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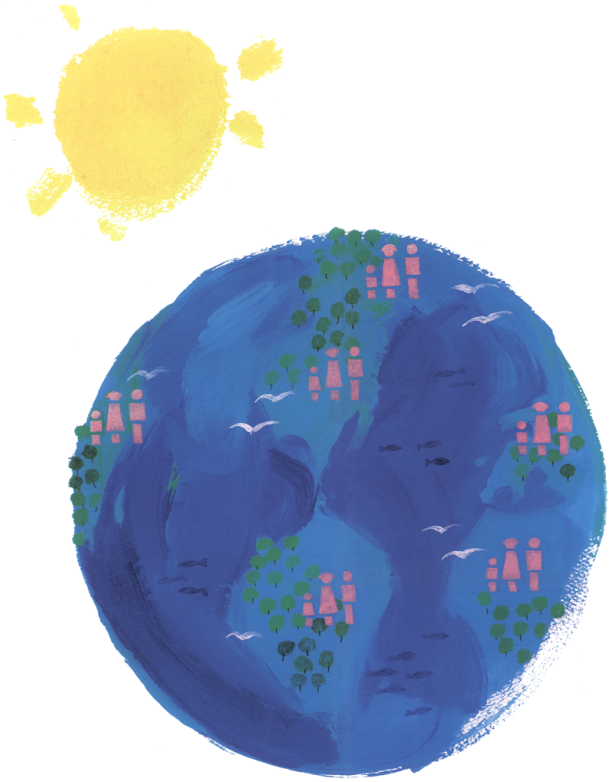
ICCVAM Biennial Progress Report

2004-2005



Prepared by 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)

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

About ICCVAM and NICEATM
In 1997, the National Institute of Environmental Health Sciences (NIEHS), one of the National Institutes of Health (NIH), established the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to:

- Coordinate interagency technical reviews of new and revised toxicological test methods, including alternative test methods that reduce, refine, or replace the use of animals

- Coordinate cross-agency issues relating to validation, acceptance, and national and international harmonization of new, modified, and alternative toxicological test methods

On December 19, 2000, the ICCVAM Authorization Act (42 U.S.C. § 2851-2, 2851-5 [2000]) established ICCVAM as a permanent interagency committee of NIEHS under the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

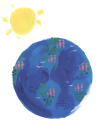
ICCVAM is comprised of representatives from 15 U.S. Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability. The Committee promotes the scientific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

The following Federal regulatory and research agencies are ICCVAM members:

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

Department of the Interior
Department of Labor
- Occupational Safety and Health Administration

Department of Transportation
Environmental Protection Agency



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

Biennial Progress Report

Interagency Coordinating Committee on the Validation of Alternative Methods

2004-2005

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

NIH Publication No.: 06-4516

Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences

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• Principal Agency Representative
◊ Alternate Principal Agency Representative
* Other Designated Agency Representative

[1]Designated agency representatives as of December 2005

LIST OF ACRONYMS AND ABBREVIATIONS

AR	Androgen receptor
BCOP	Bovine Corneal Opacity and Permeability
BET	Bacterial Endotoxin Test
BioWG	ICCVAM Biomarkers Working Group
BRD	Background Review Document
BWG	ICCVAM Biologics Working Group
COLIPA	European Cosmetic Toiletry and Perfumery Association
CTFA	Cosmetic, Toiletry, and Fragrance Association
DCIWG	ICCVAM Dermal Corrosivity and Irritation Working Group
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDWG	ICCVAM Endocrine Working Group
ELISA	Enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ER	Estrogen receptor
ESAC	ECVAM Scientific Advisory Committee
ETP	Environmental Toxicology Program
EU	European Union
FDA	U.S. Food and Drug Administration
FR	*Federal Register*
GCCP	Good Cell Culture Practices
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practices
HESI	Health and Environmental Sciences Institute
HET-CAM	Hen's Egg Test-Chorioallantoic Membrane
HSUS	Humane Society of the United States
IACUC	Institutional Animal Care and Use Committees
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated Chicken Eye
ILSI	International Life Sciences Institute
IRE	Isolated Rabbit Eye
JaCVAM	Japanese Center for the Validation of Alternative Methods
JSAAE	Japanese Society of Alternatives to Animal Experiments
LAL	Limulus amoebocyte lysate
LD_{50}	Median lethal dose
NAS	National Academy of Sciences
NCA	Netherlands Centre for Alternatives to Animal Use
NCTR	National Center for Toxicological Research
NGI	Netherlands Genomic Initiative
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
NIHS	National Institutes of Health Sciences
NRU	Neutral red uptake
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Ocular Toxicity Working Group
PBMC	Peripheral blood mononuclear cell
PPDC	Pesticide Program Dialogue Committee
PWG	ICCVAM Pyrogenicity Working Group
QSAR	Quantitative Structure Activity Relationship
R&D	Research and development
REACH	Registration, Evaluation, and Authorization of Chemicals
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
TA	Transcriptional activation
TG	Test Guideline
UN	United Nations
USDA	U.S. Department of Agriculture
WC5	5th World Congress on Alternatives and Animal Use in the Life Sciences
WC6	6th World Congress on Alternatives and Animal Use in the Life Sciences

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 an annual progress report on its first anniversary, and biennially thereafter. This report is the second ICCVAM biennial progress report and covers the period from January 2004 to December 2005.

ICCVAM's statutory 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 U.S. Federal agencies for their consideration. ICCVAM also coordinates interagency issues and provides guidance on toxicological test method development, validation, regulatory acceptance, and national and international harmonization. The Committee's overall goal is to achieve the regulatory acceptance of scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining (less pain and distress), or replacing animal use where scientifically feasible.

This report describes test method evaluation activities conducted by ICCVAM during the reporting period from January 2004 to December 2005. These include ICCVAM's review of the validation status of four *in vitro* test methods proposed to identify ocular corrosives and severe irritants. ICCVAM also developed performance standards for three types of *in vitro* test methods previously recommended for identifying chemicals that can cause chemical burns to the skin. These performance standards communicate criteria that can be used to determine if similar test methods have comparable accuracy and reliability, and allow for proprietary methods to be considered by regulatory authorities.

ICCVAM and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Methods (NICEATM) also initiated and participated in many other important activities in addition to specific test method evaluations during the reporting period. For example, ICCVAM held a strategic planning session to set its direction for the next three to five years in accordance with ICCVAM's purposes and duties as defined in the ICCVAM Authorization Act of 2000. As a result of this planning session, ICCVAM developed mission and vision statements and an implementation plan for each of the strategic priorities. These plans have specific activities that are tracked and updated on a regular basis.

ICCVAM and NICEATM continue to work closely with our European counterpart, the European Centre for the Validation of Alternative Methods (ECVAM). Collaborative activities include completion of a joint validation study on *in vitro* methods for estimating *in vivo* acute oral toxicity and organization of a joint evaluation of alternative assays for detecting ocular irritation and corrosion. In December 2005, we were pleased to participate in ceremonies establishing the Japanese Center for the Validation of Alternative Methods (JaCVAM) at the National Institute of Health Sciences in Tokyo, Japan. NICEATM and ICCVAM have already initiated collaborative activities with this new national center.

Numerous other ICCVAM initiatives to facilitate test method validation and regulatory acceptance are described in this report, including processes to develop performance standards and to consider and prioritize test method nominations and submissions.

We gratefully acknowledge the many individuals whose contributions and enthusiastic support have been invaluable to ICCVAM's success. These include the ICCVAM agency representatives, ICCVAM working group members, NICEATM and its contract support staff, Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) members, expert and peer review panel members, and many other interested stakeholders. With continued national and international cooperation and collaboration, we are confident that even greater progress will be made in the next few years to develop, validate, and gain acceptance of improved safety evaluation methods that will also provide for the refinement, reduction, and replacement of animal use.

Leonard M. Schechtman, Ph.D.
Deputy Director, FDA NCTR, Washington Operations
Chair, ICCVAM

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

ICCVAM BIENNIAL PROGRESS REPORT HIGHLIGHTS

Selected highlights of ICCVAM's progress and accomplishments during the 2004-2005 biennial reporting period are described below. More detailed information is provided in the corresponding sections of the report.

Test Method Evaluations and Related Activities

Ocular Toxicity Test Methods

- ICCVAM evaluated four *in vitro* ocular toxicity test methods for their usefulness in identifying substances that may cause irreversible damage to the eye. In collaboration with the ICCVAM Ocular Toxicity Working Group (OTWG), NICEATM prepared comprehensive Background Review Documents (BRDs) and convened an independent peer review to assess the current validation status of the four test methods. An ICCVAM Test Method Evaluation Report that contains ICCVAM's final recommendations on the usefulness of these test methods, future optimization and validation studies, standardized protocols for each test method, and recommended reference substances will be published and transmitted to ICCVAM member agencies in 2006.

- In support of the ICCVAM's evaluation of the four *in vitro* ocular toxicity test methods noted above, NICEATM compiled data from *in vivo* rabbit eye tests and clinical studies that could be used to assess the accuracy of these and other *in vitro* methods. NICEATM also analyzed the data to estimate the likelihood that ocular corrosives or severe irritants might be under- or over-classified due to variation in individual animal responses. This *in vivo* ocular testing database is the largest ever compiled and will help facilitate the validation of *in vitro* methods.

- ICCVAM, in collaboration with ECVAM and the European Cosmetic Toiletry and Perfumery Association (COLIPA), sponsored two scientific symposia on ocular toxicity at the Natcher Conference Center, National Institutes of Health (NIH) Campus, Bethesda, Maryland: (1) *Mechanisms of Chemically-Induced Ocular Injury and Recovery* and *(2) Minimizing Pain and Distress in Ocular Toxicity Testing*. The symposia identified priority research efforts that will help advance the development of more predictive *in vitro* methods for identifying ocular toxicity, and identified approaches that can significantly reduce or eliminate the pain and distress that may occur when it is necessary to conduct animal testing.

In Vitro Endocrine Disruptor Screening Methods

- NICEATM initiated standardization studies for an *in vitro* estrogen receptor (ER) transcriptional activation assay protocol that can be used to quantify the ER agonist and antagonist activity of unknown chemicals. It is anticipated that this test method will proceed to a formal international validation study in 2006. Following completion of

the validation study, it is expected that it will be considered for inclusion in the U.S. Environmental Protection Agency (EPA)'s Endocrine Disrupter Screening Battery.

Skin Corrosivity and Irritation Test Methods

- ICCVAM published recommended performance standards for three types of *in vitro* test methods for assessing the dermal corrosivity hazard of chemicals in the report *Recommended Performance Standards for In Vitro Test Methods for Skin Corrosion*. ICCVAM developed the proposed performance standards to communicate criteria that could be used to determine if similar test methods have comparable accuracy and reliability.

- The Organisation for Economic Co-operation and Development (OECD) Test Guideline 435, *In Vitro Membrane Barrier Test Method for Skin Corrosion*, gained international regulatory acceptance in June 2005. This guideline is based on Corrositex®, an *in vitro* dermal corrosivity test method previously evaluated and recommended by ICCVAM. ICCVAM and its Dermal Corrosivity and Irritation Working Group (DCIWG) developed and proposed the test guideline to the OECD Test Guidelines Program. It is the first OECD test guideline to incorporate performance standards.

Pyrogenicity Test Methods

- ICCVAM and its Pyrogenicity Working Group (PWG) determined that there are sufficient data to warrant an independent evaluation of the validation status of five *in vitro* human blood cell pyrogenicity test methods. These tests are proposed as potential replacements for the rabbit pyrogenicity test and an *in vitro* pyrogen test that uses hemolymph collected from the Limulus horseshoe crab. The test methods, submitted by ECVAM, are expected to undergo independent peer review evaluation in early 2007.

USDA Validation of Alternative Methods for Vaccine Potency

- The U.S. Department of Agriculture (USDA) is currently developing *in vitro* test methods for assessing the potency of *Leptospira* vaccines. These alternative test methods may be able to replace the use of hamsters for the USDA veterinary potency/efficacy tests for these vaccines. The USDA Center for Veterinary Biologics has initiated a pre-validation study of one *in vitro* test method. Results from this study are expected in 2006. ICCVAM established a Biologics Working Group (BWG) to provide comments on the validation study design and to coordinate future evaluation activities.

Alternative Methods for Assessing Potency of Botulinum Toxin

- ICCVAM received a nomination from the Humane Society of the United States (HSUS) in October 2005, requesting that alternative test methods to the mouse LD_{50}

assay for Botulinum toxin potency testing be assessed and prioritized for prevalidation and validation efforts. A proposed initial step in this process would be for ICCVAM to organize a workshop that would be coordinated with ECVAM and other appropriate stakeholders. In response to this nomination, ICCVAM and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) recommended that a workshop should be given a high priority. The purpose of this workshop will be to review the state-of-the-science and current knowledge of alternative methods that may reduce, replace, or refine (less pain and distress) the use of mice for Botulinum toxin testing and identify priorities for research, development, and validation efforts needed to advance the use of alternative methods. This workshop will take place in late 2006.

ICCVAM Outreach and Cooperative Activities

Interactions with ECVAM

- ICCVAM, NICEATM, and ECVAM continued to increase collaborations to evaluate test methods and to conduct validation studies. The aims of this extensive cooperation are to leverage resources and scientific expertise, maximize efficiency of evaluation/validation efforts, minimize duplication of effort, and ensure an early exchange of information concerning test method validation in order to facilitate recognition, acceptance, and implementation of scientifically valid test methods in both Europe and the United States. This cooperation promotes the international adoption of validated alternative methods by providing standardized and adequately validated test method protocols that can be expeditiously adopted as OECD international test guidelines.

- NICEATM, in collaboration with ECVAM and ICCVAM, designed and initiated a multi-laboratory, international study to evaluate the usefulness of cytotoxicity data from the BALB/c 3T3 and the Normal Human Keratinocyte (NHK) Neutral Red Uptake (NRU) assays for estimating the acute oral toxicity potential of test substances. This activity was conducted based on recommendations from the 2000 ICCVAM-sponsored *International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity*. NICEATM finalized a draft BRD for the test methods, which will be made available to the peer panel members and the public in spring 2006 along with draft ICCVAM recommendations on the usefulness of these test methods. ICCVAM will consider the conclusions and recommendations from the peer panel in developing final test method recommendations and performance standards for these test methods.

- ICCVAM and NICEATM have been collaborating with ECVAM to conduct an independent validation study on three *in vitro* test methods for assessing dermal irritation. NICEATM, in partnership with DCIWG, compiled a list of chemicals tested for skin irritancy in rabbits and/or humans. This list and supporting data were provided to ECVAM for consideration during the selection of chemicals to be used in the validation of *in vitro* test methods for dermal irritation.

- ECVAM completed the laboratory phase of this validation study to evaluate protocols for two *in vitro* dermal irritation assays. The protocols were optimized to identify chemicals that have the potential to be dermal irritants. As an additional measure to assess the acceptability of these *in vitro* assays, NICEATM undertook an effort to estimate the likelihood of under-classification of the *in vivo* test for the identification of irritants, mild irritants, and nonirritants according to the United Nations (UN) Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (UN 2003[2]).

- U.S. regulatory scientists have stated that their agencies need to know how certain corrosive substances, especially those that have produced false negative results in *in vitro* corrosivity test methods, will respond in the *in vitro* dermal irritation test method protocols. ICCVAM/NICEATM subsequently proposed a study plan to generate supplemental information deemed critical for consideration of these *in vitro* methods by regulatory authorities.

- A joint ECVAM-ICCVAM-NICEATM workshop on the validation of toxicogenomic-based test systems was held in Italy on December 11-12, 2003. Toxicogenomics offers the promise for new approaches to identify and characterize factors such as the biological activity of new and existing chemicals and drugs and 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.

- ICCVAM and ECVAM members, as part of an OECD Good Laboratory Practice (GLP) working group, contributed to publication of an OECD advisory document on the application of GLP to *in vitro* toxicology studies. This document, *Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 14, Advisory Document of the Working Group on GLP: The Application of the Principles of GLP to In Vitro Studies* (May 2004), is expected to be helpful in facilitating regulatory use of *in vitro* test methods. The necessity for carrying out validation studies under standardized conditions, i.e., in compliance with GLP and Good Cell Culture Practice (GCCP) rules, has also been recognized by national and international validation bodies.

[2] UN. 2003. Globally Harmonized System of Classification and Labeling of Chemicals (GHS). New York & Geneva: United Nations Publications. Available: http://www.unece.org/trans/danger/publi/ghs/officialtext.html [accessed 26 August 2004].

- ICCVAM, NICEATM, and ECVAM collaborated to develop a new guidance document on GCCP. The aim of this document is to reduce uncertainty in the development and application of animal and human cell and tissue culture systems, procedures, and products, by encouraging the greater international harmonization and standardization of laboratory practices, quality control systems, safety procedures, recording, reporting, and compliance with regulations and ethical principles. The report, *Guidance on Good Cell Culture Practice: A Report of the Second ECVAM Task Force on Good Cell Culture Practice,* has been published (Coecke et al. 2005[3]).

Other International Activities

- OECD Guidance Document No. 34, *The Development, Validation and Regulatory Acceptance of New and Updated Test Methods in Hazard Assessment,* was approved, declassified, and published in August 2005. The document provides internationally harmonized practical guidance on principles and processes for the validation and acceptance of animal and non-animal test methods for regulatory hazard assessment, and is based largely on principles and criteria for validation and regulatory acceptance developed by ICCVAM and ECVAM. ICCVAM representatives helped organize and participated in the *International Conference on Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment*, held March 6 - 8, 2002, in Stockholm, Sweden. Several ICCVAM publications were used as key references for the guidance document. ICCVAM and NICEATM also worked with OECD to organize and sponsor an Expert Consultation meeting to resolve several challenging issues critical to the document. The Expert Consultation meeting, hosted by the United States, was held in Bethesda, Maryland, on October 13-15, 2004. As a result of this meeting, the OECD Working Group of National Coordinators endorsed a revised version at their 17th meeting on April 12 - 14, 2005. A final version was subsequently approved and declassified by the Joint Meeting of the OECD and published in August 2005 as No. 34 in their Series on Testing and Assessment.

ICCVAM Participation in National and International Meetings

- ICCVAM members and NICEATM staff participated in over 25 national and international meetings, conferences, and workshops in 2004 and 2005. These events not only provided an opportunity for scientific interactions with the many stakeholders involved in the development and validation of alternative test methods but also served as important mechanisms for promoting and advancing the science of validation and alternative testing methods.

[3] Coecke, S., Balls, M., Bowe, G., Davis, J., Gstraunthaler, G., Hartung, T., Hay, R., (...), Stokes, W. 2005. Guidance on Good Cell Culture Practice: A Report of the Second ECVAM Task Force on Good Cell Culture Practice. ATLA 33:261-287.

ICCVAM/NICEATM Communications

- The ICCVAM/NICEATM website is a vital source of information related to ICCVAM history, legislation, organization, test methods, publications, and activities. Over the past two years, the average number of visitors was 24,200 per month, indicating a very high level of public interest. The website is currently being reworked as a database-driven site, which will allow faster retrieval of more specifically targeted information with the addition of search features, as well as choice of data format and download file type. The new site will be completed in 2006.

- During the past two years, ICCVAM/NICEATM has published nine technical reports and 20 *Federal Register* notices. Forty-five 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.

ICCVAM BIENNIAL PROGRESS REPORT

This report begins with a brief overview of the history of ICCVAM, the ICCVAM test method evaluation process, and the ICCVAM Strategic Plan. This is followed by descriptions of the activities carried out since the last ICCVAM/NICEATM biennial report (2001-2003), including test method evaluations, national and international collaborations, meetings, workshops, and conferences.

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). This act required NIEHS to establish criteria for the validation and regulatory acceptance of alternative toxicological testing methods and to recommend a process to achieve the regulatory acceptance of scientifically valid alternative test methods. The *ad hoc* ICCVAM was comprised of representatives from the 15 U.S. Federal agencies now represented on ICCVAM. In 1997, the *ad hoc* Committee published its final report, *Validation and Regulatory Acceptance of Toxicological Test Methods*. In that same year, an ICCVAM consisting of 15 U.S. Federal agencies was established as a standing committee to implement a process by which new test methods of agency interest could be evaluated and to coordinate cross-agency interactions related to issues on the development, validation, acceptance, and national and international harmonization of toxicological test methods.

1.2 The ICCVAM Authorization Act of 2000

With enactment of the ICCVAM Authorization Act of 2000 (Public Law 106-545), signed into law by the President on December 19, 2000, ICCVAM was established as a permanent interagency committee of NIEHS under NICEATM, which is located at NIEHS in Research Triangle Park. The law was enacted:

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

The Act directs that ICCVAM is composed of the heads, or their designees, of the 15 U.S. Federal agencies listed in **Annex A**.

The law's stated purposes of ICCVAM are to:

- Increase the efficiency and effectiveness of U.S. Federal agency test method review.
- Eliminate unnecessary duplication of effort and share experience among U.S. Federal regulatory agencies.

- Optimize utilization of scientific expertise outside the U.S. Federal government.
- Ensure that new and revised test methods are validated to meet the needs of U.S. Federal agencies.
- Reduce[4], refine[5], or replace[6] the use of animals in testing where feasible.

1.3 ICCVAM Duties

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

- Coordinate the technical review and evaluation of new and revised test methods.
- Submit ICCVAM test recommendations to appropriate U.S. Federal agencies.
- 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 test 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 ICCVAM progress and accomplishments under the Act and make these available to the public.

1.4 NICEATM

NICEATM is a center of the Environmental Toxicology Program (ETP) in the NIEHS Division of Intramural Research.

NICEATM was established in 1998 to:

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

The overall goal of these activities is to maximize the likelihood that test method developers will conduct appropriate validation studies and provide adequate information for U.S. Federal regulatory agencies to make scientifically sound decisions on the usefulness of new test methods. ICCVAM and NICEATM, as resources permit, also carry out activities applicable to provisions of the NIH Revitalization Act of 1993 that directs NIEHS to develop

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

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

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

and validate improved testing methods for acute and chronic toxicity, including methods that will reduce or replace animal use.

NICEATM, in providing support for ICCVAM, also: a) evaluates new test method submissions and nominations for their adherence to ICCVAM guidelines, b) assesses the completeness of test method dossiers for ICCVAM evaluation, c) determines the suitability of test methods for ICCVAM evaluation of their validation status, and d) assembles information about current best practices for the humane care and use of animals in toxicological research and testing.

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

2.0 ICCVAM PROCESSES

2.1 ICCVAM Guidelines for Nomination and Submission

ICCVAM published a revised *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods*[7] (*ICCVAM Guidelines*) in 2003. The original version of this document was published in May 1998 and revised in October 1999. The second revision reflects experience gained by ICCVAM since 1999. The document provides guidance to test method sponsors and nominators concerning the information needed by ICCVAM to evaluate the validation status of new or revised test methods at any stage from development through completion of validation studies. It also includes a framework for organizing information and data in submissions necessary to support the validity of a test method.

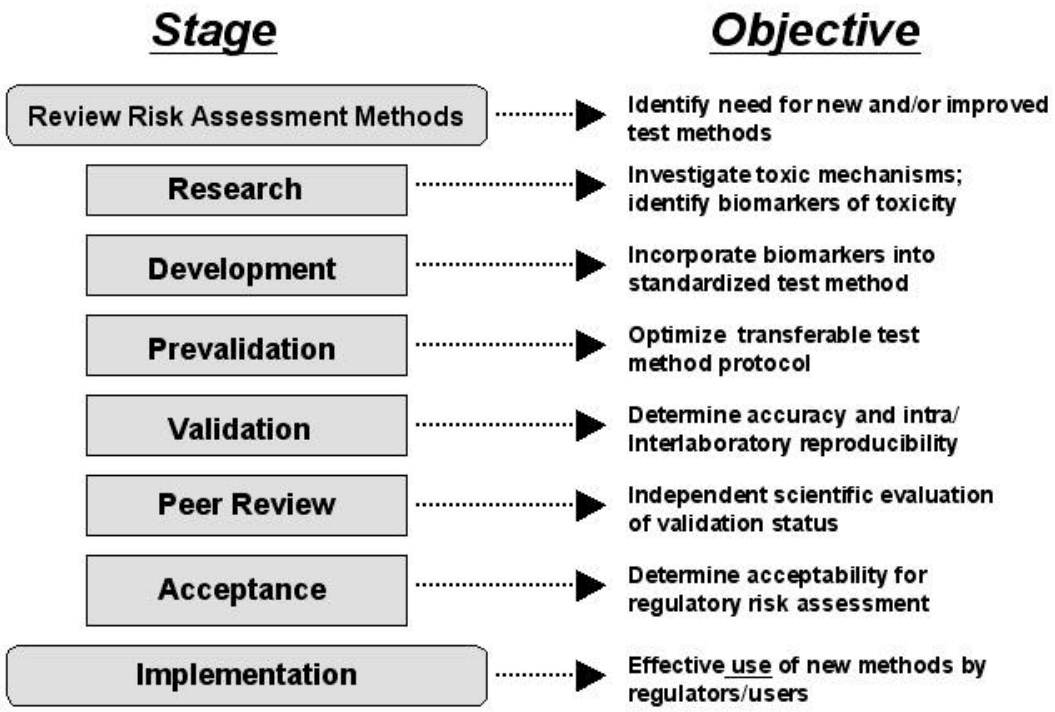
2.2 ICCVAM Test Method Evaluation Process

ICCVAM is responsible for coordinating interagency technical reviews of new or modified alternative test methods of interest and for coordinating cross-agency issues relating to the validation, acceptance, and national and international harmonization of toxicological test methods. Priority is given to test methods that may provide improved predictability of adverse human, animal, or ecological effects and to those that may reduce, refine, or replace animal use. The *ICCVAM Guidelines* identify various stages that advance a proposed test method from concept to regulatory acceptance (**Figure 1**).

The submission of a proposed test method by the sponsor or nominator to ICCVAM for consideration and review is a critical step in the test method validation process. NICEATM,

[7] NIH Publication No. 03-450.

Figure 1. Test Method Validation Process



on behalf of ICCVAM, receives nominations or submissions of proposed test methods and communicates with the submitter. Typically, the ICCVAM evaluation process involves:

- An initial assessment by NICEATM of the adequacy and completeness of the proposed test method nomination or submission.
- Prioritization of the proposed test method for technical evaluation by ICCVAM.
- Consideration of comments and recommendations from the public and from SACATM.
- Publication of an ICCVAM test method evaluation report that includes recommendations on the demonstrated usefulness and limitations of test methods for specific proposed hazard and safety assessment uses.

Once a test method has been accepted for evaluation, ICCVAM assembles an interagency working group of government scientists with expertise in the appropriate scientific disciplines and regulatory use to carry out the test method evaluation in collaboration with NICEATM. Depending on the validation status of the test method, ICCVAM develops recommendations and priorities for further efforts. Such efforts may include a workshop, an expert panel meeting, a peer review meeting, an expedited peer review process, or a validation study. Once the activity is approved, ICCVAM works in conjunction with NICEATM to organize and conduct the specific effort.

2.3 ICCVAM Test Method Nomination and Submission Process

ICCVAM has adopted a process by which test method nominations and submissions are considered and prioritized for review and evaluation. Submissions should be accompanied by all requested information; the information that should be provided in submissions and ideally in nominations is summarized in the *ICCVAM Guidelines*. Although there is no mandatory minimum requirement for information to be provided with nominations, ICCVAM's consideration of the proposed test method will be expedited by providing as much of the requested information as possible. 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 nominations and submissions, provides updates to ICCVAM, and arranges for a preliminary evaluation of nominations and submissions by NICEATM, as resources permit. Preliminary evaluations summarize the extent to which proposed test method nominations or submissions 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, reduce or replace animal use.
- The potential for the proposed test method to provide improved predictability for adverse health or environmental effects, as 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) as 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, and expedited review process) or validation studies. ICCVAM then:

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

In addition, the Director of NICEATM provides SACATM with:

- A status report on test method submissions and nominations.
- Results of NICEATM's and ICCVAM's preliminary evaluation of a test method nomination or submission.
- Draft recommendations for a test method nomination or submission regarding its priority for evaluation, the conduct of validation studies, or other activities.
- Public comments specific to these various activities.

SACATM comments on draft recommendations regarding future ICCVAM priorities and activities. ICCVAM also seeks comments from the public via public meetings, electronic methods (e.g., ICCVAM listserv groups and the ICCVAM/NICEATM website), and printed materials and publications (e.g., *Federal Register* [*FR*]). ICCVAM considers comments from SACATM and the public, develops final recommendations for future efforts connected with the nomination or submission, and prioritizes these efforts.

The Director of NICEATM estimates resource requirements for proposed evaluations and/or validation studies and forwards these, along with recommendations made by SACATM and ICCVAM, to the Associate Director of NTP. ICCVAM priorities, activities, and needed resources are considered, and the Associate Director of NTP forwards a recommendation to the Director of NIEHS and NTP. Based upon this information, the Director determines priorities and makes resource allocations for approved activities.

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

2.4 Performance Standards

Prior to the consideration of a proposed test method for regulatory testing applications, validation studies are conducted to assess reliability[8] and accuracy.[9] The purpose of performance standards is to communicate the basis by which new proprietary (i.e., copyrighted, trademarked, or registered) and nonproprietary test methods can be determined to have sufficient accuracy and reliability for specific testing purposes. Performance standards are recommended by ICCVAM as part of its evaluation of the validation status of a proposed test method. Once a proposed test method has been accepted by regulatory agencies, these performance standards can be used to evaluate the reliability and accuracy of other test methods based on similar scientific principles that measure or predict the same

[8] Reliability is a measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and inter-laboratory reproducibility and intralaboratory repeatability.

[9] Accuracy is (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. It is a measure of test method performance and one aspect of "relevance". The term is often used interchangeably with "concordance" (see also "two-by-two" table). Accuracy is highly dependent on the prevalence of positives in the population being examined.

biological or toxic effect. The ICCVAM process for developing performance standards for new test methods is described below:

- NICEATM and the appropriate ICCVAM working group develop proposed performance standards for consideration during the ICCVAM evaluation process. If performance standards are proposed by a test method sponsor, they are considered by ICCVAM at this stage. Generally, the performance standards are based on the information 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 and the test method submission are made available to the public for comment prior to and during the Peer Review Panel meeting.
- The appropriate ICCVAM working group, with assistance from 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 are 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 is announced in the *FR*, NTP newsletters, and ICCVAM/NICEATM listserv groups.

2.5 SACATM

In accordance with the ICCVAM Authorization Act, SACATM was established on January 9, 2002, to advise the NIEHS Director, ICCVAM, and NICEATM regarding statutorily mandated ICCVAM functions. SACATM also provides advice to NIEHS and NICEATM on NICEATM activities. In compliance with the Act, SACATM was established with the following members (**Annex B**):

- At least one member from each of the following stakeholders:
  - The personal care, pharmaceutical, industrial chemicals, or agriculture industry.
  - Any other industry regulated by one of the ICCVAM agencies.
  - A national animal protection organization.
- Additional representatives selected from the following:
  - Academic institutions.
  - State government agencies.
  - An international regulatory body or any corporation developing or marketing new or revised or alternative test methodologies, including contract laboratories.

SACATM has held three meetings since the last Biennial Report was published:

Meeting Date	Location	*Federal Register* Reference
March 10-11, 2004	Bethesda, MD	Vol. 69, No. 28, pp. 6674-6675, February 11, 2004
October 20, 2004	Research Triangle Park, NC	Vol. 69, No. 173, pp. 54298-54299, September 8, 2004
December 12, 2005	Alexandria, VA	Vol. 70, No. 216, pp. 68069-68070, November 9, 2005

The SACATM charter, related *FR* notices, meeting minutes, and future meeting announcements are on the NTP website (http://ntp.niehs.nih.gov, see "Advisory Committees and Boards").

2.6 ICCVAM Strategic Plan

ICCVAM held a strategic planning meeting on January 7-8, 2004, at the NIH in Bethesda, Maryland. The purpose of this meeting was to set the strategic direction of ICCVAM for the next three to five years, in accordance with its purposes and duties in the ICCVAM Authorization Act of 2000. The following Mission and Vision statements were developed during this meeting:

Mission[10]
ICCVAM's mission is to facilitate development, validation, and regulatory acceptance of new and revised regulatory test methods that reduce, refine, or replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment.

Vision
ICCVAM will:

- Be recognized as a leading authority on test method development and validation both within the U.S. Federal government and internationally.
- Play a leading role in:
  - Promoting high quality science as the basis for national and international regulatory policy.
  - Setting and harmonizing international standards for scientific validation of test methods.
  - Promoting and facilitating the development of priority alternative test methods.
  - Identifying key alternative test methods and strategies and facilitating their validation and acceptance.
  - Fostering humane and ethical approaches to testing that replace, reduce, and refine the use of animals.

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

  - Promoting awareness and adoption of scientifically validated test methods by regulatory agencies both nationally and internationally.
- Develop the internal and collaborative capacity to:
  - Ensure the scientific quality and integrity of its work.
  - Implement reliable processes and operating procedures that are credible, effective, and efficient.
  - Build national and international partnerships with governmental and non-governmental groups, including academia, industry, advocacy groups, and other key stakeholders.
  - Secure the necessary human and financial resources to effectively carry out its mission.

A Strategic Map (**Figure 2**) provides six strategic priorities that ICCVAM considers necessary to strengthen its impact both nationally and internationally. For each strategic priority, there is an accompanying brief rationale and listing of the strategic objectives that should be pursued.

ICCVAM has subsequently developed a specific implementation plan for each of these strategic priorities. These plans are reviewed and updated periodically to track the progress toward meeting the outlined objectives.

3.0 ICCVAM TEST METHOD EVALUATIONS

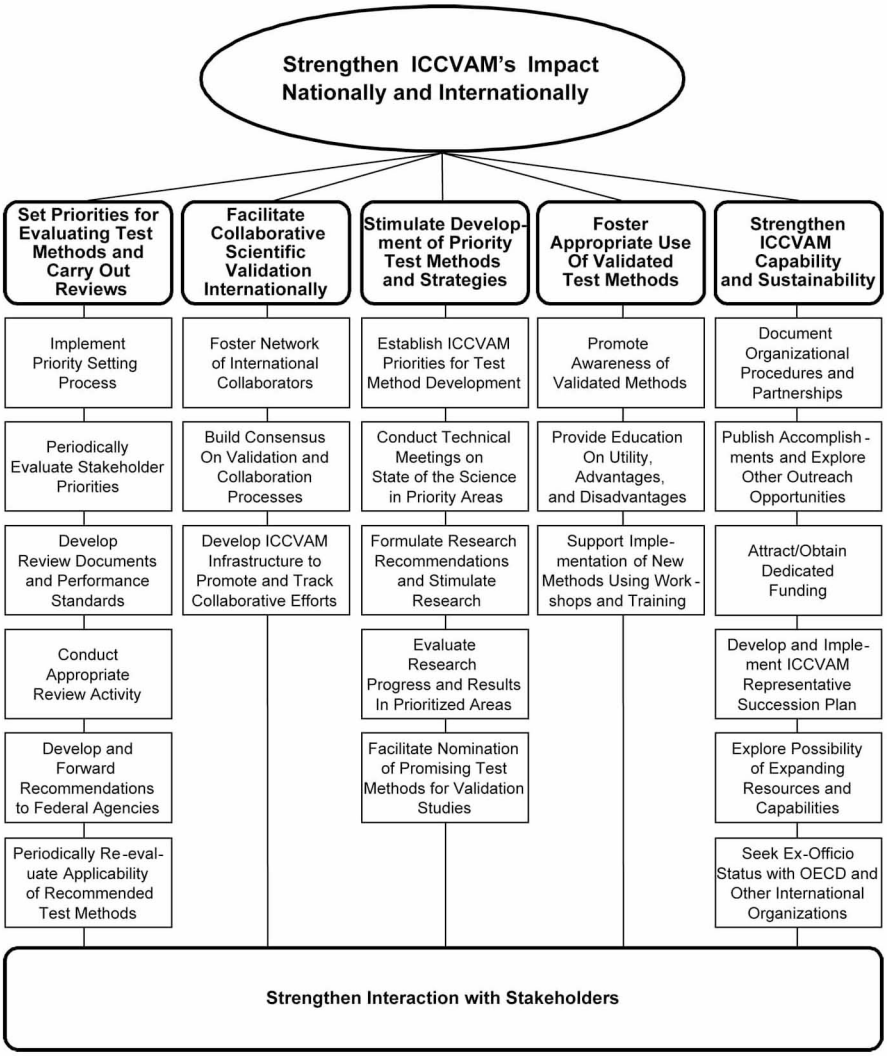
This section briefly summarizes the current status of test method evaluations and provides an update on activities during the past two years. The reports and information identified in this section are available electronically on the Internet (URLs are provided) or in hardcopy from NICEATM (NIEHS, P.O.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

3.1 Ocular Toxicity Test Methods

ICCVAM recently evaluated four *in vitro* test methods for ocular toxicity that could be used to identify substances that cause corrosive or severely irritating ocular damage. This activity was in response to a U.S. Environmental Protection Agency (EPA) nomination to ICCVAM for the following activities:

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

Figure 2. ICCVAM Strategic Map



ICCVAM and SACATM endorsed this nomination as a high priority. NICEATM published an *FR* notice (Vol. 69, No. 57, pages 13959-13861, March 24, 2004) inviting public comment on the nomination and related activities and requesting data on chemicals evaluated by *in vitro* or *in vivo* ocular irritancy test methods. The activities below have been initiated by ICCVAM in response to the EPA nomination.

3.1.1 <u>*In Vitro* Test Methods for Detecting Ocular Corrosives and Severe Irritants</u>

In collaboration with the ICCVAM Ocular Toxicity Working Group (OTWG), NICEATM prepared Background Review Documents (BRDs) that contained comprehensive summaries of available data, an analysis of the accuracy and reliability of available test method protocols, and related information characterizing the current validation status of the four *in vitro* ocular test methods. As part of this effort, and in response to the third EPA-nominated activity, NICEATM also compiled a database of high quality *in vivo* eye irritation/corrosion reference data which will continually be updated as new data are obtained.

On November 3, 2004, NICEATM released the four draft BRDs (*Federal Register*, Vol. 69, No. 212, pp. 64081-64082, November 3, 2004) for public comment. In conjunction with ICCVAM, NICEATM convened an expert panel meeting on January 11-12, 2005, to independently assess the validation status of the four *in vitro* test methods.

Public comments at the January 2005 meeting indicated that additional data could be made available that had not been provided in response to earlier requests for data announced in the *Federal Register* (Vol. 69, No. 57, pp. 13859-13861, March 24, 2004). The expert panel recommended that NICEATM conduct a reanalysis of the accuracy and reliability of each test method to include the additional data. In response to this recommendation, NICEATM published an *FR* notice (Vol. 70, No. 38, pp. 9661-9662, February 28, 2005) requesting additional *in vitro* data on these four *in vitro* ocular test methods, corresponding *in vivo* rabbit eye test method data, as well as any human ocular exposure/injury data (either from ethical human studies or accidental exposure). Subsequently, NICEATM received additional data that were used for the revised accuracy and reliability analyses and considered in revising the list of proposed reference substances.

NICEATM released the revised accuracy and reliability analyses and the revised list of proposed reference substances as an addendum to the draft BRDs and announced their availability in the *Federal Register* (Vol. 70, No. 142, pg. 43149, July 26, 2005). NICEATM subsequently announced in the *Federal Register* (Vol. 70, No. 174, pg. 53676, September 9, 2005) a second meeting of an expert panel by teleconference on September 19, 2005. As a result of this meeting, a second expert panel report was made available for public comment (*Federal Register,* Vol. 70, No. 211, pg. 66451, November 2, 2005). Both expert panel reports, the four draft BRDs, and the Addendum to the BRDs were made available to the public and SACATM in advance of their December 12, 2005, meeting in Alexandria, Virginia (*Federal Register,* Vol. 70, No. 216, pp. 68069-68070, November 9, 2005). ICCVAM will consider expert panel reports, other relevant background materials, and all comments received from the public and SACATM on this topic in finalizing its recommendations for these test methods. An ICCVAM Test Method Evaluation Report, which contains ICCVAM's final recommendations on the usefulness of these test methods, future optimization and validation studies, standardized protocols for each test method, and recommended reference substances will be published in 2006. In accordance with the ICCVAM Authorization Act of 2000, these recommendations will be forwarded to the heads of the 15 member agencies for consideration. All materials related to this evaluation are available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm.

3.1.2 Analysis of the Estimated Underclassification and Overclassification Likelihoods of the Current Rabbit Test

In support of the ICCVAM evaluation of the current validation status of the four *in vitro* test methods for detecting ocular corrosives and severe irritants, NICEATM solicited and searched for ocular data from *in vivo* rabbit eye tests and clinical studies that could be used to assess the accuracy of the *in vitro* methods. While it also would be desirable to have sufficient data to assess the reliability of the *in vivo* rabbit eye test and its accuracy for identifying substances that cause ocular corrosion and severe irritation in humans, no human

data were found to allow such assessments. However, a comprehensive review of chemically-induced human ocular injury did not reveal any chemicals that caused severe or irreversible damage in humans that did not also produce severe or irreversible effects in the animal test. NICEATM was able to analyze available animal data to estimate the likelihood that ocular corrosives or severe irritants might be under- or overclassified using the United Nations' (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UN 2003[11]) testing guidelines due to the variation in individual animal responses.

The estimated under-classification likelihoods for corrosives/severe irritants (GHS Category 1) as non-severe irritants (GHS Category 2) or non-irritants was based on 717 rabbits tested in 180 studies. The overall estimated under-classification likelihoods ranged from 4% to 13%, with the most likely estimate being 11% to 13%. Importantly, substances with any likelihood of under-classification are largely those that did not produce corrosive or severe ocular damage. Evaluation of the over-classification likelihoods of substances (i.e., the likelihood that a substance will be classified into a more hazardous irritation category than is suggested by the actual test) was based on 2563 rabbits tested in 610 studies. Based on the available data, these likelihoods were estimated to be 7% to 8% for Category 2A substances, 3% to 5% for Category 2B substances, and 0.3% to 0.5% for non-irritants. This study represents one of the largest databases of *in vivo* rabbit test studies to be evaluated and likely provides a reasonable range of estimates for the misclassification likelihood of the *in vivo* rabbit eye irritation test method.

A report on this analysis is available on the ICCVAM/NICEATM website at http://iccvam.niehs.nih.gov/methods/ocudocs/classifi/haserpt110405.pdf. Additionally, a manuscript has been prepared and will be submitted for publication in a peer-reviewed journal.

3.1.3 Ocular Toxicity Symposia

ICCVAM, in collaboration with the European Centre for the Validation of Alternative Methods (ECVAM) and the European Cosmetic Toiletry and Perfumery Association (COLIPA), sponsored two ocular toxicity symposia at the Natcher Conference Center, NIH Campus, Bethesda, Maryland, entitled:

- *Mechanisms of Chemically-Induced Ocular Injury and Recovery* (May 11-12, 2005)
- *Minimizing Pain and Distress in Ocular Toxicity Testing* (May 13, 2005)

During the symposium *Mechanisms of Chemically-Induced Ocular Injury and Recovery*, participants discussed the state-of-the-science and understanding of the pathophysiology and mechanisms of chemically-induced ocular injury and recovery (reversibility vs. irreversibility) and identified current knowledge gaps in the understanding of these mechanisms. The participants proposed research initiatives to advance the development of test systems necessary to meet regulatory testing requirements and provide for protection of human health while reducing, refining (less pain and distress), and/or replacing the use of

[11] UN. 2003. Globally Harmonized System of Classification and Labeling of Chemicals (GHS). New York & Geneva: United Nations Publications. Available: http://www.unece.org/trans/danger/publi/ghs/officialtext.html [accessed 26 August 2004].

animals. Participants recommended additional biomarkers that should be considered for inclusion in *in vitro* test systems for ocular irritation testing, such as:

- Cytotoxicity
- Cytokine measurements as potential markers of inflammatory processes
- Depth of injury
- Histopathology

Knowledge gaps for future research in regard to differences in *in vivo* and *in vitro* test systems were also identified. Finally, symposia participants distinguished quantitative, objective endpoints that were recommended for inclusion in the current *in vivo* rabbit eye test and in human chemical injuries in order to support the development and validation of predictive *in vitro* test methods and improve hazard characterization and reliability. Some of the additions to the *in vivo* rabbit eye test that were suggested include:

- Slit lamp biomicroscopy with fluorescein staining
- Pachymetry readings (corneal thickness)
- Photodocumentation of the injury
- Histopathology

Recommended additions for routine human injury assessment included a description of the offending agent, standardized/comprehensive eye exam, pachymetry readings, photodocumentation, and documentation of clinical outcomes.

During the second symposium, *Minimizing Pain and Distress in Ocular Toxicity Testing*, participants reviewed the current understanding of the sources and mechanisms of pain and distress in ocular toxicity testing. They also suggested existing best practices for preventing, recognizing, and alleviating ocular pain and distress; the use of both topical anesthesia and systemic analgesics/general anesthesia; and additional research, development, and validation studies necessary to support scientifically valid ocular testing procedures that avoid pain and distress.

A summary of both symposia and supporting information has been made available on the ICCVAM/NICEATM website (http://iccvam.niehs.nih.gov).

3.1.4 Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations

In June 2004, the EPA asked ICCVAM to evaluate the validation status of proposed non-animal approaches for determining the skin and eye irritation potential of antimicrobial cleaning product formulations for meeting regulatory hazard classification and labeling requirements. ICCVAM considered the EPA's request and recommended that the evaluation of these non-animal approaches proceed as a high priority. SACATM concurred with ICCVAM's recommendation at their October 2004 meeting. ICCVAM agreed to work with the EPA and representatives of its Pesticide Program Dialogue Committee (PPDC) to help assure that the submission provided to ICCVAM contains all relevant information, data, and appropriate analyses as described in the *ICCVAM Guidelines*. NICEATM, in collaboration with ICCVAM, will convene an independent scientific expert panel to review the submission, develop conclusions on the validation status of these methods, and make

recommendations about the usefulness and limitations of these methods for their intended purpose. The date for the expert panel meeting has not been determined but will be announced in a future *FR* notice.

3.2 *In Vitro* Endocrine Disruptor Screening Methods

In May 2003, ICCVAM published a report entitled *ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays*[12].

During its evaluation of *in vitro* endocrine disruptor screening assays, ICCVAM recommended that preference be given to development of estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays that

- do not require the use of animal tissue as the receptor source, but rather use recombinant-derived proteins, and
- do not use radioactive materials.

ICCVAM also recommended minimum procedural standards that should be incorporated in standardized ER and AR binding and TA test method protocols and minimum lists of chemicals that should be used for the validation of such assays.

ICCVAM subsequently received nominations of two methods for validation studies. The first nomination was for a biosensor system that can assess ER binding and TA. The second nomination was for a stably transfected recombinant cell-based ER TA test method. These methods met the ICCVAM recommendations for studies that do not require the use of animals as a receptor source or use radioactive materials. Both methods detect receptor agonist and antagonist activity.

ICCVAM reviewed these two nominations and unanimously recommended that "Evaluation studies for *in vitro* receptor binding and transcriptional activation test methods that do not require the use of animals should receive a high priority for support. Prior to the initiation of such studies, the proposed validation studies should be evaluated for adherence to relevant recommendations in the report, *ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays* (NIH Publication No. 03-4503), by the ICCVAM Endocrine Disruptor Working Group (EDWG) and NICEATM."

These nominations and ICCVAM's recommendation were presented to SACATM at its March 10-11, 2004, meeting. SACATM endorsed the ICCVAM recommendation that the validation of endocrine disrupting screening assays should be a priority.

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[12] ICCVAM. 2003. Evaluation of *In Vitro* Test Methods For Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays. NIH Publication No. 03-4503. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/docs.htm#endocrine [accessed 30 December 2005].

NICEATM is currently conducting standardization studies for the LUMI-CELL® assay, developed by Xenobiotic Detection Systems, Inc. The purpose of these studies is to develop a standardized test method protocol for using the LUMI-CELL® ER TA assay to quantify the ER agonist and antagonist activity of unknown chemicals. This assay uses a stable recombinant human cell line (BG1Luc4E2) that contains a reporter gene construct that expresses luciferase activity in response to exposure of the cells to estrogen and estrogen-like compounds.

A primary goal of this protocol development and standardization process is to prepare detailed, standardized protocols for detecting ER agonists and antagonists that can be easily transferred to other laboratories and used to obtain reproducible results. Elements that make protocols readily transferable include the following: a) the use of reasonably priced, commercially available products and supplies, b) detailed descriptions of the procedures involved, c) the use of objective measurements, and d) test acceptance criteria for determining whether the test system is performing adequately. This protocol standardization study is scheduled to be completed in the summer of 2006. If successful, it is anticipated that this test method will proceed to a formal validation study to determine its usefulness as a screening assay for detecting endocrine activity.

3.3 Skin Corrosivity and Irritation

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

In July 2003, an *FR* notice (Vol. 68, No. 126, pp. 39104-39105, July 1, 2003) was published announcing the availability of and inviting public comment on the ICCVAM Dermal Corrosivity and Irritation Working Group (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. Comments were considered by ICCVAM and its DCIWG during development of the final ICCVAM performance standards for these test methods. The final ICCVAM report, entitled *Recommended Performance Standards for In Vitro Test Methods for Skin Corrosion* (NIH Publication No. 04-4510), was published in May 2004 and announced in the *Federal Register* (Vol. 69, No. 104, pp. 30693-30694, May 28, 2004).

3.3.2 Organisation for Economic Co-operation and Development 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 as part of the ICCVAM evaluation process. ICCVAM agreed with the Peer Review Panel conclusions 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 at http://iccvam.niehs.nih.gov/docs/reports/corprrep.pdf.

In March 2003, a generic, international test guideline for *in vitro* membrane barrier test systems for skin corrosion was developed by ICCVAM and its DCIWG and proposed to the Organisation for Economic Co-operation and Development (OECD) Test Guidelines Program. After numerous rounds of international review and comment, OECD Test Guideline 435 (TG 435), *In Vitro Membrane Barrier Test Method for Skin Corrosion*, was endorsed at the Joint Meeting of the OECD in June 2005. Of note in this final version of TG 435 is the inclusion of performance standards for functionally and mechanistically similar assays, as introduced by ICCVAM and its DCIWG. The inclusion allows for the evaluation of newly developed alternative assays, relative to the validated reference test method, which is an important step toward ensuring that mechanistically and functionally similar test methods have been subjected to the appropriate level of scientific scrutiny prior to their acceptance. OECD is currently considering an ICCVAM proposal for the routine inclusion of performance standards as part of new OECD test guidelines.

3.3.3 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 likelihood of under-predicting corrosivity for the traditional *in vivo* rabbit skin test based on the variation of individual animal responses. With assistance from ICCVAM member agencies, NICEATM compiled relevant study results for analysis. An analysis of skin corrosivity test results obtained for 50 chemicals suggests that the *in vivo* rabbit dermal corrosivity test has an estimated under-prediction likelihood of less than 6% and that false negatives would occur only for weak corrosives (i.e., UN 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. When the analysis is completed, a manuscript on the findings will be prepared for peer-reviewed publication and its availability announced on the ICCVAM/NICEATM website.

3.4 Pyrogenicity Test Methods

In June 2005, ECVAM submitted five *in vitro* human blood cell pyrogenicity tests to ICCVAM for consideration as replacement tests for the currently required *in vivo* rabbit test or an *in vitro* test that requires the use of hemolymph collected from the Limulus horseshoe crab (Limulus amoebocyte lysate [LAL] test, also referred to as the bacterial endotoxin test [BET]). These test methods are similar in that each assay involves the measurement of cytokine levels from either human blood, peripheral blood mononuclear cells (PBMC), or a human monocytoid cell line, as a biomarker of a pyrogenic response. In each assay, cytokine levels are measured with an enzyme-linked immunosorbent assay (ELISA). The five *in vitro* pyrogenicity test methods are as follows:

- PBMC/IL-6 (The Human PBMC/IL-6 *In Vitro* Pyrogen Test)
- WB/IL-1 (The Human Whole Blood/IL-1 *In Vitro* Pyrogen Test)
- cryo WB/IL-1 (The Human Whole Blood/IL-1 *In Vitro* Pyrogen Test: Application of cryopreserved human whole blood)

- WB/IL-6 (The Human Whole Blood/IL-6 *In Vitro* Pyrogen Test)
- MM6/IL6 (An Alternative *In Vitro* Pyrogen Test Using the Human Monocytoid Cell Line MONO MAC-6 [MM6])

ICCVAM and its Pyrogenicity Working Group (PWG) determined that there are sufficient data to warrant an independent evaluation of the relevance[13] and reliability of each of the five *in vitro* pyrogenicity test methods. However, additional information is needed for the BRDs prior to a formal review by an expert peer review panel. A peer review of these methods is expected in early 2007.

3.5 Biomarkers of Toxicity

In February 2004, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) Biomarkers Committee contacted ICCVAM with their intent to submit pre-validation study plans and designs to ICCVAM for comment prior to initiating experimental work. Three ILSI HESI Working Groups indicated their desire to submit study designs for the following biomarkers:

- Serum Cardiac Troponins
- Biomarkers of Nephrotoxicity
- Inhibin B as a Biomarker of Testicular Toxicity

ILSI HESI's intent is the eventual submission of completed validation study data to ICCVAM for peer review. In anticipation of this cooperative consultation activity, ICCVAM approved creation of an ICCVAM Biomarkers Working Group (BioWG) to comment on the validation study design for new biomarkers of toxicity and to coordinate eventual peer review of any subsequent submissions of validated test methods.

In June 2004, ILSI HESI submitted the validation study design from the Inhibin B Working Group. The ICCVAM BioWG reviewed the study design and provided comments back to ILSI HESI. No other study designs have been received to date.

3.6 USDA Validation of Alternative Methods for Vaccine Potency

The U.S. Department of Agriculture (USDA) is currently developing *in vitro* test methods for assessing the potency of *Leptospira* vaccines that have the potential for large reductions in animal use. SACATM endorsed ICCVAM's recommendation that this effort should have a high priority based on the fact that animals used for this purpose account for over 30% of the animals reported to USDA as having undergone procedures involving more than momentary or slight pain and distress without pain-relieving medications. These alternative test methods may be able to replace the use of hamsters for the USDA veterinary potency/efficacy tests for these vaccines.

[13] The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the "accuracy" or "concordance" of a test method.

The USDA Center for Veterinary Biologics has initiated a pre-validation study of an alternative *in vitro* test method for this purpose. Results from this preliminary study are expected to be available in the summer of 2006. ICCVAM has established a Biologics Working Group (BWG) to provide comments on the validation study design and to coordinate any future evaluation activities.

3.7 Alternative Methods for Assessing Potency of Botulinum Toxin

On October 31, 2005, ICCVAM received a nomination from the Humane Society of the United States (HSUS) requesting that alternative test methods to the mouse LD_{50} assay for Botulinum toxin potency testing be assessed and prioritized for prevalidation and validation efforts. A proposed initial step in this process would be for ICCVAM to organize a workshop that would be coordinated with ECVAM and other appropriate stakeholders to secure the cooperation of individuals and organizations that possess relevant protocols and test data. Following this workshop, HSUS proposes that ICCVAM work with appropriate partners to validate one or more of the alternative assays as a replacement of the mouse LD_{50} test.

In response to this nomination, ICCVAM recommended that a workshop should be given a high priority. ICCVAM presented this recommendation to SACATM at their December 12, 2005, meeting. SACATM endorsed the ICCVAM recommendation that a workshop should be given a high priority. A workshop is expected to take place in 2006.

4.0 ICCVAM OUTREACH AND COOPERATIVE ACTIVITIES

4.1 Interactions with ECVAM

ICCVAM, NICEATM, and ECVAM continue to cooperate on test method evaluation and validation efforts, and the level of interaction and collaboration has continued to increase. The aim of this extensive cooperation is to a) leverage resources and scientific expertise, b) maximize efficiency of evaluation/validation efforts and minimize duplication of effort, and c) ensure an early exchange of information concerning test method validation in order to facilitate recognition, acceptance, and implementation of scientifically validated test methods in both Europe and the United States. This cooperation facilitates the OECD process in the generation of OECD test guidelines by providing harmonized, standardized protocols and promoting the international adoption of validated alternative methods. Activities where ICCVAM and ECVAM have collaborated during this report period include:

- ICCVAM and NICEATM have observer status on the ECVAM Scientific Advisory Committee (ESAC).
- The Head of ECVAM was invited in March 2004 to become a non-voting member of ICCVAM SACATM.
- ICCVAM members and ICCVAM-nominated experts participated in ECVAM taskforces, workshops, and validation management teams in 2004-2005.
- ECVAM staff participated in ICCVAM-NICEATM-sponsored workshops and validation efforts.

- ICCVAM, NICEATM, and ECVAM jointly sponsored workshops and validation studies.

4.1.1 Technical Activity Interactions

As appropriate, ICCVAM seeks to collaborate with ECVAM on methods of joint interest. ICCVAM is aware that a current priority of ECVAM is focused on identification, development, pre-validation, and validation of non-animal test methods needed to replace animal-based methods for safety testing of cosmetic ingredients, which will be banned in the European Union (EU) in January 2009 (7th Amendment to the European Cosmetics Directive 76/768/EEC). Current ECVAM programs address acute oral toxicity, reproductive and developmental toxicity, dermal irritation, dermal hypersensitivity, genotoxicity, and ocular irritation. ICCVAM and NICEATM are involved in collaborative and liaison roles with the task forces for these initiatives in order to leverage resources and avoid duplication of effort.

The European Commission (EC) is providing significant financial support for ECVAM activities in order to have valid alternatives in place by the 2009 deadline. In addition, ICCVAM and NICEATM are following ECVAM and EC efforts to respond to the recent Proposal on the Registration, Evaluation, and Authorization of Chemicals (REACH). REACH, which sets out toxicity testing requirements for the more than 30,000 chemicals currently in commerce, as well as chemicals expected to be introduced in the future, is anticipated to require the use of large numbers of animals. EC financial support also is being provided to ECVAM for the identification, development, evaluation, and validation of alternative test methods, as well as for the implementation of validated alternative methods, to reduce the number of animals required to provide the required safety data for REACH.

Additional collaborative activities:

- As recommended in October 2000 at the ICCVAM-sponsored *International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity*, NICEATM and ECVAM initiated the first joint validation study in 2002 to evaluate the usefulness of two *in vitro* basal cytotoxicity assays for estimating acute systemic toxicity. See details in **Section 4.1.2**.
- Liaisons from ICCVAM and NICEATM serve on the Management Team of the ECVAM validation study on acute skin irritation in order to provide comments and suggestions. See details in **Section 4.1.3**.
- Collaborative activities between ICCVAM and ECVAM also are ongoing in the area of *in vitro* ocular toxicity.
- The necessity for carrying out validation studies under standardized conditions, i.e., in compliance with Good Laboratory Practice (GLP) and Good Cell Culture Practice (GCCP), has been recognized by national and international validation bodies. Both ICCVAM and ECVAM members were part of a GLP working group to draft an OECD guidance document on the application of GLP to *in vitro* toxicology studies (May 2004). See details in **Section 4.2.1**.
- A joint ECVAM-ICCVAM-NICEATM workshop on the validation of toxicogenomic-based test systems was held in Italy on December 11 and 12, 2003. Toxicogenomics offers the promise for new approaches to identify and characterize factors such as the biological activity of new and existing chemicals and drugs and

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. See details in **Section 4.3**.

- ICCVAM, NICEATM, and ECVAM collaborated in drafting a new Guidance Document on GCCP. The aim of this document is to reduce uncertainty in the development and application of animal and human cell and tissue culture systems, procedures, and products, by encouraging the greater international harmonization and standardization of laboratory practices, quality control systems, safety procedures, recording, reporting, and compliance with regulations and ethical principles. A manuscript entitled *Guidance on Good Cell Culture Practice: A Report of the Second ECVAM Task Force on Good Cell Culture Practice* has been published (Coecke et al. 2005[14]).

4.1.2 NICEATM/ECVAM Joint Validation Study of *In Vitro* Cytotoxicity Assays

As a follow-up to the October 2000 ICCVAM-sponsored *International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity*, NICEATM, in collaboration with ECVAM, designed and initiated a multi-laboratory international study to evaluate the usefulness of cytotoxicity data from the BALB/c 3T3 and the Normal Human Keratinocyte (NHK) Neutral Red Uptake (NRU) assays for estimating the acute oral toxicity potential of test substances. Preliminary data indicate that using *in vitro* cytotoxicity data to estimate starting doses for rodent acute toxicity assays could 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 participated in the study.

Testing proceeded in three phases designed to facilitate standardization and optimization of the test method protocols before the majority of the chemicals were 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 in which three coded chemicals were tested at least three times each by each laboratory to assess the validity of the proposed protocols. Phase II, which involved the replicate testing of nine coded chemicals using revised protocols in each laboratory, commenced in June 2003 and was completed in November 2003. Final optimized protocols were prepared for Phase III, which involved replicate testing of the remaining 60 coded chemicals. The protocols were made available to the public via an *FR* notice (Vol. 69, No. 201, pp. 61504-61505, October 19, 2004). Phase III commenced in January 2004 and was completed in January 2005.

On behalf of ICCVAM, NICEATM requested nominees for an independent peer review panel to evaluate the validation status of these two *in vitro* cytotoxicity assays and data from

[14] Coecke, S., Balls, M., Bowe, G., Davis, J., Gstraunthaler, G., Hartung, T., Hay, R., Stokes, W. 2005. Guidance on Good Cell Culture Practice: A Report of the Second ECVAM Task Force on Good Cell Culture Practice. ATLA 33:261-287.

standard *in vivo* acute oral toxicity testing in the *Federal Register* (Vol. 70, No. 54, pp. 14473-14474, March 22, 2005). NICEATM is currently finalizing a draft BRD describing the background, methods, and results of the validation study. The draft BRD will be made available to the peer panel members and the public in Spring 2006, along with the draft ICCVAM recommendations on the validation status of these test methods and proposed performance standards. The peer panel meeting is currently scheduled for May 2006. ICCVAM will consider the conclusions and recommendations from the peer panel in developing final test method recommendations and performance standards for these test methods.

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. The NICEATM-developed *in vivo* and *in vitro* databases will also be made available for use in future studies.

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

ICCVAM and NICEATM are collaborating with ECVAM to conduct an independent validation study on three *in vitro* test methods for assessing dermal irritation. To help identify suitable reference chemicals (i.e., those with high quality rabbit or human dermal irritation data) for the validation study, ICCVAM published an *FR* 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. (High quality rabbit ocular irritation data were also requested in order to identify appropriate reference chemicals that can be used in future validation studies of *in vitro* test methods for ocular irritancy.) Selection priority was given to commercially available chemicals that have been tested for dermal irritation in both rabbits and humans. NICEATM, in collaboration with the ICCVAM DCIWG, compiled the submitted data on commercially available chemicals. A list of chemicals tested for skin irritancy in rabbits and/or humans and supporting data were provided to ECVAM in July 2004 for consideration during the selection of chemicals to be used in the validation of *in vitro* test methods for dermal irritation.

ECVAM completed the laboratory phase of this validation study to evaluate protocols for EpiDerm™ and EPISKIN™. The protocols were optimized to identify chemicals that have the potential to be dermal irritants. As an additional measure to assess the acceptability of these *in vitro* assays, NICEATM is undertaking an effort to estimate the likelihood of under-classification of the *in vivo* test for the identification of irritants, mild irritants, and non-irritants according to the UN GHS system (UN 2003[15]). Data for 164 substances tested in 197 studies were obtained from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) database for skin irritation and corrosion (ECETOC 1995[16]). Once additional data received from U.S. Federal agencies and interested stakeholders have been reviewed and accepted for inclusion in the database, a more comprehensive analysis

[15] UN. 2003. Globally Harmonized System of Classification and Labeling of Chemicals (GHS). New York & Geneva: United Nations Publications. Available: http://www.unece.org/trans/danger/publi/ghs/officialtext.html [accessed 26 August 2004].

[16] ECETOC. 1995. Skin Irritation and Corrosion – Reference Chemicals Data Bank. Technical Report No. 66. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels.

will be conducted, and the false negative analysis will be refined. When the analysis is completed, a manuscript on the findings will be prepared for peer-reviewed publication. When the manuscript is published, its availability will be announced on the ICCVAM/NICEATM website, and reprints will be provided on request.

If *in vitro* dermal corrosion and irritation methods are to be considered as replacements for animals for dermal corrosion/irritation testing, then *in vitro* testing strategies must be capable of identifying the false negative corrosive substances that are currently identified using the one to three animal dermal irritation/corrosivity protocol. Accordingly, U.S. regulatory scientists have stated that their agencies will need to know how certain corrosive substances, especially those that have produced false negative results in *in vitro* corrosivity test methods, will respond in the *in vitro* dermal irritation test method protocols. The availability of such data may allow for criteria to be established for identifying such substances as corrosives. This information, together with other relevant details regarding the limitations of the *in vitro* dermal corrosion/irritation assays, will be needed for authorities to fully consider the usefulness of these test methods for regulatory hazard classification purposes.

The need for this additional information was discussed by the ECVAM Validation Management Team for the *in vitro* dermal irritation assays, which includes one liaison from ICCVAM and one from NICEATM. The ECVAM Management Team agreed that this is an important issue to address in terms of identifying possible test method limitations, and agreed conceptually with the need to evaluate these chemicals as a follow-up to the current validation study. In July 2005, ICCVAM/NICEATM subsequently proposed a study plan to the ECVAM Management Team for their consideration. This study is intended to generate information critical for consideration of these *in vitro* methods by regulatory authorities.

4.2 Other International Activities

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

In January 2003, ICCVAM and ECVAM agreed to jointly promote the international application of GLP to *in vitro* systems. In support of this effort, ICCVAM, in February 2003, submitted to OECD a *Justification for Development of an OECD Guidance Document on the Application of Good Laboratory Practice (GLP) Principles to In Vitro Testing*. This document was discussed by participants in a March 4, 2003, consultation meeting between members of the OECD Working Group on GLP, ECVAM, and ICCVAM. They agreed on the need for further guidance on the application of the principles of GLP to *in vitro* studies. Part of ICCVAM's reasoning for developing such guidance was based on the recognition that there are increasing numbers of validation studies of *in vitro* testing methods. It is expected that the use of *in vitro* test methods will continue to increase as new science and technologies are incorporated into *in vitro* test methods and such innovative techniques enter the regulatory arena. Furthermore, with the increasing use of non-animal testing procedures, such guidance will facilitate the acceptable use of these new test methods and the proper generation and documentation 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. According to ICCVAM, ECVAM, and OECD guidances, validation studies

should ideally be conducted in accordance with GLPs. Thus, a user-friendly, clear, and concise document devoted to the application of GLPs to *in vitro* methods also could help encourage the use of GLPs for validation studies, thereby helping to facilitate and increase confidence in the validation of *in vitro* test methods.

ICCVAM and ECVAM were invited by OECD to provide joint presentations to a subcommittee of the OECD GLP Working Group concerning the need and justification for further international guidance on the application of GLPs to *in vitro* toxicological testing. It was envisioned that such a document would supplement, but not replace, the existing OECD *Principles of Good Laboratory Practice* and subsequent OECD Guidance, Advisory, and Consensus Documents. The arguments presented by ICCVAM and ECVAM were favorably received. Subsequently, the full OECD GLP Working Group endorsed the need for this additional guidance in September 2003. An OECD workshop held in May 2004 resulted in the final OECD document entitled *Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 14, Advisory Document of the Working Group on GLP, The Application of the Principles of GLP to In Vitro Studies* (available at http://appli1.oecd.org/olis/2004doc.nsf/linkto/env-jm-mono(2004)26).

4.2.2 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 unresolved 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 members served on the conference organizing committee, and several ICCVAM members were invited to serve as discussion leaders and rapporteurs. ICCVAM publications also were identified as key discussion documents. The outcome of the conference was to task a subcommittee of participants to generate a revised draft OECD Guidance Document (No. 34), which would be entitled *The Development, Validation and Regulatory Acceptance of New and Updated Test Methods in Hazard Assessment*. A revised draft document was circulated in October 2003 to OECD member countries for comment. Due to the large number and nature of the comments received, the OECD Working Group of National Coordinators at their 16th meeting on May 26-28, 2004, agreed that an Expert Consultation meeting should be held to focus on specific areas that needed particular attention and to make the appropriate changes to the document. The Expert Consultation meeting, hosted by the United States, was organized and sponsored by ICCVAM-NICEATM and held in Bethesda, Maryland, on October 13-15, 2004. As a result of this meeting, extensive revisions and additions were introduced into the draft that resulted in a third version of Guidance Document No. 34. This third version was circulated in January 2005 to OECD member countries for comment. The document was further edited and ultimately approved by the OECD Working Group of National Coordinators at their 17th meeting on April 12-14, 2005. A final version was subsequently approved and endorsed at the 38th Joint Meeting in June 2005. It was then published in the OECD Series on Testing and Assessment in August 2005. The final document provides practical guidance on

principles and processes for the validation and acceptance of animal and non-animal test methods for regulatory hazard assessment and is based to a large extent on principles and criteria for validation and regulatory acceptance developed by ICCVAM and ECVAM.

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

ICCVAM and NICEATM members participated in numerous international workshops, conferences, and meetings in 2004 and 2005. Brief descriptions of selected events are provided below. **Please note that any conclusions and recommendations outlined below are those of the respective workshop participants, and the inclusion of these conclusions and recommendations in this report should not be interpreted as an endorsement by ICCVAM or any of its member agencies.**

4.3.1 ECVAM-ICCVAM/NICEATM Workshop on the Validation of Toxicogenomic-Based Test Systems

A workshop on the *Validation of Toxicogenomic-Based Test Systems*, co-organized and co-sponsored by ECVAM and ICCVAM/NICEATM was held on December 11 and 12, 2003, in Ispra, Italy. The purpose of the workshop 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. New and innovative approaches will likely be necessary to standardize and evaluate the scientific validity and regulatory applicability of test methods based on toxicogenomics. It is envisioned that the entire validation process will be different and more complex than that for conventional alternative methods because both the predictive test system and the applied new technology itself will need to be validated. Because data are already being generated using this technology, it was considered 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. By addressing the critical validation issues early on, and in parallel with the evolution of the technological development of toxicogenomic-based methods, it was reasoned that it should be possible to pre-empt many potential pitfalls and data gaps encountered with retrospective method evaluations that could impede validation of, buy-in, and confidence in this promising research and regulatory tool.

A report from this workshop has been published in *Environmental Health Perspectives* and is available at http://ehp.niehs.nih.gov/docs/2005/8247/abstract.html.

4.3.2 Insuring Animal Welfare in Research: Coexistence of Toxicology Studies with Humane Endpoints

A workshop on *Insuring Animal Welfare in Research: Coexistence of Toxicology Studies with Humane Endpoints* was conducted at the 43rd annual meeting of the Society of Toxicology, Baltimore, Maryland, March 21-25, 2004. Subjects addressed included:

- The types of data required for regulatory approval and their necessity with respect to refining, reducing, and replacing animal use.
- The development and utilization of relevant humane endpoints and the criteria for determining when study interventions are necessary.

- The role of the Institutional Animal Care and Use Committees (IACUC) in assisting the investigator in the refinement of the experimental design and in the optimization of the numbers of animals used in a manner consistent with sound scientific practices and in consideration of humane endpoints.
- The conduct of toxicological studies with regard to balancing regulatory, scientific, and humane factors while producing sound and reliable data.
- How the EU addresses regulatory and scientific issues and humane endpoints in the care and use of laboratory animals.

Among the presentations were those by Dr. Schechtman (*Issues in Toxicity Testing for Regulatory Purposes*) and Dr. Stokes (*Veterinary Medicine and Animal Welfare Issues*).

4.3.3 International Workshop on Weight-of-Evidence Approaches to Validation

An ECVAM *International Workshop on Weight-of-Evidence Approaches to Validation* was held on May 5-7, 2004, in Ispra, Italy. The objectives of the workshop were to define weight-of-evidence approaches to validation and to develop criteria for validation based on weight-of-evidence approaches. Discussions involved:

- The meaning of weight of evidence in different contexts
- The pitfalls surrounding the approach
- The types of evidence that would be considered (e.g., pharmacotoxicology, human, animal, *in vitro*, *in silico*, and quantitative structure-activity relationships [QSARs])
- The quality of evidence, its relevance, and its evaluation
- The need for better evidence and evidence gathering
- The need for a way of evaluating a test/system in the absence of *in vivo* data of sufficient relevance, quality, and/or quantity
- The gold standard approach versus the weight-of-evidence approach
- The need for mechanistically-based systems/tests
- Regulatory consideration in the use of weight-of-evidence approaches.

A key area addressed was the retrospective evaluation of available data to substantiate the scientific validity of proposed new test methods. At issue was how to ensure that all available existing data are considered while appropriately weighing the value of data for which complete supporting information and/or original raw data may no longer be available or which may not have been generated in accordance with GLPs. ICCVAM and NICEATM members shared their considerable experience with this approach. Another important issue was how to set criteria for data that will be used to develop and validate QSAR models, since interest in validating these models for regulatory safety decision-making is increasing. A workshop report is currently being drafted which conveys examples of retrospective weight-of-evidence evaluations, conclusions, and recommendations. Since the EC has committed significant resources to validate new *in vitro* regulatory testing methods that will eventually impact U.S. government regulatory agencies, ICCVAM and NICEATM continue to actively collaborate with ECVAM to maximize the likelihood that validation studies and processes are consistent with agencies' regulatory and scientific needs.

4.3.4 Genomics and Alternatives to Animal Use 2004

A priority of the Dutch government is to advance laboratory animal welfare by applying new technologies such as genomics. The Netherlands Genomics Initiative (NGI) was established by the Dutch government to coordinate research, development, and application of genomics-based technologies. Accordingly, a conference was co-organized by the NGI with the Netherlands Centre for Alternatives to Animal Use (NCA) and held on May 31-June 5, 2004, to develop a strategy for the highest priority genomics research and development (R&D) areas that may directly result in replacement, reduction, and/or refinement (the 3Rs) of laboratory animal use in research and testing. A major driver for this conference was the recognition that genomics technologies will continue to have a major impact on biomedical research and testing. Invitations to attend the conference were extended to both the Chair of ICCVAM and the Director of NICEATM. The Director of NICEATM participated in this conference as an invited workshop leader.

Specific recommendations were developed for future research, development, validation, and implementation of genomics-based alternatives to animal use. The conference specifically addressed the potential beneficial and adverse consequences of these technologies on laboratory animal use and discussed opportunities and challenges for animal reduction, refinement, and replacement. The Dutch NGI is clearly committed to devoting financial resources to 3Rs projects that will likely benefit animal welfare.

There was strong agreement that the internationally harmonized validation and regulatory acceptance criteria developed by ICCVAM, ECVAM, and OECD are fully applicable to genomics-based test methods. There was also agreement that targeted 3Rs genomics research, development, and validation may lead to earlier, more predictive biomarkers of toxicity that will refine animal tests by reducing the need to produce overt clinical toxicity and that will serve as more humane endpoints for toxicity studies. A workshop report will be submitted for publication in *Environmental Health Perspectives*. Future workshops will be convened to review progress and explore new opportunities for research in this area.

4.3.5 The ECVAM Consultation Meeting on the Validation of QSARs for ER and AR Binding Assays

The ECVAM *Consultation Meeting on the Validation of QSARs for ER and AR Receptor Binding Assays* was held on September 2-3, 2004. Attendees developed recommendations for an international validation study of QSARs. QSARs that might predict whether and to what extent chemicals might bind to ERs and ARs were discussed. Because of concerns about potential adverse health effects of chemicals that can disrupt the normal endocrine system, the United States and other governments have mandated that chemicals should now be evaluated for this potential. Therefore, a critical need exists to have a rapid and accurate way to triage the tens of thousands of existing chemicals in commerce so that definitive screening and testing can be conducted on those most likely to cause adverse effects. The development and validation of accurate and reliable QSARs is one possible approach to accomplish this.

The participants agreed that it is essential that data used to create QSARs must be generated from standardized and adequately validated ER and AR binding and TA assays, which

currently do not exist. Therefore, the experts agreed that validation studies for these assays are a high priority, and that these should be accomplished in accordance with ICCVAM recommendations in the report *ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays*. Once the assays are adequately validated and performance standards established, these i*n vitro* data may be used to develop and validate QSARs for endocrine disruptors. The validated QSARs may then be used to evaluate large numbers of chemical structures that fit within the respective applicability domain. The group recommended that independent validation studies of the *in vitro* methods should be undertaken by ECVAM and ICCVAM/NICEATM, with consideration of advice from OECD.

4.3.6 OECD Meeting on the Current Status of Non-Animal Methods for Screening Chemicals for Endocrine Disruptor Activity

This meeting was held in November 2004 to review the current status of international efforts to develop and validate non-animal methods for screening chemicals for endocrine disruptor activity. High throughput methods discussed included TA reporter gene and receptor-binding assays for estrogen and androgen activity, aromatase and steroidogenesis assays, and QSAR models. Results of completed work and plans for future work were presented and discussed, with emphasis on international collaboration for future work. The recommended essential test method components and validation reference chemicals for ER and AR methods developed by ICCVAM were presented and discussed as minimum standards for all future studies on these methods.

4.3.7 Toxicological Research and Testing: Best Practices and Opportunities for Laboratory Animal Refinement, Reduction, and Replacement

A workshop on *Toxicological Research and Testing: Best Practices and Opportunities for Laboratory Animal Refinement, Reduction, and Replacement*, sponsored by NICEATM and co-chaired by the Director of NICEATM, was held at the 44th annual meeting of the Society of Toxicology, New Orleans, Louisiana, March 6-10, 2005. This workshop was conducted in recognition that it is becoming increasingly important to adopt practices and approaches that refine, reduce, and replace the numbers of laboratory animals utilized in the performance of toxicology studies, irrespective of purpose. Adoption of best practices will help ensure that animals are used in the most humane and judicious manner while still fulfilling research or testing objectives. The topics covered included:

- Consideration and appropriate incorporation of *in vitro* methods, humane endpoints, and tiered testing strategies
- Current best practices for housing and providing environmental enrichment for study animals and factors that might potentially influence study outcomes
- GLP requirements for pre-clinical safety studies and optimal animal welfare practices consistent with GLP compliance
- Application of toxicogenomics to pre-clinical safety studies involving animals and potential opportunities for these methodologies to refine, reduce, and replace animal use
- The potential impact of EU animal welfare laws on harmonization of animal care programs and toxicological research.

4.3.8 Workshop on Validation of Toxicogenomic Technologies: A Focus on Chemical Classification Strategies

A *Workshop on Validation of Toxicogenomic Technologies: A Focus on Chemical Classification Strategies* was convened by the Committee on Emerging Issues and Data on Environmental Contaminants of the National Research Council of the National Academy of Sciences (NAS) and was held at the NAS, Washington, DC, July 7-8, 2005. The Chair of ICCVAM was invited to discuss ICCVAM fundamentals for validation and regulatory acceptance as it might impact toxicogenomics.

The workshop sought to examine the need for and approaches that might be useful in the validation of toxicogenomic technologies. The need for validation of toxicogenomic technologies is an issue of concern to both regulators and regulated parties and fundamental to determining the relevance, reliability, and appropriate use of data derived from these technologies. While often considered, validation of these methods is rarely addressed adequately.

The goal of this workshop was to evaluate current validation strategies in the field of toxicogenomics and to facilitate the development of other appropriate validation approaches as necessary. The workshop focused on technology-based validation strategies employed in gene expression studies to classify drugs or chemicals based on potential mode of action (classification studies). This effort involved examining the state of the science, evaluating the benefits and limitations of current strategies, and considering mechanisms to strengthen their use in classification studies and potentially other toxicogenomic applications. The workshop is anticipated to result in a report that summarizes the approaches presented and discussed that might be useful to assess the validity of toxicogenomic applications, with specific focus on classification studies and on the principles, methods, and technologies employed in successful validation strategies.

4.3.9 Tools of the Trade: Common Ground in the Application of Genomics in Regulatory Decision-Making

A meeting on *Tools of the Trade: Common Ground in the Application of Genomics in Regulatory Decision-Making*, co-organized and co-sponsored by the U.S. Food and Drug Administration (FDA) and EPA, was held at the EPA, Research Triangle Park, North Carolina, on August 4, 2005. The purpose of the meeting was to assess the status of genomics and its applicability for regulatory decision-making in the FDA and the EPA, to identify common agency goals related to genomics, and to distinguish information, tools, and nomenclature (particularly related to validation) held in common and/or that can be shared between the agencies. Among the primary goals of this effort was to establish the basis for interagency coordination and collaboration and to work with ICCVAM to help ensure consistency in validation exercises, taxonomy, standards, and quality control. Also identified as essential was the need for appropriate training (e.g., for reviewers) in both agencies.

Training goals would include providing an overview of genomics, genomic technologies and their potential capabilities, data/information handling and repositories, data interpretation tools, and data analysis. Training would take place at various levels:

1. Introductory genomics training
2. Training of senior managers
3. Training of non-technical personnel
4. Advanced training for scientists and those likely to use genomics data (e.g., risk assessors).

The following presentations were given by ICCVAM members:

- ICCVAM Guidelines for Validation and Regulatory Acceptance: Criteria and Processes for New, Revised, and Alternative Test Methods
- ICCVAM Test Method Nomination and Submission Guidelines and Terminology
- ECVAM-ICCVAM/NICEATM Workshop on Validation of Toxicogenomics-Based Test Systems.

4.3.10 The International Workshop on *In Vitro* Alternative Methods for Assessing Ocular Irritancy/Corrosion

The *International Workshop on In Vitro Alternative Methods for Assessing Ocular Irritancy/Corrosion* was held on August 26, 2005, in Berlin, Germany. The workshop was organized by the German Center for Alternatives and hosted by the Federal Institute for Risk Assessment. As of 2004, positive results from four *in vitro* methods can be used in the EU to classify substances as severe eye irritants/corrosives, although the methods are not yet validated. There were presentations and/or demonstrations by the developers of the methods. The Director of NICEATM presented an overview of the recent NICEATM and ICCVAM review of the validation status of the methods, including their demonstrated usefulness and limitations. Other ICCVAM members also were in attendance. This workshop contributed to increased understanding of the methods by potential users and regulatory authorities and can be expected to facilitate their implementation and use. ICCVAM and NICEATM historically have organized implementation workshops for all newly recommended test methods. Such a workshop for ocular irritancy/corrosion test methods may be held in the United States after the ICCVAM recommendations on these methods are finalized.

4.3.11 2nd International Conference on the Use of Humane Endpoints in Animal Experiments for Biomedical Research

The *2nd International Conference on Humane Endpoints in Animal Experiments for Biomedical Research* was held on August 20-21, 2005, and was attended by over 76 participants from 24 countries. The Director of NICEATM presented an overview of current related policies and recent progress in the United States. Humane endpoints are now routinely considered by investigators for research and testing studies where animals are likely to experience unrelieved pain and distress (estimated to exceed 10% of all animals used). Safety testing accounts for over 75% of animals that experience unrelieved pain and distress. For toxicology testing, the collection of additional quantitative objective measurements will facilitate the identification of earlier endpoints that reduce or avoid pain.

4.3.12 5th World Congress on Alternatives and Animal Use in the Life Sciences

Members of NICEATM and ICCVAM participated in the *5th World Congress on Alternatives and Animal Use in the Life Sciences* (WC5) held in Berlin, Germany, from August 21-25, 2005. The World Congress on Alternatives is an international conference that focuses on the development, validation, and evaluation of alternative test methods that might be used to reduce, refine, and/or replace the use of animals in regulatory testing strategies. The Chair of ICCVAM was involved in the organization and scientific planning of the WC5 and co-chaired the largest of the scientific themes (i.e., Toxicology, Safety Evaluation, and Policy). ICCVAM and NICEATM members participated extensively in many of the theme sessions as invited co-chairs, speakers, panel discussants, and poster presenters. Seven members of NICEATM and 13 members of ICCVAM, representing other Federal agencies, attended the Congress, contributing to 18 platform and 13 poster presentations. The oral presentations by ICCVAM and NICEATM scientists included the following topics:

- Mechanisms of chemically-induced ocular injury and recovery: Current understanding and knowledge gaps
- *In vitro* models for ocular injury: Current and potential biomarkers
- *In vivo* models of ocular injury and recovery: Current and potential biomarkers to support development and validation of predictive *in vitro* models
- *In vitro* methods for the evaluation of hypersensitivity
- Research directions in toxicology at the U.S. National Toxicology Program
- USDA 3R initiatives in veterinary biologics
- NIH funding of the 3Rs
- Ensuring quality of *in vitro* alternative test methods
- ICCVAM's role in validating *in vitro* test methods for endocrine disruptor screening
- Sound science: A prerequisite for advancing alternative methods and protecting public health
- ICCVAM progress in evaluating *in vitro* test methods for identifying severe ocular irritants/corrosives
- Minimizing pain and distress in ocular safety testing: Current best practices and research needs
- Streamlining the validation process: The ICCVAM nomination and submission process and guidelines for new, revised, and alternative test methods
- The use of test method performance standards to streamline the validation process
- Special resources supporting the 3Rs at the U.S. National Library of Medicine
- Alternative search methods to retrieve information on the Web
- Overview of regulatory requirements for the consideration of alternatives
- Humane Endpoints for Animals Used in Biomedical Research and Testing: Best Practices, Policies, and Progress in the United States
- NIEHS: Program in alternative test method research, development, and validation
- ICCVAM's role in validating *in vitro* test methods for endocrine disruptor screening
- Analysis of the correlation between *in vitro* cytotoxicity data and acute toxic effects in humans
- The importance of Good Cell Culture Practice (GCCP)
- Standardization in cell and tissue culture - The need for specific GLP guidelines in the cell culture laboratory (GCCP)

Poster topics included the following:

- Results of a joint NICEATM/ECVAM-sponsored validation study of two *in vitro* cytotoxicity assays for potentially estimating rodent and human acute systemic toxicity
- ICCVAM's recommended reference chemicals for the validation of *in vitro* ER and AR binding and TA assays
- Assessment of the performance of four different *in vitro* test methods (BCOP, IRE, ICE, and HET-CAM) for the detection of ocular corrosives and severe irritants
- Proposed reference substances for optimization and validation studies for *in vitro* ocular test methods
- Evaluation of the under- and over-classification rates of a one to three rabbit sequential Draize rabbit eye test
- Current understanding and knowledge gaps of chemically-induced ocular injury and recovery
- Current and potential biomarkers of ocular injury and recovery that might be used to support the development and validation of predictive *in vitro* model systems.

Copies of NICEATM poster presentations at this Congress can be found at http://iccvam.niehs.nih.gov.

4.3.13 ECVAM Workshop on the Roles of Validation and Invalidation in the Acceptance and Rejection of Test Methods

The ECVAM *Workshop on the Roles of Validation and Invalidation in the Acceptance and Rejection of Test Methods* was held in Ispra, Italy, on September 21-24, 2005. The objective of the workshop was to consider the role of independence in the validation process, in the light of experience in practical validation studies and weight-of-evidence evaluations and their consequences in terms of the acceptance or rejection of test methods that either have or have not undergone validation. The workshop was based on a commentary by Balls and Combes[17]. Participation was limited to specific European and U.S. experts in the areas of validation of alternatives, independent peer review, regulatory acceptance, animal and non-animal test methods, and animal welfare; invitations were extended to the Chair of ICCVAM and the Director of NICEATM. A summary of the workshop deliberations will be published in a future issue of *ATLA*.

4.3.14 Occupational Toxicology Roundtable

A member of NICEATM was invited to speak to the *Occupational Toxicology Roundtable* at their annual meeting in Williamsburg, Virginia, on October 12, 2005. The Occupational Toxicology Roundtable is an informal group of occupational toxicologists, physicians, and hygienists that meet yearly to discuss common workplace exposure issues. The use of alternative assays, especially those used to identify skin and eye irritants is a topic of high interest to this group. A presentation was given on the progress ICCVAM has made on evaluating alternative assays for identifying eye irritants.

[17] Balls M, Combes R. 2005. The need for a formal invalidation process for animal and non-animal tests. ATLA 33:299-308.

4.3.15 European Union ACuteTox Consortium Meeting

The Director of NICEATM was invited to participate on the advisory board of the ACuteTox Consortium, an integrated project funded by the EC. The Consortium consists of 35 partners from academia, industry, partners from small and medium enterprises, governmental institutions, and partners from international organizations, including the Joint Research Centre of the European Commission, the European *In Vitro* Testing Industrial Platform, the European Society of Toxicology *In Vitro*, and the European Consensus-Platform for Alternatives. A NICEATM representative participated in the ACuteTox Consortium meeting held in Alicante, Spain, on October 26-28, 2005, and gave a presentation on the status of the NICEATM/ECVAM Validation Study of *In Vitro* Cytotoxicity Assays.

4.3.16 Animal Alternatives in Cosmetic Safety Testing

The Cosmetic, Toiletry, and Fragrance Association (CTFA), invited a member of NICEATM to attend a one-day symposium entitled *Animal Alternatives in Cosmetic Safety Testing* on November 11, 2005, in Newark, New Jersey. A presentation was given that reviewed the purpose and goals of NICEATM and ICCVAM and summarized the current status of their activities in regard to the validation of alternative test methods. The symposium also included a discussion of the animal use implications of the 7th Amendment of the Cosmetics Directive in Europe and ECVAM's activities and validation efforts. Additionally, information was provided on animal alternatives for evaluating dermal, ocular, systemic and developmental toxicity.

4.3.17 Progress and Barriers to Incorporating Alternative Toxicological Methods in the U.S.

ICCVAM/NICEATM members participated in the workshop *Progress and Barriers to Incorporating Alternative Toxicological Methods in the U.S.*, on November 17-18, 2005, in Baltimore, Maryland. The objective of this workshop was to summarize progress in implementing new, revised, and alternative toxicological test methods across regulatory evaluation frameworks and decision-making programs in the United States; identify technical, regulatory, economic, or societal barriers; and explore potential bridges to facilitate overcoming such barriers to progress. The workshop produced:

- Better understanding of current alternatives research and validation efforts in the United States (i.e., who, what, objectives of research, and availability of resources)
- Enhanced understanding of the environment motivating alternatives research and how this is or is not linked with the regulatory communities who rely on test methods
- Identification and understanding of barriers that impede progress
- Recommendations to overcome these barriers.

A summary of the workshop proceedings, including the recommendations of the panel discussions, will be published in *Regulatory Toxicology and Pharmacology.*

4.3.18 Memorial Conference of the Japanese Center for Alternatives and Animal Experiments (JaCVAM) at the 19th Annual Meeting of the Japanese Society of Alternatives to Animal Experiments (JSAAE)

The inauguration of the newly established JaCVAM took place November 29-December 3, 2005, at an international conference held in Tokyo, Japan, in conjunction with the JSAAE. Participants included members of the Japanese National Institutes of Health Sciences

(NIHS), ECVAM, ICCVAM, NICEATM, and JaCVAM, as well as scientists involved in the field of alternatives in Korea and China. The Chair of ICCVAM was invited to co-chair a plenary session on the establishment of JaCVAM, focusing on the International Partnership on Alternatives. The Chair of ICCVAM also was invited to present a memorial lecture on *International Cooperation of the Validation and Evaluation of Alternative Methods*, and the Director of NICEATM was invited to present a memorial lecture on the *Development of Alternative Methods in the U.S.*

4.3.19 6th World Congress on Alternatives and Animal Use in the Life Sciences
In addition, to commemorate the creation of JaCVAM, Japan has proposed to host an interim *6th World Congress on Alternatives and Animal Use in the Life Sciences* (WC6) in Tokyo in August 2007. For this occasion, the Chair of ICCVAM and the Director of NICEATM have been invited to be members of the WC6 Program Planning Committee. The first meeting of this committee was held at the WC5 in August 2005, and the next meeting was held in Tokyo on December 3, 2005, following the JaCVAM/JSAAE conference. As in past WCs, ICCVAM and NICEATM will play major roles in the planning, organizing, sponsorship, chairmanship, and speaking at the WC6.

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, publications, and activities. It provides user-friendly 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. Not only is information disseminated through the site, but the contact page also 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 number of visitors was 24,200 per month, indicating a very high level of public interest. The website is currently being reworked as a database-driven site, which will allow faster retrieval of more specifically targeted information with the addition of search features, as well as choice of data format and download file type. The new site will be completed in 2006.

5.2 Reports, Federal Register Notices, Publications, and Presentations

5.2.1 Reports
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Test Method. Available: http://iccvam.niehs.nih.gov/methods/ocudocs/ocu_brd.htm [accessed 30 December 2005].

5.2.2 Federal Register Notices

NIEHS. 2004. Notice of Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) on March 10-11, 2004. *Federal Register* 69:6674-6675. Available: http://a257.g.akamaitech.net/7/257/2422/14mar20010800/edocket.access.gpo.gov/2004/04-2931.htm [accessed 30 December 2005].

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5.2.3 Publications and Presentations

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ANNEX A: SACATM MEMBERS

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

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

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

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

Grantley D. Charles, Ph.D.
Research Specialist
Toxicology & Environmental Research Consulting
Dow Chemical Company
Midland, MI
Appointment ends: 6/30/2009

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

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

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

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

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

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

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

Peter Theran, V.M.D.
Consultant, MSPCA
Novato, CA
Appointment ends: 6/30/2006

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

ANNEX B: ICCVAM AUTHORIZATION ACT

Public Law 106–545
106th Congress

An Act

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

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

SECTION 1. SHORT TITLE.
This Act may be cited as the ''ICCVAM Authorization Act of 2000.''

SEC. 2. DEFINITIONS.
In this Act:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(1) Agency for Toxic Substances and Disease Registry.

(2) Consumer Product Safety Commission.

(3) Department of Agriculture.

(4) Department of Defense.

(5) Department of Energy.

(6) Department of the Interior.

(7) Department of Transportation.

(8) Environmental Protection Agency.

(9) Food and Drug Administration.

(10) National Institute for Occupational Safety and Health.

(11) National Institutes of Health.

(12) National Cancer Institute.

(13) National Institute of Environmental Health Sciences.

(14) National Library of Medicine.

(15) Occupational Safety and Health Administration.

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

(d) SCIENTIFIC ADVISORY COMMITTEE.—

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

(2) MEMBERSHIP.—

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

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

from each of—

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

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

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

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

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

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

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

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

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

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

(5) Consider for review and evaluation, petitions received from the public that—

(A) identify a specific regulation, recommendation, or guideline regarding a regulatory mandate; and

(B) recommend new or revised or alternative test methods and provide valid scientific evidence of the potential of the test method.

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

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

SEC. 4. FEDERAL AGENCY ACTION.

(a) IDENTIFICATION OF TESTS.—With respect to each Federal agency carrying out a program that requires or recommends acute or chronic toxicological testing, such agency shall, not later than 180 days after receiving an ICCVAM test recommendation, identify and

forward to the ICCVAM any relevant test method specified in a regulation or industry-wide guideline which specifically, or in practice requires, recommends, or encourages the use of an animal acute or chronic toxicological test method for which the ICCVAM test recommendation may be added or substituted.

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

(c) TEST METHOD VALIDATION.—Each Federal agency carrying out a program described in subsection (a) shall ensure that any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, is determined to be valid for its proposed use prior to requiring, recommending, or encouraging the application of such test method.

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

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

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

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

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

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

SEC. 5. APPLICATION.

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

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

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

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

Approved December 19, 2000.

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ANNEX C: 1993 NIH REVITALIZATION ACT, SECTIONS 1301 AND 205

Official Title (caption):
A bill to amend the Public Health Service Act to revise and extend the programs of the National Institutes of Health, and for other purposes.

Item 81: (34) TITLE XIII--NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Item 82: (32) SEC. 1301. APPLIED TOXICOLOGICAL RESEARCH AND TESTING PROGRAM

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

APPLIED TOXICOLOGICAL RESEARCH AND TESTING PROGRAM

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

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

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

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

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

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

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

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

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

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

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

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

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

PLAN FOR THE USE OF ANIMALS IN RESEARCH

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(3) The Committee shall be composed of--

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

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

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

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