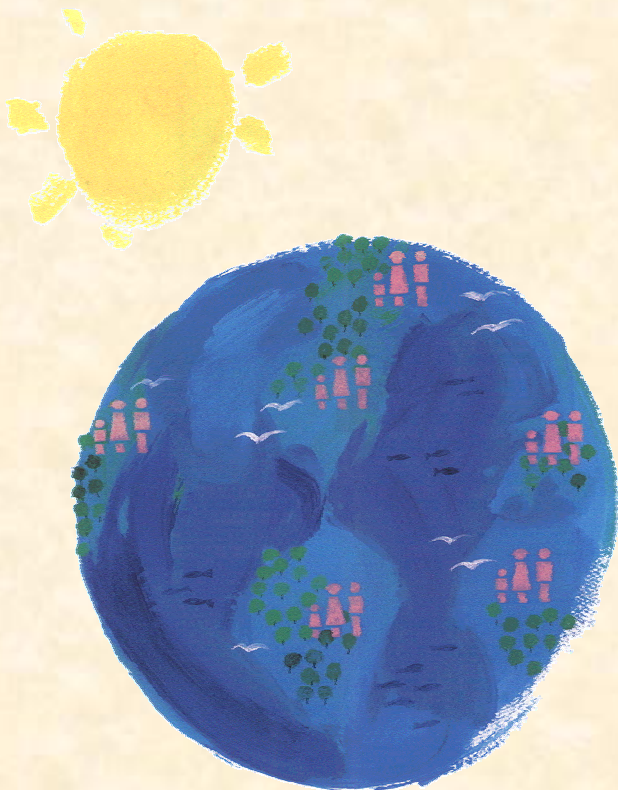


NICEATM

National Toxicology Program Interagency
Center for the Evaluation Of Alternative
Toxicological Methods

ICCVAM

Interagency Coordinating Committee
on the Validation of Alternative
Methods



ICCVAM Test Method Nominations

William S. Stokes, D.V.M., D.A.C.L.A.M.

**NTP Interagency Center for the Evaluation of
Alternative Toxicological Methods**

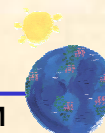
**Meeting of the Scientific Advisory Committee
on Alternative Toxicological Methods**

March 11, 2004

Bethesda, Maryland

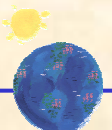


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NICEATM



Outline

- I. Overview of ICCVAM Nomination Process and Prioritization Criteria**
- II. In Vitro Endocrine Disruptor Nominations**
- III. In Vitro Ocular Irritation Test Methods**
- IV. Other Pending Test Method Nominations**



I. Overview of ICCVAM Nomination Process and Prioritization Criteria

ICCVAM Guidelines

NIH Publication No: 03-4508



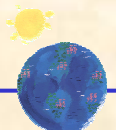
ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods

Prepared by the
Interagency Coordinating Committee on the
Validation of Alternative Methods (ICCVAM)
and the
National Toxicology Program (NTP) 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

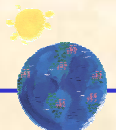
- Published September 2003
- Available on Internet at:

[http://iccvam.niehs.nih.gov/
docs/guidelines/subguide.htm](http://iccvam.niehs.nih.gov/docs/guidelines/subguide.htm)



Test Method Nominations

- **Test methods proposed to ICCVAM for consideration but for which a test method submission is not available. Examples include:**
 - **Test methods for which adequate validation studies have been conducted but which lack a complete submission package**
 - **Test methods that appear promising based on limited prevalidation or validation studies and are proposed for additional validation efforts**
 - **Test methods proposed for prevalidation or validation studies**
 - **Test methods recommended for workshops or other activities**
- **Following receipt, NICEATM conducts a preliminary evaluation to summarize the extent to which nomination addresses ICCVAM prioritization criteria**



ICCVAM Nomination Process

NICEATM (<http://iccvam.niehs.nih.gov>)

- Solicits, receives, and tracks nominations and submissions
- Conducts preliminary evaluation of each nomination or submission
 - determines completeness of each nomination or submission
 - summarizes findings
 - proposes appropriate future efforts (e.g., workshop, expert panel meeting, peer review meeting, expedited review, validation study)



ICCVAM

- Reviews NICEATM preliminary evaluation report
- Develops draft recommendations on priority for future efforts
- Seeks comment from the public on the nominated or submitted test method (via NICEATM)



SACATM

- Considers public comments on the nominated or submitted test method
- Comments on NICEATM and ICCVAM draft recommendations



ICCVAM

- Considers SACATM and public comments
- Finalizes recommendations and priorities
- NICEATM estimates resource requirements



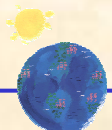
Director, ETP/NIEHS

- Responds to NICEATM resource requests for proposed test method activities



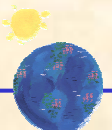
Director, NICEATM

- Informs ICCVAM of availability of resources for activities recommended for nominated or submitted test methods
- If appropriate, ICCVAM Working Group established
- If appropriate, test method evaluations or validation studies organized in conjunction with ICCVAM Working Group



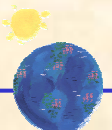
ICCVAM Prioritization Criteria (1)

- **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**
- **Potential for the proposed test method, compared to current test methods accepted by regulatory agencies, to:**
 - **Refine animal use (decrease or eliminate pain and distress)**
 - **Reduce animal use**
 - **Replace animal use**

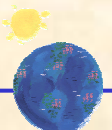


ICCVAM Prioritization Criteria (2)

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



II. In Vitro Endocrine Disruptor Nominations



In Vitro Endocrine Disruptor Nominations

NIH Publication No: 03-4503



ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors:

Estrogen Receptor and Androgen Receptor Binding
and Transcriptional Activation Assays

Interagency Coordinating Committee on the Validation of Alternative Methods
(ICCVAM)

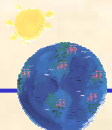
National Toxicology Program (NTP) Interagency Center for the Evaluation of
Alternative Toxicological Methods (NICEATM)

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

- **Expert Panel Convened
May 21-22, 2002 in RTP,
NC**
- **Panel evaluated and
made recommendations
on ER and AR Binding
and Transcriptional
Activation Assays**
- **Final ICCVAM Report
published in May 2003**
- **Available on Internet at:**

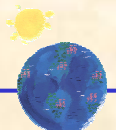
**[http://iccvam.niehs.nih.gov/
methods/endocrine.htm](http://iccvam.niehs.nih.gov/methods/endocrine.htm)**

ICCVAM
NICEATM



ICCVAM Recommendations: Endocrine Disruptor Test Methods

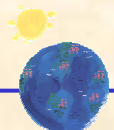
- Preference should be given to development and validation of assays that:
 - Do not require the use of animal tissue/surgical procedures for the receptor source, but rather use recombinant-derived proteins
 - Do not use radioactive materials
- Performance Standards will be developed following validation studies on at least one test method



Nominated Endocrine Disruptor Test Methods

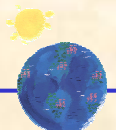
- Method 1: Biosensor system that can assess estrogen receptor binding and transcriptional activation**
- Pre-validation data is expected by June 2004
 - Developer intends to submit data and request funding of multi-lab validation study

- Method 2: Stably transfected recombinant cell-based transcriptional method**
- Developer has completed pre-validation using 120 chemicals
 - NICEATM has requested data



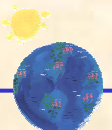
Nominated Test Methods' Adherence to ICCVAM Prioritization Criteria

<u>Criteria</u>	<u>Adherence</u>
1. Applicable to regulatory testing needs	YES
2. Applicable to multiple agencies/programs	YES
3. Warranted for use/application and impact	YES
4. Refine/Reduce/Replace Animal Use	YES/YES/YES
5. Improved Prediction	YES
6. Other Advantages	Reduced time/cost
7. Completeness of Submission	TBD



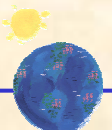
ICCVAM Draft Recommendation: Endocrine Disruptor Nominations

- *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*

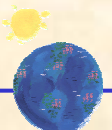


ICCVAM ED Nominations: Next steps

- ***Federal Register* notice**
 - Request for comments
 - Request for other nominations with pre-validation data
- **NICEATM request from Sponsors:**
 - Pre-validation study results
 - Proposed standardized protocol
 - Proposed validation study design
- **ICCVAM/EDWG review and draft recommendations**
- **SACATM comments on ICCVAM recommendations**
- **ICCVAM Final Recommendations**
- **NICEATM request for funding of recommended studies to Director, ETP**



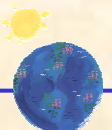
III. In Vitro Ocular Irritation Test Methods



Ocular Irritation Nominations

Background:

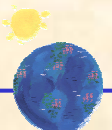
- SACATM Meeting, August 12-13, 2003
 - EPA plans to nominate *in vitro* ocular toxicity tests to ICCVAM for evaluation
 - Emphasis on those that may be able to identify severe irritants without animal testing
 - SACATM unanimous approval with high priority:
ICCVAM and NICEATM should review the validation status of these test methods and carry out a workshop or expert meeting, as appropriate
 - Public Comment
HET-CAM used routinely in-house by one large chemical company before any substance is tested on an animal eye



Ocular Irritation Nominations

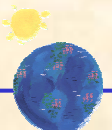
Received from EPA on October 27, 2003
(Request for 4 different test method activities)

1. Nominated evaluation of *in vitro* ocular toxicity test methods as screening assays to identify severe ocular irritants/corrosives
2. Requested that ICCVAM evaluate the state-of-the-science of *in vitro* test methods for assessing nonirritants and mild or moderate ocular irritants, with the goal of identifying research, development, and validation priorities that might advance the usefulness of such methods



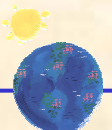
EPA Nominations (Cont'd)

3. Requested that ICCVAM explore ways of obtaining existing and generating, if necessary, good quality *in vivo* eye irritation/corrosion reference data to assess interlaboratory variability and support validation of *in vitro* tests
4. Requested that ICCVAM explore ways of alleviating pain and suffering which might arise from administration of mild to moderate irritants in current *in vivo* eye irritation tests



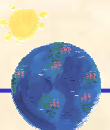
ICCVAM Recommendations

- Unanimously recommended all four activities with high priority
- Highest priority:
 - Preparation of Background Review Documents and review of test methods that can identify severe ocular irritants/corrosives
 - Review of existing *in vivo* data to identify appropriate reference chemicals for validation studies



Current Activities for All Ocular Nominations

- ICCVAM Ocular Toxicity Working Group established
- *Federal Register* notice submitted for publication:
 - Request for public comment on the nominations
 - Request for data and information on all four activities
 - *In Vitro* data
 - Human and animal data
- Collaborations with ECVAM
 - Leverage resources and coordinate review efforts



Ocular Toxicity Nominations

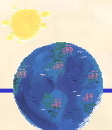
Nomination #1:

In Vitro Test Methods for Identifying
Substances Causing Severe/Irreversible
Ocular Damage



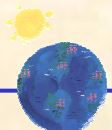
***In Vitro* Methods to Identify Severe Ocular Irritants/Corrosives**

- **Bovine Corneal Opacity and Permeability Test Method (BCOP)**
- **Hen's Egg Test on Chorioallantoic Membrane (HET-CAM)**
- **Isolated Rabbit Eye Test Method (IRE)**
- **Isolated Chicken Test Method (ICE)/ Chicken Enucleated Eye Test Method (CEET)**



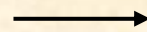
Current Status of *In Vitro* Ocular Screening Methods

- BCOP, HET-CAM, ICE/CEET, and IRE test methods accepted on case-by-case basis by some European Union countries
 - Hazard identification of severe ocular irritants without animal testing
 - Belgium, France, Germany, Ireland, Netherlands, United Kingdom
- Not yet adopted into European Guidelines (Annex V of Directive 67/548/EEC)
- GHS (UN 2003) allows for use of validated and accepted *in vitro* methods to identify severe ocular irritants/corrosives without further testing

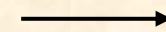


Testing and Evaluation Strategy

(5a) If a valid *in vitro* test is available to assess severe damage to eyes



Severe damage to eyes



Category 1

Not a severe eye irritant

(6a) If a valid *in vitro* test is available For eye irritation



Eye irritant



Category 2

No indication of eye irritant properties

(7) Experimentally assess skin corrosion potential



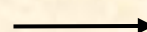
Skin corrosive



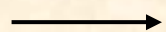
No evaluation of effects on eyes

Not corrosive

(8) 1 rabbit eye test



Serious damage to eyes



Category 1

No serious damage



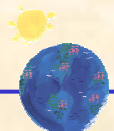
Eye irritant



Category 2

**(9) 1 or 2 further rabbits
Not an eye irritant**

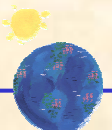
From UN 2003. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). [ST/SG/AC.10/30]. United Nations, New York and Geneva.



NICEATM Preliminary Evaluation

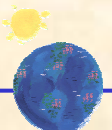
- **Publications**

- **BCOP assay: 18**
- **HET-CAM assay: 11**
- **IRE assay: 11**
- **ICE/CEET assay: 3**



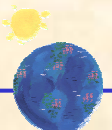
***In Vitro* Ocular Irritation Studies Completed (1991 - 1997)**

- **European Commission/Home Office (EC/HO) study**
- **Cosmetics, Toiletries and Fragrance Association (CTFA) study**
- **Bundesgesundheitsamt/German Department of Research and Technology (BGA/BMBF) study**
- **European Cosmetic, Toiletry and Perfumery Association (COLIPA) study**
- **Japanese Ministry of Health and Welfare/Japanese Cosmetic Industry Association (MHW/JCIA) study**



Previous Studies

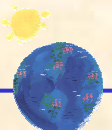
- Protocols for the same test method varied among studies
- All studies except EC/HO assessed test method accuracy, but:
 - Analysis based on the correlation of *in vitro* “scores” with *in vivo* MAS or MMAS scores
 - Different *in vivo* ocular irritancy classification systems were used by different investigators
 - No assessment for current US or GHS classification schemes
- Individual animal data (i.e., raw scores for corneal opacity, iritis, conjunctival redness, and chemosis) not available
 - Required to assess accuracy for predicting GHS and U.S. hazard categories



Background Review Document on Screening Methods: Current Activities

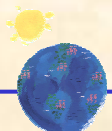
- Contact study authors to obtain original *in vivo* and *in vitro* data and identity of test substances
- *Federal Register* notice requesting
 - data on all four *in vitro* test methods
 - *in vivo* data for potential reference substances
- Re-assess performance of standardized protocols based on additional information
- Assess predictivity in terms of current U.S. and international ocular irritancy classification categories
- Coordinate activities with ECVAM*

*EC/HO study data were obtained from ECVAM on Feb. 12, 2004, so performance of the 4 test methods can be re-evaluated in terms of U.S. and international ocular irritancy classification systems



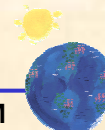
Current Evaluation Plan

- Complete background review document for each of the 4 test methods
- Identify or develop recommended:
 - essential test method components
 - standardized protocols
 - list of reference substances with high quality *in vivo* ocular irritation data
 - If adequate data exists, develop proposed performance standards that screening or replacement *in vitro* ocular toxicity test methods should meet or exceed
- Convene an expert panel to review and comment on NICEATM and ICCVAM OTWG recommendations



Tentative Timeline

- **March - October 2004:**
 - *Federal Register* notice requesting information and data
 - Contact acknowledged experts for information and data
 - Complete four draft BRDs, including OTWG review, data reanalysis, etc.
 - Organize and plan expert panel meeting
- **November 1, 2004:**
 - *Federal Register* notice announcing availability of the draft BRDs for public review and comment and the expert panel meeting
- **January 2005:**
 - Ocular Toxicity Expert Panel Review Meeting
- **April 2005:**
 - Publication of expert panel report; request for public comment
- **July 2005:**
 - Publication of ICCVAM final recommendations



Ocular Toxicity Nominations

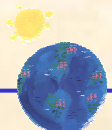
Nomination #2:

**Review State-of-the-Science and Availability
of *In Vitro* Test Methods for Assessing
Moderate, Mild, or Non-Irritation**



Proposed NICEATM-ICCVAM Activities (Nomination #2)

- Literature search for relevant *in vitro* test methods
- *Federal Register* Notice requesting information on and data generated by relevant *in vitro* test methods
- Contact experts for data
- Develop criteria to identify promising *in vitro* test methods for detecting negative and mild to moderate ocular irritants
- Collaborate with ECVAM in its review and evaluation of promising assays
- As appropriate, convene a workshop, expert panel meeting, or peer panel meeting to review findings and recommendations



Ocular Toxicity Nominations

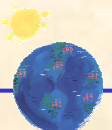
Nomination #3:

**Obtain Existing and/or Generate
Good Quality *In Vivo* Eye
Irritation/Corrosion Reference Data**



Proposed NICEATM-ICCVAM Activities (Nomination #3)

- Evaluate ECETOC database (N = 132 chemicals)
- *Federal Register* Notice requesting *in vivo* ocular irritation data
 - Review data received
- Collaborate with EPA to access the TSCA database for test substances assessed for ocular irritancy
 - Records exist for 2557 chemicals
 - Identify commercially available test substances
- Query authors of studies for test substance information and original *in vivo* data
- Collaborate with ECVAM to obtain European data
- Collaborate with NIHS (Japan) to obtain data



Ocular Toxicity Nominations

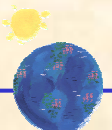
Nomination #4:

Explore Ways of Alleviating Pain and Suffering for Animals Used in Ocular Irritancy Testing



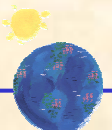
Proposed NICEATM-ICCVAM Activities (Nomination #4)

- Literature search for methods or procedures that reduce or alleviate pain and suffering
 - e.g., use of local anesthetics, systemic analgesics, the low volume eye test (LVET)
- *Federal Register* request for relevant information and data
- Contact industry/government experts
- Prepare a Background Review Document

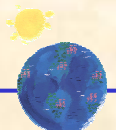


Proposed NICEATM-ICCVAM Activities (2) (Nomination #4)

- **Expert workshop**
 - review available procedures and methods
 - develop recommendations for research, development, and validation efforts for procedures that might further reduce or eliminate pain and suffering
- **Collaborate with ECVAM on its evaluation of the low volume eye test (LVET) method**
 - Assist with data collection



IV. Other Pending Test Method Nominations



Other Pending Test Method Nominations

- **USDA *In Vitro* Leptospira Vaccine Potency Test Methods**
 - ICCVAM unanimously approved a request by USDA to create an ICCVAM Working Group to:
 - provide comments on proposed validation studies
 - coordinate eventual peer review
 - **USDA:**
 - currently refining the *Leptospira* cultures
 - will present study designs to ICCVAM when available
 - Dr. Kulpa-Eddy will provide status update as next agenda item
 - **Formal nomination is expected in the near future**

