



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

Biennial Progress Report 2012–2013

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

July 2014



About ICCVAM and NICEATM

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is a permanent committee of the National Institute of Environmental Health Sciences (NIEHS) under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

ICCVAM is composed of representatives from 15 U.S. Federal regulatory and research agencies that generate or use toxicological and safety testing information:

- Agency for Toxic Substances and Disease Registry (ATSDR)
- Consumer Product Safety Commission (CPSC)
- Department of Agriculture (USDA)
- Department of Defense (DOD)
- Department of Energy (DOE)
- Department of the Interior (DOI)
- Department of Transportation (DOT)
- Environmental Protection Agency (EPA)
- Food and Drug Administration (FDA)
- National Cancer Institute (NCI)
- National Institute for Occupational Safety and Health (NIOSH)
- National Institute of Environmental Health Sciences (NIEHS)
- National Institutes of Health (NIH)
- National Library of Medicine (NLM)
- Occupational Safety and Health Administration (OSHA)

The ICCVAM Authorization Act (42 U.S.C. 285f-3) formally established ICCVAM in 2000. 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.

NICEATM is located within the Division of the National Toxicology Program (DNTP) at NIEHS. NICEATM's responsibilities include:

- Providing scientific and operational support for ICCVAM activities
- Conducting and publishing analyses and evaluations of data from new testing approaches
- Providing information to test method developers, regulators, and regulated industry
- Organizing workshops and symposia on topics of interest
- Conducting independent validation studies of priority alternative testing approaches
- Providing bioinformatics and computational toxicology support to the DNTP Biomolecular Screening Branch for studies conducted for the Tox21 collaboration

More information about NICEATM and ICCVAM can be found at <http://ntp.niehs.nih.gov/go/niceatm> or obtained by contacting NICEATM (telephone: [919] 316-4729; email: niceatm@niehs.nih.gov or iccvam@niehs.nih.gov).



Interagency Coordinating Committee on the Validation of Alternative Methods

Biennial Progress Report 2012–2013

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

July 2014

This document is available electronically at <http://ntp.niehs.nih.gov/go/iccvam-bien>

Table of Contents

Abbreviations and Acronyms.....	6
A Message from NIEHS and NTP.....	7
A Message from NICEATM and ICCVAM.....	8
Safety Testing, Alternative Methods, and the Role of ICCVAM	9
ICCVAM Was Established to Coordinate Evaluation of Alternative Test Methods.....	10
ICCVAM Activities.....	10
How NICEATM Supports ICCVAM.....	11
Scientific Advisory Committee on Alternative Toxicological Methods.....	11
2013: A New Vision and Direction for ICCVAM.....	13
ICCVAM Agency Activities 2012–2013.....	16
Eye Safety Testing.....	17
Acute Systemic Toxicity Testing.....	20
Biologics and Vaccine Testing.....	22
Immunotoxicity Testing: Allergic Contact Dermatitis.....	25
Endocrine Disruptor Testing.....	28
Pyrogen Testing.....	31
Reproductive and Developmental Toxicity Testing.....	32
Photosafety Testing.....	33
Ecotoxicity Testing.....	34
Research and Development Activities Supporting Alternative Methods Development.....	36
Other ICCVAM Agency Activities Promoting Alternative Methods.....	39
Outreach and Collaborative Activities	41
International Cooperation on Alternative Test Methods.....	42
Collaborations with International Validation Organizations.....	43
ICCVAM Contributions to OECD Test Guidelines and Guidance Documents.....	44
Meetings of the Scientific Advisory Committee on Alternative Toxicological Methods.....	45
ICCVAM Participation in National and International Workshops, Conferences, and Meetings.....	46
References Cited in this Report.....	49

Appendices50

Appendix A: ICCVAM Agency Representatives in 2012 and 2013 50

Appendix B: NICEATM and ICCVAM Publications, 2012–2013..... 51

 NICEATM and ICCVAM Reports.....51

Federal Register Notices51

 Abstracts and Manuscripts52

 Articles in the National Institute of Environmental Health Sciences *Environmental Factor* Newsletter53

Appendix C: Scientific Advisory Committee on Alternative Toxicological Methods 55

Acknowledgements 57

Abbreviations and Acronyms

3Rs	Principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing	ILS	Integrated Laboratory Systems, Inc.
ACD	Allergic contact dermatitis	JaCVAM	Japanese Center for the Validation of Alternative Methods
ALTBIB	National Library of Medicine Alternatives to Animal Testing Web portal	KoCVAM	Korean Center for the Validation of Alternative Methods
ATSDR	Agency for Toxic Substances and Disease Registry	LAL	<i>Limulus</i> amoebocyte lysate
CFR	U.S. Code of Federal Regulations	LD ₅₀	In traditional acute systemic toxicity tests, the dose that produces lethality in 50% of the animals tested
CPSC	U.S. Consumer Product Safety Commission	LLNA	Murine local lymph node assay
CVB	Center for Veterinary Biologics (U.S. Department of Agriculture)	NCATS	National Center for Advancing Translational Sciences (U.S. National Institutes of Health)
CYP	Cytochrome P450 enzymes	NCI	National Cancer Institute (U.S. National Institutes of Health)
DNTP	Division of the National Toxicology Program (National Institute of Environmental Health Sciences)	NICEATM	U.S. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
DOD	U.S. Department of Defense	NIEHS	National Institute of Environmental Health Sciences (U.S. National Institutes of Health)
DOE	U.S. Department of Energy	NIOSH	U.S. National Institute for Occupational Safety and Health
DOI	U.S. Department of the Interior	NIH	U.S. National Institutes of Health
DOT	U.S. Department of Transportation	NLM	National Library of Medicine (U.S. National Institutes of Health)
DREAM	Dialogue for Reverse Engineering Assessments and Methods	NTP	U.S. National Toxicology Program
EASA	Electrophilic allergen screening assay	OECD	Organisation for Economic Co-operation and Development
EDSP	Endocrine Disruptor Screening Program (U.S. Environmental Protection Agency)	OSHA	U.S. Occupational Safety and Health Administration
ELISA	Enzyme-linked immunosorbent assay	ROBatt	Replacement Ocular Battery
EPA	U.S. Environmental Protection Agency	SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
ER	Estrogen receptor	SOT	Society of Toxicology
ER-Bla	Estrogen receptor β -lactamase test method	STE	Short time exposure test
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing	TA	Transactivation
FDA	U.S. Food and Drug Administration	Tox21	U.S. government interagency high-throughput screening initiative
FHSA	Federal Hazardous Substances Act (U.S.)	VS	Veterinary Services office of the Center for Veterinary Biologics (U.S. Department of Agriculture)
FR	<i>Federal Register</i>	U.S.C.	United States Code
HIST	Murine histamine sensitization test	USDA	U.S. Department of Agriculture
ICATM	International Cooperation on Alternative Test Methods	USGS	U.S. Geological Survey (Department of the Interior)
ICCR	International Cooperation on Cosmetics Regulation		
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		



A Message from NIEHS and NTP

The purpose of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) is to promote safety testing methods that protect human and animal health and the environment while replacing, reducing, and refining animal tests (3Rs). The National Institute of Environmental Health Sciences' (NIEHS) mission is to discover how the environment affects people in order to promote healthier lives. Identifying the most effective tests to characterize hazards presented by chemicals currently in use is consistent with the NIEHS' mission. NIEHS is proud to be one of the 15 ICCVAM member agencies and to support the committee through the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

ICCVAM's 15th anniversary coincided with an opportunity to evaluate ICCVAM's progress and implement a new approach towards fulfilling its mission. ICCVAM is moving forward with a new vision that is driven by member agency needs and priorities. This new vision focuses on priority areas for immediate resource investment including exploring new paradigms for the validation and utilization of alternative methods and improving communications with stakeholders and the public. In addition, NICEATM has broadened its scope of activities to support NIEHS Tox21 projects. Increased interaction between NICEATM and Tox21 will better position ICCVAM to integrate new toxicological testing approaches into the existing regulatory framework. ICCVAM stakeholders have welcomed and support the new vision, which promises to increase ICCVAM's effectiveness in implementing improved safety tests that incorporate the 3Rs.

We would like to close by noting the retirement of Rear Admiral William Stokes, DVM, who led NICEATM and represented NIEHS on ICCVAM through December 2012. We express our sincere appreciation to Bill for his dedication and leadership during the early years of NICEATM and ICCVAM. We also welcome former NICEATM Deputy Director Warren Casey, PhD, as the new Director of NICEATM and pledge him our support as NICEATM and ICCVAM move forward.

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 the 2012–2013 ICCVAM Biennial Progress Report. This report describes activities of ICCVAM and its 15 member agencies that support the ICCVAM mission of facilitating development, validation, and regulatory acceptance of new testing approaches.

ICCVAM is advancing a new philosophy whereby its activities are driven by member agency priorities and better reflect the current direction of modern toxicology. This report reflects this new philosophy by including ICCVAM member agency-specific research activities supporting toxicology innovation, new agency rules and guidance supporting the reduction and refinement of animal use, and information about the implementation of *in vitro* methods for safety testing. NICEATM is increasing its involvement in the development of high-throughput and computational approaches to safety testing to better assist ICCVAM in evaluating the potential regulatory applications of these methods.

ICCVAM continues to foster strong collaborations with our international partners, recognizing that effective interactions enhance progress towards our mutual goals. During 2012 and 2013, NICEATM and ICCVAM member agency scientists participated on teams to validate studies coordinated by our international counterparts. Scientists from our international partner organizations also participated in planning ICCVAM sponsored workshops.

We gratefully acknowledge the contributions of the representatives and interagency working group members from the 15 ICCVAM member agencies. We also acknowledge the contributions from NICEATM and its contract staff, the Scientific Advisory Committee on Alternative Toxicological Methods, and our many other stakeholders. In particular, we recognize and thank Rear Admiral William Stokes, DVM, who retired from the National Institute of Environmental Health Sciences in 2012, for his leadership roles in NICEATM and ICCVAM during their early years. We also thank Jodie Kulpa-Eddy, DVM, for her 15 years of service to ICCVAM as the principal agency representative from the U.S. Department of Agriculture and her service as ICCVAM Chair during 2011 and 2012.

The activities summarized in this report exemplify ICCVAM's ongoing 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 greater progress and more effective interactions with our stakeholders in the coming years.

Abby Jacobs, PhD
Co-Chair, ICCVAM
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

Anna Lowit, PhD
Co-Chair, ICCVAM
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency

Warren Casey, PhD, DABT
Director, NICEATM
Administrative Director, ICCVAM
National Institute of Environmental Health Sciences



1

Safety Testing, Alternative Methods, and the Role of ICCVAM



ICCVAM WAS ESTABLISHED TO COORDINATE EVALUATION OF ALTERNATIVE TEST METHODS

U.S. regulatory agencies are charged to protect human and animal health and the environment. To do this, agencies must determine the hazards presented by substances such as pesticides, consumer products, and workplace chemicals. Testing these substances provides information about possible hazards and enables informed decisions about responsible use, storage, and disposal.

Many currently accepted safety-testing methods use laboratory animals. Alternative test methods are methods that *replace* animal use with nonanimal test systems or lower species, *reduce* the number of animals required for a specific test procedure, or *refine* animal use to enhance animal well-being and lessen or avoid pain and distress. Collectively, the principles of [replacement](#), [reduction](#), or [refinement](#) of animal use for scientific research or product safety testing are referred to as the “[3Rs](#).”

The ICCVAM Authorization Act of 2000 (42 U.S.C. 285f-3) created the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) 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

ICCVAM is a permanent interagency committee of the National Institute of Environmental Health Sciences under the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Methods (NICEATM).

ICCVAM ACTIVITIES

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 replacing, reducing, and refining 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

A [table on pages 14–15](#) notes how selected 2012–2013 ICCVAM and ICCVAM agency activities align with the ICCVAM duties as outlined in the ICCVAM Authorization Act.

Since its establishment, ICCVAM and ICCVAM member agencies have contributed to the development and regulatory acceptance of a number of [alternative methods](#) that address a variety of regulatory applications. These methods are listed on the NTP website at <http://ntp.niehs.nih.gov/go/regaccept>.

HOW NICEATM SUPPORTS ICCVAM

[NICEATM](#), an office within the Division of the NTP (DNTP) at NIEHS, provides technical and scientific support for ICCVAM and ICCVAM working group activities, peer review panels, expert panels, workshops, and validation efforts.



In addition to providing scientific and operational support for ICCVAM test method evaluations, NICEATM:

- Supports DNTP activities, especially those contributing to the U.S. government’s interagency initiative known as Tox21
- Conducts analyses and evaluations and coordinates independent validation studies on novel and high-priority [alternative testing approaches](#)
- Provides information to test method developers, regulators, and regulated industry through its website and workshops on topics of interest

SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) was established in 2002 in accordance with the ICCVAM Authorization Act. SACATM advises the NIEHS Director, NICEATM, and ICCVAM about NICEATM and ICCVAM activities.

The ICCVAM Authorization Act states that SACATM must include:

- At least one member from each of the following stakeholder groups:
 - 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 methods, including contract laboratories

SACATM, which is directed by its charter to meet at least once each fiscal year, met in September 2012 and September 2013. Summaries of these meetings are provided on [page 45](#).

The SACATM charter, related *Federal Register* notices, current roster, past meeting materials, and future meeting announcements can be found at <http://ntp.niehs.nih.gov/go/167>. SACATM members during 2012 and 2013 are listed in [Appendix C](#).

ABOUT THE TOX21 COLLABORATION

Most traditional toxicity testing methods involve treating a laboratory animal and observing any adverse effects on health. This approach is expensive and time-consuming, and the use of animals raises ethical concerns and issues of interspecies extrapolation.

Tox21 is a collaborative effort among four U.S. Federal government agencies to develop more efficient approaches to predict how chemicals may affect human health. In Tox21 studies, substances are tested using *in vitro* rodent and human cell-based and biochemical assays and lower organisms as model systems. These assays are run at higher throughput and lower cost than animal tests; in some cases, many thousands of chemicals can be tested in a few days. Data from these assays can potentially be used to prioritize substances for further evaluation, inform our understanding of mechanisms of action, and develop improved predictive models for toxicity.

Ultimately, test approaches developed and data collected via the Tox21 initiative may enable agencies to reduce their reliance on animal data for establishing regulations for safe handling of chemicals. ICCVAM will evaluate testing approaches developed through the Tox21 collaboration that show promise for regulatory applications and make recommendations on their use to Federal agencies.

The four agencies participating in the Tox21 collaboration are all ICCVAM members:

- Environmental Protection Agency
- Food and Drug Administration
- National Institute of Environmental Health Sciences
- National Institutes of Health (National Center for Advancing Translational Sciences)



2013: A NEW VISION AND DIRECTION FOR ICCVAM

During its first 15 years, ICCVAM evaluated many [alternative methods](#) for regulatory use and made a number of formal recommendations to Federal agencies. However, ICCVAM stakeholders raised concerns that many methods recommended by ICCVAM were not being used for regulatory decision-making. There was little evidence that ICCVAM recommendations on alternative test methods were leading to meaningful reductions in animal use for toxicity testing.

In an *Environmental Health Perspectives* editorial (Birnbaum 2013), NIEHS and NTP Director Dr. Linda Birnbaum announced that NIEHS would move forward with a different philosophy toward ICCVAM whereby the partner regulatory agencies would drive ICCVAM's activities. At the same time, NICEATM would expand its scope to provide bioinformatic and computational toxicology support to the DNTP overall and the DNTP's Biomolecular Screening Branch. The goal of these activities would be to better position ICCVAM to address how data from the Tox21 collaboration (see [sidebar page 12](#)) could be integrated into the existing regulatory framework.

In response to this new philosophy, ICCVAM developed a draft document titled "A New Vision and Direction for ICCVAM." The document presents ICCVAM's (1) areas of priority and scientific focus for immediate resource investment; (2) plans to improve communications with stakeholders and the public; and (3) interest in exploring new paradigms for the validation and utilization of alternative toxicological methods. This document was released for public comment in August 2013 and discussed at the September 2013 SACATM meeting. ICCVAM is carefully considering public and SACATM comments on the draft document as it develops new operating procedures and plans activities for the future.

DEFINITIONS OF KEY TERMS

3Rs: the principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing

Alternative methods: testing methods that replace, reduce, or refine animal use

Harmonization: the act of making systems or laws similar among different companies, countries, etc. so the organizations using those systems or laws can operate more easily within the different venues

High-throughput screening: a practice that uses robotics, liquid-handling devices, detectors, and associated software to quickly conduct a large number of chemical or biochemical tests

Reduction alternative: a test method that requires fewer animals

Refinement alternative: a test method that modifies procedures to enhance animal well-being and lessen or avoid pain and distress in animals

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

Validation: a process by which the reliability and relevance of a test method are established for its intended application

Selected ICCVAM and ICCVAM Agency Activities in 2012–2013 and Alignment with ICCVAM Duties

ICCVAM Duty	Activity
Coordinate the technical review and evaluation of new, revised, or alternative test methods	<ul style="list-style-type: none"> Reviewed the short time exposure method for eye safety testing (NIEHS; see page 18)
Foster interagency and international harmonization of test protocols that encourage reducing, refining, and replacing animal test methods	<ul style="list-style-type: none"> Participated in the International Cooperation on Alternative Test Methods (ICCVAM; see page 42) Participated on international validation management teams for <i>in vitro</i> assays for evaluating human liver metabolism and identifying potential skin sensitizers (NIEHS and ICCVAM; see page 20) Sponsored workshop on alternatives to the HIST (ICCVAM; see page 22) Issued guidance on photosafety testing (FDA; see page 33) Participated in development of International Cooperation on Cosmetics Regulation report on animal testing (FDA; see page 39)
Assist with and provide guidance on validation criteria and processes	<ul style="list-style-type: none"> Provided test substances for a validation study on the OptiSafe method for eye safety testing and comments on the validation study report (NIEHS; see page 17) Issued guidance on the definition of “strong sensitizer” (CPSC; see page 26) Created reference databases of <i>in vivo</i> immunotoxicology (NIEHS; see page 27) and endocrine disruptor data (NIEHS; see page 29) Organized workshop on reproductive and developmental toxicity testing (FDA; see page 32) Issued guidance on toxicity data requirements (EPA; see page 40)
Promote the acceptance of scientifically valid test methods	<ul style="list-style-type: none"> Sponsored workshop on alternatives for <i>Leptospira</i> vaccine testing (ICCVAM; see page 22) Issued guidance on use of humane endpoints and methods in biologics testing (USDA; see page 24) Developed alternate testing protocol for tuberculin testing reagents (USDA; see page 23)
Promote awareness of accepted test methods	<ul style="list-style-type: none"> Issued policy on alternate framework for eye irritation testing of pesticide products (EPA; see page 18) Launched a thorough update of its Alternatives to Animal Testing web portal (NLM; see page 39)
Submit ICCVAM test method recommendations to appropriate U.S. Federal agencies; make ICCVAM’s final test recommendations available to the public	<ul style="list-style-type: none"> Developed recommendations on using fewer animals for eye safety testing (NIEHS; see page 17) Recommended murine local lymph node assay for identification of strong sensitizers (ICCVAM; see page 25) Recommended using the BG1Luc estrogen receptor transactivation test method to identify substances with the potential to induce or inhibit activation of the estrogen receptor (ICCVAM; see page 28)

Selected ICCVAM and ICCVAM Agency Activities in 2012–2013 and Alignment with ICCVAM Duties

ICCVAM Duty	Activity
Consider requests from the public to review and evaluate new, revised, or alternative test methods that have evidence of scientific validity	<ul style="list-style-type: none"> • Received nomination of the electrophilic allergen screening assay for identification of potential skin sensitizers (ICCVAM; see page 25)
Prepare reports on ICCVAM progress and accomplishments under the Act and make them available to the public	<ul style="list-style-type: none"> • Prepared and published 2010–2011 ICCVAM Biennial Report and prepared 2012–2013 ICCVAM Biennial Report (NIEHS and ICCVAM) • Published summaries of workshops on alternatives for testing of <i>Leptospira</i>, pertussis, and rabies vaccines (NIEHS and ICCVAM; see pages 22, 23, and 24) • Prepared and published a test method evaluation report on using fewer animals for identification of chemical eye hazards (NIEHS; see page 17)

2

ICCVAM Agency Activities 2012–2013



EYE SAFETY TESTING

Manufacturers test personal care products, household cleaning products, and other substances to determine if they could cause temporary or permanent eye damage. Test results are used to classify these substances using appropriate national and international [hazard classification](#) systems. [Hazard classification](#) systems direct how substances must be packaged, labeled, and handled in order to prevent exposure and injury to the eyes.

Nearly all eye (ocular) safety testing has been conducted using the rabbit eye test. Evaluation of alternatives to animal use for eye safety testing is a high priority for ICCVAM agencies. Past ICCVAM eye safety test method evaluations identified *in vitro* test methods that could replace animal use in some applications. For applications where animal use is still necessary, ICCVAM recommended approaches to reduce the number of animals used and minimize pain and distress in those animals.



ICCVAM and ICCVAM Agency Activities

- **National Institute of Environmental Health Sciences (NIEHS) and ICCVAM:** OptiSafe is an *in vitro* eye irritation test method in which a test substance is applied to a [semipermeable membrane](#). Damage to [macromolecules](#) in the membrane is measured to assess the test substance's potential to cause eye irritation.

In 2013, at the request of the test method developer, Lebrun Labs, NICEATM selected test substances against which to [validate](#) the OptiSafe test method. The test substances represented a range of physical and chemical properties and hazard classes. NICEATM provided these test substances in coded form to Lebrun Labs, which used them to conduct a validation study on OptiSafe. An initial NICEATM review of the validation study data indicated that the OptiSafe method may compare favorably to other *in vitro* eye safety testing methods.

ICCVAM will reactivate its Ocular Toxicity Working Group in 2014 to more thoroughly evaluate this test method to determine its potential regulatory utility and whether additional activities are necessary.

- **NIEHS:** Although currently approved *in vitro* test methods can identify some eye hazards, they are not sufficiently [validated](#) and accepted to completely replace all animal testing. In 2012 NICEATM prepared a report describing a [hazard classification](#) approach for *in vivo* eye irritation testing with the potential to reduce animal use. When it is necessary to use animals for eye safety testing, this approach may allow for

DEFINITIONS OF KEY TERMS

“Bottom-up” screen: a test method used before running any other tests to identify substances that will not require hazard labeling

Corneal epithelial cells: structural cells from the cornea, the transparent front part of the eye

Decision-tree approach: an approach that uses a defined group of test methods in a sequential manner and defines the action to be taken on a given outcome of each test

Hazard classification: assignment of a substance to a category according to results of toxicity testing, most often for labeling purposes

Macromolecule: a large molecule, such as a protein, that consists of many smaller molecules linked together

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

use of fewer animals and harmonizes the number of animals used for this testing across U.S. regulatory agencies and international test guidelines.

The NICEATM evaluation is described in the *Test Method Evaluation Report: Identifying Chemical Eye Hazards with Fewer Animals*, available at <http://ntp.niehs.nih.gov/go/40530>.

- **NIEHS:** In 2013, NICEATM prepared a summary review document on the short time exposure (STE) test. The document summarized a NICEATM evaluation of data provided by Kao Corporation, the test method sponsor. The STE (Takahashi et al. 2008) is an *in vitro* test proposed to identify the eye injury hazard potential of chemicals and products by measuring cultured rabbit [corneal epithelial cell](#) viability following test substance exposure.

In its evaluation, NICEATM compared data from the STE test to *in vivo* test results and described the usefulness and limitations of the STE as a [screening](#) approach to identify severe [irritants](#) and [corrosives](#) (“[top-down](#)” screen) as well as substances that do not require hazard labeling (“[bottom-up](#)” screen).

The National Toxicology Program (NTP) provided the NICEATM summary review document to four external reviewers, who concluded that the database of compounds tested was generally sufficient and the review of the STE was thorough. The final document (http://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/STE-SRD-NICEATM-508.pdf) includes a summary of reviewer comments.

Kao Corporation submitted the final NICEATM summary review document and other documents to the Organisation for Economic Co-operation and Development (OECD) for consideration of the STE in 2014 as an *in vitro* alternative to current ocular hazard identification tests. The OECD made a draft test guideline for the STE test method available for public comment in November 2013.

- **Environmental Protection Agency (EPA):** In May 2013, the EPA Office of Pesticide Programs issued the policy “Use of an Alternate Testing Framework for Classification of Eye Irritation Potential of EPA Pesticide Products.” This document describes the use of three nonanimal tests—bovine corneal opacity and permeability test, EpiOcular test, and cytosensor microphysiometer test—in a [decision-tree approach](#). This approach, used for antimicrobial cleaning products in general and for other classes of pesticide products on a case-by-case basis, enables test substance evaluations under the EPA classification and labeling system without the use of animals. The data used to support the EPA policy were drawn from a 2010 ICCVAM evaluation of the approach and an EPA pilot study initiated in 2009.

The policy is available at <http://www.epa.gov/pesticides/science/eye-irritation.html>.

- **National Institutes of Health (NIH), Food and Drug Administration (FDA), and EPA:** The ROBatt (Replacement Ocular Battery) is a [tiered-testing strategy](#) that uses a battery of four *in vitro* eye irritation tests

DEFINITIONS OF KEY TERMS

Ocular irritant: a substance that causes temporary eye tissue damage. A severe irritant produces damage persisting 21 days after application or causes serious physical decay of vision.

Screening test: a rapid, simple test conducted for preliminary decision-making and to set priorities for definitive testing

Semipermeable membrane: a barrier that allows some molecules to pass through but not others

Tiered-testing strategy: an approach that uses multiple tests sequentially to characterize the potential hazard of a test substance

“Top-down” screen: a test method used before running any other tests to identify substances likely to cause severe illness or injury

Validation: a process by which the reliability and relevance of a test method are established for its intended application

as a replacement for the rabbit eye test. MB Research Labs developed ROBatt with a 2010 grant from the NIH Common Fund Grant Regulatory Science Program. The NIH and FDA established the Regulatory Science Program as a partnership to support development of new methods to evaluate product safety, efficacy, and quality.

MB Research Labs conducted a validation study on the ROBatt using a panel of 52 chemicals selected by the EPA and FDA that covered the full range of eye irritancy from nonirritating to [corrosive](#). The results from the ROBatt testing and data evaluation are being compared to *in vivo* eye irritation reference data.

ACUTE SYSTEMIC TOXICITY TESTING

Chemicals, drugs, and natural substances have potential toxic effects that can be identified by appropriate testing. [Acute systemic toxicity](#) tests identify short-term effects that appear soon after a single exposure, and are the most frequently performed safety tests. These tests measure the toxicity of a substance when swallowed (oral toxicity tests), absorbed through the skin (dermal toxicity tests), or inhaled (inhalation toxicity tests). If appropriate, data from [acute systemic toxicity](#) tests are used to develop warning labels, protective packaging, requirements for workers to wear personal protective equipment, and environmental release limits.

Traditional [acute systemic toxicity](#) tests yield an LD_{50} value, or the dose that causes death in 50% of the animals tested. The LD_{50} value is used to categorize toxic substances and determine the hazard classification used on product labels. The currently applied alternatives (up-and-down procedure, acute toxic class method, and fixed dose procedure) reduce the number of animals used for classification and labeling compared to the traditional [acute systemic toxicity](#) test. However, ICCVAM member agencies are actively seeking additional methods that can replace these tests with nonanimal alternatives or further reduce the number of animals required for acute toxicity testing.



ICCVAM and ICCVAM Agency Activities

- **NIEHS and ICCVAM:** Representatives from NICEATM and ICCVAM participated on the management team for a [validation](#) study conducted by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM). The aim of this study was to assess an *in vitro* assay for evaluating human liver [metabolism](#) and toxicity. Advancing novel *in vitro* platforms for assessing [metabolism](#) and toxicity is a key step in developing *in vitro* alternatives for *in vivo* toxicity studies.

The [validation](#) study used a human liver cancer cell line (HepaRG[®]) and cryopreserved human liver cells to assess the potential to [induce liver enzymes](#) at clinically relevant doses of pharmaceuticals. In humans and other animals, the liver [cytochrome P450 \(CYP\) enzymes](#) play a major role in [biotransformation](#), the process that converts a substance into a chemically different substance. [Biotransformation](#) in the liver can potentially increase or decrease chemical toxicity. A stable *in vitro* model with functional CYP enzyme activity is important for a nonanimal assessment of the contribution of [biotransformation](#) to toxicity.

The [validation](#) study was completed in 2013 and a draft report of the study will be submitted to the EURL ECVAM scientific advisory committee for peer review in 2014. EURL ECVAM also submitted a proposal to OECD in 2013 to support the development of a performance-based test guideline for establishment of human-derived liver cell systems to investigate [biotransformation](#) and toxicity of compounds by evaluating CYP induction.

More information about the study is available from the European Union Institute for Health and Consumer Protection at <http://ihcp.jrc.ec.europa.eu/our-labs/eurl-ecvam/validation-regulatory-acceptance/toxicokinetics/toxicokinetics#2-test-methods-under>



- **NIEHS and EPA:** Data from dermal systemic toxicity tests measure the toxicity of a substance when absorbed through the skin and are used to determine appropriate hazard classification of pesticides and other substances. These categorizations in turn are



used to develop product labels and define personal protective equipment requirements for occupational users. Since 2008, NICEATM has been collecting oral and dermal toxicity data to evaluate if acute oral toxicity data could be reliably used to assign EPA acute dermal hazard classifications, potentially reducing the number of animals needed for pesticide testing.

The EPA is providing NICEATM with acute oral toxicity data for both pesticide active ingredients and pesticide formulations. NICEATM is reviewing the data for quality control purposes and using modified Klimisch criteria (Schneider et al. 2009) to evaluate the reliability of the data.

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: by ingestion (oral), through the skin (dermal), or by inhalation.

Biotransformation: the process in a living system of converting a substance to a different substance

Cytochrome p450 enzymes: a group of enzymes that alter the structure of drugs and other molecules

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

LD₅₀: in traditional animal tests, the dose that causes death in 50% of the animals tested; a value used to categorize toxic substances and determine the hazard phrases used on product labels

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

Validation: a process by which the reliability and relevance of a test method are established for its intended application

BIOLOGICS AND VACCINE TESTING

Biologics are products derived from biological sources and used as medicines in humans or animals. Biologics include viruses, substances derived from blood and serum, toxins, antitoxins, vaccines, and other substances such as insulin or antihemophilic factor.

Regulatory agencies such as the FDA and U.S. Department of Agriculture (USDA) require testing of biologics to develop appropriate labeling, ensure potency of the product when used as labeled, and evaluate the safety and potency of manufactured vaccines prior to sale. The testing requirements of these applications necessitate the use of large numbers of animals, and the specific procedures used may cause the animals to experience significant pain and distress. ICCVAM agencies are working to identify methods that will refine existing testing procedures or reduce or eliminate the need for animal testing for biologics.



ICCVAM and ICCVAM Agency Activities

- **ICCVAM, NIEHS, and USDA:** NICEATM worked with ICCVAM member agencies and international partners to organize the “International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing” (September 2012). Workshop participants reviewed available alternative methods and defined efforts necessary to achieve global acceptance and implementation of the methods.

Specific actions for both regulators and industry were identified to facilitate broader use of USDA-validated ELISA [antigen quantification methods](#), which do not use animals. Workshop participants also identified applications for which the use of the ELISA methods would not be appropriate. In those cases, alternatives to reduce and refine animal use in *Leptospira* vaccine potency testing include: (1) [serological assays](#), (2) potential elimination of [back-titrations](#) for calculation of LD₅₀, and (3) [harmonization](#) of regulatory requirements for the number of animals used for testing. Methods to reduce pain and distress through the use of analgesics and [humane endpoints](#) were also discussed.

Proceedings of the workshop were published in 2013 as a dedicated issue of *Biologicals* (volume 41, issue 5; available at <http://www.sciencedirect.com/science/journal/10451056/41/5>). A link to the proceedings, workshop summary, workshop presentations, and other information are available at <http://ntp.niehs.nih.gov/go/leptowksp>. In response to the workshop recommendations, the USDA Center for Veterinary Biologics (CVB) issued Veterinary Services (VS) Memorandum No. 800.102 (http://www.aphis.usda.gov/animal_health/vet_biologics/publications/memo_800_102.pdf). This document provides guidance for vaccine manufacturers on obtaining an exemption that allows use of the nonanimal ELISA test instead of the traditional hamster test for *Leptospira* vaccine potency testing. The USDA is committed to the promotion of the nonanimal ELISA test. The agency will monitor trends in hamster usage over the next five years to assess the impact of this test and report the findings annually to ICCVAM.

- **ICCVAM, NIEHS, and FDA:** NICEATM worked with ICCVAM member agencies and international partners to organize the “International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines” (November 2012). Workshop participants reviewed and discussed data generated by an international study that compared the performance of 12 *in vitro* assays.

Participants concluded that none of the alternative methods is sufficiently developed to justify progression to a validation study at this time. Participants also agreed that no single *in vitro* assay would be applicable to all vaccine formulations. Two cell-based assays that appeared most promising for future acceptance or adoption were recommended for further development and optimization. Workshop participants also agreed

that development of a standardized Chinese hamster ovary cell aggregation assay should be pursued as an alternative to the HIST for calibration of pertussis toxin reference standards.

An international collaborative study of the Chinese hamster ovary cell assay is currently in progress. Data from this study and other recent advances in method development will be reviewed at an international workshop planned for mid-2015 in London, England. In addition, a workshop to consider implementation and regulatory acceptance of *in vitro* alternatives to the HIST will be convened on August 24, 2014, as a satellite meeting of the Ninth World Congress on Alternatives and Animal Use in the Life Sciences in Prague, Czech Republic.

A report of the 2012 workshop is in press in *Biologicals* and will be published in early 2014 (Isbrucker et al. 2014). A link to the report, workshop summary, workshop presentations, and other information are on the NTP website at <http://ntp.niehs.nih.gov/go/HISTwksk>.

- **USDA:** In April 2012, CVB issued an alternate testing protocol for reagents used for tuberculin testing. VS Memorandum No. 800.114 (http://www.aphis.usda.gov/animal_health/vet_biologics/publications/memo_800_114.pdf) describes the protocol, which reduces the number of guinea pigs (from 43 to 15) required for testing lots of purified protein derivative used in tuberculosis skin tests.
- **FDA:** In 2012, the FDA Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research issued "Guidance for Industry: S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals." This document complements and updates the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use document on this topic. Application of procedures in this guidance should potentially reduce animal use.

The ICH document and the FDA addendum are both available at <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm304390.htm>.

- **FDA:** In 2013, the FDA Center for Biologics Evaluation and Research issued "Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products." This document provides comprehensive recommendations for preclinical testing in this emerging medical product area. Included are

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

Back-titration: inoculation of an animal with a virus preparation to assess the potency of the preparation for use in vaccine testing

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

Harmonization: the act of making systems or laws similar among different companies, countries, etc. so the organizations using those systems or laws can operate more easily within the different venues

Humane endpoint: a clear and predictable criterion set prior to an animal test that will allow the test to be stopped once experimental objectives have been met and before animals experience significant pain or distress

Serological assay: 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

Validation: a process by which the reliability and relevance of a test method are established for its intended application

recommendations for the replacement, reduction, or refinement of animal use (the “3Rs”) in the overall design of proof-of-concept and toxicity testing programs during development of cell and gene therapy products. Consideration of the 3Rs during preclinical testing should minimize animal use for testing as the field matures.

The guidance is available at <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm376136.htm>

- **NIEHS, ICCVAM, and USDA:** NICEATM staff, ICCVAM committee members, and ICCVAM member agency scientists prepared a report on the ICCVAM-sponsored 2011 “International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing” (Stokes et al. 2012). Conclusions of the workshop included:
 - When conducting the mouse rabies [challenge test](#), general anesthesia should be used for intracerebral virus injections. [Humane endpoints](#) should be used routinely as the basis for euthanizing animals.
 - Manufacturers of rabies vaccines should collaborate with regulatory authorities to initiate product-specific validation of the serum neutralization test. While this test still uses animals, it reduces the number of animals used and eliminates animal pain and distress compared to the mouse rabies [challenge test](#).
 - The possibility of replacing the mouse rabies [challenge test](#) with a nonanimal [antigen quantification method](#) for monovalent nonadjuvanted vaccines should be explored.

After the workshop, CVB issued policies reflecting the recommendations and goals of the workshop.

- CVB Notice No. 1212 (http://www.aphis.usda.gov/animal_health/vet_biologics/publications/notice_12_12.pdf), issued in May 2012, contains specific guidance on implementing [humane endpoints](#) in testing, including the rabies [challenge test](#). The guidance strongly encourages the use of anesthesia for intracerebral virus injections during rabies vaccine testing and the use of analgesics in animal studies and potency testing when shown that it does not affect the study outcome.
- CVB Notice No. 1310 (http://www.aphis.usda.gov/animal_health/vet_biologics/publications/notice_13_10.pdf), issued in July 2013, revises the procedure for the rabies vaccine potency test described in Supplemental Assay Method 308 to eliminate the LD₅₀ upper limit for challenge virus as a validity requirement. The previous procedure required repeating otherwise satisfactory tests if the challenge LD₅₀ was greater than the upper limit, a requirement CVB found was not necessary for test validity. The revised procedure is expected to reduce animal use for rabies vaccine testing.



IMMUNOTOXICITY TESTING: ALLERGIC CONTACT DERMATITIS



Allergic contact dermatitis (ACD) is a skin reaction characterized by localized redness, swelling, blistering, or itching after direct contact with a skin allergen. ACD develops in workers and consumers exposed to skin-sensitizing chemicals and products. To prevent such exposure, regulatory agencies require the testing of chemicals and products to determine their potential to cause ACD. Examples of sensitizers include chemicals such as formaldehyde, formulations such as pesticides, and metals such as nickel.

The traditional test methods for detecting ACD hazard potential of chemicals use guinea pigs. Based on a 1998 evaluation (ICCVAM 1999), ICCVAM recommended the murine local lymph node assay (LLNA) as a valid alternative to guinea pig tests. The LLNA eliminates pain and distress experienced by the test animal, requires less time to perform, uses fewer animals, and provides dose–response information. More recently, ICCVAM evaluated new applications and versions of the LLNA that should promote more widespread use.

While the LLNA has advantages over guinea pig methods, there is a growing international need for nonanimal test methods to identify skin sensitizers. ICCVAM is developing a strategy to evaluate new test methods for identifying potential skin sensitizers, with a focus on methods that avoid the use of animals.

ICCVAM and ICCVAM Agency Activities

- **ICCVAM:** In 2011, ICCVAM recommended the LLNA to Federal agencies for use as a screening test to identify substances that should be categorized as strong skin sensitizers (substances considered to have a significant potential for causing hypersensitivity). However, substances not identified as strong sensitizers by the LLNA require additional study. In early 2012, ICCVAM member agencies responded that they accepted or endorsed the ICCVAM recommendations.

A summary of the ICCVAM recommendations on using the LLNA for potency categorization and agency responses are available at <http://ntp.niehs.nih.gov/go/40447>.

- **ICCVAM:** In 2012, ICCVAM received a nomination of the electrophilic allergen screening assay (EASA) for the identification of potential skin sensitizers. The test method developers at the National Institute for Occupational Safety and Health proposed collaborations with NICEATM to conduct validation studies on the EASA. NICEATM conducted a preliminary evaluation of the EASA and concluded that, based on the information provided by the test method developer and consideration of the ICCVAM prioritization criteria, the nomination should be selected for the proposed studies. The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) concurred with NICEATM's assessment at their 2012 meeting. ICCVAM is considering the EASA nomination in the context of ongoing international evaluations of test method approaches that replace animal use in skin sensitization testing (see following item).
- **ICCVAM:** In 2013, ICCVAM announced that it would develop a U.S. plan for the evaluation of alternative skin sensitization test methods and testing strategies. Activities proposed as part of the plan included collaboration with international partners to support ongoing development and validation of *in vitro* skin sensitization



test methods; evaluation of alternative test method and testing strategy submissions; and promotion of validated methods through workshops, webinars, and guidance documents. In addition to discussing further development and evaluation of alternative [skin sensitization](#) test methods and testing strategies at the September 2013 SACATM meeting, ICCVAM also solicited public comments on the plan via a *Federal Register* notice (78 FR 68076). The newly established ICCVAM Skin Sensitization Working Group is considering comments received in response to the FR notice and will advance recommendations for appropriate ICCVAM activities for the next several years. More information about the ICCVAM plan to evaluate alternative [skin sensitization](#) test methods is available at <http://ntp.niehs.nih.gov/go/40498>.

- **Consumer Product Safety Commission (CPSC):** In 2013, the CPSC issued guidance to clarify the definition of “strong sensitizer” as the term applies to substances and products it regulates. The document helps chemical manufacturers understand how the CPSC determines that products are strong sensitizers and thus require cautionary labeling under the Federal Hazardous Substances Act. The guidance describes the types of data CPSC considers in making those determinations and the available methods for generating such data. Of primary importance are data from “well-controlled [human] clinical and diagnostic studies” and epidemiological studies; data from “well-conducted *in vitro* test studies” may also be considered and are weighted more heavily than case studies.

The CPSC also issued a proposal to update the supplemental definition of “strong sensitizer” and related definitions under the Federal Hazardous Substances Act. As stated in the announcement, the goals of the updated definitions are to

“eliminate redundancy, remove certain subjective factors, incorporate new and anticipated technology, rank the criteria for classification of strong sensitizers in order of importance, define criteria for ‘severity of reaction,’ and indicate that a [weight-of-evidence approach](#) will be used to determine the strength of the sensitizer.”

The CPSC will issue a final rule updating the Federal Hazardous Substances Act in early 2014.

- **CPSC, FDA, and NIEHS:** EURL ECVAM conducted a study from 2009 to 2012 to evaluate three *in vitro* test methods proposed for use in an integrated approach to identify potential [skin sensitization](#) hazards. Scientists

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.

Bayesian network: a graphical model created to explore probabilistic relationships among variables of interest

Immunotoxicity: adverse effects caused by a chemical or substance (an “immunotoxicant”) that disrupts the normal function of the immune system

Integrated testing strategy: a testing strategy that considers all available relevant information about a substance to determine the appropriate hazard classification

Ontology: a vocabulary of defined names and terms relevant to a specific topic area (in this case, identification of potential skin sensitizers)

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

from CPSC, FDA, and NICEATM participated on the EURL ECVAM management team for the study.

EURL ECVAM finalized its test method recommendations for the direct peptide reactivity assay in 2013. Draft recommendations on the human cell line activation test will be issued in early 2014. Both methods are expected to be suitable for use in a [weight-of-evidence approach](#) to identify potential skin sensitizers. The protocol for the myeloid U937 [skin sensitization](#) test requires additional development to improve the interlaboratory reproducibility.

Test method recommendations issued by EURL ECVAM are available at <http://ihcp.jrc.ec.europa.eu/our-labs/eurl-ecvam/eurl-ecvam-recommendations>.

- **NIEHS:** Scientists within NICEATM and NIEHS are collaborating with scientists at the Procter & Gamble Company (P&G) to develop an approach that can identify potential skin sensitizers and characterize [skin sensitization potency](#) without conducting animal tests.

P&G scientists developed an [integrated testing strategy](#) (Jaworska et al. 2011, 2013) that uses a [Bayesian network](#) to analyze all available relevant substance information, including nonanimal tests, *in silico* models, and other information such as chemical structure and solubility, to produce a numerical probability of [skin sensitization potency](#). Using the available information, the [Bayesian network](#) can indicate the subsequent test that will best inform the [skin sensitization potency](#) prediction of a substance. The calculated probability could be used to make a hazard labeling decision without animal testing.

The software used by P&G for these analyses is patented. The 2013 collaboration between the NIEHS and P&G was initiated to develop an open-source tool using free, publicly available software to make the [integrated testing strategy](#) approach more widely available. The open-source [integrated testing strategy](#) will be described in a short communication to the journal *ALTEX* in 2014. Downloadable files to run the analysis, along with documentation and sample data, are available at <http://ntp.niehs.nih.gov/go/its>. A user community and listserv are being formed to encourage collaboration on improvement and refinement of integrated testing strategies for skin sensitizers.

- **NIEHS and EPA:** During 2013, NICEATM scientists worked with immunotoxicologists from EPA, the NIEHS Division of the National Toxicology Program (DNTP), and other academic institutions and international organizations to create a standardized [ontology](#) for *in vivo* [immunotoxicity](#) data. Using the standardized [ontology](#), NICEATM is working with EPA to create a comprehensive database consisting of high-quality data from *in vivo* testing of potential immunotoxicants. This database will support the validation of high-throughput *in vitro* test methods for prediction of [allergic contact dermatitis](#) and other immunotoxic effects.

The initial literature review for the database focused on 52 reference chemicals selected by the EPA and NIEHS/DNTP. Studies are being selected that include relevant endpoints (thymus weight, spleen weight, skin and respiratory sensitization, etc.) for these chemicals. NICEATM is collaborating with EPA and DNTP scientists to analyze high-throughput screening data for these chemicals from the ToxCast and Tox21 testing programs and to identify small organism model systems such as zebrafish for followup testing of potential immunotoxicants.

- **EPA:** Scientists in the EPA National Health and Environmental Effects Research Laboratory developed a refined version of the LLNA that eliminates the injection of animals and the use of radioisotopes. The assay accurately predicted the [skin sensitization potential](#) of a small group of known sensitizers and nonsensitizers (Williams et al. 2014). These results lay the foundation for the additional assay development and evaluation needed for acceptance of this test for regulatory purposes.

DEFINITIONS OF KEY TERMS

Skin sensitization potential: the likelihood that a substance may cause skin sensitization; also referred to as “ACD hazard potential”

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

ENDOCRINE DISRUPTOR TESTING

The endocrine system is one of the body's main communication networks. Hormones produced by glands throughout the body act as chemical messengers to control a variety of body functions. Examples of endocrine hormones include [estrogens](#), androgens, and thyroid hormones.

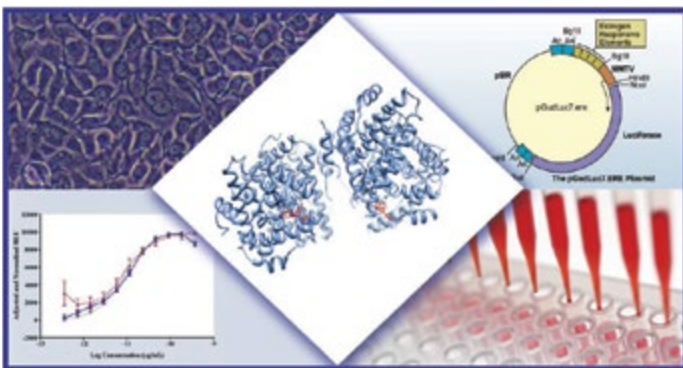
[Endocrine disruptors](#) include a wide range of compounds that interfere with normal hormone function by mimicking or blocking their action and may thereby cause adverse health effects. Evidence suggests that environmental exposure to [endocrine disruptors](#) may cause reproductive and developmental problems in animals; the effect of [endocrine disruptors](#) in humans is less clear.

Congress passed the Food Quality Protection Act (7 U.S.C. 136) in 1996, which directed the EPA to screen pesticides and other substances for their potential to affect the endocrine systems of humans. The EPA subsequently initiated the Endocrine Disruptor Screening Program (EDSP) and began efforts to standardize and validate test methods to include in the program. In support of these efforts, ICCVAM evaluated the [validation](#) status of *in vitro* test methods to identify potential [endocrine disruptors](#) and sponsored [validation](#) studies on test methods for this purpose.

ICCVAM agencies are currently exploring how high-throughput screening approaches can be used to identify potential [endocrine disruptors](#) without using animals.



ICCVAM and ICCVAM Agency Activities



- **ICCVAM:** In 2012, ICCVAM made recommendations to Federal agencies on the use of the *in vitro* BG1Luc [estrogen receptor \(ER\)](#) transactivation (TA) test method, developed by XDS, Inc., as an initial screen for identifying substances with the potential to induce or inhibit activation of the [ER](#). These recommendations were made on the basis of data from a NICEATM-sponsored international [validation](#) study of the BG1Luc ER TA test method, completed in 2010.

In response to the ICCVAM recommendations, the EPA accepted the BG1Luc ER TA test method as an alternative to the ER TA test method currently used in the EDSP.

Several other Federal agencies indicated that they would communicate the ICCVAM recommendations to stakeholders and encourage appropriate use of the recommended method.

More information about the NICEATM-sponsored [validation](#) study, the ICCVAM recommendations, and the agency responses can be found at <http://ntp.niehs.nih.gov/go/40416>.

- **ICCVAM:** NICEATM coordinated an international interlaboratory [validation](#) study of a MCF-7 [cell proliferation](#) test method for the detection of estrogenic activity. CertiChem, Inc., the test method developer, nominated the MCF-7 [cell proliferation](#) test method for a [validation](#) study in response to an ICCVAM call for nominations of *in vitro* test methods to identify potential [endocrine disruptors](#).

DEFINITIONS OF KEY TERMS

Bioconcentration potential: the likelihood of a substance to reach a higher concentration in the tissues of an exposed animal than would be observed in the surrounding environment

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

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

ER agonist: a substance that increases activity of the estrogen receptor

ER antagonist: a substance that decreases activity of the estrogen receptor

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

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

Ontology: a vocabulary of defined names and terms relevant to a specific topic area (in this context, testing of chemicals for endocrine disruptor activity)

The MCF-7 [cell proliferation](#) test method [validation](#) study, completed in 2011, was jointly sponsored by ICCVAM, the Japanese Center for the Validation of Alternative Methods, and the Korean Center for the Validation of Alternative Methods, and included laboratories located in each of the sponsoring countries. Although the accuracy of the [ER agonist](#) protocol was high at the U.S. laboratory and sufficient in the partner labs, interlaboratory reproducibility was considered inadequate. The test method protocols, especially the antagonist protocol, require additional development to enhance interlaboratory reproducibility before this method can be used for the EDSP or other regulatory applications.

A [validation](#) study report was prepared in 2012 and is available at <http://ntp.niehs.nih.gov/iccvam/methods/endocrine/MCF7/MCF7-ValStudyReport-19Jun12-WCv2-draft.pdf>.

- **NIEHS and NIH:** NICEATM nominated the BG1Luc ER TA test method for use in the Tox21 high-throughput screening program. The assay was adapted to a high-throughput format using 1536-well plates by the NIH National Center for Advancing Translational Sciences (NCATS) and used to screen the Tox21 10K chemical library. NICEATM analyzed the results and determined that the high-throughput BG1Luc ER TA test method performed well when compared against the validated manual method. A poster describing the NICEATM analysis (<http://ntp.niehs.nih.gov/iccvam/meetings/TERA-EDSP-2013/TERA-EDSP-Ceger-BG1.pdf>) was presented in 2013 at the workshop “Lessons Learned, Challenges, and Opportunities: The U.S. Endocrine Disruptor Screening Program.”
- **NIEHS:** NICEATM compared data from the BG1Luc ER TA test method to an ER β -lactamase (ER-Bla) method already being used in the Tox21 program. The ER-Bla method differs from the BG1Luc ER TA method in that the cell line used includes a partial ER receptor that contains only the ligand-binding site of the ER. Results from the BG1Luc ER TA and ER-Bla were compared both to each other and to reference data. The study found that the two methods produced data of acceptable quality and results that agreed mostly but not completely with each other and with the reference data. Understanding the factors contributing to differences in performance of these assays is critical to their regulatory acceptance and utilization.

A poster on this project will be presented at the 2014 Annual Meeting of the Society of Toxicology and can be found at <http://ntp.niehs.nih.gov/go/41297>.
- **NIEHS and EPA:** High-throughput screening and computational toxicology tools can quickly and cost-effectively identify and prioritize potential [endocrine](#)

DEFINITIONS OF KEY TERMS

Reverse toxicokinetic model: a mathematical model created to estimate the amount of a toxic substance an animal or human was exposed to based on measurements such as concentration of the substance or its metabolites in blood or urine

Validation: a process by which the reliability and relevance of a test method are established for its intended application

[disruptors](#) for further testing. In 2013, NICEATM began creating a comprehensive database of high-quality *in vivo* testing data on 52 chemicals selected by the EPA and DNTP to support future [validation](#) of high-throughput *in vitro* test methods and *in silico* models of estrogenic activity. Studies selected included data for a number of different endpoints that indicate estrogenic activity. Information from references is being extracted and compiled using a standardized [ontology](#). A semiautomated graphical user interface is being developed to evaluate the quality of the data in an efficient and standardized manner according to modified Klimisch criteria (Schneider et al. 2009). The NICEATM database will be made available on the NTP website.

Other ongoing NICEATM activities initiated in 2013 to support risk-based chemical testing prioritization include construction of [reverse toxicokinetic models](#) to assist *in vitro* to *in vivo* extrapolation and development of approaches to estimate [bioconcentration potential](#) and exposure.

Posters on these projects will be presented at the 2014 Annual Meeting of the Society of Toxicology and can be found at <http://ntp.niehs.nih.gov/go/41297>.



PYROGEN TESTING

Pyrogens are substances that can cause inflammation and fever when introduced into the body via injectable drugs or implanted medical devices. Contamination with bacteria, fungi, and viruses can introduce pyrogens into these products, and the pyrogens may persist even after the contaminating organisms are killed. The inflammatory reaction to pyrogens can be severe and sometimes fatal.

Regulatory agencies require testing of medical devices and pharmaceutical products administered by injection before they are sold to demonstrate that they are free of pyrogen contamination. One test method for this purpose, the rabbit pyrogen test, measures body temperature in rabbits after intravenous injection of a test solution. Use of rabbits for pyrogen testing substantially decreased with the acceptance of the *Limulus* amoebocyte lysate (LAL) test by the FDA in the 1980s. The LAL test is an *in vitro* test method that measures coagulation of a blood-like substance from the horseshoe crab. Other *in vitro* methods are needed, however, for cases in which the LAL test is not appropriate.

Alternative *in vitro* test systems based on the activation of specific human blood cells or cell lines derived from these cells take advantage of the role of these cells in the fever response. 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 Agency Activities

- **FDA:** In 2012, the FDA issued “Guidance for Industry: Pyrogens and Endotoxin Testing: Questions and Answers.” This document (<http://www.fda.gov/drugs/guidancecomplianceregulatoryinformation/guidances/ucm314718.htm>) is addressed to biological product, drug, and device manufacturers and is meant to clarify FDA’s current position on pyrogen testing and acceptance criteria. The guidance discusses approaches, such as pooling samples for testing, that could reduce animal use, and states that *in vitro* monocyte activation pyrogen tests may be used in lieu of the rabbit pyrogen test or the LAL test given appropriate product-specific validation.

A monocyte activation test was nominated to ICCVAM in 2011 for evaluation of its usefulness in detecting nonendotoxin pyrogens. ICCVAM determined that the monocyte activation test was addressed under the FDA’s guidance, and a separate evaluation by ICCVAM was not necessary.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY TESTING

Pesticides, food additives, drugs, and other substances are tested for their potential to cause reproductive or developmental toxicity. Reproductive toxicity tests assess a substance's tendency to cause reproductive system effects, while developmental toxicity testing evaluates the extent to which exposure to a substance may harm a developing embryo or fetus.

Reproductive and developmental toxicity tests are required by multiple regulatory agencies and can use large numbers of animals. The complexity of these endpoints makes it unlikely that any single alternative test method will serve all regulatory needs. ICCVAM agencies such as the FDA are working with industry partners to explore alternative tests that can be used in combination to provide the information needed to make accurate developmental and reproductive safety assessments.



ICCVAM Agency Activities

- **FDA, NIH, and NIEHS:** In April 2012, the FDA convened a workshop titled “Reproductive and Developmental Toxicology: From *In Vivo* to *In Vitro*.” ICCVAM member agencies NIH and NIEHS were among the cosponsors of the workshop.

Speakers from the FDA discussed the regulatory requirements for reproductive and developmental toxicity testing and the challenges involved in implementing alternative approaches that meet regulatory needs. Pharmaceutical industry representatives presented approaches using genomics, cell culture, computational tools, and nonmammalian animal models that are currently being explored to address these challenges. Panel discussions addressed the strengths, weaknesses, and validation of these novel approaches and what steps are needed for implementation.

An archived webcast and other materials from the workshop are available at <http://www.fda.gov/scienceresearch/aboutscienceresearchatfda/ucm305348.htm>.

Issues discussed at the 2012 workshop will be addressed by an ICH expert working group to evaluate the use of batteries of *in vitro* assays for assessment of embryofetal toxicity.



PHOTOSAFETY TESTING

Photosafety testing is conducted to determine if a substance reacts with light to cause a skin reaction. For example, drugs such as tetracycline antibiotics and sulfonamides can cause skin irritation in response to light, even when taken by mouth. Traditionally, animals have been used to evaluate photosafety. ICCVAM agencies such as the FDA are exploring the use of accepted alternative tests to reduce or replace animal use for photosafety testing.

ICCVAM Agency Activities

- **FDA:** In 2013, “ICH Harmonised Tripartite Guideline: Photosafety Evaluation of Pharmaceuticals S10” was developed by FDA regulators and collaborators in the ICH. The guidance (<http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html>) discusses *in vitro* test methods for photosafety assessment that can reduce or replace animal use.

ECOTOXICITY TESTING

[Ecotoxicity testing](#) refers both to the assessment of chemical effects on fish, birds, or other wild organisms and the testing of soil, sediment, or effluents for the presence of toxic compounds. To fulfill their mandates to protect the environment, several ICCVAM member agencies, including the Department of the Interior (DOI) and the EPA, require manufacturers of pesticides and other chemical products to conduct [ecotoxicity testing](#).

[Ecotoxicity testing](#) can require animal testing using either the species of interest or animals representative of the species of interest. ICCVAM member agencies are exploring ways to reduce or replace animal use for ecotoxicity studies.

ICCVAM Agency Activities

- **DOI:** In 2013, the U.S. Fish and Wildlife Service revised the existing protocol for the evaluation and approval of nontoxic replacements for lead shot (as specified in 50 CFR 20.134) for hunting of migratory birds. A March 2013 *Federal Register* notice (78 FR 14060) described the revised protocol and requested public comment. Comments received on the revised protocol suggested reducing the number of test subjects (mallard ducks, *Anas platyrhynchos*) required for testing tiers 2 and 3. The final rule, issued in December 2013 (78 FR 78275) made a number of revisions to the regulation, including a requirement that manufacturers conduct *in vitro* testing in tier 1 of the approval process, which may decrease the need for the *in vivo* testing required in tiers 2 and 3.
- **DOI:** [Anticoagulant rodenticides](#) are used throughout the world for control of rodent pests. However, there is growing concern about their effects on nontarget wildlife species. In 2013, the U.S. Geological Survey (USGS) submitted a proposal to the OECD for development of an [adverse outcome pathway](#) for poisoning of nontarget wildlife by [anticoagulant rodenticides](#). Development of the [adverse outcome pathway](#) will facilitate risk assessments and help to better define the mechanism by which [anticoagulant rodenticides](#) cause toxicity in nontarget species. This activity may inform predictions of comparative sensitivity among exposed species, as well as encourage generation of relative potency information for the [anticoagulant rodenticides](#).
- **DOI:** In 2013, the USGS initiated new studies to increase the screening and testing efficiency for candidate [fishery management chemicals](#) using a three-tiered approach.
 - A fish [toxicant](#) database is being developed that will include data from the EPA ECOTOX Database, the Pesticide Action Network Database, and the National Library of Medicine (NLM) ChemIDplus Advanced website. [Physicochemical data](#) on available fish [toxicants](#) were analyzed to identify [structure–activity relationships](#) and trends and common features of the [toxicants](#). This database will be accessible on the web in 2014.
 - Use of *in vitro* fish cell lines from target and nontarget species is being investigated to replace *in vivo* testing as a screening tool for the identification of candidate [toxicants](#).
 - Genomic analysis is being used to assess the molecular responses of resistant and nonresistant fish to fish [toxicants](#). The initial focus is on two registered fish [toxicants](#), rotenone and antimycin-A, but the



DEFINITIONS OF KEY TERMS

Adverse outcome pathway: a conceptual framework constructed from existing knowledge that relates exposure of a type of toxic substance to subsequent steps that result in illness or injury

Anticoagulant rodenticides: chemicals that inhibit blood clotting that are sold for the purpose of killing rodents

Biomarker: a biological molecule found in blood, other body fluid, or tissues that can be measured and that may provide a sign of toxicity or disease

Ecotoxicity testing: refers both to the assessment of chemical effects on fish, birds, or other wild organisms and testing of soil, sediment, or effluents for the presence of toxic compounds

Fishery management chemicals: toxicants or other chemicals added to an aquatic or marine ecosystem to achieve a specific objective, such as increasing or decreasing the population of a particular fish species

Physicochemical data: data related to the physical or chemical properties of a substance

Structure–activity relationship: a model that relates the physical and chemical properties of a substance to its biological activity

Toxicant: a toxic or poisonous substance

project will be expanded as additional candidates are identified from the [toxicant](#) database. Once resistance [biomarkers](#) have been identified, resource managers can use them to help determine the appropriate [toxicant](#) to be used for a specific application and fish population, minimizing the need for repetitive *in vivo* testing.

- **DOI:** Scientists of the USGS routinely seek out and use alternative methods that reduce animal use or replace animals with *in vitro* methods. Examples include:
 - Yeast and cell culture assays for screening environmental sample extracts and individual chemicals (e.g., endocrine disruptors) for their potency and toxicity
 - *In vitro* reporter gene assays and transgenic zebrafish strains to screen water samples for endocrine activity, including samples from sites near hydraulic fracturing operations
 - The up-and-down procedure (EPA 2012; OECD 2008) in upcoming work on the effects of [anticoagulant rodenticides](#) in triggerfish to minimize the number of test subjects

RESEARCH AND DEVELOPMENT ACTIVITIES SUPPORTING ALTERNATIVE METHODS DEVELOPMENT

ICCVAM member agencies work to promote the regulatory acceptance of new, scientifically valid toxicological tests that protect human and animal health and the environment while replacing, reducing, or refining animal tests. To achieve this goal, many ICCVAM member agencies engage in research activities that focus both on developing new test methods and exploring new technologies that may support future test method development. Effective translation of technological advances into new test methods should allow better protection of public health while addressing animal use and welfare concerns.



ICCVAM Agency Activities

- NIEHS, NIH, EPA, and FDA:** The interagency Tox21 research initiative uses *in vitro* [high-throughput screens](#) to test a broad variety of substances and considers data from those screens collectively to assess effects on biological pathways related to toxicity. Data from Tox21 testing will be used to develop a better understanding of these toxicity or “adverse outcome” pathways, enabling the eventual use of *in vitro* assay data to predict the adverse effects of chemical exposures *in vivo*. The goals of Tox21 are to efficiently prioritize chemicals for *in vivo* testing and to use results from validated HTS assays to identify endpoints for targeted *in vivo* testing.

In 2013, the NTP solicited nominations of [environmentally responsive genes](#) to be screened using mid- to [high-throughput](#) targeted [transcriptomics](#) platforms in the Tox21 program, with the goals of better understanding the effect of chemical exposure on gene regulation and transcription and supporting [biomarker](#) development. The NTP also solicited nominations of *in vitro* assays and assays using lower organisms that might be used to prioritize substances for *in vivo* neurotoxicity and *in vivo* carcinogenicity testing.

The OECD Adverse Outcome Pathway (AOP) Development Programme represents a key resource to support discovery of new *in vitro* test methods. Data from Tox21 projects contributed to the development of [adverse outcome pathways](#) for several toxicities such as embryonic vascular disruption leading to adverse prenatal outcomes or neurodevelopmental toxicity. OECD accepted these [adverse outcome pathways](#) into the current AOP Development Programme workplan (<http://www.oecd.org/env/ehs/testing/listsofprojectsontheaopdevelopmentprogrammeworkplan.htm>). Data from Tox21 projects will also help prioritize chemical testing for these toxicities.



DEFINITIONS OF KEY TERMS

Adverse outcome pathway: a

conceptual framework constructed from existing knowledge that relates exposure to a type of toxic substance to subsequent steps that result in illness or injury

Biomarker: a biological molecule found

in blood, other body fluid, or tissues that can be measured to provide a sign of toxicity or disease

Cytotoxic: describing a chemical or condition that kills cells

Environmentally responsive genes:

genes that exhibit changes in expression or activity in response to environmental changes or stressors

Genomic: referring to the hereditary information of an organism encoded in its DNA

High-throughput screening: a

practice that uses robotics, liquid-handling devices, detectors, and associated software to quickly conduct a large number of chemical or biochemical tests

Transcriptomics: referring to the set of all transcripts in one or a population of cells for a given set of environmental circumstances; a characterization of gene expression

Validation: a process by which the reliability and relevance of a test method are established for its intended application

More information about Tox21 can be found on participating agencies' websites:

- **NIEHS/NTP:** <http://ntp.niehs.nih.gov/go/tox21>
 - **NIH/NCATS:** <http://www.ncats.nih.gov/research/reengineering/tox21/tox21.html>
 - **EPA/National Center for Computational Toxicology:** <http://www.epa.gov/ncct/Tox21/>
- **NIEHS and NIH:** Genetic variation among individuals can influence [cytotoxic](#) response in human cells following chemical exposure. To better understand the effects of genetic variation, NIEHS and NCATS teamed with the University of North Carolina, Sage Bionetworks, and DREAM (Dialogue for Reverse Engineering Assessments and Methods) to sponsor the NIEHS-NCATS-UNC-DREAM Toxicogenetics Challenge (<http://www.niehs.nih.gov/funding/challenges/index.cfm>). This crowdsourced computational project provided participants with testing data from cell lines that had been treated with 156 drugs and environmental chemicals. The 884 human cell lines represented nine geographically distinct human subpopulations. Participants were asked to use the data to develop models that would accurately predict either individual responses to compound exposure based on [genomic](#) information or population-specific responses to certain types of chemicals.

The Challenge was launched in June 2013 and closed in November 2013. The winning teams, both from the University of Texas Southwestern Medical Center, presented their prediction models at the Sixth Annual RECOMB/ISCB Conference on Regulatory and Systems Genomics in November 2013. Dr. Hao Tang, leader of the team that won the chemical-focused challenge, presented the team's findings and lessons learned in a seminar at NIEHS in December 2013.

- **NIH and FDA:** Many promising medications fail in human clinical trials because they are either not effective or exhibit some unforeseen toxicity. These failures occur even when prior studies in animal models suggest that the medication will be safe and effective in humans. Other potentially promising drugs never enter clinical trials because animal models predict that they will be toxic or ineffective. Tissue chips incorporating human cells from multiple organs may more accurately predict drug efficacy or toxicity, thereby improving the selection of clinical trial candidate drugs.

In 2012, NIH/NCATS awarded grants to support development of three-dimensional chips with living cells and tissues that accurately model the structure and

function of human organs such as the lung, liver, and heart (<http://www.ncats.nih.gov/research/reengineering/tissue-chip/tissue-chip.html>). Once developed, these tissue chips will be evaluated with compounds of known human safety or toxicity. Data from these evaluations should help to identify reliable drug safety signals and support development of faster, more cost-effective, and more accurate predictive drug development approaches. The FDA will help explore how this new technology might be used to assess drug safety prior to first-in-human studies.

- **NIH:** A series of workshops focused on “Validation and Qualification of New *In Vitro* Tools and Models for the Pre-clinical Drug Discovery Process” is being organized by the American Institute for Medical and Biological Engineering and cosponsored by the National Institute of Biomedical Imaging and Bioengineering. ICCVAM member Dr. Christine Kelley (NIH/National Institute of Biomedical Imaging and Bioengineering) is a co-chair of the workshop series (<http://www.nibib.nih.gov/news-events/meetings-events/fourth-aimbenih-workshop-validation-and-qualification-new-vitro-tools>).

The first three workshops were held in March and September 2012 and March 2013. A fourth workshop (March 2014) will build on the results and recommendations of the first three workshops and begin to develop guidelines for the [validation](#) and qualification of new model systems for the preclinical drug discovery process. Specific emphasis will be on model systems that may augment or replace existing models, especially animal models, in the FDA drug approval process.



OTHER ICCVAM AGENCY ACTIVITIES PROMOTING ALTERNATIVE METHODS

- **CPSC:** A rule published by the CPSC in December 2012 codified the animal testing policy statement that provides guidance regarding replacement, reduction, and refinement of animals to manufacturers of products subject to the Federal Hazardous Substances Act (FHSA). Codification of this policy is intended to make CPSC's animal testing policy and ICCVAM test method recommendations accepted by CPSC more transparent and accessible to interested parties. The CPSC also amended its regulations on animal testing methods under the FHSA.



The FHSA (15 U.S.C. 1261–1278) requires that certain hazardous household products be appropriately labeled to alert consumers to their potential hazards. The changes to the FHSA clarified the criteria used for classification of substances as “highly toxic,” “toxic,” “corrosive,” “irritant,” “primary irritant,” and “eye irritant.” The changes emphasize that the use of prior human experience and *in vitro* and other alternative test methods, including weight-of-evidence approaches, are recommended over *in vivo* animal tests wherever possible. Furthermore, the CPSC reiterates its preference for reliable human experience over animal test data.

CPSC also established a page on its website (<http://www.cpsc.gov/en/Business--Manufacturing/Testing-Certification/Recommended-Procedures-Regarding-the-CPSCs-Policy-on-Animal-Testing/>) regarding ICCVAM recommendations and new developments in test methods that avoid or further reduce or refine animal testing.

- **FDA:** The FDA participates in the International Cooperation on Cosmetics Regulation (ICCR), a body of cosmetics regulatory authorities that aims to maintain the highest level of global consumer protection while minimizing barriers to trade. In 2012, the ICCR issued the report “Applicability of Animal Testing in Regulatory Frameworks Within ICCR Regions.” The report “provides an overview of processes and mechanisms for the use of alternatives in human safety assessments of cosmetic products and ingredients in the four ICCR jurisdictions.” (<http://www.fda.gov/downloads/cosmetics/internationalactivities/iccr/ucm386297.pdf>)
- **NLM:** In 2012, the NLM launched an update of its Alternatives to Animal Testing (ALTBIB) portal (<http://toxnet.nlm.nih.gov/altbib.html>). ALTBIB provides access to PubMed®/MEDLINE® citations relevant to alternatives to the use of live vertebrates in biomedical research and testing.

The ALTBIB topics and subtopics are aligned with current approaches ([see sidebar page 40](#)). For example, information is provided on *in silico*, *in vitro*, and refined or improved animal testing methods. Strategies that incorporate validated methods and other approaches are also covered. In addition to the topic areas for PubMed searches, the ALTBIB portal includes a searchable bibliographic collection of alternatives to animal testing, including citations from published articles, books, book chapters, and technical reports published from 1980 to 2000. Many citations provide access to free full text. ALTBIB also has an extensive collection of links to key organizations providing information on alternatives to animal testing, and provides access to animal alternatives news sources.

ALTBIB TOPIC AREA SEARCHES

General Topics on Alternatives to Animal Testing

Hepatic/Renal Toxicity

Pulmonary Toxicity

Biologics and Vaccines

Immunotoxicity/Immunology

Carcinogenesis

Quantitative Structure Activity Relationship (QSAR)

Neurotoxicity

Reproductive and Developmental Toxicity

Cytotoxicity

Ocular Toxicity

Skin Toxicity

Ecotoxicity

Pharmacokinetic/Mechanistic Studies

Welfare (Animal)

Genotoxicity

Pyrogenicity

- **EPA:** In 2012, the EPA Office of Pesticide Programs issued “Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests for Pesticides and Pesticide Products.” This document (<http://www.epa.gov/pesticides/science/acute-data-waiver-guidance.pdf>) consolidated guidance from various sources on waivers for acute toxicity testing and criteria for bridging acute toxicity data, providing a single source for this information. Application of these waivers or bridging criteria could potentially reduce animal use for acute systemic toxicity testing; eye irritation and corrosion testing; and skin irritation, corrosion, and sensitization testing.
- **EPA:** In 2013, the EPA Office of Pesticide Programs updated its webpage on “Test Guidelines for Data Requirements.” This page (<http://www.epa.gov/pesticides/science/guidelines.htm>) summarizes the safety requirements addressed by pesticide testing and EPA’s efforts to harmonize test guidelines and minimize animal use. A number of key documents relevant to these topics are linked to this page. Two of these were issued in 2013:
 - “Guiding Principles for Data Requirements”: Federal regulations (40 CFR 158) describe toxicity data for certain types of pesticide ingredients that the EPA requires for adequate protection of public health and the environment. “Guiding Principles for Data Requirements” (<http://www.epa.gov/pesticides/regulating/data-require-guide-principle.pdf>) is intended to guide the identification of data needs and reduce unnecessary testing.
 - “Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal, and Immunotoxicity Studies”: This document (<http://www.epa.gov/pesticides/regulating/part158-tox-data-requirement.pdf>) describes circumstances under which waivers can be granted for certain types of tests covered under the “Part 158 data requirements,” thus reducing the need for animal testing.



3

Outreach and Collaborative Activities

INTERNATIONAL COOPERATION ON ALTERNATIVE TEST METHODS

The International Cooperation on Alternative Test Methods (ICATM) was established in 2009 to promote consistent and enhanced voluntary international cooperation, collaboration, and communication among national validation organizations. The goals of ICATM are to:

- Ensure optimal design and conduct of validation studies
- Ensure high-quality independent scientific peer reviews of alternative test methods
- Ensure consistent and transparent stakeholder involvement
- Achieve greater efficiency and effectiveness by internationally leveraging limited resources and avoiding duplication of effort
- Support the timely international adoption of alternative test methods



This cooperation enables scientifically valid alternative methods or strategies to be more readily accepted worldwide for regulatory use.

ICATM currently includes member organizations from the European Union, United States, Japan, Canada, and South Korea (see sidebar).

ICATM coordination meetings take place several times a year and provide an opportunity for the five organizations to discuss activities in the major areas of cooperation. Regular interactions allow the ICATM partners to develop good communications and working relationships in support of collaborative test method development. ICCVAM representatives participated in six ICATM coordination meetings in 2012 and 2013.

PARTICIPANT ORGANIZATIONS: INTERNATIONAL COOPERATION ON 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.
- **EURL ECVAM** (European Union Reference Laboratory for Alternatives to Animal Testing) is a unit within the Institute of Health and Consumer Protection in the European Union's Joint Research Centre. EURL ECVAM coordinates the validation of alternative test methods in the European Union.
- **JaCVAM** (Japanese Center for the Validation of Alternative Methods) coordinates the evaluation of alternative test methods for the Japanese National Institute of Health Sciences, its parent organization.
- Health Canada's **Environmental Health Science and Research Bureau** coordinates the evaluation of alternative test methods in Canada.
- **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.

COLLABORATIONS WITH INTERNATIONAL VALIDATION ORGANIZATIONS

ICCVAM interacted with EURL ECVAM, JaCVAM, Health Canada, or KoCVAM in the following activities:

- NICEATM and ICCVAM collaborated with EURL ECVAM, JaCVAM, and KoCVAM in conducting international validation studies. ([See sidebar below](#) for list)
- Representatives from EURL ECVAM, JaCVAM, KoCVAM, and Health Canada participated in the 2012 meeting of the Scientific Advisory Committee on Alternative Toxicological Methods as nonvoting liaison members.
- The Director of NICEATM participated in the March 2012 meeting of the EURL ECVAM Scientific Advisory Committee as an official observer.
- The Acting Director of NICEATM participated in a meeting of the JaCVAM advisory council in February 2013.
- EURL 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.
- ICCVAM nominated experts to participate on EURL ECVAM Scientific Advisory Committee peer reviews for the 3T3 neutral red uptake assay for the identification of chemicals that do not require hazard classification for acute oral toxicity and for the KeratinoSens (Givaudan SH) assay for identification of sensitizers.
- ICATM partner organizations cosponsored the September 2012 “International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing” and the November 2012 “International Workshop on Alternatives to the Murine Histamine Sensitization Test for Acellular Pertussis Vaccines.”

INTERNATIONAL COOPERATION ON VALIDATION STUDIES

NICEATM or ICCVAM scientists participated on the management teams for the following validation studies coordinated by international partners during 2012 and 2013.

Eye Safety Testing

- Validation study on the rabbit cornea derived cell line (SIRC-CVS) assay for identification of ocular irritants (coordinated by JaCVAM)
- Validation study on the Vitrigel-EIT method for identifying potential eye irritants (coordinated by JaCVAM)

Acute Systemic Toxicity Testing

- International validation study in the field of toxicokinetics and metabolism: human cryopreserved HepaRG and cryopreserved hepatocytes cytochrome P450 induction test methods (coordinated by EURL ECVAM)

Immunotoxicity Testing: Allergic Contact Dermatitis

- Evaluation of *in vitro* tests for assessing skin sensitization potential of chemicals (coordinated by EURL ECVAM)
- Validation study of the IL-8 luciferase skin sensitization assay (coordinated by JaCVAM)

Developmental Toxicity Testing

- Validation study on the Hand-1 luciferase assay for identification of substances with potential embryotoxicity (coordinated by JaCVAM)

ICCVAM CONTRIBUTIONS TO OECD TEST GUIDELINES AND GUIDANCE DOCUMENTS



During 2012 and 2013, ICCVAM member agencies 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.

ICCVAM member Dr. Christine Olinger of the Environmental Protection Agency (EPA) serves as the U.S. National Coordinator for the OECD Test Guidelines Programme. In that role, she solicits and collates U.S. comments on draft test guidelines and other documents of the Test Guidelines Programme. Dr. Olinger represents the United States at the annual meeting of the National Coordinators of the Test Guidelines Programme and in other test guideline development activities.

MEETINGS OF THE SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) is a federally chartered advisory group that advises NICEATM, ICCVAM, and the Director of the National Institute of Environmental Health Sciences (NIEHS) about ICCVAM activities. SACATM held public meetings on September 5–6, 2012 (announced in 77 FR 40358) and September 24, 2013 (announced in 78 FR 45253) at NIEHS in Research Triangle Park, North Carolina.

At the 2012 meeting, NICEATM Director Dr. William Stokes presented an update on NICEATM and ICCVAM activities. NICEATM Deputy Director Dr. Warren Casey presented an overview of the adaptation of the BG1Luc ER TA test method to a high-throughput screening platform for use in the Tox21 program. ICCVAM Vice-Chair Dr. Joanna Matheson, Consumer Product Safety Commission, and Dr. Paul Siegel, National Institute for Occupational Safety and Health, summarized the nomination of the electrophilic allergen screening assay for identification of potential skin sensitizers. Representatives from the



National Institutes of Health (NIH), NIEHS, and EPA reported on research activities at their agencies relevant to the 2008–2012 NICEATM-ICCVAM Five-Year Plan. ICCVAM Chair Dr. Jodie Kulpa-Eddy, U.S. Department of Agriculture (USDA), and ICCVAM member Dr. Richard McFarland, FDA, presented a summary of the October 2011 workshop on alternative methods for rabies vaccine testing and information about workshops planned for 2012. The SACATM Implementation Working Group presented an assessment of the status of implementation of ICCVAM-recommended alternative methods. The working group recommended that ICCVAM collect data on the current implementation status of ICCVAM-recommended methods and on reductions in animal use resulting from future test method recommendations. The working group also recommended that regulatory agencies engage stakeholders and be more proactive about supporting the use of alternatives. Representatives from the ICATM partner organizations presented updates on their activities.

At the 2013 meeting, Acting NICEATM Director Dr. Warren Casey and Acting ICCVAM Co-Chair Dr. Anna Lowit of the EPA presented an overview of the new vision and procedures for ICCVAM, which received a positive response from SACATM. Dr. Casey also provided an update on NICEATM activities. Dr. Matheson discussed the skin sensitization adverse outcome pathway and an ICCVAM strategy for skin sensitization projects. Dr. Nicole Kleinstreuer, Integrated Laboratory Systems, Inc., (ILS, NICEATM contractor) presented a summary of current NICEATM projects related to skin sensitization. ICCVAM member Dr. Raymond Tice, head of the NIEHS Biomolecular Screening Branch, provided an update on the Tox21 collaboration. Dr. Geetha Srinivas, USDA, presented a report from the September 2012 workshop on alternative methods for *Leptospira* vaccine potency testing, and Dr. McFarland presented a report from the November 2012 workshop on alternatives to the murine histamine sensitization test for acellular pertussis vaccine testing.

[Appendix C](#) lists SACATM members during 2012 and 2013. Materials from past SACATM meetings are available at <http://ntp.niehs.nih.gov/go/8202>.

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

NICEATM and ICCVAM scientists participated in numerous international workshops, conferences, and meetings in 2012 and 2013. 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.

World Congress on In Vitro Biology

The Society for In Vitro Biology sponsored the 2012 World Congress on In Vitro Biology, held on June 3–7 in Bellevue, Washington. NICEATM Deputy Director Dr. Warren Casey co-chaired a session titled “Identifying Environmental Endocrine Disruptors: Do *In Vitro* Models Predict Relevance?” His presentation in the session was titled “Validating High-Throughput Test Methods for Tox21; the Technology Has Changed, but the Objective Remains the Same.”

Workshop on Adverse Outcome Pathways for Skin Sensitization Testing

Regulatory authorities worldwide require testing of chemicals and products for their potential to cause allergic skin reactions. The biological pathway leading to skin sensitization is well-characterized and thus is a promising area for the near-term development of testing strategies that do not require the use of animals. JaCVAM convened a workshop on September 13, 2012, in which participants considered methods to identify skin allergy hazards based on key events in the skin sensitization adverse outcome pathway.

NICEATM Director Dr. William Stokes gave a presentation at the workshop titled “Adverse Outcome Pathways for Skin Sensitization in the USA,” which focused on the NICEATM development of an integrated testing and decision strategy.

Workshop on Stem Cell-Derived Cardiomyocytes as Models of Cardiac Pathobiology and Toxicology

Cardiac cells are targets for environmental toxicants and therapeutic drugs; cardiotoxicity is also a major reason for drug development failure. The use of cultured heart cells, or cardiomyocytes, derived from stem cells holds promise for research to understand how chemicals can affect heart muscle function. The Health and Environmental Sciences Institute held a workshop on March 18–19, 2013, to evaluate how such technologies might be used to evaluate risks to human cardiac health from pharmaceuticals and environmental chemicals.

Acting NICEATM Director Dr. Warren Casey joined an international group of presenters representing research institutions, pharmaceutical companies, and government agencies at the workshop. His presentation, titled “ICCVAM and Cell-Based Assay Systems,” described how testing approaches employing cultured cells might be used in regulatory or safety decision-making contexts. Dr. Casey and ICCVAM Committee member Dr. Donna Mendrick, FDA/ National Center for Toxicological Research, participated in a panel on structural cardiotoxicity.

Workshop on Lessons Learned, Challenges, and Opportunities: The U.S. Endocrine Disruptor Screening Program

The EPA's Endocrine Disruptor Screening Program (EDSP) has produced data on 50 pesticide actives and two inert ingredients using the tier 1 battery of five *in vitro* and six *in vivo* screening assays. This workshop on April 23–24, 2013, brought together over 240 scientists from government, industry, academia, and nonprofit organizations to collect the insight of multiple stakeholders involved in the EDSP and provide a framework for retrospective analysis of the data generated.

Speakers at the workshop gave presentations in three sessions titled (1) Performance of the EDSP Tier 1 Screening Assays, (2) Practical Applications of Tier 1 Data, and (3) Considerations in the Future of Endocrine Testing. Dr. Casey co-chaired Session 3, and Ms. Patricia Ceger, ILS NICEATM contractor, presented a poster on the adaptation of the BG1Luc ER TA test method to a quantitative high-throughput screening format. Dr. Casey and ILS NICEATM contractors Drs. Lori Rinckel and Brett Jones were among the coauthors of the workshop report (Juberg et al. 2013).

53rd Annual Meeting of the Teratology Society

The theme of the Teratology Society's 2013 Annual Meeting, held June 22–26, was "Application of Cutting-Edge Technologies to Improve Assessment, Treatment, Prevention, and Communication Regarding Birth Defects." Dr. Nicole Kleinstreuer, ILS NICEATM contractor, gave a presentation titled "Adverse Outcome Pathways in Computational Toxicology."

XIII International Congress of Toxicology

The XIII International Congress of Toxicology was held June 30–July 4, 2013, in Seoul, South Korea. The theme for the conference was "From Basic Science to Clinical and Environmental Outcomes" and its goal was to consider novel approaches and technologies used to properly assess safety, toxicity, and risk to human health.

Dr. Casey was among the six NIEHS scientists who attended the conference. He co-chaired a session on "Alternative Test Methods and International Regulatory Perspectives" and gave a presentation titled "A New Strategic Direction for ICCVAM and NICEATM: Future Plans for the Validation and Acceptance of Alternative Test Methods in the U.S."

Workshop on Integrated Testing Strategies for Safety Assessment

The Center for Alternatives for Animal Testing–Europe and the Transatlantic Think Tank for Toxicology held a July 8–10, 2013, workshop on "Integrated Testing Strategies for Safety Assessment" in Varese, Italy. The goal of the workshop was to bring together experts to discuss the practical use of integrated testing strategies and provide guidance on integrating data from different sources to inform chemical risk assessments. A workshop report will be published in the journal ALTEX in 2014. Dr. Kleinstreuer was an invited participant in the workshop and the sole NICEATM representative.

Workshop on Assessing the Carcinogenic Potential of Low Dose Exposures to Chemical Mixtures in the Environment

The Halifax Project, an initiative of Getting to Know Cancer, promotes cancer biology research and communication of scientific knowledge in order to develop better cancer prevention and treatment strategies. The Halifax Project organized an August 8–9, 2013, workshop in Halifax, Nova Scotia, Canada, on "Assessing the Carcinogenic Potential of

Low Dose Exposures to Chemical Mixtures in the Environment.” NIEHS was a sponsor of the workshop. A workshop report will be published as a special issue of *Carcinogenesis* in 2014. Dr. Kleinstreuer gave a presentation on “Predicting Chemical Carcinogenesis from High-Throughput Screening Data.”

Adverse Outcome Pathways Framework: Development and Uses for Chemical Assessment

There is a worldwide effort to move away from observational animal testing of chemicals toward a mechanism-based understanding of chemical hazard and risk, with the goal of increasing testing efficiency and relevance to human health while reducing our reliance on animal tests. Development of adverse outcome pathways as models of chemical toxicity mechanisms has become a cornerstone of this effort.

The California Environmental Protection Agency organized a September 9, 2013, seminar on adverse outcome pathways for its scientists and regulators. The goal was to discuss current U.S. and international regulatory and scientific activities aimed at their development and use. Dr. Kleinstreuer gave a presentation on “Adverse Outcome Pathways and Computational Toxicology.”

51st and 52nd Annual Meetings of the Society of Toxicology

NICEATM and ICCVAM participated in the 2012 and 2013 annual meetings of the Society of Toxicology (SOT).

The 51st Annual SOT Meeting was held on March 11–15, 2012, in San Francisco, California. Fourteen NICEATM scientists and seven members of ICCVAM and ICCVAM interagency working groups contributed to eight poster presentations. Details of poster presentations are included in the list of publications in [Appendix B](#).

The 52nd Annual SOT Meeting was held on March 10–14, 2013, in San Antonio, Texas. Seven NICEATM scientists contributed to two poster presentations. Details of poster presentations are included in the list of publications in [Appendix B](#).

REFERENCES CITED IN THIS REPORT

- Birnbaum L. 2013. 15 years out: reinventing ICCVAM. *Environ Health Perspect* 121: a40. Available: <http://ehp.niehs.nih.gov/1206292/> [accessed 23 July 2014].
- Department of the Interior. 1997. Nontoxic Shot. 50 CFR 20.134.
- Department of the Interior. 2013. Migratory Bird Hunting; Revision of Language for Approval of Nontoxic Shot for Use in Waterfowl Hunting (Proposed Rule). *Federal Register* 78: 14060–14069.
- Department of the Interior. 2013. Migratory Bird Hunting; Revision of Language for Approval of Nontoxic Shot for Use in Waterfowl Hunting (Final Rule). *Federal Register* 78: 78275–78284.
- Environmental Protection Agency. 2012. Acute Oral Toxicity Up-and-Down Procedure [Internet]. Available: <http://www.epa.gov/oppfead1/harmonization/> [accessed 12 May 2014].
- Environmental Protection Agency. 2013. Data Requirements for Pesticides. 40 CFR 158.
- Federal Hazardous Substances Act. 15 U.S.C. 1261–1278, Public Law 86-613.
- Food Quality Protection Act of 1996. 7 U.S.C. 136, Public Law 104-170.
- Isbrucker R, Arciniega J, McFarland R, Chapsal J-M, Xing D, Bache C, et al. 2014. Report on the International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines: State of the Science and the Path Forward [published online ahead of print 4 January 2014]. *Biologicals*. Available: <http://www.sciencedirect.com/science/article/pii/S1045105613001462> [accessed 12 May 2014].
- ICCVAM. 1999. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. The Results of an Independent Peer Review Evaluation Coordinated by ICCVAM and NICEATM. NIH Publication No. 99-4494. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://ntp.niehs.nih.gov/iccvam/docs/immunotox_docs/lna/lnarep.pdf [accessed 12 May 2014].
- ICCVAM Authorization Act of 2000. 42 U.S.C. 285f-3, Public Law 106-545.
- Jaworska J, Dancik Y, Kern P, Gerberick F, Natsch A. 2013. Bayesian integrated testing strategy to assess skin sensitization potency: from theory to practice. *J Appl Toxicol* 33: 1353–1364.
- Jaworska J, Harol A, Kern PS, Gerberick GF. 2011. Integrating non-animal test information into an adaptive testing strategy — skin sensitization proof of concept case. *ALTEX* 28: 211–225.
- Juberg DR, Borghoff SJ, Becker RA, Casey W, Hartung T, Holsapple MP, et al. 2013. t4 workshop report: lessons learned, challenges and opportunities: the U.S. Endocrine Disruptor Screening Program [published online ahead of print 10 October 2013]. *ALTEX*. Available: http://www.altex.ch/resources/WR_Juberg_epub.pdf [accessed 12 May 2014].
- NIEHS. 2012. Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). *Federal Register* 77: 40358–40359.
- NIEHS. 2013. National Toxicology Program Scientific Advisory Committee on Alternative Toxicological Methods; Announcement of Meeting; Request for Comments. *Federal Register* 78: 45253–45254.
- NIEHS. 2013. Request for Information on Alternative Skin Sensitization Test Methods and Testing Strategies and for Comment on ICCVAM's Proposed Activities. *Federal Register* 78: 68076–68077.
- OECD. 2008. Test No. 425. Acute Oral Toxicity — Up-and-Down Procedure [adopted 3 October 2008]. In: *OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects*. Paris: OECD Publishing. Available: http://www.oecd-ilibrary.org/environment/test-no-425-acute-oral-toxicity-up-and-down-procedure_9789264071049-en [accessed 12 May 2014].
- Schneider K, Schwarz M, Burkholder I, Kopp-Schneider A, Edler L, Kinsner-Ovaskainen A, et al. 2009. “ToxRTool”, a new tool to assess the reliability of toxicological data. *Toxicol Lett*. 189(2): 138–44.
- Stokes W, McFarland R, Kulpa-Eddy J, Gatewood D, Levis R, Halder M, et al. 2012. Report on the International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward. *Biologicals* 40: 369–381.
- Takahashi Y, Koike M, Honda H, Ito Y, Sakaguchi H, Suzuki H, et al. 2008. Development of the short time exposure (STE) test: an *in vitro* eye irritation test using SIRC cells. *Toxicol In vitro* 22: 760–770.
- Williams WC, Copeland C, Boykin E, Quell SJ, Lehmann DM. 2014. Development and utilization of an *ex vivo* bromodeoxyuridine local lymph node assay protocol for assessing potential chemical sensitizers [published online ahead of print 14 Feb 2014]. *J Appl Toxicol*. Available: <http://onlinelibrary.wiley.com/doi/10.1002/jat.2983/abstract> [accessed 12 May 2014].

Appendix A

ICCVAM Agency Representatives in 2012 and 2013

The individuals listed below served as designated representatives from ICCVAM member agencies in 2012 and 2013. Asterisks indicate members who were serving as of January 2014.

Agency for Toxic Substances and Disease Registry

- ★ Moiz Mumtaz, PhD*
- Ed Murray, PhD*
- Eric Sampson, PhD

Consumer Product Safety Commission

- ★ Joanna Matheson, PhD* (Vice-Chair through December 2012)
- ◆ Kristina Hatlelid, PhD*

Department of Agriculture

- ★ Carol Clarke, DVM, DAACLAM*
- ◆ Donna Malloy, DVM, DAACLAM*
- Tim Allen*
- Elizabeth Goldentyer, DVM
- Jodie Kulpa-Eddy, DVM (Chair through October 2012)

Department of Defense

- ★ Cheng Cao, PhD*
- ◆ Dawn Fitzhugh*
- Kevin Nemelka, DVM, DAACLAM
- Terry Besch, DVM, DAACLAM, DACVPM
- Patty Decot
- Annette Hildabrand, DVM, MPH, DAACLAM, DACVPM
- Patrick Mason, PhD, SES

Department of Energy

- ★ Michael Kuperberg, PhD*

Department of the Interior

- ★ Barnett A. Rattner, PhD*

Department of Transportation

- ★ Steve Hwang, PhD*

Environmental Protection Agency

Office of Pesticide Programs

- ★ Anna Lowit, PhD* (Acting Co-Chair from January 2013)
- Vicki Dellarco, PhD

Office of Research and Development

- ◆ Stephanie Padilla, PhD*

Food and Drug Administration

Center for Drug Evaluation and Research

- ★ Abigail C. Jacobs, PhD* (Acting Co-Chair from January 2013)
- Paul C. Brown, PhD*

Center for Food Safety and Nutrition

- ◆ Suzanne Fitzpatrick, PhD, DABT*
- David G. Hattan, PhD*
- Diego Rua, PhD*

Center for Biologics Evaluation and Research

- Richard McFarland, PhD, MD*
- Ying Huang, PhD*

Center for Devices and Radiological Health

- Vasant Malshet, PhD, DABT*

Center for Veterinary Medicine

- M. Cecilia Aguila, DVM*
- Li You, PhD*

National Center for Toxicological Research

- Paul Howard, PhD*
- Donna Mendrick, PhD*

National Cancer Institute

- ★ T. Kevin Howcroft, PhD*
- ◆ Chand Khanna, DVM, PhD*

National Institute for Occupational Safety and Health

- ★ Paul Nicolaysen, VMD*

National Institute of Environmental Health Sciences

- ★ Raymond R. Tice, PhD*
- ◆ Elizabeth Maull, PhD*
- Warren Casey, PhD, DABT*
- Rajendra S. Chhabra, PhD, DABT
- Jerrold J. Heindel, PhD
- William S. Stokes, DVM, DAACLAM

National Institutes of Health

- ★ Christine Kelley, PhD*
- ◆ Harold Watson, PhD*
- Margaret D. Snyder, PhD

National Library of Medicine

- ★ Pertti (Bert) Hakkinen, PhD*
- ◆ Jeanne Goshorn, MS*

Occupational Safety and Health Administration

- ★ Surender Ahir, PhD*

U.S. National Coordinator for OECD Test Guidelines Programme

- Christine Olinger, PhD*

★ Principal agency representative

◆ Alternate principal agency representative

Appendix B

NICEATM and ICCVAM Publications, 2012–2013

NICEATM AND ICCVAM REPORTS

ICCVAM. 2012. ICCVAM Test Method Evaluation Report: Identifying Chemical Eye Hazards with Fewer Animals. NIH Publication No. 12-7930. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: <http://ntp.niehs.nih.gov/go/40530>.

ICCVAM. 2012. Biennial Progress Report 2010–2011: Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 12-7873. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: <http://ntp.niehs.nih.gov/iccvam/docs/annrpt/Biennial2012-508.pdf>.

FEDERAL REGISTER NOTICES

All *Federal Register* notices issued by NICEATM can be found on the NTP website at <http://ntp.niehs.nih.gov/go/frn>.

NIEHS. 2012. Availability of the Report on the International Workshop on Alternative Methods To Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions. *Federal Register* 77: 2064–2065.

NIEHS. 2012. Availability of ICCVAM Evaluation Report and Recommendations on the Usefulness and Limitations of the LUMI-CELL® ER (BG1Luc ER TA) Test Method, an *In Vitro* Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals. *Federal Register* 77: 8258–8260.

NIEHS. 2012. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Recommendations on Use of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis: Availability of Federal Agency Responses. *Federal Register* 77: 11536–11538.

NIEHS. 2012. National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods: Call for Nominations of High-Throughput Screening (HTS) Assays for the Tox21 Initiative. *Federal Register* 77: 22321–22322.

NIEHS. 2012. Biennial Progress Report of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). *Federal Register* 77: 35393–35394.

NIEHS. 2012. Draft Five-Year Plan for the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods and the Interagency Coordinating Committee on the Validation of Alternative Methods. *Federal Register* 77: 35395–35396.

NIEHS. 2012. Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). *Federal Register* 77: 40358–40359.

NIEHS. 2012. Evaluation of *In Vitro* Tests for Identifying Eye Injury Hazard Potential of Chemicals and Products: Request for Nominations for an Independent Expert Panel and Submission of Relevant Data. *Federal Register* 77: 41406–41408.

NIEHS. 2012. Nomination of an *In Vitro* Test Method for the Identification of Contact Allergens: Request for Comments and Data. *Federal Register* 77: 43087–43089.

NIEHS. 2012. Evaluation of an Up-and-Down Procedure for Acute Dermal Systemic Toxicity Testing: Request for Nominations for an Independent Expert Panel and Submission of Relevant Data. *Federal Register* 77: 43089–43090.

NIEHS. 2012. International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and the Way Forward. *Federal Register* 77: 43827–43828.

NIEHS. 2012. Federal Agency Responses to ICCVAM Recommendations on the Usefulness and Limitations of the LUMI-CELL® ER (BG1Luc ER TA) Test Method, an *In Vitro* Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals. *Federal Register* 77: 50510–50511.

NIEHS. 2012. International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines: State of the Science and the Path Forward. Federal Register 77: 52333–52334.

NIEHS. 2012. ICCVAM Evaluation Report and Recommendations for Identifying Chemical Eye Hazards With Fewer Animals; Availability of Report; Notice of Transmittal to Federal Agencies. Federal Register 77: 61610–61611.

NIEHS. 2013. National Toxicology Program Scientific Advisory Committee on Alternative Toxicological Methods; Announcement of Meeting; Request for Comments. Federal Register 78: 45253–45254.

NIEHS. 2013. Request for Information on Alternative Skin Sensitization Test Methods and Testing Strategies and for Comment on ICCVAM's Proposed Activities. Federal Register 78: 68076–68077.

ABSTRACTS AND MANUSCRIPTS

2012

Burns T, Allen D, Strickland J, Salicru E, Stokes W. 2012. Updated NICEATM evaluation of the reduced murine local lymph node assay [Abstract No. 1857]. Presented at the 51st Annual Meeting of the Society of Toxicology; San Francisco, CA; 11–15 March 2012. *Toxicol Sci/Toxicologist* 126(1): 399.

Casey W, Hattan D, Carlson K, Jacobs A, Bray J, Hamm J, et al. 2012. ICCVAM performance standards for the BG1Luc ER TA test method [Abstract No. 1823]. Presented at the 51st Annual Meeting of the Society of Toxicology; San Francisco, CA; 11–15 March 2012. *Toxicol Sci/Toxicologist* 126(1): 391.

Hattan D, Carlson K, Jacobs A, Bray J, Casey W, Stokes W. 2012. ICCVAM recommendations and limitations of the BG1Luc ER TA test method for identifying estrogen receptor agonists and antagonists [Abstract No. 1822]. Presented at the 51st Annual Meeting of the Society of Toxicology; San Francisco, CA; 11–15 March 2012. *Toxicol Sci/Toxicologist* 126(1): 391.

McFarland R, Kulpa-Eddy J, Levis R, Gatewood D, Halder M, Pulle G, et al. 2012. International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing [Abstract No. 2739]. Presented at the 51st Annual Meeting of the Society of Toxicology; San Francisco, CA; 11–15 March 2012. *Toxicol Sci/Toxicologist* 126(1): 391.

Paris M, Strickland J, Stack F, Allen D, Casey W, Stokes W. 2012. Analysis to determine if acute oral systemic toxicity data can be used to estimate and avoid acute dermal systemic toxicity testing [Abstract No. 1856]. Presented at the 51st Annual Meeting of the Society of Toxicology; San Francisco, CA; 11–15 March 2012. *Toxicol Sci/Toxicologist* 126(1): 399.

Stokes W, Burns T, Strickland J, Rinckel L, Allen D. 2012. Comparison of the DPRA with a three-test battery for *in vitro* evaluation of skin sensitization [Abstract No. 1853]. Presented at the 51st Annual Meeting of the Society of Toxicology; San Francisco, CA; 11–15 March 2012. *Toxicol Sci/Toxicologist* 126(1): 398.

Stokes W, McFarland R, Kulpa-Eddy J, Gatewood D, Levis R, Halder M, et al. 2012. Report on the International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward. *Biologicals* 40: 369–381.

Stokes WS, Strickland J, Casey W. 2012. Validation of the 21st century toxicology toolbox: challenges, opportunities, and the way forward. In: Proceedings of the 8th World Congress on Alternatives and Animal Use in the Life Sciences. ALTEX Proceedings 1: 323–328.

Strickland J, Stack F, Burns T, Allen D, Casey W, Stokes W. 2012. Evaluation of two nonradiolabeled murine local lymph node assays (LLNA) for potency categorization of substances causing allergic contact dermatitis in humans [Abstract No. 1858]. Presented at the 51st Annual Meeting of the Society of Toxicology; San Francisco, CA; 11–15 March 2012. *Toxicol Sci/Toxicologist* 126(1): 399.

2013

Casey W, Ceger P, Strickland J, Rinckel L, Grignard E, Bremer S, et al. 2013. Regulatory acceptance of the BG1Luc estrogen receptor transactivation test method [Abstract No. 1574]. Presented at the 52nd Annual Meeting of the Society of Toxicology; San Antonio, TX; 10–14 March 2013. *Toxicol Sci/Toxicologist* 132(1): 337.

Ceger P, Strickland J, Rinckel L, Casey W. 2013. Adaptation of the BG1Luc estrogen receptor transactivation test method to qHTS: comparison of results from both methods. Presented at Lessons Learned, Challenges, and Opportunities: The U.S. Endocrine Disruptor Screening Program; Research Triangle Park, NC; 23–24 April, 2013. Available: <http://ntp.niehs.nih.gov/iccvam/meetings/TERA-EDSP-2013/TERA-EDSP-Ceger-BG1.pdf> [accessed 12 May 2014].

Juberg DR, Borghoff SJ, Becker RA, Casey W, Hartung T, Holsapple MP, et al. 2013. Lessons learned, challenges, and opportunities: the U.S. Endocrine Disruptor Screening Program [published online ahead of print 23 Oct 2013]. ALTEX.

Kolle SN, Basketter DA, Casati S, Stokes WS, Strickland J, van Ravenzwaay B, et al. 2013. Performance standards and alternative assays: practical insights from skin sensitization. *Regul Toxicol Pharmacol* 65(2): 278–285.

Stokes W, Srinivas G, McFarland R, Kulpa-Eddy J, Casey W, Walker A, et al. 2013. Report on the International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and the Way Forward. *Biologicals* 41(5): 279–294.

Stokes W, Strickland J, Morris R, Ho L, Wilkerson J, Stack F, et al. 2013. The dermal up-and-down procedure: an alternative test method for acute dermal systemic toxicity testing [Abstract No. 1576]. Presented at the 52nd Annual Meeting of the Society of Toxicology; San Antonio, TX; 10–14 March 2013. *Toxicol Sci/Toxicologist* 132(1): 338.

ARTICLES IN THE NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES ENVIRONMENTAL FACTOR NEWSLETTER

2012

Mackay R. October 2012. Moving forward with alternative test methods [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/10/spotlight-sacatm/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. January 2012. NICEATM workshop report on vaccine testing now available [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/1/science-niceatm/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. January 2012. Stokes builds international partnerships for advancing alternative testing [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/1/science-stokes/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. January 2012. Casey presents at international meeting in Hungary [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/1/science-casey/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. March 2012. Federal agencies respond to test method recommendations [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/3/science-method/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. June 2012. Upcoming workshop on *Leptospira* vaccine potency testing [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/6/science-vaccine/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. July 2012. NICEATM seeks comment on draft plan for 2013–2017 [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/7/Spotlight-NICEATM/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. September 2012. NICEATM publishes biennial report [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/9/science-biennial/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. September 2012. Federal agencies accept ICCVAM-recommended testing methods [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/9/science-iccvam/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. October 2012. Upcoming workshop on innovative safety tests for pertussis vaccines [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/10/science-upcoming/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. November 2012. Committee recommends using fewer animals in eye hazard testing [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/11/science-committee/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. December 2012. Fall NICEATM activities focus on international collaborations [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2012/12/science-NICEATM/index.htm> [accessed 12 May 2014].

2013

Mackay R. October/November 2013. Advisors learn about the reinvention of ICCVAM [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2013/10/spotlight-iccvam/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. January 2013. NICEATM holds workshop on new safety tests for pertussis vaccines [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2013/1/science-niceatm/index.htm> [accessed 12 May 2014].

McCarley D, Sprankle C. January 2013. Casey meets with international experts to assess endocrine disruptor testing [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2013/1/science-casey/index.htm> [accessed 12 May 2014].

NICEATM. May 2013. Casey presents at workshop on stem cells in cardiotoxicity testing [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2013/5/science-casey/index.htm> [accessed 12 May 2014].

Sprankle C. July 2013. NICEATM/EPA workshop on evaluating cardiovascular safety [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2013/7/science-cvworkshop/index.htm> [accessed 12 May 2014].

Sprankle C, Burns T. July 2013. Industry scientist discusses statistical approach to safety testing [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2013/7/science-industry/index.htm> [accessed 12 May 2014].

Sprankle C. August 2013. Promoting alternative testing on an international level (sidebar article) [Internet]. Environmental Factor. Available: <http://www.niehs.nih.gov/news/newsletter/2013/8/spotlight-east/index.htm> [accessed 12 May 2014].

Appendix C

Scientific Advisory Committee on Alternative Toxicological Methods

This appendix lists all members of the Scientific Advisory Committee on Alternative Toxicological Methods during 2012 and 2013. Ending dates of appointments are indicated.

Laura Andrews, PhD, DABT
Vice President, Pharmacology and Toxicology
Genzyme Corporation
Framington, MA
Appointment ended 2012

Lauren E. Black, PhD
Senior Scientific Advisor, Navigators Services
Charles River Laboratories
Reno, NV
Appointment ends 2016

Tracie E. Bunton, DVM, PhD
President and Founder
Eicarte LLC
Gettysburg, PA
Appointment ends 2015

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

Joan M. Chapdelaine, PhD
Senior Immunologist
Calvert Laboratories, Inc.
Tunkhannock, PA
Appointment ends 2015

Mark G. Evans, DVM, PhD, ACVP
Research Fellow, Drug Safety Research and Development
Pfizer Global Research and Development La Jolla Laboratories
San Diego, CA
Appointment ends 2015

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

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

Michael D. Kastello, DVM, PhD
Vice President and Global Head, Animal Research and Welfare
Sanofi
Bridgewater, NJ
Appointment ends 2016

Safdar A. Khan, DVM, MS, PhD, DABVT
Senior Toxicologist and Senior Director of Toxicology Research
ASPCA Animal Poison Control Center
Urbana, IL
Appointment ends 2016

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

Steven M. Niemi, DVM (Chair 2011–2013)
Director, Office of Animal Resources
Harvard University
Charlestown, MA
Appointment ended 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 ended 2013

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

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

Marilyn Wind, PhD
Bethesda, MD
Appointment ends 2015

Acknowledgements

The Interagency Coordinating Committee on the Validation of Alternative 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

Warren Casey, PhD, DABT
Senior Toxicologist; Director

Elizabeth Maull, PhD
Toxicologist; Project Officer

NICEATM Contract Staff (Integrated Laboratory Systems, Inc.)

David Allen, PhD,
Principal Investigator

Steven Morefield, MD
Project Manager

Patricia Ceger, MS

Xiaoqing Chang, PhD, DABT

Neepa Choksi, PhD

Jon Hamm, PhD

Brett Jones, PhD

Nicole Kleinstreuer, PhD

Linda Litchfield

Michael Paris

Catherine Sprankle, MS

Judy Strickland, PhD, DABT

NICEATM reports, *Federal Register* notices, and other information about NICEATM are available at <http://ntp.niehs.nih.gov/go/niceatm>. Information about ICCVAM and ICCVAM activities is available at <http://ntp.niehs.nih.gov/go/iccvam>.



AGENCIES

Agency for Toxic Substances and Disease Registry

Consumer Product Safety Commission

Department of Agriculture

Department of Defense

Department of Energy

Department of the Interior

Department of Transportation

Environmental Protection Agency

Food and Drug Administration

National Cancer Institute

National Institute for Occupational Safety and Health

National Institute of Environmental Health Sciences

National Institutes of Health

National Library of Medicine

Occupational Safety and Health Administration

July 2014



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