

**Biennial Progress Report
of the
Interagency Coordinating Committee on the
Validation of Alternative Methods
(ICCVAM)**

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

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

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LIST OF ACRONYMS AND ABBREVIATIONS

AR	Androgen receptor
BRD	Background review document
DCIWG	Dermal Corrosivity and Irritation Working Group
ECVAM	European Centre for the Validation of Alternative Methods
EDSP	Endocrine Disruptor Screening Program
EPA	Environmental Protection Agency
ER	Estrogen receptor
ETP	Environmental Toxicology Program
HPV	High production volume
GLP	Good Laboratory Practices
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
LD ₅₀	Median lethal dose
LLNA	Local Lymph Node Assay
NHK	Normal human keratinocyte
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NRU	Neutral red uptake
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
TER	Transcutaneous electrical resistance
TA	Transcriptional activation
UDP	Up-and-Down Procedure

PREFACE

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was formally authorized and designated as a permanent committee by the ICCVAM Authorization Act of 2000 (Public Law 106-545) on December 19, 2000. The Act directs ICCVAM to prepare reports on its progress and to make them available to the public. The first report was published in December 2001. This second report complies with the requirement for subsequent biennial progress reports.

ICCVAM's duties include the technical evaluation of new, revised, and alternative testing methods, development of test recommendations based on those technical evaluations, and the forwarding of test method recommendations to ICCVAM agencies for their consideration. The ICCVAM also coordinates interagency issues and provides guidance on toxicological test method development, validation, regulatory acceptance, and national and international harmonization. The objective is to achieve the regulatory acceptance of scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining (less pain and distress), and replacing animal use where scientifically feasible.

During the past two years, ICCVAM has reviewed and recommended test methods that significantly reduce, refine, and replace animal use. The success of these efforts is best exemplified by the first two test methods forwarded to ICCVAM agencies in accordance with the ICCVAM Authorization Act. These methods, the revised Up-and-Down Procedure for estimating the acute oral toxicity of chemicals and *in vitro* methods for estimating the starting dose for such studies, have now been endorsed by U.S. Federal regulatory agencies. When used in place of one of the most commonly performed animal testing procedures, the traditional LD₅₀ test, these methods reduce the number of animals required for acute toxicity determinations by 60-70%. Other test methods recommended by ICCVAM are described in the report.

ICCVAM and the NTP Interagency Center for the Evaluation of Alternative Methods (NICEATM) embarked on many other important activities during the past two years. For example, ICCVAM and NICEATM established close working relationships with our European counterpart, the European Center for the Validation of Alternative Methods (ECVAM). Collaborative activities include the initiation of a joint validation study on *in vitro* methods for acute oral toxicity, organization of a joint workshop on validation of toxicogenomic-based test methods, and a joint initiative to develop international guidance on the application of Good Laboratory Practices to *in vitro* studies. Numerous other initiatives to facilitate test method validation and regulatory acceptance are described in this report, including the development of processes for development of performance standards, and consideration and prioritization of test method nominations and submissions.

We gratefully acknowledge the ICCVAM agency representatives, ICCVAM working group members, NICEATM contract support staff, expert and peer review panel members, and many other interested stakeholders for their contributions and enthusiastic support that has been invaluable to ICCVAM's success. Finally, we want to acknowledge the vision, wisdom, and leadership of our dear friend and colleague, Dr. Richard N. Hill, who retired this year after an outstanding career of public service with the EPA. Dick was instrumental in establishing the ICCVAM and

its predecessor, the Interagency Regulatory Alternatives Group, and served as the co-chair of the ICCVAM for eight years. As a physician and scientist, he worked tirelessly to ensure the use of good science to protect public health and the environment, and was an ardent advocate for the judicious and humane use of laboratory animals. He will be greatly missed by all.

Leonard M. Schechtman, Ph.D.
Chair, ICCVAM

William S. Stokes, D.V.M., D.A.C.L.A.M.
Director, NICEATM
Executive Director, ICCVAM

December 15, 2003

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ICCVAM BIENNIAL PROGRESS REPORT HIGHLIGHTS

Highlights of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) accomplishments and progress during the 2001-2003 biennial reporting period are described below. More detailed information is provided in the narrative that follows.

Test Method Evaluations and Related Activities

Acute Systemic Toxicity Test Methods

- In accordance with the ICCVAM Authorization Act, ICCVAM forwarded recommendations on two alternative acute systemic toxicity test methods to ICCVAM agencies.
 1. ICCVAM recommended the revised Up-and-Down Procedure (UDP) as a replacement for the conventional LD₅₀¹ test for regulatory hazard classification and labeling purposes. All Federal regulatory agencies that require acute oral toxicity testing now have accepted and adopted the revised UDP, which is expected to reduce the use of animals for this purpose by 60 to 70%.
 2. ICCVAM recommended that *in vitro* toxicity test methods should be considered as one way to estimate the starting dose for acute oral toxicity studies, thereby reducing the number of animals required by up to 40%. Use of this approach also will significantly reduce the number of animals that become severely ill or die when a test article is highly toxic. U.S. Federal agencies support ICCVAM's recommendations on the use of these *in vitro* methods and will make this information available to their respective stakeholders. In addition, the U.S. Environmental Protection Agency (EPA) recommended this test method for use in the high production volume (HPV) challenge program.
- ICCVAM, in partnership with the EPA and the International Life Sciences Institute, held a training workshop on acute toxicity testing methods from February 19 through 21, 2002, at the National Institutes of Health in Bethesda, MD. The workshop provided practical information and case studies to facilitate the understanding and implementation of the UDP and other *in vivo* and *in vitro* alternative methods for acute toxicity.

Skin Corrosivity and Irritation Test Methods

- ICCVAM completed an expedited review and published a comprehensive technical report on three alternative *in vitro* test methods - EpiDerm™ (EPI-200), EPISKIN™, and the Rat Skin Transcutaneous Electrical Resistance (TER) assay -for assessing skin corrosivity. ICCVAM recommended that these test methods be used as screening assays for corrosive chemicals. Chemicals that are predicted to cause skin corrosion by these test methods do not have to be tested in animals.

¹Median Lethal Dose

- Corrositex®, an *in vitro* test method for assessing the dermal corrosivity potential of chemicals, was recommended by ICCVAM and accepted by U.S. Federal regulatory agencies in 2000. A generic, international test guideline for similar *in vitro* membrane barrier test systems for skin corrosion was developed by ICCVAM and its Dermal Corrosivity and Irritation Working Group and proposed to the Organisation for Economic Co-operation and Development (OECD) Test Guidelines Program in March 2003.
- In response to a request from the EPA, ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) developed proposed performance standards for three types of *in vitro* dermal corrosivity test methods (human skin model systems, membrane barrier test systems, skin TER assays) previously reviewed by ICCVAM. Final ICCVAM performance standards will be published as addendums to previously published ICCVAM reports on these test methods and will be forwarded to ICCVAM agencies for consideration early in 2004.

In Vitro Endocrine Disruptor Screening Methods

- NICEATM completed a comprehensive evaluation of four *in vitro* test methods under consideration by the EPA for identifying potential endocrine disrupting chemicals. Comprehensive review documents were prepared that include over 4,000 test results for more than 1,000 substances evaluated in estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays.
- NICEATM and ICCVAM convened an international Expert Panel to evaluate the validation status of the *in vitro* ER and AR binding and TA assays. Based on the Expert Panel's report, ICCVAM recommended reference chemicals and minimum procedural standards to facilitate standardization and scientific validation of these assays.

ICCVAM Processes

- ICCVAM adopted a process by which test method nominations and test method submissions to ICCVAM are considered and prioritized for review and evaluation. This process allows for any new, revised, or alternative test method to be nominated for evaluation by any organization or individual.
- ICCVAM revised the "ICCVAM Guidelines for Nomination and Submission of New, Revised, and Alternative Test Methods." This document provides improved guidance on the information needed by ICCVAM to evaluate the validation status of new or revised test methods at any stage of development. It also includes a framework for organizing the information supporting the validity of a test method.
- ICCVAM developed a process for establishing performance standards for validated and accepted test methods. The purpose of performance standards is to communicate the basis by which new proprietary and non-proprietary test methods have been determined to have sufficient accuracy and reliability for specific testing purposes. Performance standards

can be used to evaluate the reliability and accuracy of other test methods that are based on similar scientific principles and measure or predict the same biologic or toxic effect. The three elements of performance standards are (1) essential test method components (i.e., structural, functional, and procedural elements of a validated test method that a proposed mechanistically and functionally similar test method should adhere to), (2) a minimum list of reference chemicals that is used to assess the accuracy and reliability of the proposed test method, and (3) the accuracy and reliability values that should be achieved by the proposed test method when evaluated using the minimum list of reference chemicals.

- In accordance with the ICCVAM Authorization Act, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) was chartered on January 9, 2002, to advise the NIEHS Director, NICEATM, and ICCVAM regarding statutorily mandated ICCVAM function. The SACATM also provide advice to he NIEHS Director and NICEATM on NICEATM activities. SACATM held its first meeting in Crystal City, VA, on December 5, 2002, and its second meeting in Research Triangle Park, NC, on August 12 and 13, 2003.

International Activities

- In April 2002, the OECD adopted a new internationally harmonized test guideline (TG 429) on skin sensitization using the mouse Local Lymph Node Assay (LLNA). The LLNA reduces and refines animal use compared to the traditional guinea pig test methods it replaces. Another advantage over the traditional test is that the LLNA also provides dose-response information. The validity of the new guideline was supported by the report of the independent scientific peer review evaluation of the LLNA coordinated by ICCVAM and NICEATM.
- In collaboration with the European Commission's European Center for the Validation of Alternative Methods (ECVAM), NICEATM designed and initiated a multi-laboratory international study to evaluate the usefulness of cytotoxicity data from the BALB/c 3T3 Neutral Red Uptake (NRU) and the Normal Human Keratinocyte (NHK) NRU assays for estimating the acute oral toxicity potential of substances. Completion of the validation study is anticipated in 2004.
- ICCVAM and NICEATM are collaborating with ECVAM to conduct a validation study to evaluate three *in vitro* test methods for assessing dermal irritation. NICEATM has requested data from stakeholders for substances that can be considered as potential reference chemicals for the validation study.
- In 2002 and 2003, ICCVAM representatives participated in three workshops convened by ECVAM:
 1. The ECVAM Status Meeting, which reviewed the progress made during the first 10 years of ECVAM, and provided perspectives on future opportunities.

2. The ECVAM Workshop on Strategies to Replace *In Vivo* Acute Systemic Toxicity Testing, the purpose of which was to establish the state-of-the-art in the field and to develop a strategy toward the replacement of *in vivo* testing for acute systemic toxicity.
 3. The ECVAM Workshop on Validation Principles and Approaches for Toxicogenomics-Based Test Systems, the purpose of which was to develop a new generation of alternative predictive testing and screening methods that can reduce, refine, and replace animal usage.
- In March 2003, ICCVAM and ECVAM made joint presentations to an OECD Good Laboratory Practices (GLP) Working Group on the need for further international guidance on the application of GLPs to *in vitro* toxicological testing. With the increasing use of non-animal testing procedures, such guidance will facilitate the acceptable use of new test methods and the generation of data in accordance with the requirements of GLPs. The full OECD GLP Working Group endorsed and initiated planning to develop this additional guidance in September 2003.
 - ICCVAM representatives served on the Organizing Committee for the International Conference on Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment, held in Stockholm, Sweden, from March 6 through 8, 2002. Several ICCVAM representatives also served as invited discussion leaders and rapporteurs, and ICCVAM publications were used as key discussion documents. The conference report was used to revise a draft OECD Guidance Document (No. 34) entitled, "The Development, Validation and Regulatory Acceptance of New and Updated Test Methods in Hazard Assessment." The final document will provide practical guidance on principles and processes for the validation and acceptance of animal and non-animal test methods for regulatory hazard assessment.

ICCVAM/NICEATM Communications

- The ICCVAM/NICEATM website is a vital source of information related to the ICCVAM history, legislation, organization, test methods and types, publications, and activities. It provides easy access to the latest information on validation processes and the most up-to-date status of alternative test methods previously reviewed and those currently under review. Not only is information disseminated through the site, the contact page serves as a portal for inquiries or submission of comments to NICEATM. A combination of e-mail and website announcements informs the public of the availability of newly published *Federal Register* notices, documents, and upcoming events. The average website visitors numbered more than 23,500 each month over the past two years, indicating a high level of public interest.
- During the past two years, ICCVAM/NICEATM has published nine technical reports, 10 *Federal Register* notices, and seven articles. Eighteen oral and poster presentations also have been made by members of ICCVAM and NICEATM staff at national and international

meetings in an effort to communicate ICCVAM processes, progress on validation studies, and test method recommendations to stakeholders.

BIENNIAL PROGRESS REPORT OF THE INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS (ICCVAM)

1.0 HISTORY AND ORGANIZATION OF ICCVAM

1.1 History of ICCVAM

The Director of the National Institute of Environmental Health Sciences (NIEHS) established an ad hoc ICCVAM in September 1994 to develop a report responsive to requirements in the National Institutes of Health (NIH) Revitalization Act of 1993 (Public Law 103-43). The Act required NIEHS to establish criteria for the validation and regulatory acceptance of alternative testing methods and to recommend a process through which scientifically valid alternative test methods could be accepted for regulatory use. The ad hoc ICCVAM was comprised of representatives from the 15 U.S. Federal agencies now represented on ICCVAM. The ad hoc Committee published its final report, "Validation and Regulatory Acceptance of Toxicological Test Methods," in 1997. A standing ICCVAM consisting of 15 U.S. Federal agencies was established in 1997 to implement a process by which new test methods of agency interest could be evaluated and to coordinate cross-agency issues on the development, validation, acceptance, and national and international harmonization of toxicological test methods. ICCVAM gained permanent status with enactment of the ICCVAM Authorization Act of 2000 (Public Law 106-545).

1.2 The ICCVAM Authorization Act of 2000

The ICCVAM Authorization Act of 2000 was signed into law by the President on December 19, 2000. The law was enacted "to establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness." The stated 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
- reduce², refine³, or replace⁴ the use of animals in testing where feasible

The Act established ICCVAM as a permanent interagency committee of NIEHS under the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological

²Reduction alternative: A new or modified test method that reduces the number of animals required.

³Refinement alternative: A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.

⁴Replacement alternative: A new or modified test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one.

Methods (NICEATM), which is located at NIEHS in Research Triangle Park, NC. ICCVAM is composed of the heads or their designees from the following 15 U.S. Federal agencies (**Annex I**):

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

1.3 ICCVAM Functions

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

- coordinate the technical review and evaluation of new and revised test methods of interagency interest
- submit ICCVAM test recommendations to each appropriate U.S. Federal agency
- facilitate interagency and international harmonization of test protocols that encourage the reduction, refinement, and replacement of animal test methods
- facilitate and provide guidance on validation criteria and processes
- facilitate the acceptance of scientifically valid test methods
- facilitate awareness of accepted methods
- consider petitions from the public for review and evaluation of new and revised test methods for which there is evidence of scientific validity
- make ICCVAM final test recommendations available to the public
- prepare reports on the progress of this Act and make these available to the public

This report briefly reviews the history of ICCVAM and the test method evaluation process used by ICCVAM. This is followed by a description of the activities that have been carried out during the past two years by ICCVAM and NICEATM.

1.4 NICEATM

NICEATM was established in 1998 to administer ICCVAM and its advisory committee, to provide scientific and operational support for ICCVAM, and to organize committee-related activities, such as peer reviews and workshops for test methods of interest to U.S. Federal agencies. NICEATM is

a component of the Environmental Toxicology Program (ETP) in the NIEHS Division of Intramural Research. NICEATM provides administrative, technical, and scientific support and coordination for ICCVAM, ICCVAM working groups, peer review panels, expert panels, workshops, validation efforts, and the scientific advisory committee. These efforts include actions necessary to comply with applicable provisions of the NIH Revitalization Act of 1993 and the ICCVAM Authorization Act of 2000.

NICEATM also provides a mechanism for interagency communication with test method developers. The goal of these interactions is to maximize the likelihood that test method developers will conduct appropriate validation studies and provide adequate information for agencies to make scientifically sound decisions on the usefulness of a new test method. In addition, NICEATM evaluates new test method submissions and nominations for their compliance with ICCVAM guidelines, and assembles information about current best practices for the humane care and use of animals in toxicological research and testing.

As resources allow, NICEATM conducts validation studies to evaluate potential new alternative methods that may provide improved predictions of chemical toxicity for humans or animals or of adverse ecological effects and that may reduce, replace, or refine animal use for toxicity testing.

2.0 ICCVAM PROCESSES

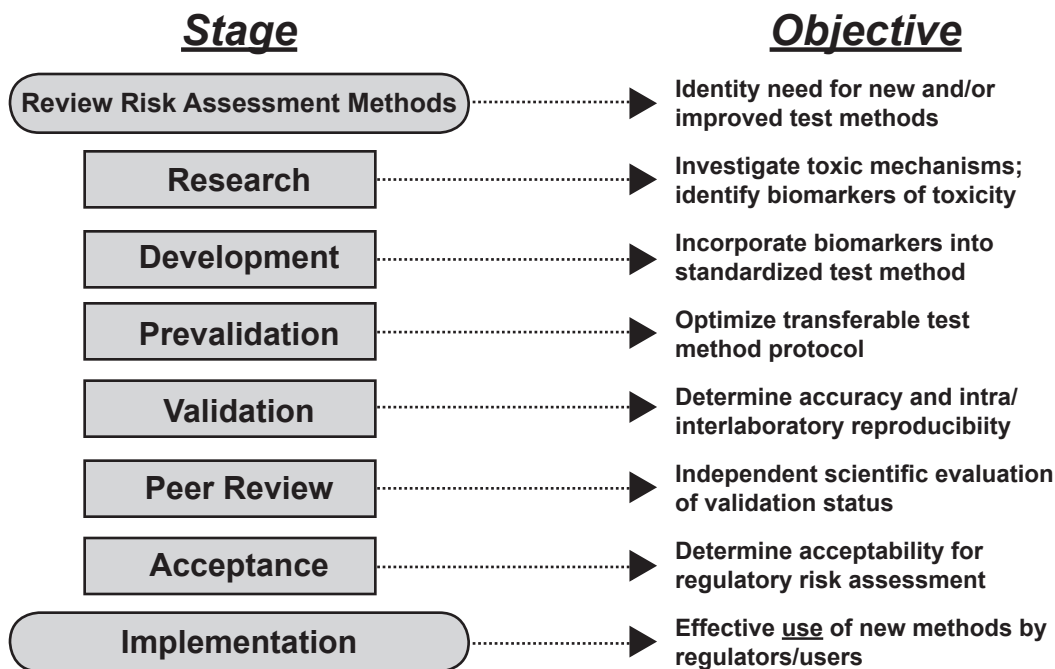
2.1 ICCVAM Revised Guidelines for Nomination and Submission

ICCVAM recently has revised the “ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods” (NIH Publication No. 03-4508) (*ICCVAM Guidelines*). The original version of this document was published in May 1998 and revised in October 1999. This second revision reflects experience gained by ICCVAM since 1999. The document provides guidance to test method sponsors and nominators on the information needed by ICCVAM to evaluate the validation status of new or revised test methods at any stage of development and after the completion of validation studies. It also includes a framework for organizing the information supporting the validity of a test method. A *Federal Register* notice was recently published (Vol. 68, No. 220, p. 64636, November 14, 2003) announcing the availability of the revised ICCVAM guidelines and requesting nominations and submissions of alternative test methods for evaluation by ICCVAM.

2.2 ICCVAM Test Method Evaluation Process

ICCVAM is responsible for coordinating the interagency technical review of new or modified alternative test methods of interagency interest, and for coordinating cross-agency issues relating to the validation, acceptance, and national and international harmonization of toxicological test methods throughout the U.S. Federal government. Priority is given to test methods that may provide improved prediction of adverse human, animal, or ecological effects and to those that may reduce refine, or replace animal use. In the newly revised *ICCVAM Guidelines*, various stages were identified in advancing a proposed test method from concept to regulatory acceptance (**Figure 1**).

Figure 1. Test Method Validation Process

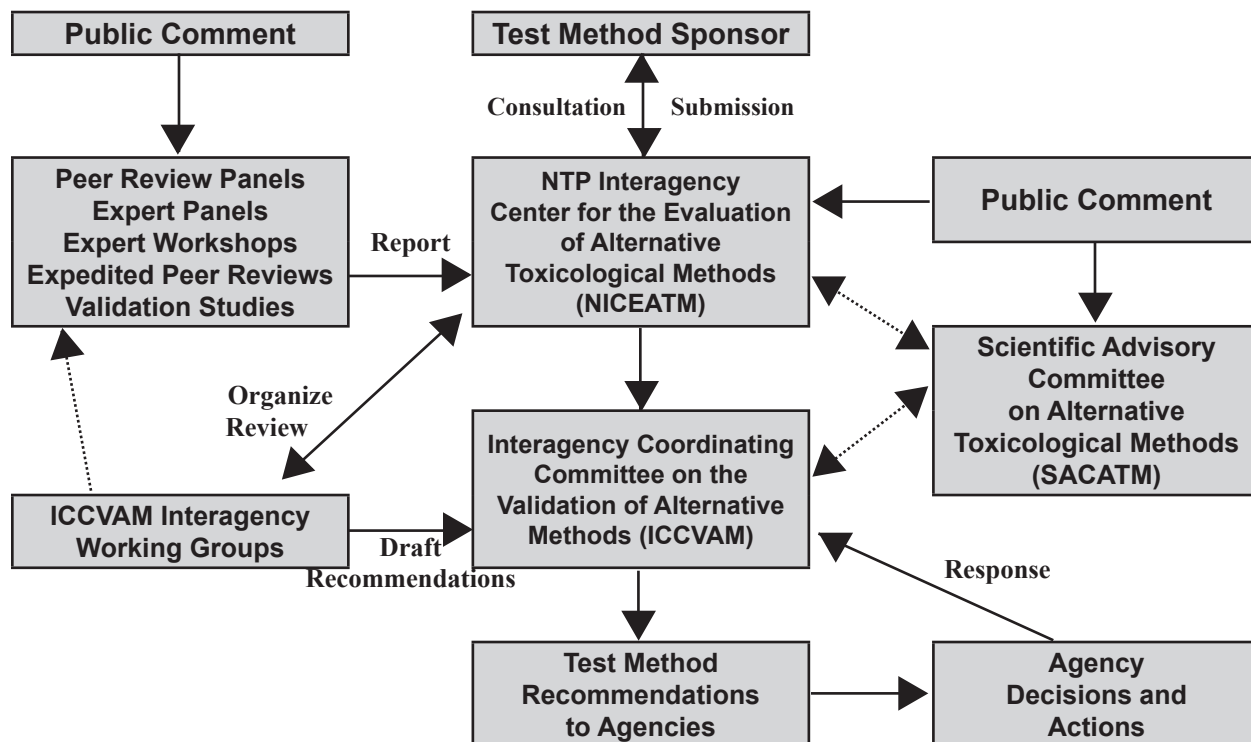


A critical stage is the communication of a proposed test method by the sponsor or nominator to ICCVAM for consideration and review. NICEATM, on behalf of ICCVAM, receives proposed test method submissions or nominations and communicates with the submitting organization or individual (Figure 2). Typically, the ICCVAM evaluation process involves an initial assessment by NICEATM of the adequacy and completeness of the proposed test method submission or nomination, and a determination by ICCVAM of the priority that the proposed test method will have for technical evaluation, taking into consideration comments and recommendations from the public and from the ICCVAM Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). Once a proposed test method has been accepted for evaluation, ICCVAM assembles an interagency working group of government scientists with scientific and regulatory expertise in the appropriate scientific disciplines to collaborate with NICEATM on the evaluation process. Depending on the validation status of the proposed test method, ICCVAM, in conjunction with NICEATM, develops recommendations and priorities for further efforts. Such efforts may include an expert workshop, an expert panel meeting, a peer review meeting, an expedited peer review process, or a validation study.

2.3 ICCVAM Test Method Nomination and Submission Process

ICCVAM recently adopted a process by which test method nominations and test method submissions to ICCVAM are considered and prioritized for review and evaluation (Figure 3). Submissions should be accompanied by all requested information. Although there is no mandatory minimum requirement for information to provide with nominations, ICCVAM consideration of the proposed test method will be expedited by providing as much of the requested information as

Figure 2. ICCVAM Test Method Evaluation Process

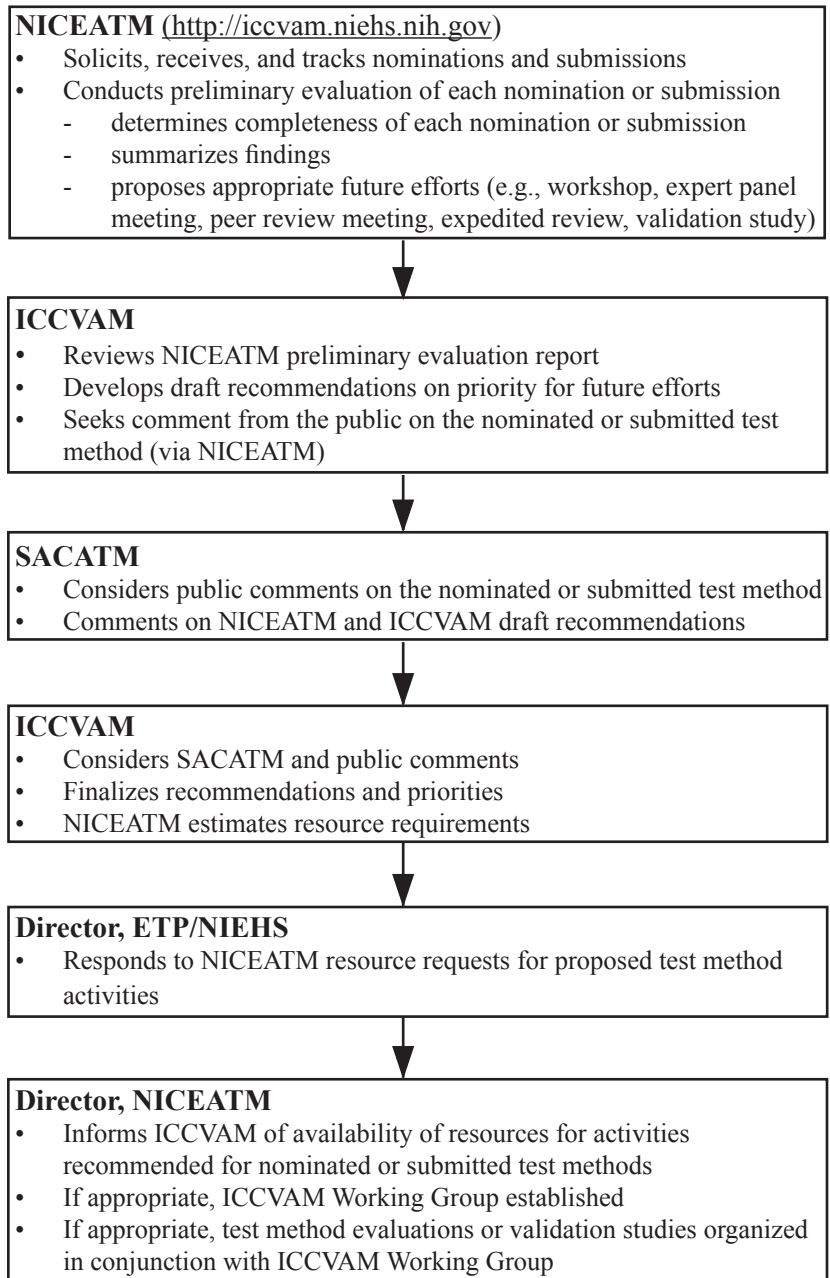


possible. The minimum information required for submissions and recommended to accompany nominations is summarized in the *ICCVAM Guidelines*. Areas where the requested information is unavailable or incomplete should be indicated, along with the scientific approach(es) planned to generate those data.

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

- The extent to which the proposed test method is:
 - applicable to regulatory testing needs
 - applicable to multiple agencies/programs
 - warranted, based on the extent of expected use or application and impact on human, animal, or ecological health
- The potential for the proposed test method, compared to current test methods accepted by regulatory agencies, to:
 - refine animal use
 - reduce animal use
 - replace animal use

Figure 3. ICCVAM Test Method Submission and Nomination Process



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

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

- reviews the NICEATM preliminary evaluation report
- determines whether the test method is of sufficient interest and applicability to one or more agencies to warrant further evaluation
- develops draft recommendations regarding priority for evaluation, the conduct of validation studies, or other activities

The Director of NICEATM provides SACATM with a status report on test method submissions and nominations, the results of NICEATM and ICCVAM preliminary evaluations, and any draft recommendations. SACATM comments on the draft test method evaluations and recommendations in terms of future ICCVAM efforts. ICCVAM also seeks comment from the public, using electronic methods (e.g., ICCVAM listserv groups and the ICCVAM/NICEATM website) and printed materials and publications (e.g., *Federal Register*). ICCVAM considers comments from SACATM and the public, develops final recommendations, and prioritizes future evaluation and validation efforts.

The Director of NICEATM estimates resource requirements for proposed evaluations and/or validation studies and forwards these, along with ICCVAM, NICEATM, and SACATM recommendations, to the Director of the ETP/NIEHS with a request for funding, when necessary. The ETP Director responds with information on the availability of the requested resources for the recommended activity.

The Director of NICEATM informs ICCVAM of the availability of funding from NIEHS, other ICCVAM agencies, or other stakeholders that can be used to support the recommended activities. When resources are available to support a recommended activity (e.g., workshop, expert panel meeting, independent peer review, expedited review, validation study), ICCVAM establishes a U.S. Federal interagency working group of knowledgeable scientists to work with NICEATM in organizing the appropriate evaluation or validation study. In collaboration with ICCVAM and the appropriate working group, NICEATM organizes the recommended activity to evaluate the validation status of the proposed test method.

2.4 Performance Standards

Prior to the acceptance of a proposed test method for regulatory testing applications, validation studies are conducted to assess reliability and accuracy. The purpose of performance standards is to communicate the basis by which new proprietary (i.e., copyrighted, trademarked, registered) and nonproprietary test methods can be determined to have sufficient accuracy and reliability for specific testing purposes. Performance standards may be recommended by ICCVAM as part of its evaluation of the validation status of a proposed test method. These performance standards, based on test methods accepted by regulatory agencies, can be used to evaluate the reliability and accuracy of other test methods that are based on similar scientific principles and measure or predict the same biological or toxic effect.

The three elements of performance standards are:

1. **Essential test method components:** These consist of essential structural, functional, and procedural elements of a validated test method that should be included in the protocol of a proposed, mechanistically and functionally similar test method. These components include unique characteristics of the test method, critical procedural details, and quality control measures. Adherence to essential test method components will help to assure that a proposed test method is based on the same concepts as the corresponding validated test method.
2. **Minimum list of reference chemicals:** These are used to assess the accuracy and reliability of a proposed, mechanistically and functionally similar test method. These chemicals are a representative subset of those used to demonstrate the reliability and the accuracy of the validated test method. To the extent possible, these reference chemicals should:
 - be representative of the range of responses that the validated test method is capable of measuring or predicting
 - have produced consistent results in the validated test method and in the *in vivo* reference test method and/or the species of interest
 - reflect the accuracy of the validated test method
 - have well-defined chemical structures
 - be readily available
 - not be associated with excessive hazard or prohibitive disposal costs

These reference chemicals are the minimum number that should be used to evaluate the performance of a proposed, mechanistically and functionally similar test method. These chemicals should not be used to develop the prediction model for the proposed test method. If any of the recommended chemicals are unavailable, other chemicals for which adequate reference data are available could be substituted. To the extent possible, the substituted chemical(s) should be of the same chemical class as the original chemical(s). If desired, additional chemicals representing other chemical or product classes and for which adequate reference data are available can be used to more comprehensively evaluate the accuracy of the proposed test method. However, these additional chemicals should not include any that had been used to develop the proposed test method.

3. **Accuracy and reliability values:** These are the comparable performance characteristics that should be achieved by the proposed test method when evaluated using the minimum list of reference chemicals.

The ICCVAM process for developing performance standards for new test methods is described below.

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

Performance standards recommended by ICCVAM would be incorporated into ICCVAM test method evaluation reports, which are provided to U.S. Federal agencies and made available to the public. Regulatory authorities then can reference the performance standards in the ICCVAM report when they communicate their acceptance of a new test method. In addition, performance standards adopted by regulatory authorities could be provided in guidelines issued for new test methods. Availability of ICCVAM test method evaluation reports are announced routinely in the *Federal Register*, NTP newsletters, and ICCVAM/NICEATM listserv groups.

2.5 ICCVAM SACATM

In accordance with the ICCVAM Authorization Act, SACATM was established to advise the NIEHS Director, ICCVAM, and NICEATM regarding statutorily mandated ICCVAM functions. SACATM also provides advice to NIEHS and NICEATM on NICEATM's activities. The SACATM also provides advice to the NIEHS Director and NICEATM on NICEATM activities. SACATM was established on January 9, 2002. The establishment of SACATM was announced in a *Federal Register* Notice (Vol. 67, No. 49, p. 11358, March 13, 2002). In compliance with the ICCVAM Authorization Act of 2000, the SACATM was established with the following members (**Annex 2**).

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

- Additional representatives are selected from an academic institution, a State government agency, an international regulatory body, or any corporation developing or marketing new or revised or alternative test methodologies, including contract laboratories.

The SACATM held its first meeting on December 5, 2002, in Crystal City, VA, and their second meeting on August 12 and 13, 2003, in Research Triangle Park, NC. Both meetings were announced in *Federal Register* notices (Vol. 67, No. 213, pp. 67203-67204, November 4, 2002; Vol. 68, No. 136, pp. 42066-42067, July 16, 2003). The SACATM charter, related *Federal Register* notices, and future meeting announcements can be found on the ICCVAM/NICEATM website (<http://iccvam.niehs.nih.gov/about/sacatm.htm>).

3.0 ICCVAM TEST METHOD EVALUATIONS

This section briefly summarizes the current status of test method evaluations and provides an update on activities during the past two years. The reports and information identified in this section are available electronically on the Web (URLs are provided) or in hard copy from NICEATM (NIEHS, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709; telephone: 919-541-2384; fax: 919-541-0947; e-mail: niceatm@niehs.nih.gov).

3.1 Acute Systemic Toxicity Test Methods

3.1.1 Up-and-Down Procedure for Acute Oral Toxicity

An independent Peer Review Panel evaluated the validation status of a revised Up-and-Down Procedure (UDP) for assessing acute oral toxicity, which was proposed as a replacement for the conventional LD₅₀ test used to evaluate the acute oral toxicity potential of chemicals for hazard classification and labeling purposes. The Peer Review Panel concluded that the revised UDP Primary Test, the revised UDP Limit Test, and the proposed UDP Supplemental Test for slope and confidence interval should be used for acute toxicity testing. The Peer Review Panel concluded also that an EPA-developed computational software program to aid in dose selection, test-stopping decisions, and calculation of an estimated LD₅₀ value and its associated confidence interval was appropriate for its intended purpose. When used in place of the conventional LD₅₀ test, the revised UDP will reduce animal use by 60 to 70%, and reduce the numbers of animals that become severely ill or die when a test substance is highly toxic. Results of the peer review were considered during finalization of an internationally harmonized OECD UDP Test Guideline. The OECD's updated UDP was formally adopted as Test Guideline 425 in December 2001. Additionally, EPA announced incorporation of the UDP in their revised final test guideline for acute oral toxicity (Vol. 67, No. 241, pp. 77064-77065, December 16, 2002). The EPA strongly recommends the use of the revised UDP to meet the testing requirements for industrial chemicals and registration of pesticides, and recommends that acute oral toxicity studies using the UDP that are initiated after December 17, 2002, should be in accordance with the UDP described in the revised EPA guideline. The UDP report entitled "The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals" (NIH No. 02-4501) and all related materials are available on the ICCVAM/NICEATM website (<http://iccvam.niehs.nih.gov/methods/udp.htm>). The availability of the report and a request for public comment were announced in a *Federal Register* notice (Vol. 67, No. 26, pp. 5842-5844, February 7, 2002).

In accordance with the ICCVAM Authorization Act of 2000, ICCVAM's recommendation that the revised UDP be used instead of the conventional LD₅₀ test to determine the acute oral toxicity hazard of chemicals was sent to the heads of the 15 member Federal agencies of ICCVAM on March 21, 2003. U.S. Federal testing regulations for which the UDP may be applicable are provided in the UDP report. Pursuant to Sections 4(a) and 4(d) of the ICCVAM Authorization Act of 2000, agencies are required to review ICCVAM test recommendations and notify ICCVAM in writing of their findings, including identification of relevant test methods for which the ICCVAM test recommendations may be added or substituted, no later than 180 days after receipt of recommendations. All U.S. Federal agencies that require the acute toxicity testing have accepted and adopted the revised UDP. The test recommendations and agency responses are available on the ICCVAM/NICEATM website (<http://iccvam.niehs.nih.gov/methods/udp.htm>).

3.1.2 In Vitro Test Methods to Estimate Starting Doses for Acute Oral Toxicity Studies

Also in accordance with the ICCVAM Authorization Act of 2000, an ICCVAM recommendation concerning *in vitro* test methods that can be used to estimate starting doses for acute oral toxicity studies was sent to the heads of the 15 member Federal agencies of ICCVAM on March 21, 2003. The *in vitro* methods are described in two ICCVAM Reports, "Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity" (NIH No. 01-4499) and the "Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity" (NIH No. 01-4500). It has been estimated that use of these *in vitro* toxicity test methods could reduce the number of animals required for acute toxicity testing by up to 40%. Use of this approach also will reduce significantly the number of animals that become severely ill or die when a test article substance is highly toxic. U.S. Federal agencies supported the ICCVAM recommendations on the use of these *in vitro* methods and will make this information available to their respective researchers and contractors. In addition, EPA recommended this test method for use in the high production volume (HPV) challenge program. This test recommendation and the responses from the agencies are available on the ICCVAM/NICEATM website (<http://iccvam.niehs.nih.gov/methods/invitro.htm>).

3.1.3 Training Workshop on Acute Toxicity Test Methods

ICCVAM, in partnership with EPA and the International Life Sciences Institute, held a training workshop on acute toxicity testing methods. The workshop provided practical information and case studies to facilitate the understanding and implementation of the UDP and other *in vivo* and *in vitro* alternative methods for acute toxicity. The workshop was held February 19 through 21, 2002, at NIH in Bethesda, MD.

3.2 **Skin Corrosivity and Irritation**

3.2.1 Review of Three In Vitro Test Methods for Assessing Skin Corrosivity

ICCVAM completed an expedited review of three alternative *in vitro* test methods for assessing skin corrosivity: EpiDerm™ (EPI-200), EPISKIN™, and the Rat Skin Transcutaneous Electrical Resistance (TER) Assay. EpiDerm™ (EPI-200) and EPISKIN™ utilize a three-dimensional tissue culture model of human skin comprised of a reconstructed epidermis and a functional stratum corneum. The test substance is applied and cell viability assessed over a defined exposure period. The Rat Skin TER assay measures the extent to which a test substance alters the transcutaneous

electrical resistance of a skin disk during a defined exposure period. The European Commission's European Centre for the Validation of Alternative Methods (ECVAM) had previously conducted validation studies on these test methods, which subsequently were accepted by the European Commission as alternatives to the rabbit dermal corrosivity test. NICEATM prepared a background review document (BRD) summarizing the available data and prior reviews of the three test methods. ICCVAM and its Corrosivity Working Group reviewed the BRD and determined that the test methods should be reviewed using an expedited review process. Draft ICCVAM test recommendations were prepared and made available with the BRD for public comment in a *Federal Register* notice (Vol. 66, No. 189, pp. 49686-49687, September 28, 2001). Public comments were received and considered by ICCVAM during finalization of the test method recommendations. In July 2002, ICCVAM published a comprehensive report on the ICCVAM-expedited review of these three *in vitro* corrosivity test methods; availability of the report was announced in a *Federal Register* notice (Vol. 67, No. 147, pp. 49706-49707, July 31, 2002).

In its report, ICCVAM recommended that these test methods be used in a weight-of-evidence approach that is part of an internationally harmonized, integrated, and tiered testing scheme. In the weight-of-evidence approach, positive *in vitro* corrosivity responses do not generally require further testing and can be used for classification and labeling, whereas negative *in vitro* corrosivity responses should be followed by *in vivo* dermal corrosion/irritation testing. This recommendation was based, in part, on the 12 to 21% false-negative rates of these *in vitro* assays for identifying corrosive substances. Because a substance incorrectly labeled as noncorrosive could cause irreversible and permanent injuries to exposed persons or animals, this level of error was considered to provide inadequate protection for human or animal health and safety. All materials related to this expedited review can be found on the ICCVAM/NICEATM website (<http://iccvam.niehs.nih.gov/methods/epiderm.htm>).

3.2.2 OECD Test Guideline for an *In Vitro* Membrane Barrier Test System for Skin Corrosion

Corrositex®, an *in vitro* method for assessing the dermal corrosivity potential of substances, was reviewed by an independent Peer Review Panel in 1999. The Peer Review Panel concluded that Corrositex® could be used to assess the corrosivity potential of substances in certain chemical classes and could be used in a tiered approach for the testing of substances in some additional chemical classes. When used in this manner, the test method provides for the refinement, reduction, and partial replacement of animal use. The final peer review report was published in 1999, and acceptance by regulatory agencies was announced in 2000. The Peer Review Panel's report is available on the Internet at: <http://iccvam.niehs.nih.gov/docs/reports/corprprep.htm>. In March 2003, a generic, international test guideline for similar *in vitro* membrane barrier test systems for skin corrosion was developed by ICCVAM and its Dermal Corrosivity and Irritation Working Group (DCIWG) and proposed to the OECD Test Guidelines Program.

3.2.3 Performance Standards for Three *In Vitro* Test Methods for Assessing the Dermal Corrosivity Hazard of Chemicals

In July 2003, a *Federal Register* notice (Vol. 68, No. 126, pp. 39104-39105, July 1, 2003) was published announcing the availability of and inviting public comment on the DCIWG-proposed performance standards (called minimum performance standards in the draft report) for three types of *in vitro* test methods for assessing the dermal corrosivity hazard of chemicals. ICCVAM

developed the proposed performance standards to communicate criteria that could be used to determine if similar test methods have comparable accuracy and reliability. All written comments received were posted on the ICCVAM/NICEATM website and were considered by DCIWG and ICCVAM during development of the final ICCVAM performance standards for these test methods. Final ICCVAM performance standards will be published as addendums to previously published ICCVAM reports on these test methods and will be forwarded to U.S. Federal agencies for their consideration. Availability of the final performance standards will be announced in a *Federal Register* notice.

3.2.4 ICCVAM Review of the *In Vivo* Rabbit Dermal Corrosivity Test

Public comments received in response to the ICCVAM recommendations on the three *in vitro* dermal corrosivity methods led ICCVAM to initiate a project to evaluate the false-negative rate for the traditional *in vivo* rabbit skin test used to assess dermal corrosivity. With assistance from ICCVAM member agencies, NICEATM is compiling relevant study results for analysis. An analysis of the first 50 chemicals identified suggests that the *in vivo* rabbit dermal corrosivity test has an estimated false-negative rate of less than 6%, and that false negatives would occur only for weak corrosives (i.e., United Nations Packing Group III). A more comprehensive analysis will be conducted once additional data received from U.S. Federal agencies and interested stakeholders have been reviewed and accepted for inclusion in the database. Once the analysis is completed, a manuscript on the findings will be prepared for peer-reviewed publication. Once the manuscript is published, its availability will be announced on the ICCVAM/NICEATM web site and reprints will be available on request.

3.3 *In Vitro* Endocrine Disruptor Screening Methods

ICCVAM and NICEATM held an international Expert Panel Review meeting on May 21 and 22, 2002, to assess the validation status of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays proposed for use in EPA's Endocrine Disruptor Screening Program. The Expert Panel concluded that none of the *in vitro* ER and AR binding and TA assays were sufficiently validated for use in the regulatory decision-making process. Considering the lack of defined protocols and statistical methods for data analysis, the Expert Panel recommended that pre-validation studies, using a relatively small number of selected substances, be conducted to demonstrate the adequacy of those test methods deemed to have merit for further evaluation. To facilitate the expert review, NICEATM prepared comprehensive BRDs on *in vitro* ER and AR binding and TA assays. The documents reviewed available data and related information necessary to evaluate the validation status of these assays. Following the meeting, the four draft BRDs were edited and revised to include additional information recommended by the Expert Panel. Final BRDs were published in 2003. A report outlining all the conclusions and recommendations of the Expert Panel was prepared and made available to the public for comment in a *Federal Register* notice (Vol. 67, No. 204, pp. 64902-64903, October 22, 2002). NICEATM and the ICCVAM Endocrine Disruptor Working Group developed a list of chemicals for validation studies of these test methods and proposed essential test method components (known as minimum procedural standards in the BRDs and the Expert Panel's report). Public comment also was sought on the proposed list of chemicals and the proposed essential test method components. ICCVAM considered the public comments and finalized its recommendations, which were included in a

report published in June 2003 and announced in a *Federal Register* notice (Vol. 68, No. 106, pp. 33171-33172, June 3, 2003). All relevant materials from this review can be found on the ICCVAM/NICEATM website (<http://iccvam.niehs.nih.gov/methods/endocrine.htm>).

4.0 INTERNATIONAL ACTIVITIES

4.1 Local Lymph Node Assay: International Adoption of an OECD Test Guideline

A new internationally harmonized test guideline (TG 429) on skin sensitization, using the mouse Local Lymph Node Assay (LLNA), was officially adopted by the OECD in April 2002. The LLNA reduces and refines animal use compared to the traditional guinea pig test methods for skin sensitization. Another advantage over the traditional test is that the LLNA provides dose-response information. The validity of the new guideline was supported by the report of the independent scientific peer review evaluation of the LLNA coordinated by ICCVAM and NICEATM.

4.2 NICEATM/ECVAM Validation Study of *In Vitro* Cytotoxicity Assays

In collaboration with ECVAM, NICEATM designed and initiated a multi-laboratory international study to evaluate the usefulness of cytotoxicity data from the BALB/c 3T3 Neutral Red Uptake (NRU) and the Normal Human Keratinocyte (NHK) NRU assays for estimating the acute oral toxicity potential of test substances. Preliminary data indicates that using *in vitro* cytotoxicity data to estimate starting doses for rodent acute toxicity assays can reduce significantly the number of animals required and the number of deaths that occur. A priority list of 72 chemicals was identified for the study. These chemicals represent the five globally harmonized acute toxicity hazard categories and unclassified, relatively nontoxic chemicals. Two laboratories in the United States and one in Europe are participating in the study. Testing is proceeding in three phases that are designed to facilitate standardization and optimization of the test method protocols before the majority of the chemicals are tested. Phase I was initiated in August 2002 and completed in May 2003. During this phase, acceptable positive control ranges were established for each laboratory, based on a series of sequential positive control testing, and three coded chemicals were tested at least three times by each laboratory to assess the validity of the proposed protocols. Phase II, which involves the replicate testing of nine coded chemicals using revised protocols in each laboratory, commenced in June 2003. On completion of Phase II, final optimized protocols will be prepared and used for Phase III, which will involve replicate testing of the remaining 60 coded chemicals. The basal cytotoxicity data from this study also will serve as a high-quality database that will support the development of additional specialized *in vitro* tests needed to increase the accuracy of *in vitro* predictions of acute toxicity. Completion of this validation study is expected to occur in 2004.

4.3 ECVAM Validation Study on *In Vitro* Methods for Dermal Irritation

ICCVAM and NICEATM are collaborating with ECVAM to conduct a validation study on three *in vitro* test methods for assessing dermal irritation. In July 2003, to help identify suitable reference chemicals (i.e., those with high quality rabbit or human dermal irritation data) for the validation study, ICCVAM published a *Federal Register* notice (Vol. 68, No. 136, pp. 42067-42068,

July 16, 2003) requesting data on commercially available chemicals used for dermal irritancy in rabbits and/or humans using standardized testing methods. Selection priority is being given to commercially available chemicals that have been tested for dermal irritation in both rabbits and humans. High quality rabbit ocular irritation data also was requested in order to identify appropriate reference chemicals that can be used in future validation studies of *in vitro* test methods for ocular irritancy. NICEATM, in collaboration with the DCIWG, is currently compiling the submitted data on commercially available chemicals. Once the data are compiled, a list of chemicals tested for skin irritancy in rabbits and/or humans and supporting data will be provided to ECVAM for consideration in the upcoming validation of *in vitro* test methods for dermal irritation. NICEATM continued to accept dermal and ocular irritation data as described in the *Federal Register* notice for inclusion in the database until the end of 2003.

4.4 International Guidance on the Application of Good Laboratory Practices to *In Vitro* Testing

In March 2003, ICCVAM and ECVAM made joint presentations to a subcommittee of the OECD Good Laboratory Practice (GLP) Working Group on the need for further international guidance on the application of GLPs to *in vitro* toxicological testing. With the increasing use of nonanimal testing procedures, such guidance will facilitate the acceptable use of new test methods and generation of data in accordance with the requirements of GLPs. This should help ensure that *in vitro* data are of acceptable quality for consideration by regulatory authorities. The full OECD GLP Working Group endorsed the need for this additional guidance in September 2003, and a workshop is planned for 2004.

4.5 International Conference and Guidance Document on Validation and Regulatory Acceptance

In response to an OECD draft guidance document on the validation of new test methods, entitled “Draft Guidance Document on the Development, Validation, and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment,” ICCVAM submitted extensive comments, including a recommendation that an international conference be held to address validation and regulatory acceptance issues. The International Conference on Validation and Regulatory Acceptance was held in Stockholm, Sweden, from March 6 through 8, 2002. ICCVAM representatives served on the conference organizing committee, and several ICCVAM representatives were invited to serve as discussion leaders and rapporteurs. ICCVAM publications also were identified as key discussion documents. The conference report was by a subcommittee of participants to revise the draft OECD Guidance Document (No. 34) entitled, “The Development, Validation and Regulatory Acceptance of New and Updated Test Methods in Hazard Assessment.” The final document will provide practical guidance on principles and processes for the validation and acceptance of animal and non-animal test methods for regulatory hazard assessment. The revised draft document was circulated in October 2003 to OECD member countries for comment.

4.6 Workshops

ICCVAM representatives participated in three workshops convened by ECVAM in 2002 and 2003:

1. The ECVAM Status Seminar, held June 4 through 6, 2002. The purpose of this meeting was to review the progress made during the first 10 years of ECVAM and to provide perspectives on future opportunities. The meeting proceedings were published in the journal *ATLA (Alternatives To Laboratory Animals)*.
2. The ECVAM Workshop on Strategies to Replace *In Vivo* Acute Systemic Toxicity Testing, held September 15 through 18, 2003. This workshop built on the progress made at the October 2000 ICCVAM workshop on *in vitro* methods for assessing acute *in vitro* toxicity testing. The purpose was to establish the state-of-the-art in the field and to develop a strategy toward the replacement of *in vivo* testing for acute systemic toxicity.
3. The Workshop on Validation Principles and Approaches for Toxicogenomics-Based Test Systems, held December 11 and 12, 2003. Toxicogenomics, an emerging field in molecular toxicology, offers the promise for new approaches to identify and characterize such factors as the biological activity of new and existing chemicals and drugs, and it could play an important role in the hazard assessment for human health. This revolutionary technology potentially can affect many scientific and medical areas, including the development of a new generation of alternative predictive testing and screening methods that could reduce, refine, and replace animal usage.

The purpose of the workshop, a collaborative effort between ECVAM, ICCVAM, and NICEATM, was to bring experts together to discuss and define principles applicable to the validation of toxicogenomics platforms, as well as validation of specific toxicological test methods that incorporate toxicogenomic assessments. Because data are already being generated using this technology, it is important to address this issue now, with the aim of establishing the foundation that will facilitate future regulatory acceptance of scientifically valid toxicogenomics-based test methods. A workshop report will be published and a summation of this meeting will be provided in the next ICCVAM biennial report.

5.0 ICCVAM/NICEATM COMMUNICATIONS

5.1 ICCVAM/NICEATM Website

The ICCVAM/NICEATM website is a vital source of information related to the ICCVAM history, legislation, organization, test methods and types, publications, and activities. It provides easy access to the latest information on validation processes and the most up-to-date status of the alternative test methods previously reviewed and those currently under review. This site has been used as the means of providing information ranging from a method-specific software package to a listing of public comments received by ICCVAM. Not only is information disseminated through the site, but the contact page serves as a portal for inquiries or submission of comments to NICEATM. As new

items are placed on the website, older information is archived for easy retrieval. A combination of e-mail and website announcements informs the public of the availability of newly published *Federal Register* notices and documents, and of upcoming events. Over the past two years, the average website visitors numbered more than 23,500 each month, indicating the very high level of public interest.

5.2 Reports, *Federal Register* Notices, Publications, and Presentations

5.2.1 Reports

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5.2.2 *Federal Register* Notices

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5.2.3 Publications

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