The NICEATM-ICCVAM Five Year Plan: Creating a Path Forward to Reduce, Refine, and Replace Animal Testing

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Abstract

NICEATM and ICCVAM have developed a five-year plan that builds on the ICCVAM mission, vision, and strategic priorities detailed in the 2004 Strategic Plan. Implementing the five-year plan involves addressing four key challenges. The first is to identify priority toxicity testing areas for the next five years, and to conduct and facilitate activities in those areas. Currently, the four highest-priority toxicity testing areas are ocular, dermal, acute, and biologics. Other priority areas include immunotoxicity, endocrine disruptors, pyrogenicity, reproductive and developmental toxicity, and chronic toxicity/carcinogenicity. Neurotoxicity is also an area of interest. The second challenge is to identify and promote research initiatives that are expected to support future development of innovative alternative test methods. These new methods might incorporate techniques such as high throughput screening, computer modeling, informatics, and biomarkers. The third challenge is for NICEATM and ICCVAM to foster the acceptance and appropriate use of alternative test methods through outreach and communication. This will be accomplished through sponsorship and participation in workshops, the NICEATM-ICCVAM website, and the development and publication of standardized test method protocols. Finally, ICCVAM and NICEATM will develop partnerships and strengthen interactions with their stakeholders to facilitate meaningful progress. The plan presents a clear vision of how NICEATM and ICCVAM will promote research, development, translation, and validation of relevant and reliable non-animal and other alternative assays that can be integrated into Federal agency testing programs. Contract support provided by NIEHS contract N01-ES-35504.

Introduction

U.S. laws, regulations and policies require that alternatives must be considered before using animals for research and testing (e.g., Public Law 99-158). Such alternatives include new or revised test methods that:

- **Reduce** the number of animals to the minimum required to obtain scientifically valid data
- **Refine** procedures to lessen or eliminate pain and distress to animals
- **Replace** animals with non-animal systems or one animal species with a phylogenetically lower animal species

Reduction, refinement, and replacement alternatives are commonly referred to as "the 3Rs" of alternatives.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is a permanent interagency committee administered by the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), a component of the National Institute of Environmental Health Sciences (NIEHS). The committee is composed of members from 15 Federal agencies (**see list at right**). ICCVAM's mission is to

- Facilitate development, validation, and regulatory acceptance of new, revised, and alternative test methods that reduce, refine, and replace the use of animals in testing
- Maintain and promote scientific quality and the protection of human health, animal health, and the environment

NICEATM and ICCVAM work with a broad range of stakeholders to fulfill this mission:

- Federal agencies
- National and international validation and test guideline organizations
- Industry
- Academia

These interactions have resulted in the review of 185 test methods by NICEATM and ICCVAM (**See Table 1**). To date, ICCVAM has recommended alternative methods for the four most commonly used toxicity tests. These have been adopted by national and international regulatory authorities.

ICCVAM Five-Year Plan Subcommittee

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Table 1

Test Methods Reviewed or Under Consideration by ICCVAM

Toxicity Area	No.	Test Method (No.)	Regulatory Application and ICCVAM Recommendations
Acute Systemic Toxicity	3	Up-and-Down Procedure (UDP) *In vitro* basal cytotoxicity methods (2)	In 2001, recommended as replacement alternative for OECD TG 401, the traditional *in vivo* rodent LD_{50} test for assessing acute oral systemic toxicity, and adopted by OECD as TG 425; in 2003, accepted by U.S. agencies. In 2007, both *in vitro* test methods recommended as reduction alternatives to estimate the starting dose in the UDP and Fixed Dose Procedure (FDP) for assessing acute oral systemic toxicity.
Biologics Testing	23[2]	*In vivo* alternatives *Ex vivo* alternatives *In vitro* cell-based methods *In vitro* enzymatic alternatives	In 2006, various reduction, refinement, and replacement alternatives to the mouse LD_{50} assay for botulinum toxin detection and potency testing reviewed at a NICEATM-ICCVAM/ECVAM-sponsored workshop; future activities recommended.
Developmental Toxicity	1	Frog Embryo Teratogenesis Assay: Xenopus (FETAX)	In 2000, reviewed at a NICEATM-ICCVAM sponsored workshop as a reduction or replacement alternative to assess the developmental toxicity of chemicals and mixtures; data gaps and inadequacies identified, future activities recommended.
Endocrine Disruptors	138	*In vitro* androgen receptor (AR) binding (11) *In vitro* AR transcriptional activation (TA) (18) *In vitro* estrogen receptor (ER) binding (14) *In vitro* ER TA (95)	In 2002, evaluated as screens for identifying potential endocrine-disrupting chemicals; to be included in EPA's Endocrine Disruptor Screening Program; in 2003, report with guidance for protocol standardization and validation studies released; in 2006, revised reference substance list released. Same as for *in vitro* AR assays.
Eye Corrosion/ Irritation	4	Bovine Corneal Opacity and Permeability (BCOP) Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) Isolated Chicken Eye (ICE) Isolated Rabbit Eye (IRE)	In 2007, BCOP and ICE recommended as screening tests for identifying corrosives and severe irritants, with certain limitations; HET-CAM and IRE not recommended for regulatory hazard classification purposes until further developed and evaluated.
Pyrogenicity	5	*In vitro* pyrogenicity	In 2007, *in vitro* pyrogenicity test methods measuring cytokine release from human cells recommended as replacements for the rabbit test, subject to product specific validation, to detect endotoxin contamination in parenteral drugs.
Skin Corrosion	4	Corrositex® EpiDerm™ EPISKIN™ Rat Trancutaneous Electrical Resistance (TER) Assay	In 1999, Corrositex® recommended as a stand-alone assay for evaluating acids, bases, and acid derivatives for DOT; otherwise, recommended as part of a tiered testing strategy; in 2000, accepted by U.S. agencies; in 2006, adopted by OECD as TG 435. In 2002, TER and human skin models (EPISKIN™, EpiDerm™) recommended as part of a tiered testing strategy; in 2004, adopted by OECD as TG 430/431.
Skin Sensitization	7	Murine Local Lymph Node Assay (LLNA) LLNA limit dose approach Non-radiolabeled LLNA methods (5)	In 1999, LLNA recommended and accepted by regulatory agencies as alternative for guinea pig tests for allergic contact dermatitis; in 2002, adopted by OECD as TG 429. LLNA performance standards, LLNA limit dose approach, non-radiolabeled LLNA methods currently under review.
Total	**185**		

No. = Number of methods reviewed in each toxicity area, OECD = Organisation for Economic Co-operation and Development; TG = test guideline.
[1]These methods were reviewed and discussed at an ICCVAM-NICEATM/ECVAM sponsored workshop to review the state-of-the-science and current knowledge of alternatives that may reduce, replace, and refine (less pain and distress) the use of mice for botulinum toxin testing (see: http://iccvam.niehs.nih.gov/methods/biologics/bot_workshop.htm)

The NICEATM-ICCVAM Five-Year Plan

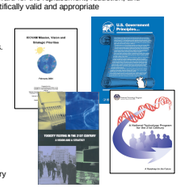
NICEATM and ICCVAM, working in partnership with relevant Federal agency program offices, have developed a five-year plan (ICCVAM 2008) to:

- Research, develop, translate, and validate new and revised non-animal and other alternative assays for integration of relevant and reliable methods into Federal agency testing programs
- Identify areas of high priority for new and revised non-animal and alternative assays or batteries of those assays to create a path forward for the replacement, reduction, and refinement of animal tests, when this is scientifically valid and appropriate

An overall goal is for ICCVAM to assume a greater leadership role in promoting research, development, translation, validation, and regulatory acceptance of alternative test methods.

This five-year plan builds on and supports the objectives of:

- The ICCVAM Mission, Vision, and Strategic Priorities (ICCVAM 2004)
- The NTP's Roadmap for the 21st Century (NTP 2004)
- The U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training
- The recent report by the National Research Council on Toxicity Testing in the 21st Century (NRC 2007)



Four Key Challenges Addressed in the Plan

- Identify Priorities, and Conduct and Promote Alternative Test Method Activities
- Promote New Science and Technology
- Foster Regulatory Acceptance and Appropriate Use of Alternative Methods
- Develop Partnerships and Strengthen Interactions with ICCVAM Stakeholders

Key Challenge 1: Identify Priorities, and Conduct and Promote Alternative Test Method Activities

Prioritization criteria for ICCVAM test method activities include:

- The potential impact that alternative test methods may have on reducing, refining, or replacing the use of animals for testing, taking into consideration the severity of pain and distress and numbers of animals involved
- The potential for the proposed test method(s) to provide improved prediction of adverse health or environmental effects
- The applicability of testing alternatives across agencies

The four highest priorities:
- Ocular toxicity
- Dermal toxicity
- Acute toxicity
- Biologics

Other priority areas:
- Immunotoxicity
- Endocrine disruption
- Pyrogen testing
- Reproductive/developmental toxicity
- Chronic toxicity/carcinogenicity

ICCVAM and NICEATM Planned Activities in Priority Test Method Areas

Ocular Toxicity

- Carry out activities to improve the usefulness and applicability of two recommended *in vitro* test methods (ICCVAM 2006a) for identification of ocular corrosives and severe irritants
- Work with the European Centre for the Validation of Alternative Methods (ECVAM) to evaluate the use of these methods for characterization of the potential for substances to cause reversible ocular damage
- Evaluate *in vitro* approaches to assess the ocular irritation potential of antimicrobial cleaning product formulations
- Facilitate the submission of *in vivo* reference data to be added to a database for use in expanding the development and applicability of new alternative ocular test methods
- Conduct a comprehensive review of the use of topical anesthetics and systemic analgesics to reduce pain and distress in *in vivo* testing
- Encourage stakeholders to carry out studies recommended in recent symposia on "Mechanisms of Chemically-Induced Ocular Injury and Recovery," and "Minimizing Pain and Distress in Ocular Toxicity Testing."

Biologics Testing

- Evaluate alternative test methods and testing strategies for vaccine potency testing, with particular focus on the *in vitro* vaccine potency test being developed by the USDA to reduce the numbers of animals required to evaluate the potency of a common veterinary bacterial vaccine for Leptospirosis
- Facilitate the acceptance of adequately validated test methods and humane endpoints found to be sufficiently accurate and reliable

Dermal Toxicity Testing

- Evaluate alternative dermal irritation test methods, including the use of a combination of *in vitro* test methods for both corrosivity and irritation to reduce or replace animals
- Evaluate non-animal methods and approaches for determining the skin irritation potential of antimicrobial cleaning products

Acute Toxicity Testing

Organize an international workshop to:

- Identify standardized procedures for collecting mechanistic information from acute oral toxicity testing to aid in developing batteries of predictive *in vitro* test methods that can further reduce and eventually replace animals for acute toxicity testing
- Seek more predictive and more humane endpoints that may be used to terminate studies earlier in order to further reduce pain and distress
- Conduct a study to determine how two recommended cell culture test methods (ICCVAM 2006b) can be used to set the starting dose for acute toxicity tests of mixtures
- Assemble high quality rodent acute oral toxicity data from previous studies or future required regulatory studies, and make this reference database available for the development and validation of other new *in vitro* tests (or batteries of tests) to more accurately predict oral acute systemic toxicity
- Collaborate with the ECVAM ACuteTox Project to develop and evaluate a battery of *in vitro* tests for predicting acute oral toxicity hazard classification categories

Other Priority Testing Areas

- ***Immunotoxicity:***
  - Evaluate whether the Murine Local Lymph Node Assay (LLNA) can be used as a stand-alone method for the determination of potency (including severity)
  - Evaluate the possible expansion of the scope of substances and mixtures for which the LLNA may be used
  - Evaluate modifications to the LLNA that may further reduce the number of animals used, or that may eliminate the need to use radioactive materials in the protocol
- ***Endocrine Disruptor Testing***
  - Lead a joint international study with ECVAM and the Japanese Center for the Validation of Alternative Methods (JaCVAM) to evaluate the usefulness and limitations of an *in vitro* test method to identify estrogen-like chemicals that does not require the use of animals as donors for test components
  - Increase involvement in Organization for Economic Cooperation and Development (OECD) test guideline activities related to endocrine disruptors
- ***Pyrogen Testing***
  - Issue recommendations on the current usefulness of recently evaluated test methods and for future studies that may support their expanded use.
- ***Reproductive and Developmental Toxicity Testing***
  - Explore, and promote where appropriate, possible revisions to existing *in vivo* testing protocols to reduce the overall number of animals required without compromising assay performance
  - Closely follow the ECVAM ReProTect project
- ***Chronic Toxicity/Carcinogenicity Testing***
  - Facilitate efforts towards developing alternative models for the multiple mechanisms associated with these endpoints that better simulate living organisms
  - Participate in a JaCVAM-sponsored international validation study of the ability of the *in vitro* alkaline Comet assay to measure the induction of DNA damage in cells of multiple organs

Key Challenge 2: Promote New Science and Technology

NICEATM and ICCVAM will identify and promote research incorporating new science and technologies that can be expected to support the future development of new test methods and approaches to reduce and eliminate the need for animals. These efforts will support the goals outlined in the National Research Council's recently published *Toxicity Testing in the 21st Century: A Vision and a Strategy* (NRC 2007), as well as the National Toxicology Program's *Roadmap for the Future* (NTP 2004).

Planned activities in this area include:

- **High Throughput Screening (HTS):** Facilitate reviews of the usefulness and limitations of defined HTS approaches with regulatory applicability, and also assist in the identification of assays and endpoints that are relevant for alternative test methods that have already been adopted
- **Other Animal Systems**: Evaluate the validation status of test methods with applicability for regulatory testing and that use *C. elegans*, fish, amphibians and other non-mammalian species as test systems
- **Computational Approaches**: Monitor ongoing projects at EPA, ATSDR and DOE to develop computational methods for modeling of biological effects and testing prioritization
- **Biomarkers of Toxicity**: Follow progress of efforts within NIEHS and FDA to use biomarkers to predict damage to a specific organ, and to identify how sets of biomarkers might be more predictive of exposure risks than single biomarkers
- **Toxicology Databases**: Promote the availability of data from searchable toxicology databases at NIEHS for use in the development of alternative test methods
- **Nanomaterials Testing**: Work with regulators and stakeholders to identify tests that effectively characterize the potential hazards of nanomaterials while also addressing the 3Rs

References and Additional Information

Animal Welfare Act, as amended. 1990. Public Law 89-544. Available: http://www.aphis.usda.gov/ac/publications/AWA/AWAINDEX.HTML.

Health Research Extension Act of 1985. 1985. Public Law 99-158. Available: http://grants2.nih.gov/grants/olaw/references/hrea1985.htm.

ICCVAM. 1997. Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. Available: http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf.

ICCVAM 1999. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. NIH Publication Number 99-4494. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna_PrePanel98.htm.

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. Available: http://iccvam.niehs.nih.gov/SuppDocs/SubGuidelines/SD_subg034508.pdf.

ICCVAM 2004. ICCVAM: Mission, Vision and Strategic Priorities. Available: http://iccvam.niehs.nih.gov/docs/about_docs/MisVisStrat.pdf.

ICCVAM 2006a. ICCVAM Test Method Evaluation Report: *In Vitro* Ocular Toxicity Test Methods for Identifying Ocular Corrosives and Severe Irritants. NIH Publication 07-4517. Available: http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_timer.htm.

ICCVAM 2006b. ICCVAM Test Method Evaluation Report: *In Vitro* Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests. NIH Publication Number 07-4519. Available: http://iccvam.niehs.nih.gov/methods/acutetox/ivlv_nru_timer.htm.

ICCVAM 2008. The NICEATM-ICCVAM Five-Year Plan (2008-2012). NIH Publication Number 08-6410. Available: http://iccvam.niehs.nih.gov/docs/5yearplan.htm.

National Research Council. 2007. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Washington, DC: National Academy of Sciences. Available: http://books.nap.edu/catalog.php?record_id=11970.

National Toxicology Program. 2004. Toxicology in the 21st Century: The Role of the National Toxicology Program. Available: http://ntp.niehs.nih.gov/ntp/main_pages/NTPVision.pdf.

A full listing of all ICCVAM publications can be found on the NICEATM-ICCVAM website at http://ntp-apps.niehs.nih.gov/iccvamlp/searchDoc.cfm

Key Challenge 3: Foster Acceptance and Appropriate Use of Alternative Test Methods

While NICEATM and ICCVAM promote and employ good science in determining the validation status of alternative test methods, Federal agencies make the acceptance decisions for their respective programs. However, once an alternative test method is accepted, it has little impact unless it is actually used. Therefore, NICEATM and ICCVAM will work to promote acceptance and use of scientifically valid alternative test methods by:

- Communicating the outcomes of ICCVAM review activities and/or workshops via the *Federal Register*, at national or international scientific meetings, via peer reviewed journal publications, and at training courses
- Promoting active communication and outreach efforts with both government and non-government stakeholders
- Implement improvements to the NICEATM-ICCVAM website:
  - Develop lists of relevant Frequently Asked Questions
  - Maintain a summary of test methods that have been, or are currently undergoing, ICCVAM review
  - Provide links to webpages of member agencies dedicated to alternative test methods research, development, translation and validation
- Sponsor and participate in workshops that include both government and non-government stakeholders to increase the acceptance and use of new alternative test methods
- Facilitate the international adoption of valid alternative test methods by providing standardized protocols, comprehensive test method background review documents, and the results of independent scientific peer reviews

The NICEATM-ICCVAM website is a key resource for ICCVAM stakeholders

ICCVAM Member Agencies

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

Key Challenge 4: Develop Partnerships And Strengthen Interactions With ICCVAM Stakeholders

NICEATM and ICCVAM recognize that effective interactions with stakeholders are an essential component of successfully protecting human and animal health and the environment while implementing the 3Rs. We will seek to develop partnerships and strengthen interactions with our stakeholders by:

- Proactively identifying research needs and promising methods that should be priorities for further development, translation, validation, or ICCVAM evaluation
- Fostering interagency collaboration among Federal research and regulatory agencies, including opportunities for test method validation activities
- Collaborating with government and non-governmental organizations, where appropriate, to co-sponsor workshops to
  - Evaluate the state-of-the-science related to the development and validation of alternative toxicological test methods
  - Identify high priority research, development, translation, and validation activities necessary to advance and characterize the usefulness of such methods
- Including experts from the international scientific community on expert panels and workshops
- Strengthening international relationships with appropriate organizations to foster the validation and evaluation of alternative test methods
- Participating, as appropriate, in the development of performance standards for international test guidelines
- Engaging interested stakeholders in assessing how to efficiently meet Federal peer review requirements

Monitoring Progress

ICCVAM and NICEATM have a number of mechanisms in place for providing periodic updates to the public:

- The ICCVAM Biennial Progress Report - to be next published later in 2008
- The NICEATM-ICCVAM website
- Periodic meetings of the Scientific Advisory Committee on Alternative Toxicological Methods
  - Next meeting scheduled for June 18-19, 2008 in Research Triangle Park, NC
  - See the NTP website at ntp.niehs.nih.gov, "About the NTP > Advisory Boards and Committees" for more information

ICCVAM and NICEATM will continue to be committed to an interactive and transparent process in their test method evaluation activities.

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