTS 870.1100 (2002) | OECD TG 425 (2001) | Via OECD |
| 4 | Fixed dose procedure for acute oral toxicity | ICCVAM working group contributed to test guideline development | Accepted by U.S. via OECD TG 420 | OECD TG 420 (2001) | Via OECD |
| 5 | Acute toxic class method for acute oral toxicity | ICCVAM working group contributed to test guideline development | Accepted by U.S. via OECD TG 423 | OECD TG 423 (2001) | Via OECD |
| 6 | ELISA test for batch potency testing of human tetanus vaccines (refinement: antibody quantification) | ICCVAM agency consideration | 21 CFR 610.10; use reviewed on a case-by-case basis | NA | Published in European Pharmacopoeia (2003) |
| 7 | ToBI test for batch potency testing of human tetanus vaccines (refinement: antibody quantification) | ICCVAM agency consideration | 21 CFR 610.10; use reviewed on a case-by-case basis | NA | Published in European Pharmacopoeia (2003) |
| 8 | EpiSkin™ *in vitro* human skin model skin corrosivity test | ICCVAM review and report; recommended in 2002 | Accepted by U.S. via OECD TG 431 | OECD TG 431 (2004) | Via OECD |
| 9 | EpiDerm™ *in vitro* human skin model skin corrosivity test | ICCVAM review and report; recommended in 2002 | Accepted by U.S. via OECD TG 431 | OECD TG 431 (2004) | Via OECD |

*(continued on next page)*

| No. | Method | ICCVAM and ICCVAM Agency Contributions | U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance | OECD/Other Adoption | EU Regulatory Acceptance/ Endorsement |
|---|---|---|---|---|---|
| 10 | SkinEthic™ *in vitro* human skin model skin corrosivity test | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 431 (meets performance standards 2006) | OECD TG 431 (2004) | Via OECD |
| 11 | Rat TER *in vitro* skin corrosivity test | ICCVAM review and report; recommended in 2002 | Accepted by U.S. via OECD TG 430 | OECD TG 430 (2004) | Via OECD |
| 12 | 3T3 NRU phototoxicity test for skin photo-irritation | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 432 | OECD TG 432 (2004) | Via OECD |
| 13 | 3T3 NRU phototoxicity test: application to UV filter chemicals | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 432 | OECD TG 432 (2004) | Via OECD |
| 14 | *In vitro* dermal absorption methods | ICCVAM contributed to U.S. OECD test guideline review, expert consultation meetings | Accepted by U.S. via OECD TG 428 | OECD TG 428 (2004) | Via OECD |
| 15 | Use of humane endpoints in animal testing of biological products | ICCVAM agency initiative | Addressed in 9 CFR 117.4e, CVB Notice No. 04-09 (2004) | NA | |
| 16 | Rabies vaccine, humane endpoints | ICCVAM agency initiative | Addressed in 9 CFR 117.4e, CVB Notice No. 04-09 (2004) | NA | |
| 17 | Relevance of the target animal safety test for batch safety testing of vaccines for veterinary use | ICCVAM agency consideration | 9 CFR 113.4 provides for authorizing exemptions from standard requirements | NA | Published in European Pharmacopoeia (2004) |
| 18 | Uterotrophic bioassay in rodents: a short-term screening test for estrogenic properties | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 440; EPA 890.1600 (2009) | OECD TG 440 (2007) | Via OECD |
| 19 | Bovine corneal opacity and permeability *in vitro* test method to identify severe eye irritants/ corrosives | ICCVAM review and report; recommended in 2007 | Accepted by U.S. agencies in 2008 | OECD TG 437 (2009) | Via OECD |

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| No. | Method | ICCVAM and ICCVAM Agency Contributions | U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance | OECD/Other Adoption | EU Regulatory Acceptance/ Endorsement |
|---|---|---|---|---|---|
| 20 | Isolated chicken eye *in vitro* test method to identify severe eye irritants/corrosives | ICCVAM review and report; recommended in 2007 | Accepted by U.S. agencies in 2008 | OECD TG 438 (2009) | Via OECD |
| 21 | Acute toxicity *in vitro* starting dose procedure, 3T3 cells | ICCVAM 2001 workshop report; ICCVAM 2006 peer review and report; recommended in 2008 | Accepted by U.S. agencies in 2008 | OECD GD 129 (2010) | Via OECD |
| 22 | Acute toxicity *in vitro* starting dose procedure, NHK cells | ICCVAM 2001 workshop report; ICCVAM 2006 peer review and report; recommended in 2008 | Accepted by U.S. agencies in 2008 | OECD GD 129 (2010) | Via OECD |
| 23 | ELISA test for batch potency testing of *Leptospira interrogans* serovar *pomona* (replacement: antigen quantification) | ICCVAM agency initiative | USDA SAM 624 (2008) | NA | |
| 24 | ELISA test for batch potency testing of *Leptospira interrogans* serovar *canicola* (replacement: antigen quantification) | ICCVAM agency initiative | USDA SAM 625 (2008) | NA | |
| 25 | ELISA test for batch potency testing of *Leptospira interrogans* serovar *icterohaemorrhagiae* (replacement: antigen quantification) | ICCVAM agency initiative | USDA SAM 627 (2008) | NA | |
| 26 | ELISA test for batch potency testing of erysipelas vaccines (replacement: antigen quantification) | ICCVAM agency initiative | USDA SAM 613 (2008) | NA | Published in European Pharmacopoeia |

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| No. | Method | ICCVAM and ICCVAM Agency Contributions | U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance | OECD/Other Adoption | EU Regulatory Acceptance/ Endorsement |
|---|---|---|---|---|---|
| 27 | ELISA test for batch potency testing of *Leptospira kirschneri* serovar *grippotyphosa* (replacement: antigen quantification) | ICCVAM agency initiative | USDA SAM 626 (2009) | NA | |
| 28 | Human whole blood/ interleukin-1β *in vitro* pyrogen test | ICCVAM peer review and report; recommended in 2008 | Accepted by FDA in 2009 | NA | Published in European Pharmacopoeia |
| 29 | Human whole blood/ interleukin-1β *in vitro* pyrogen test: application of cryopreserved human whole blood | ICCVAM peer review and report; recommended in 2008 | Accepted by FDA in 2009 | NA | Published in European Pharmacopoeia |
| 30 | Human whole blood/ interleukin-6 *in vitro* pyrogen test | ICCVAM peer review and report; recommended in 2008 | Accepted by FDA in 2009 | NA | Published in European Pharmacopoeia |
| 31 | Human peripheral blood mononuclear cell/ interleukin-6 *in vitro* pyrogen test | ICCVAM peer review and report; recommended in 2008 | Accepted by FDA in 2009 | NA | Published in European Pharmacopoeia |
| 32 | Monocytoid cell line Mono Mac 6/interleukin-6 *in vitro* pyrogen test | ICCVAM peer review and report; recommended in 2008 | Accepted by FDA in 2009 | NA | Published in European Pharmacopoeia |
| 33 | Inhalation toxicity—acute toxic class method | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 436 | OECD TG 436 (2009) | Via OECD |
| 34 | Hershberger bioassay in rats: a short-term screening assay for (anti) androgenic properties | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 441; EPA OPPTS 890.1400 (2009) | OECD TG 441 (2009) | Via OECD |
| 35 | Stably transfected human estrogen receptor-α *in vitro* transcriptional activation assay for the detection of estrogenic agonist-activity of chemicals | ICCVAM contributed to U.S. OECD test guideline review, expert consultation meetings | Accepted by U.S. via OECD TG 455; EPA OPPTS 890.1300 (2009) | OECD TG 455 (2009) | Via OECD |
| 36 | EST-1000 *in vitro* test method for skin corrosivity testing | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 431 (meets performance standards 2009) | OECD TG 431 (2004) | Via OECD |

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| No. | Method | ICCVAM and ICCVAM Agency Contributions | U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance | OECD/Other Adoption | EU Regulatory Acceptance/ Endorsement |
|---|---|---|---|---|---|
| 37 | Updated LLNA protocol (requires 20% fewer animals) | ICCVAM peer review and report; recommended in 2009 | Accepted by U.S. agencies in 2010; EPA updated policy on the use of the LLNA for end-use pesticide products in 2011 | OECD TG 429 (2010) | Via OECD |
| 38 | Reduced LLNA protocol (requires 40% fewer animals by using only the high dose group) | ICCVAM peer review and report; recommended in 2009 | Accepted by U.S. agencies in 2010; EPA adopted the rLLNA in 2011 | OECD TG 429 (2010) | Via OECD |
| 39 | LLNA: DA for skin sensitization testing (a nonradioisotopic LLNA test method) | ICCVAM peer review and report; recommended in 2010 | Accepted by U.S. agencies in 2010 | OECD TG 442A (2010) | Via OECD |
| 40 | LLNA: BrdU-ELISA for skin sensitization testing (a nonradioisotopic LLNA test method) | ICCVAM peer review and report; recommended in 2010 | Accepted by U.S. agencies in 2010 | OECD TG 442B (2010) | Via OECD |
| 41 | EpiSkin™ *in vitro* human skin model skin irritation test | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 439 | OECD TG 439 (2010) | Via OECD |
| 42 | EpiDerm™ *in vitro* human skin model skin irritation test | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 439 | OECD TG 439 (2010) | Via OECD |
| 43 | SkinEthic™ *in vitro* human skin model skin irritation test | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 439 | OECD TG 439 (2010) | Via OECD |
| 44 | *In vitro* mammalian cell micronucleus test | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 487 | OECD TG 487 (2010); included in 2011 ICH harmonized guideline for testing human pharmaceuticals | Via OECD |
| 45 | Avian acute oral toxicity test (reduction of animal use) | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. via OECD TG 223 | OECD TG 223 (2010) | Via OECD |

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| No. | Method | ICCVAM and ICCVAM Agency Contributions | U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance | OECD/Other Adoption | EU Regulatory Acceptance/ Endorsement |
|---|---|---|---|---|---|
| 46 | Cytosensor microphysiometer *in vitro* test method for eye safety testing | ICCVAM peer review and report; recommendations in 2010 | Accepted by U.S. agencies in 2011 | *New OECD test guideline considered by Working Group of National Coordinators in 2011* | |
| 47 | Use of anesthetics, analgesics, and humane endpoints for *in vivo* ocular safety testing | ICCVAM peer review and report; recommendations in 2010 | Accepted by U.S. agencies in 2011 | *OECD TG 405 considered by Working Group of National Coordinators in 2011* | |
| 48 | Cell-based potency assay for stability and potency of botulinum neurotoxin type A products | ICCVAM workshop in 2006 | Allergan, Inc., method accepted by FDA in 2011 | NA | |
| 49 | USDA guidelines on master reference qualification and requalification for vaccine potency assays (reduction of animal use) | ICCVAM agency initiative | Addressed in 9 CFR 113.8(d)(2), Veterinary Services Memorandum 800.211 (2011) | NA | |
| 50 | *In vitro* H295R steroidogenesis assay | ICCVAM contributed to U.S. OECD test guideline review | Accepted by U.S. agencies via OECD TG 456 | OECD TG 456 (2011) | Via OECD |
| Totals | | 50 | 50 | 31 | 40 |

## *Abbreviations:*

CFR = U.S. Code of Federal Regulations; CVB = Center for Veterinary Biologics (USDA); ELISA = enzyme-linked immunosorbent assay; EPA = U.S. Environmental Protection Agency; EU = European Union; FDA = U.S. Food and Drug Administration; GD = guidance document; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; ICH = International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; ISO = International Organization for Standardization; LLNA = murine local lymph node assay; NA = not applicable; NHK = normal human keratinocyte; NRU = neutral red uptake; OECD = Organisation for Economic Co-operation and Development; OPPTS = Office of Prevention, Pesticides, and Toxic Substances (EPA); rLLNA = reduced LLNA; SAM = Supplemental Assay Method; TER = transcutaneous electrical resistance; TG = Test Guideline; USDA = U.S. Department of Agriculture; UV = ultraviolet.


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## NICEATM AND ICCVAM REPORTS

ICCVAM. 2011. ICCVAM Test Method Evaluation Report. The LUMI-CELL® ER (BG1Luc ER TA) Test Method: An *In Vitro* Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals. NIH Publication No. 11-7850. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/endocrine/ERTA-TMER.htm.

ICCVAM. 2011. ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans. NIH Publication No. 11-7709. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-pot/TMER.htm.

ICCVAM. 2011. Independent Scientific Peer Review Panel Report. Evaluation of the LUMI-CELL® ER (BG1Luc ER TA) Test Method. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/endo_docs/EDPRPRept2011.pdf.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products. NIH Publication No. 10-7553. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Recommendation to Discontinue Use of the Low Volume Eye Test for Ocular Safety Testing. NIH Publication No. 10-7515. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/ocutox/LVET.htm.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Recommendations for Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing. NIH Publication No. 10-7514. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/ocutox/OcuAnest-TMER.htm.

ICCVAM. 2010. 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 Cleaning Products. NIH Publication No. 10-7513. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/ocutox/AMCP-TMER.htm.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: BrdU-ELISA, a Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 10-7552. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/TMER.htm.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: DA, a Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 10-7551. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/TMER.htm.

ICCVAM. 2010. 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. NIH Publication No. 10-7512. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-app/TMER.htm.

ICCVAM. 2010. Biennial Progress Report 2008-2009: Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 10-7612. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/annrpt/BiennialRpt2010-508.pdf.

## *FEDERAL REGISTER* NOTICES

NIEHS. 2011. Update of the NICEATM-ICCVAM Five-Year Plan: Request for Comments. *Federal Register* 76:71977–71978. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-30001.pdf.

NIEHS. 2011. Availability of Draft ICCVAM Recommendations on Using Fewer Animals to Identify Chemical Eye Hazards: Revised Criteria Necessary to Maintain Equivalent Hazard Classification; Request for Comments. *Federal Register* 76:50220–50221. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-20537.pdf.

NIEHS. 2011. International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward. *Federal Register* 76:50221–50223. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-20540.pdf.

NIEHS. 2011. Report and Recommendations on the Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans. *Federal Register* 76:45254–45256. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-18639.pdf.

NIEHS. 2011. Nomination of *In Vitro* Test Methods for Detection and Quantification of Botulinum Neurotoxins and Detection of Non-Endotoxin Pyrogens; Data Request for Substances Evaluated by These Test Methods. *Federal Register* 76:29752–29754. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-12627.pdf.

NIEHS. 2011. Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of an *In Vitro* Estrogen Receptor Transcriptional Activation Test Method for Endocrine Disruptor Chemical Screening: Notice of Availability and Request for Public Comments. *Federal Register* 76:28781–28782. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-12264.pdf.

NIEHS. 2011. Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). *Federal Register* 76:23323–23324. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-10020.pdf.

NIEHS. 2011. Recommendations on *In Vitro* Ocular Safety Testing Methods and Strategies and Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints for Ocular Safety Testing. *Federal Register* 76:20672–20673. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-8938.pdf.

NIEHS. 2011. Independent Scientific Peer Review Panel Meeting on an *In Vitro* Estrogen Receptor Transcriptional Activation Test Method for Endocrine Disruptor Chemical Screening; National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Announcement of an Independent Scientific Peer Review Panel Meeting on an *In Vitro* Estrogen Receptor Transcriptional Activation Test Method for Endocrine Disruptor Chemical Screening; Availability of Draft Background Review Document (BRD); Request for Comments. *Federal Register* 76:4113–4115. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-1329.pdf.

NIEHS. 2011. Federal Agency Responses to Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Recommendations on Two Nonradioactive Versions of the Murine Local Lymph Node Assay (LLNA) for Assessing Allergic Contact Dermatitis (ACD) Hazard Potential of Chemicals and Products, and Expanded Uses of the LLNA for Pesticide Formulations and Other Products; Notice of Availability. *Federal Register* 76:2388–2390. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2011-669.pdf.

NIEHS. 2010. Workshop Series on Best Practices for Regulatory Safety Testing: Assessing the Potential for Chemically Induced Eye Injuries and Chemically Induced Allergic Contact Dermatitis (ACD). *Federal Register* 75:62845–62847. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2010-25676.pdf.

NIEHS. 2010. Availability of Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Test Method Evaluation Reports: *In Vitro* Ocular Safety Testing Methods and Strategies, and Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints for Ocular Safety Testing; Notice of Transmittal to Federal Agencies. *Federal Register* 75:57027–57029. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2010-23262.pdf.

NIEHS. 2010. Availability of Interagency Coordinating Committee on the Validation of Alternative Methods Test Method Evaluation Reports on Two Nonradioactive Versions of the Murine Local Lymph Node Assay for Assessing Allergic Contact Dermatitis Hazard Potential of Chemicals and Products, and Expanded Uses of the Local Lymph Node Assay for Pesticide Formulations and Other Products; Notice of Transmittal to Federal Agencies. *Federal Register* 75:37443–37445. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2010-15777.pdf.

NIEHS. 2010. Availability of the Biennial Progress Report of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). *Federal Register* 75:32942. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2010-13952.pdf.

NIEHS. 2010. Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). *Federal Register* 75:26757–26759. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2010-11318.pdf.

NIEHS. 2010. International Workshop on Alternative Methods To Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions. *Federal Register* 75:25867–25869. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2010-10958.pdf.

NIEHS. 2010. Federal Agency Responses to Interagency Coordinating Committee on the Validation of Alternative Methods Recommendations on the Murine Local Lymph Node Assay, An Alternative Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability. *Federal Register* 75:25866–25867. Available: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2010-10954.pdf.

## ABSTRACTS AND MANUSCRIPTS

**2011**

Allen D, Burns T, Strickland J, Salicru E, Stokes W. 2011. Updated NICEATM evaluation and international acceptance of the reduced murine local lymph node assay [Abstract No. I-11-446]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 141.

Arciniega JL, Corvette L, Hsu H, Lynn F, Romani T, Dobbelaer R. 2011. Target alternative vaccine safety testing strategies for pertussis toxin. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 248-260.

Arciniega JL, Dominguez-Castillo RI. 2011. Development and validation of serological methods for human vaccine potency testing: case study of an anthrax vaccine. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 213-220.

Brown P, Matheson J, Jacobs A, McMahon T, Germolec D, Wind M, Stokes W. 2011. ICCVAM evaluation of the local lymph node assay (LLNA) for potency categorization of chemicals causing allergic contact dermatitis in humans [Abstract No. 1519]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/ Toxicologist 150(S-2): 326.

Brown P, Matheson J, Jacobs A, Ward R, Margosches E, Salicru E, Allen D, Stack F, Stokes W. 2011. International acceptance of the nonradioactive LLNA: DA for evaluating allergic contact dermatitis hazards [Abstract No. I-11-575]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 145.

Casey W, Ceger P, Allen D, Stokes W. 2011. BG1Luc ER TA test method: results of an international validation study and proposed performance standards [Abstract No. I-4-130]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 54.

Casey W, Ceger P, Deal F, Allen D, Clark G, Pazos P, Grignard E, de Lange J, Bremer S, Nakamura M, Kojima H, Stokes W. 2011. Final results of an international validation study of an *in vitro* ER TA test method in BG-1 cells [Abstract No. 1017]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/ Toxicologist 150(S-2): 217.

Casey W, Schmitt M, McFarland R, Isbrucker R, Levis R, Arciniega JL, Descamps J, Finn T, Hendriksen C, Horiuchi Y, Keller J, Kojima H, Sesardic D, Stickings P, Johnson NW, Lipscomb E, Allen D. 2011. Improving animal welfare and reducing animal use for human vaccine potency testing: state-of-the-science and future directions. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 33-46.

Deal F, Casey W, Ceger P, Allen D, Yang C, Nakamura M, Kojima H, Ono A, Yoon H, Han S, Stokes W. 2011. International validation study of an *in vitro* cell proliferation test method for screening potential estrogenic agonists and antagonists in MCF-7 cells [Abstract No. 1016]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/Toxicologist 150(S-2): 217.

Haseman JK, Allen DG, Lipscomb EA, Truax JF, Stokes WS. 2011. Using fewer animals to identify chemical eye hazards: revised criteria necessary to maintain equivalent hazard classification. Regul Toxicol Pharmacol 61(1): 98–104.

Haseman JK, Strickland J, Allen D, Salicru E, Paris M, Tice RR, Stokes WS. 2011. Safety assessment of allergic contact dermatitis hazards: an analysis supporting reduced animal use for the murine local lymph node assay. Regul Toxicol Pharmacol 59: 191–196.

Isbrucker R, Levis R, Casey W, McFarland R, Schmitt M, Arciniega JL, Descamps J, Finn T, Hendriksen C, Horiuchi Y, Keller J, Kojima H, Sesardic D, Stickings P, Johnson NW, Allen D. 2011. Alternative methods and strategies to reduce, refine, and replace animal use for human vaccine post-licensing safety testing: state of the science and future directions. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 47-59.

Jacobs A, Matheson J, Malshet V, Toy J, Strickland J, Allen D, Burns T, Stack F, Stokes W. 2011. International acceptance of the nonradioactive LLNA: BrdU-ELISA for evaluating allergic contact dermatitis hazards [Abstract No. I-11-574]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 145.

Jacobs A, Matheson J, Wind M, Malshet V, Toy J, Stokes W. 2011. ICCVAM evaluation and recommendations on the nonradioactive LLNA: BrdU-ELISA for evaluating allergic contact dermatitis hazards [Abstract No. 1518]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/Toxicologist 150(S-2): 326.

King-Herbert A, Tubbs J, Chhabra R, Harry J, Stokes W. 2011. Environmental enrichment for NTP studies [Abstract No. IV-1-467]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 247.

Kulpa-Eddy J, Dusek D. 2011. Application of the consistency approach to reduce animal use in vaccine potency testing. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 232-235.

Kulpa-Eddy J, McFarland R, Isbrucker R, Halder M, Kojima H, Jones B, Johnson N, Allen D, Lipscomb E, Morefield S, Casey W, Stokes W. 2011. International workshop on alternative methods to reduce, refine, and replace the use of animals in vaccine potency and safety testing [Abstract No. 2581]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/Toxicologist 150(S-2):552.

Kulpa-Eddy J, McFarland R, Isbrucker R, Halder M, Kojima H, Srinivas G, Brown K, Jones B, Johnson N, Allen D, Casey W, Stokes W. 2011. International workshop on alternative methods to reduce, refine, and replace the use of animals in veterinary vaccine potency and safety testing [Abstract No. I-18-570]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 159.

Kulpa-Eddy J, Srinivas G. 2011. Approaches to reducing animal numbers in vaccine potency testing. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 227-231.

Kulpa-Eddy J, Srinivas G, Halder M, Brown K, Draayer H, Galvin J, Claassen I, Gifford G, Woodland R, Doelling V, Jones B, Stokes WS. 2011. Alternative methods and strategies to reduce, refine, and replace animal use for veterinary vaccine post-licensing safety testing: state of the science and future directions. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 106-119.

Kulpa-Eddy J, Srinivas G, Halder M, Hill R, Brown K, Roth J, Draayer H, Galvin J, Claassen I, Gifford G, Woodland R, Doelling V, Jones B, Stokes WS. 2011. Non-animal replacement methods for veterinary vaccine potency testing: state of the science and future directions. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 60-83.

Matheson J, Jacobs A, Wind M, Brown P, Ward R, Margosches E, Stokes W. 2011. ICCVAM evaluation and recommendations on the nonradioactive LLNA: DA for evaluating allergic contact dermatitis hazards [Abstract No. 1520]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/Toxicologist 150(S-2): 326.

McFarland R, Kulpa-Eddy J, Isbrucker R, Halder M, Kojima H, Verthelyi D, Schmitt M, Levis R, Johnson N, Jones B, Allen D, Casey W, Stokes W. 2011. International workshop on alternative methods to reduce, refine, and replace the use of animals in human vaccine potency and safety testing [Abstract No. I-18-569]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue):158.

McFarland R, Verthelyi D, Casey W, Arciniega JL, Isbrucker R, Schmitt M, Finn T, Descamps J, Horiuchi Y, Sesardic D, Stickings P, Johnson NW, Lipscomb E, Allen D. 2011. Non-animal replacement methods for human vaccine potency testing: state of the science and future directions. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 16-32.

McMahon T, McCall D, Matheson J, Jacobs A, Burns T, Allen D, Stokes W. 2011. Evaluation of the murine local lymph node assay (LLNA) for assessing the allergic contact dermatitis hazard potential of pesticide formulations [Abstract No. I-11-568]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 144.

Merrill J, Lowther D, Layton A, Redden J, Wind M, Stokes W. 2011. ICCVAM recommendations on the usefulness and limitations of the Cytosensor microphysiometer (CM) test method for ocular safety testing [Abstract No. 1553]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/Toxicologist 150(S-2): 333.

Stokes WS. ICATM: translating science to provide improved public health safety assessment tools [Abstract No. 418]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/Toxicologist 150(S-2): 89.

Stokes WS. 2011. Validation of the 21st century toxicology toolbox: challenges, opportunities, and the way forward [Abstract No. II-5-561]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue):196.

Stokes WS. 2011. Best practices for use of animals in toxicological research and testing. Ann N Y Acad Sci 1245(1): 17–20.

Stokes WS, Brown K, Kulpa-Eddy J, Srinivas G, Halder M, Draayer H, Galvin J, Claassen I, Gifford G, Woodland R, Doelling V, Jones B. 2011. Improving animal welfare and reducing animal use for veterinary vaccine potency testing: state-of-the-science and future directions. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 84-105.

Stokes W, Haseman J, Lipscomb E, Truax J, Johnson N, Jones B, Allen D. 2011. Using fewer animals to identify chemical eye hazards: revised classification criteria necessary to maintain equivalent hazard labeling [Abstract No. 1521]. Presented at the 50th Annual Meeting of the Society of Toxicology; Washington, DC; March 6–10, 2011. Toxicol Sci/Toxicologist 150(S-2): 327.

Stokes WS, Kulpa-Eddy J, McFarland R. 2011. The International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: introduction and summary. In: International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions (Kulpa-Eddy J, McFarland R, Stokes WS, eds). Procedia Vaccinol 5: 1-15.

Stokes W, Lowther D, Layton A, Redden J, Lipscomb E, Truax J, Johnson N, Allen D. 2011. Usefulness and limitations of the Cytosensor® microphysiometer (CM) test method for ocular safety testing [Abstract No. I-8-576]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue):85.

Stokes W, Merrill J, Lowther D, McMahon T, Chen J, Hashim M, Lewis M, Jones B, Lipscomb E, Allen D. 2011. Eliminating pain and distress in ocular safety testing: use of topical anesthetics, systemic analgesics, and humane endpoints [Abstract No. I-8-576]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 85.

Strickland J, Brown P, Matheson J, Jacobs A, McMahon T, Germolec D, Allen D, Salicru E, Burns T, Stack F, Stokes W. 2011. Evaluation of the murine local lymph node assay (LLNA) for potency categorization of chemicals causing allergic contact dermatitis in humans [Abstract No. I-11-567]. Presented at the 8th World Congress on Alternatives and Animal Use in the Life Sciences; Montréal, Canada; August 21–25, 2011. ALTEX 28(Spec. Issue): 144.

**2010**

Birnbaum LS, Stokes WS. 2010. Safety testing: moving toward alternative methods. Environ Health Perspect 118(1): A12–13.

Burns T, Strickland J, Salicru E, Allen D, Stokes W. 2010. Comparison of LLNA responses between CBA and BALB/c mouse strains [Abstract No. 300]. Presented at the 49th Annual Meeting of the Society of Toxicology; Salt Lake City, UT; March 7–11, 2010. Toxicol Sci/Toxicologist 114(1): 65.

Ceger P, Deal F, Allen D, Clark G, Pazos P, de Lange J, Bremer S, Nakamura M, Kojima H, Ono A, Tice R, Stokes W. 2010. Testing of coded substances in the NICEATM/ECVAM/JaCVAM LUMI CELL® STTA multiphase international validation study [Abstract No. 101]. Presented at the 49th Annual Meeting of the Society of Toxicology; Salt Lake City, UT; March 7–11, 2010. Toxicol Sci/Toxicologist 114(1): 21.

Fitzpatrick S, Wind M, Jacobs A, Hattan D, Kulpa-Eddy J, Malshet V, Mumtaz M, Snyder M, McCarley D, Morefield S, Sprankle C, Allen D, Stokes W. 2010. ICCVAM implementation of the NICEATM-ICCVAM Five-Year Plan: advancing the development, validation, acceptance, and appropriate use of alternative test methods [Abstract No. 1808]. Presented at the 49th Annual Meeting of the Society of Toxicology; Salt Lake City, UT; March 7–11, 2010. Toxicol Sci/Toxicologist 114(1): 385.

Matheson J, Jacobs A, Wind M, Chen J, Hashim M, Lewis M, Margosches E, McCall D, McMahon T, Redden J, Ward R, Stokes W. 2010. ICCVAM recommendations for use of the LLNA for evaluating the allergic contact dermatitis potential of pesticide formulations [Abstract No. 1789]. Presented at the 49th Annual Meeting of the Society of Toxicology; Salt Lake City, UT; March 7–11, 2010. Toxicol Sci/Toxicologist 114(1): 380.

Salicru E, Haseman J, Paris M, Strickland J, Allen D, Stokes W. 2010. Impact of reducing the sample size on the performance of the LLNA [Abstract No. 1806]. Presented at the 49th Annual Meeting of the Society of Toxicology; Salt Lake City, UT; March 7–11, 2010. Toxicol Sci/Toxicologist 114(1): 384.

Scott L, Eskes C, Hoffmann S, Adriaens E, Alepée N, Bufo M, Clothier R, Facchini D, Faller C, Guest R, Harbell J, Hartung T, Kamp H, Varlet BL, Meloni M, McNamee P, Osborne R, Pape W, Pfannenbecker U, Prinsen M, Seaman C, Spielmann H, Stokes W, Trouba K, Berghe CV, Goethem FV, Vassallo M, Vinardell P, Zuang V. 2010. A proposed eye irritation testing strategy to reduce and replace *in vivo* studies using Bottom-Up and Top-Down approaches. Toxicol *In Vitro* 24: 1–9.

Stokes WS, Wind M. 2010. Recent progress and future directions at NICEATM-ICCVAM: validation and regulatory acceptance of alternative test methods that reduce, refine, and replace animal use. ALTEX 27 (Spec. Issue):221–232.

Stokes WS, Wind M. 2010. Validation of innovative technologies and strategies for regulatory safety assessment methods: challenges and opportunities. ALTEX 27 (Spec. Issue): 87–95.

Stokes WS, Wind M. 2010. NICEATM and ICCVAM participation in the International Cooperation on Alternative Test Methods. ALTEX 27 (Spec. Issue): 211–219.

Stokes WS, Wind M, Blakey D, Kreysa J, Kojima H, Anklam E. 2010. Establishment of the International Cooperation on Alternative Test Methods (ICATM) and its role in the validation and regulatory acceptance of globally harmonized safety assessment methods [Abstract No. 1810A]. Presented at the 49th Annual Meeting of the Society of Toxicology; Salt Lake City, UT; March 7–11, 2010. Toxicol Sci/Toxicologist 114(1): 385.

Strickland J, Matheson J, Wind M, Jacobs A, Stack F, Allen D, Stokes W. 2010. Using the murine local lymph node assay to categorize strong skin sensitizers [Abstract No. 1807]. Presented at the 49th Annual Meeting of the Society of Toxicology; Salt Lake City, UT; March 7–11, 2010. Toxicol Sci/Toxicologist 114(1): 384.

Wind M, Stokes WS. 2010. Developing performance standards to expedite validation of innovative and improved test methods. ALTEX 27 (Spec. Issue): 97–102.

Wind M, Stokes WS. 2010. The International Cooperation on Alternative Test Methods (ICATM). ALTEX 27 (Spec. Issue): 207–210.

## ARTICLES IN THE NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES ENVIRONMENTAL FACTOR NEWSLETTER THAT DESCRIBE NICEATM-ICCVAM ACTIVITIES

**2011**

Casey W. March 2011. Alternative testing panel to meet in Bethesda. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/march/science-alternative.

Hood E. February 2011. Steady progress reported at SACATM. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/july/science-steady/index.cfm.

McCarley D, Sprankle C. February 2011. NTP alternatives center holds workshops on best safety testing. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/february/science-alternatives.

NICEATM. January 2011. Casey presents at international meeting on alternatives for endocrine disruptors. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/january/science-casey.cfm.

NICEATM. January 2011. New green safety testing methods available to identify ACD. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/january/science-new.cfm.

NICEATM. January 2011. Stokes presents at international meeting on alternatives for vaccine testing. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/january/science-stokes.cfm.

NICEATM. October 2011. Committee recommends alternative method for product safety testing. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/august/science-committee/index.cfm.

NICEATM. October 2011. NTP alternatives center showcases accomplishments. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/october/science-ntp/index.cfm.

NICEATM. November 2011. Stokes presents at international conference on animal models and drug testing. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/november/science-stokes/index.cfm.

NICEATM. November 2011. NICEATM convenes international workshop on rabies vaccine testing. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2011/november/science-niceatm/index.cfm.

**2010**

Ball E. January 2010. Stokes helps build Pacific rim partnerships for advancing alternative testing. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2010/january/spotlight-pacific.cfm.

Mackar R. July 2010. Committee advises on alternative toxicological methods. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2010/july/spotlight-committee.cfm.

McCarley D. October 2010. New eye safety test methods proposed by interagency group. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2010/october/science-eyesafety.cfm.

McCarley D. October 2010. NICEATM convenes international workshop on vaccine safety testing. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2010/october/spotlight-niceatm.cfm.

McCarley D. July 2010. NTP hosts international visitors. Environmental Factor [Internet]. Available: http://www.niehs.nih.gov/news/newsletter/2010/july/spotlight-ntp.cfm.

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| ACD | Allergic contact dermatitis |
| AMCP | Antimicrobial cleaning product |
| BCOP | Bovine corneal opacity and permeability |
| BET | Bacterial endotoxin test |
| BRD | Background review document |
| BrdU | Bromodeoxyuridine |
| CFR | U.S. Code of Federal Regulations |
| CM | Cytosensor microphysiometer |
| CPSC | U.S. Consumer Product Safety Commission |
| CVB | Center for Veterinary Biologics (U.S. Department of Agriculture) |
| ECVAM | European Centre for the Validation of Alternative Methods |
| ELISA | Enzyme-linked immunosorbent assay |
| EPA | U.S. Environmental Protection Agency |
| ER | Estrogen receptor |
| EU | European Union |
| FDA | U.S. Food and Drug Administration |
| FR | *Federal Register* |
| GD | Guidance Document (OECD) |
| ICATM | International Cooperation on Alternative Test Methods |
| ICCVAM | Interagency Coordinating Committee on the Validation of Alternative Methods |
| ICE | Isolated chicken 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 |
| NCTT | U.S. National Institutes of Health Center for Translational Therapeutics |
| NHK | Normal human keratinocyte |
| NICEATM | U.S. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods |
| NIEHS | U.S. National Institute of Environmental Health Sciences |
| NIOSH | U.S. National Institute for Occupational Safety and Health |
| NIH | U.S. National Institutes of Health |
| NRU | Neutral red uptake |
| NTP | U.S. National Toxicology Program |
| OECD | Organisation for Economic Co-operation and Development |
| rLLNA | Reduced murine local lymph node assay |
| RPT | Rabbit pyrogen test |
| SACATM | Scientific Advisory Committee on Alternative Toxicological Methods |
| SAM | Supplemental Assay Method (USDA) |
| SOT | Society of Toxicology |
| TA | Transactivation |
| TG | Test Guideline (OECD) |
| U.S.C. | United States Code |
| USDA | U.S. Department of Agriculture |
| VMT | Validation management team |
| WC8 | 8th World Congress on Alternatives and Animal Use in the Life Sciences |

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

This appendix lists all members of the Scientific Advisory Committee on Alternative Toxicological Methods during 2010 and 2011. Ending dates of appointments are indicated.

Laura Andrews, PhD, DABT
VP Pharmacology and Toxicology
Genzyme Corporation
Framington, MA
Appointment ends 2012

Karen K. Brown, PhD
President, Pair O'Docs Enterprises
Parkville, MO
Appointment ended 2011

Joy Cavagnaro, PhD, DABT,
RAC, ATS, RAPS
President and Founder Access BIO, LC
Boyce, VA
Appointment ends 2014

George B. Corcoran, PhD, ATS
Chairman and Professor, Dept. of
Pharmaceutical Sciences
Eugene Applebaum College of
Pharmacy and Health Sciences
Wayne State University
Detroit, MI
Appointment ended 2011

Helen E. Diggs, DVM
Director, Office of Laboratory
Animal Care
University of California
Berkeley, CA
Appointment ended 2010

Marion F. Ehrich, PhD
Professor, Dept. of Biomedical Sciences
& Pathobiology/Laboratory for
Neurotoxicity Studies
VA-MD Regional College of
Veterinary Medicine
Blacksburg, VA
Appointment ended 2010

Eugene L. Elmore, PhD
Senior Project Scientist
Dept. of Radiation Oncology
University of California, Irvine
Irvine CA
Appointment ends 2012

James Freeman, PhD
(Chair through 2010)
Section Head, Global Product
Stewardship and Regulatory Affairs
ExxonMobil Biomedical Sciences, Inc.
Annandale, NJ
Appointment ended 2010

Steven R. Hansen, DVM, MS, MBA,
DABT, ABVT
ASPCA Poison Control Center
Urbana, IL
Appointment ends 2012

Gwendolyn Y. McCormick, DVM, MS,
DACLAM
Attending Veterinarian,
Distinguished Research Fellow
Animal Resources Dept.
Boehringer Ingelheim Pharmaceuticals,
Inc.
Ridgefield, CT
Appointment ends 2012

Sharon Meyer, PhD
Professor, Dept. of Toxicology
University of Louisiana at Monroe
Monroe, LA
Appointment ended 2011

Steven M. Niemi, DVM (Chair since 2011)
Director, Center for Comparative
Medicine
Massachusetts General Hospital
Charlestown, MA
Appointment ends 2013

Ricardo Ochoa, DVM, PhD, ACVP
President and Principal
Pre-Clinical Safety, Inc.
Niantic, CT
Appointment ends 2014

Michael J. Olson, PhD, ATS
Director, Occupational Toxicology
Corporate Environment, Health, Safety
and Sustainability
GlaxoSmithKline
Research Triangle Park, NC
Appointment ends 2013

Annie (Peiyong) Qu, PhD
Assoc. Professor, Dept. of Statistics
Oregon State University
Corvallis, OR
Appointment ended 2010

Linda A. Toth, DVM, PhD
Assoc. Dean for Research
and Faculty Affairs
Professor, Dept. of Pharmacology
Southern Illinois University School
of Medicine
Springfield, IL
Appointment ends 2013

Daniel M. Wilson, PhD, DABT
Mammalian Toxicology Consultant
Toxicology and Environmental Research
and Consulting
The Dow Chemical Company
Midland, MI
Appointment ends 2014

Gary Wnorowski, MBA, LAT
President, Eurofins/Product Safety
Laboratories
Dayton, NJ
Appointment ended 2011

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.

The Interagency Coordinating Commitee on the Validation of Alternative Toxicological Methods (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**

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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/*.
Paper copies of NICEATM–ICCVAM reports are also available on request from NICEATM:

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**Notes**

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National Toxicology Program
U.S. Department of Health and Human Services