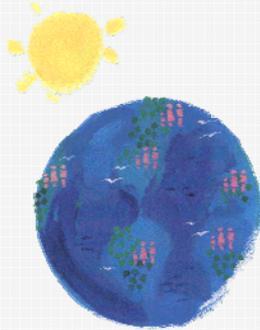


NICEATM

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

ICCVAM

*Interagency Coordinating Committee on
the Validation of Alternative Methods*



NICEATM and ICCVAM Update

William S. Stokes, DVM, DACLAM
RADM, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

6th Meeting of the Scientific Advisory Committee on
Alternative Toxicological Methods
November 30, 2006
Research Triangle Park, NC



Outline

■ Updates

- Botulinum Toxin Testing
- Acute Systemic Toxicity
- Ocular Toxicity
- Pyrogenicity Test Methods
- Endocrine Disruptor Test Methods
- Skin Irritation Assays
- Genetic Toxicity
- Immunotoxicity
- ICCVAM Biennial Report

■ Separate Agenda Items

- Nominations
- Planned Future Activities
- NICEATM-ICCVAM 5-year Plan

Scientific Workshop on Alternative Methods for Botulinum Toxin Testing



- Co-sponsored by ICCVAM, NICEATM, and ECVAM
- Nov. 13-14, 2006
- 110 participants
 - 10 countries
- 10 poster presentations
- Workshop Goals:
 - Review the state-of-the-science and current knowledge of alternatives that may reduce, replace, and refine the use of mice for botulinum toxin testing
 - Identify priorities for research, development, and validation efforts needed to advance the use of alternative methods

<http://iccvam.niehs.nih.gov/methods/biolodocs/biolowkshp/wkshpinfo.htm>

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Botulinum Toxin 3Rs Workshop Agenda

- Introduction: Public Health and Regulatory Testing Needs
 - Food safety: outbreak investigations
 - Drug safety and efficacy
 - Therapeutic products
 - Vaccine potency testing
 - Wildlife: outbreak investigations
- Sessions and Panel Discussions
 - Current Understanding and Knowledge Gaps
 - Replacement
 - Cell-based assays
 - Endopeptidase assays
 - Refinement
 - *Ex vivo* methods
 - Assays with nonlethal endpoints
 - Humane endpoints for the standard assay
 - Reduction

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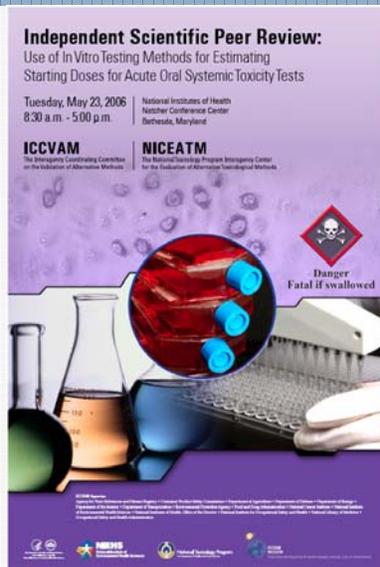
Botulinum Toxin 3Rs Workshop

- A detailed workshop report will be prepared
- General overview
 - Replacement
 - Significant progress has been made on *in vitro* methods
 - Use as initial screens and tiered testing has reduced animal use
 - Further development and validation efforts needed for a full replacement
 - Refinement
 - *Ex vivo* methods are promising, need to complete validation studies
 - Humane endpoints have been identified and are in use by some labs, need further evaluation in additional labs
 - Moribund euthanasia can be performed in nearly all situations
 - Reduction
 - A reduction in animal use has been achieved by various approaches
 - Further reductions may be supported by assay standardization, use of reference standards, and *in vitro* screening

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Cytotoxicity Methods for Estimating Acute Oral Systemic Toxicity



- ICCVAM Peer Review Panel Meeting
 - May 23, 2006
 - National Institutes of Health, Bethesda, Maryland, U.S.A.
- Peer Review Panel
 - 16 scientists
 - 6 countries
- Purpose: Review the validation status of two cytotoxicity test methods

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ICCVAM *In Vitro* Acute Toxicity Peer Panel

- **David H. Blakey, D.Phil.**
Health Canada, Ontario, Canada
- **June Bradlaw, Ph.D.**
International Foundation for Ethical Research (IFER)
- **Robert Copeland, Ph.D.**
Howard University College of Medicine
- **Gianni Dal Negro, D.V.M., Ph.D.**
GlaxoSmithKline Medicine Research Centre, Verona, Italy
- **Marion Ehrich, Ph.D., RPh., DABT**
Virginia-Maryland Regional College of Veterinary Medicine
- **Eugene Elmore, Ph.D.**
University of California, Irvine
- **Benjamin Gerson, M.D.**
Thomas Jefferson University School of Medicine
- **Michael Greene, Ph.D.**
U.S. Consumer Product Safety Commission
- **Janice Kuhn, Ph.D., DABT**
Stillmeadow Inc.
- **Daniel Marsman, D.V.M., Ph.D., DABT**
Procter & Gamble Company
- **Andrew Rowan, Ph.D.**
Humane Society of the United States
- **Hasso Seibert, Ph.D.**
University Medical School Schleswig, Kiel, Germany
- **Nigel Stallard, Ph.D.**
The University of Warwick, Coventry, United Kingdom
- **Katherine Stitzel, D.V.M. (Panel Chair)**
Consultant
- **Shinobu Wakuri, MSc.**
Hatano Research Institute, Japan
- **Daniel Wilson, Ph.D., DABT**
The Dow Chemical Company

7



Timeline

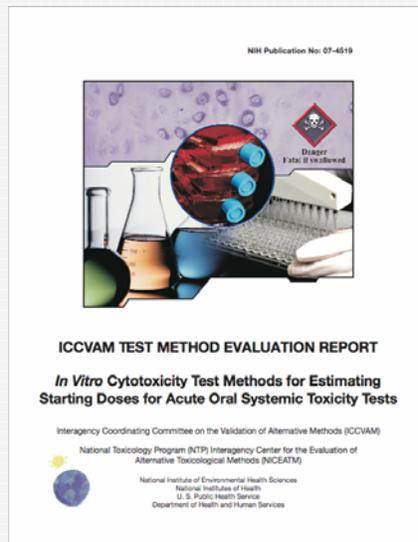
2006

- May 23** Peer Review Panel Meeting
- July** Peer Review Panel Report published (*FR Notice*)
- Aug 3** SACATM public teleconference meeting: comments on Peer Review Panel Report
- Oct** ICCVAM finalizes Recommendations
 - Approves Test Method Evaluation Report and final BRD
- Nov** Publication of ICCVAM Test Method Evaluation Report and final BRD
- Dec** ICCVAM Test Method Recommendations forwarded to Federal Agencies per Public Law 106-545
 - Responses due 180 days after receipt

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Cytotoxicity Methods for Estimating Acute Oral Systemic Toxicity

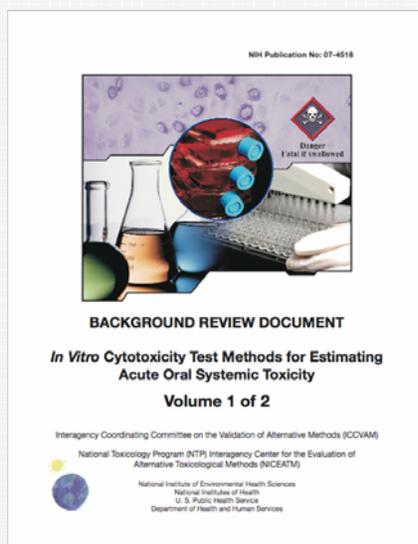


- **ICCVAM Test Method Evaluation Report**
- Provides ICCVAM Recommendations on:
 - Current test method uses and limitations
 - Standardized test method protocols
 - Performance standards
 - Future studies
- Peer Review Panel Report included as an appendix
- Recommendations will be forwarded to federal agencies per ICCVAM Authorization Act

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Final ICCVAM Background Review Document



- Describes the international validation study results and analyses
 - Reference LD₅₀ values
 - Reproducibility of IC₅₀
 - 2 IC₅₀-LD₅₀ regressions
 - Animal savings when used to estimate starting doses for acute oral toxicity
 - Accuracy for predicting GHS hazard categories

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Overall ICCVAM Recommendations: Cytotoxicity Test Method Uses

- Currently not sufficiently accurate to predict regulatory hazard categories for acute oral toxicity
- May be used in a weight-of-evidence approach to determine starting doses for current *in vivo* acute oral toxicity protocols
 - Up-and-Down Procedure
 - Acute Toxic Class Method
- Should be considered and used where appropriate before testing is conducted using animals
 - In accordance with:
 - U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
 - U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals
- For some types of substances, this approach will reduce the number of animals needed
- In some testing situations, the approach may also reduce the numbers of animals that die or need to be humanely killed

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ICCVAM Recommendations

- Test Method Protocols
 - Based on the GLP-compliant optimized test method protocols used in Phase III of the NICEATM-ECVAM validation study
 - Protocols for:
 - BALB/c 3T3 NRU Cytotoxicity Test Method
 - NHK NRU Cytotoxicity Test Method
- Test Method Performance Standards
- Future Studies

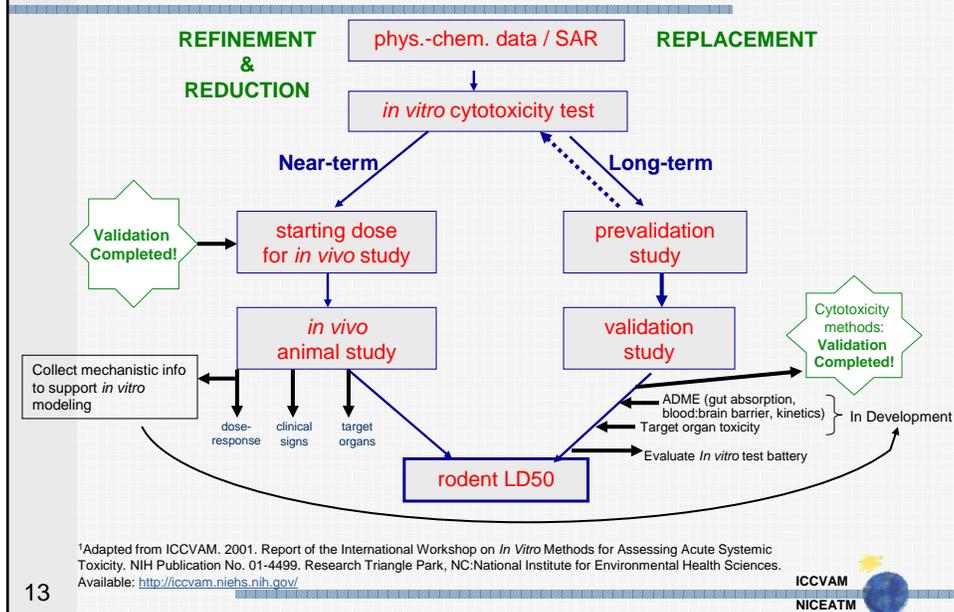
**Please refer to the Test Method Evaluation Report
for the detailed recommendations**

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Strategy for Reduction, Refinement, and Replacement of Animals in Acute Oral Toxicity Testing¹



Acute Toxicity Working Group (ATWG)

- **Consumer Product Safety Commission (CPSC)**
 - Kailash Gupta, Ph.D., D.V.M.
 - Cassandra Prioleau, Ph.D.
 - Marilyn Wind, Ph.D. (ATWG chair, ICCVAM Vice Chair)
- **Department of Energy (DOE)**
 - Po-Yung Lu, Ph.D.
- **Environmental Protection Agency (EPA)**
 - Karen Hamernik, Ph.D.
 - Masih Hashim, Ph.D.
 - Marianne Lewis
 - Elizabeth Margosches, Ph.D.
 - Debbie McCall
 - John Redden, Ph.D.
 - Amy Rispin, Ph.D.
- **Food and Drug Administration (FDA)**
 - Leonard Schechtman, Ph.D. (ICCVAM Chair)
 - Abigail Jacobs, Ph.D.
 - Suzanne Morris, Ph.D.
 - David Morse, Ph.D.
 - Thomas Umbreit, Ph.D.
- **National Institute for Occupational Safety & Health (NIOSH)**
 - Steven Reynolds, Ph.D.
- **National Institute of Environmental Health Sciences (NIEHS)**
 - Rajendra Chhabra, Ph.D.
 - William Stokes, D.V.M., DACLAM
 - Raymond Tice, Ph.D.

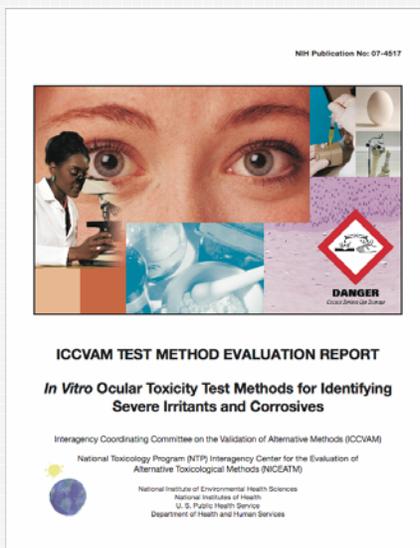
***In Vitro* Ocular Toxicity Test Methods for Identifying Corrosives and Severe Irritants**

- 2003: EPA nominated four *in vitro* test methods proposed for identifying potential ocular corrosives and severe irritants
 - Bovine Corneal Opacity and Permeability (BCOP) assay
 - Hen's Egg Test Chorioallantoic Membrane (HET-CAM) assay
 - Isolated Chicken Eye (ICE) assay
 - Isolated Rabbit Eye (IRE) assay
- 2004: NICEATM and ICCVAM prepared comprehensive background review documents (BRDs)
 - Each BRD described the current validation status of each of the nominated methods
- 2005: Independent Expert Peer Review Panel convened to assess validation status of the 4 methods (2 meetings); SACATM comments
- 2006
 - ICCVAM Test Method Evaluation Report
 - Final Background Review Documents (4)

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ICCVAM Test Method Evaluation Report

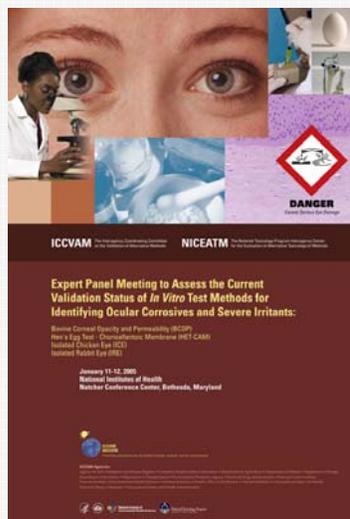


- Provides ICCVAM Recommendations on:
 - Current test method usefulness and limitations
 - A standardized protocol for each test method
 - Reference substances for validation studies
 - Future studies
- Peer Review Panel Report included as an appendix
- Recommendations will be forwarded to agencies per ICCVAM Authorization Act

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ICCVAM Expert Panel Meeting



- January 11-12, 2005
National Institutes of Health
Bethesda, Maryland
- Independent Scientific
Expert Panel
 - 23 scientists
 - 6 countries
- Purpose: Evaluate the
validation status of four *in vitro*
cytotoxicity test
methods for identifying
ocular corrosives and
severe irritants

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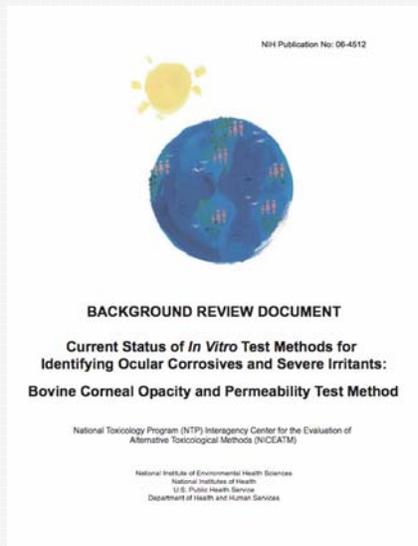
ICCVAM *In Vitro* Ocular Toxicity Expert Panel

- | | | |
|---|---|---|
| ■ Sally Atherton, Ph.D.
<i>Medical College of Georgia</i> | ■ Shayne Gad, Ph.D.
<i>Gad Consulting Services</i> | ■ Lionel Rubin, V.M.D.
<i>University of Pennsylvania</i> |
| ■ Roger Beuerman, Ph.D.
<i>Louisiana State University</i> | ■ Sidney Green, Ph.D.
<i>Howard University</i> | ■ Robert Scala, Ph.D. (<u>Panel Chair</u>)
<i>Consultant</i> |
| ■ June Bradlaw, Ph.D.
<i>International Foundation for
Ethical Research</i> | ■ Frederick Guerriero, M.S.
<i>GlaxoSmithKline</i> | ■ Horst Spielmann, Dr. Med.
<i>ZEBET, Germany</i> |
| ■ Ih Chu, Ph.D.
<i>Health Canada, Canada</i> | ■ A. Wallace Hayes, Ph.D.
<i>Harvard School of Public
Health</i> | ■ Martin Stephens, Ph.D.
<i>Humane Society of the United States</i> |
| ■ Henry Edelhauser, Ph.D.
<i>Emory University</i> | ■ Hiroshi Itagaki, Ph.D.
<i>Shiseido Co., Ltd., Japan</i> | ■ Katherine Stitzel, D.V.M.
<i>Consultant</i> |
| ■ Donald Fox, Ph.D.
<i>University of Houston</i> | ■ David Lovell, Ph.D.
<i>University of Surrey, UK</i> | ■ Peter Theran, V.M.D.
<i>Massachusetts Society for the
Prevention of Cruelty to Animals</i> |
| ■ James Freeman, Ph.D.
<i>ExxonMobil Biomedical
Sciences Inc.</i> | ■ Yasuo Ohno, Ph.D.
<i>National Institute of Health
Sciences - Japan</i> | ■ Scheffer Tseng, M.D.
<i>Ocular Surface Research &
Education Foundation</i> |
| | ■ Robert Peiffer, Ph.D.
<i>Merck Research Laboratories</i> | ■ Philippe Vanparys, Ph.D.
<i>Johnson and Johnson, The
Netherlands</i> |

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ICCVAM Background Review Documents



- Comprehensive review and analysis of available information and data
- Describes the current validation status of the *in vitro* methods including:
 - Reliability and accuracy
 - Substances tested
 - Protocols used

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ICCVAM General Recommendations: Use of *In Vitro* Ocular Test Methods

- All four *in vitro* test methods should be considered prior to conducting *in vivo* ocular testing and an alternative test method should be used where determined appropriate for the specific testing situation
 - Consideration necessary for compliance with:
 - U.S. Animal Welfare Act regulations
 - Public Health Service Policy on the Humane Care and Use of Laboratory Animals
 - U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
- Use results in a weight-of-evidence decision making process in accordance with EPA and EU ocular testing regulations and GHS tiered-testing strategy

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ICCVAM General Recommendations: Use of *In Vitro* Ocular Test Methods

- Users should consider chemical and physical properties of test articles to determine whether and which of these *in vitro* test methods are appropriate to use as a screening test for ocular corrosion or severe irritation
 - Performance varies widely for each method based on physical and chemical properties

For full and complete test method recommendations, please refer to the ICCVAM Test Method Evaluation Report

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ICCVAM Evaluation of *In Vitro* Skin Irritation Test Methods

- ECVAM Skin Irritation Validation Study
 - Validation study completed 2006
 - EpiDerm™ and EPISKIN™
 - Performance analysis based for the EU hazard classification system
- ICCVAM future evaluation
 - Review usefulness for relevant U.S. hazard classification schemes
 - Environmental Protection Agency (EPA)
 - Federal Hazardous Substances Act (FHSA)
 - UN Globally Harmonized System (GHS)

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ICCVAM Test Method Evaluation: *In Vitro* Pyrogenicity Test Methods

- 5 *in vitro* test methods proposed as replacements for the *in vivo* rabbit pyrogen test (RPT)
 - PBMC/IL-6
 - The Human Peripheral Blood Mononuclear Cell [PBMC]/IL-6 *In Vitro* Pyrogen Test
 - WB/IL-1
 - The Human Whole Blood [WB]/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
 - *In Vitro* Pyrogen Test Using the Human Monocytoid Cell Line MONO MAC-6 [MM6]

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ICCVAM *In Vitro* Pyrogenicity Test Method Review

2006

Dec *FR* notice announcing availability of BRDs and draft ICCVAM test method recommendations

2007

Feb 6 ICCVAM Peer Review Panel Meeting

- National Institutes of Health, Bethesda, Maryland

Mar-Apr Release of Expert Panel Report and publication of an *FR* notice requesting public comment on the report

July-Aug *Publish Final ICCVAM Test Method Evaluation Report*

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Endocrine Disruptor Test Method Activities

- Nomination for Validation Studies: Estrogenic Activity Cell Proliferation Assay
- Comments on Draft OECD Test Guideline: “The Uterotrophic Bioassay in Rodents: a short-term screening test for (anti)estrogenic properties”
- Updated List of Reference Substances Recommended for *In Vitro* ED Validation Studies
- International Validation Study

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Updated Reference Substances Recommended for *In Vitro* ED Validation Activities

- Originally proposed in ICCVAM Test Method Evaluation Report (NIH Pub. 03-4503)
 - Based on 2002 Expert Panel Meeting
 - Common list of 78 substances for both ER and AR methods
 - **ER methods: 53 minimum substances**
 - **AR methods: 44 minimum substances**
- ICCVAM revised to address availability and cost issues
 - 6 of 78 substances replaced
 - FR Notice requesting comments on the proposed revisions (71 (51):56997-56998, 2006)
 - FR Notice announcing the availability of a revised reference substances list (71(188):60748-60749, 2006)

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International Validation Study: ER Transcriptional Activation Assay

- LUMI-CELL[®] ER Screening Assay
 - Stably-transfected transcriptional activation assay
 - Detects agonist and antagonist activity
- Standardization study
 - Completed: July, 2006
 - Standardized GLP-compliant test method protocols for detecting ER agonists and antagonists for the international validation study
- 3 laboratories: US, Japan, Europe
 - Study Management Team: NICEATM, ECVAM and JaCVAM

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ICCVAM International Collaborations: Genetic Toxicity Test Methods

- ICCVAM Genetic Toxicity Working Group established
- Will provide comments on JaCVAM planned international validation studies on *in vivo* and *in vitro* Comet assays
 - First phase:
 - Evaluate *in vivo* rodent Comet assay as an alternative to the *in vivo* rat liver unscheduled DNA synthesis (UDS) assay
 - Second phase:
 - Evaluate *in vitro* Comet assay as an alternative to the *in vivo* Comet assay

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Murine Local Lymph Node Assay (LLNA)

- ICCVAM Planned Review Activities
 - Evaluate the validation status of the LLNA for determining potency categories
 - Evaluate a “Limit dose” proposal
 - Evaluation of non-radioactive LLNA method
 - BRDU; any others
 - Develop LLNA performance standards

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ICCVAM Biennial Report: 2004-05

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

- Describes ICCVAM and NICEATM activities
- Test method evaluations
- Workshops
- International collaborations and achievements
 - ECVAM
 - JaCVAM
 - OECD
 - World Congress on Alternatives
- Communications
 - Updated bibliography

<http://iccvam.niehs.nih.gov/home.htm>

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ICCVAM Agency Representatives

<u>ATSDR</u>	Moiz Mumtaz	<u>FDA</u>	Leonard Schechtman, NCTR (Chair) Suzanne Fitzpatrick, OS Abigail Jacobs, CDER Raju Kammula, CDRH Melvin Stratmeyer, CDRH Richard McFarland, CBER Ying Huang, CBER David Hattan, CFSAN Robert Bronaugh, CFSAN Devaraya Jagannath, CVM M. Cecilia Auguila, CVM William Allaben, NCTR Lawrence D'Hoostelaere, ORA
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<u>DOD</u>	Robert E. Foster Patty Decot Harry Salem		
<u>DOE</u>	Michael Kuperberg Marvin Stodolsky		
<u>DOI</u>	Barnett Rattner Sarah Gerould	<u>NCI</u>	Alan Poland T. Kevin Howcroft
<u>DOT</u>	George Cushmac Steve Hwang	<u>NIEHS</u>	William Stokes John Bucher Rajendra Chhabra Jerrold Heindel
<u>EPA</u>	Karen Hamernik, OSCP Julian Preston, ORD Suzanne McMaster, ORD Jerry Smrchek, OECD TGP Amy Rispin, OPP Deborah McCall, OPP	<u>NIOSH</u>	Paul Nicolaysen K. Murali Rao
		<u>NIH</u>	Margaret Snyder
		<u>NLM</u>	Vera Hudson Jeanne Goshorn
		<u>OSHA</u>	Surrender Ahir

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 - Debbie McCarley Special Assistant; Asst. Project Officer
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 - Thomas Burns, M.S. Sr. Project Coordinator/Technical Writer
 - Patricia Ceger, M.S. Project Coordinator/Technical Writer
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 - Frank Deal, M.S. Staff Toxicologist
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 - Jim Truax, M.S. Project Coordinator/Technical Writer

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