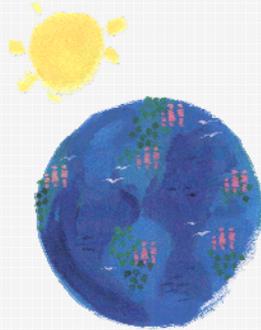


**NICEATM**

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

**ICCVAM**

Interagency Coordinating Committee on  
the Validation of Alternative Methods



## ICCVAM Nominations

Raymond Tice, Ph.D.  
Deputy Director, NICEATM

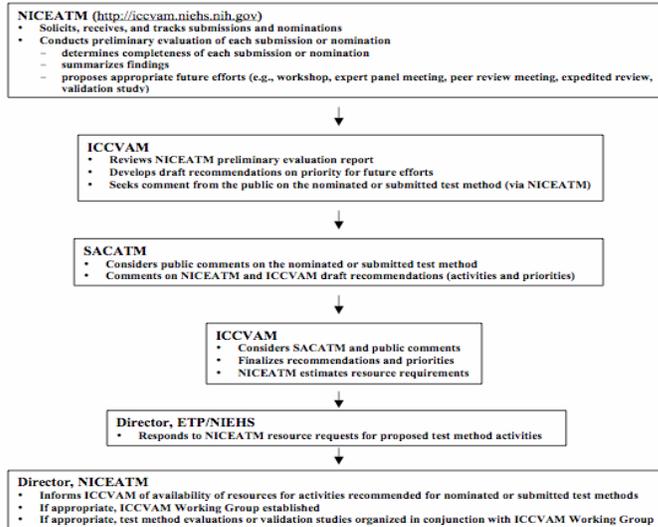
SACATM Meeting  
Research Triangle Park, NC  
November 30, 2006



## ICCVAM Nominations

- Nomination from CertiChem, Inc. (CCi) for the validation of a cell-based estrogen receptor (ER) transcriptional activation (TA) test method measuring cell proliferation in MCF-7 cells
- An ICCVAM nominated future study of *in vitro* cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity testing of mixtures
- An ICCVAM nominated future study of the most appropriate corneal holder and test substance solvent for the Bovine Cornea Opacity and Permeability (BCOP) test method

## ICCVAM Nomination and Submission Process



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\* ICCVAM (2003) *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods*. NIH Publication No. 03-4508, 50 pp. Research Triangle Park, NC, USA.



## ICCVAM Criteria for Nomination Priority Setting

1. The extent to which the proposed test method is:
  - Applicable to regulatory testing needs
  - Applicable to multiple agencies/program
2. The extent of expected use or application and impact on human, animal, or ecological health
3. The potential for the test method, compared to current methods, to:
  - Refine animal use (i.e., decrease or eliminate pain and distress)
  - Reduce animal use
  - Replace animal use
4. The potential for the test method to provide improved prediction of an adverse health or environmental effect, compared to current methods
5. The extent to which the test method provides other advantages, such as reduced cost and time to perform, compared to current methods

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## CCi Nomination: Validation of a Cell-Based ER TA Test Method Measuring Cell Proliferation in MCF-7 Cells

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### CCi ER Cell Proliferation Assay

- **Jun 4, 2005:** NICEATM received a nomination from CCi for the validation of a cell-based ER TA test method. The proposed test method evaluates the potential estrogenic activity of substances by measuring to what extent a substance induces cell proliferation in MCF-7 cells via an ER-dependent pathway.

**Robotic  
EpMotion  
5070 used  
in assay**



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## The CCI ER TA Assay

- To measure proliferative response:
  - Cells are incubated in the presence of a test substance for one week
  - The amount of DNA per well is quantified
  - Significant increases in the amount of DNA is indicative of ER agonist activity
- ER specificity is demonstrated by testing for the ability of the known estrogen antagonist ICI 182,780 to inhibit test substance-induced proliferation
- A version of the same assay to detect ER antagonist activity is in development

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## Accuracy of the CCI ER TA Assay

- CCI tested 40 substances on the ICCVAM recommended reference substances list and compared ICCVAM published ER activities to their experimental results:
  - Concordance = 93% (37/40)
  - Sensitivity = 93% (27/29)
  - Specificity = 91% (10/11)
  - False Negative Rate = 7% (2/29)
  - False Positive Rate = 9% (1/11)

		ICCVAM Classification		
		Positive	Negative	Total
CCI Classification	Positive	27	1	28
	Negative	2	10	12
	Total	29	11	40

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## CCI Timeline

- **Jan 2006:** NICEATM received a submission from CCI entitled, “Test Method Nomination: MCF-7 Cell Proliferation Assay of Estrogenic Activity.”
- **Apr 2006:** NICEATM received a revised BRD from CCI
- **Aug 2006:** The EDWG and ICCVAM recommend a high priority for validation of the CCI test method
- **16 Oct 06:** *Federal Register* notice requesting public comment on the CCI test method and proposed activity published
- **30 Nov 06:** Six public comments received recommend validation of the test method  
  
SACATM considers nomination and proposed priority

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## Recommended Priority is “High”

1. The proposed test method is:
  - Applicable to regulatory testing needs
  - Applicable to EPA and potentially other regulatory agencies
2. The extent of expected use or application and impact on human, animal, or ecological health
  - EPA is required to identify endocrine disruptors and there are a large number of products and pollutants that may exhibit such activity
3. The potential for the method, compared to current methods, to refine, reduce, and replace animal use
  - Accurate *in vitro* methods that do not use animal tissues will significantly reduce the use of animals
4. The potential for the method to provide improved prediction of an adverse health or environmental effect, compared to current methods
  - Currently, there are no adequately validated ED test methods
5. The extent to which the test method provides other advantages, such as reduced cost and time to perform, compared to current methods
  - A robotics based assay offers throughput advantages

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## ICCVAM Nominated Future Study: Evaluation of the Applicability of *In Vitro* Cytotoxicity Test Methods to Determine Starting Doses for Acute Oral Toxicity Testing of Chemical Mixtures

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## ICCVAM Nominated Activity

- **03 Aug 06:** SACATM considers conclusions and recommendations of the Independent Scientific Peer Review on the Use of *In Vitro* Testing Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests (May 23, 2006)
- **Nov 2006:** ICCVAM (in its Test Method Evaluation Report recommends:
  - consideration of cytotoxicity methods to estimate the starting doses for acute oral toxicity studies
  - future studies to further expand the use of in vitro cytotoxicity test methods
- **Study Objective:** To determine the usefulness of the 3T3 NRU basal cytotoxicity test method for reducing and refining the use of animals for the acute oral toxicity testing of chemical mixtures

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## Proposed Validation Approach

- Collect historical rat acute oral LD<sub>50</sub> values for mixtures from standardized rat acute oral toxicity tests (data to be provided by regulatory agencies and/or chemical manufacturers)
  - Test mixtures using the 3T3 NRU test method
  - No additional animal testing
- Prospectively test mixtures using the *in vitro* 3T3 NRU test method as they undergo mandatory *in vivo* safety testing
- Evaluate animal savings and reduction of animal deaths produced by using the 3T3 NRU IC<sub>50</sub> values to predict starting doses for computer simulated acute oral toxicity testing

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## Recommended Priority is “High”

1. The proposed test method is:
  - Applicable to regulatory testing needs
  - Applicable to all regulatory agencies that require the acute oral toxicity testing of mixtures
2. The extent of expected use or application and impact on human, animal, or ecological health
  - Acute oral toxicity testing of mixtures is widely used
3. The potential for the method, compared to current methods, to refine, reduce, and replace animal use
  - Will reduce and refine the use of animals in acute oral toxicity testing and will set the stage for the development of more accurate batteries of *in vitro* toxicity tests
4. The potential for the method to provide improved prediction of an adverse health or environmental effect, compared to current methods
  - No
5. The extent to which the test method provides other advantages, such as reduced cost and time to perform, compared to current methods
  - Amenable to high throughput – cost reduction

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## ICCVAM Nominated Future Studies for the Bovine Cornea Opacity and Permeability (BCOP) Test Method: Evaluation of the Optimal Corneal Holder and Vehicle for the BCOP Test Method

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## Expert Panel and ICCVAM Recommendations on the BCOP Test Method

- In reviewing the validation status of the BCOP test method, the Ocular Expert Panel stated:
  - "For the purposes of detecting severe eye irritants in the tiered testing scheme outlined in the BRD, the proposed BCOP test method protocol is useful for identification of severe corrosive ocular irritants with the following caveats:
    - 0.9% NaCl should be used instead of distilled water as the test substance diluent."
  - "The following are recommended as modifications that might improve the accuracy and reliability (repeatability/reproducibility) of the BCOP test method:
    - Use of the larger holder as suggested by Ubels et al. (2002, 2004)"
- 12 Dec 05 - SACATM agrees with conclusions and recommendations of the Independent Scientific Peer Review on the Use of In Vitro Methods to Identify Ocular Corrosives and Severe Irritants
- ICCVAM Ocular Test Method Evaluation Report states:
  - Studies should be conducted to evaluate the impact of using a corneal holder that maintains normal corneal curvature (e.g., the corneal mounting system designed by Ubels et al. 2002) on the accuracy and/or reliability of the BCOP test method
  - The effect of modifying various test method protocol components (e.g., changing the duration of exposure) on the accuracy and/or reliability of the BCOP test method should be evaluated

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## Reported Problems with the Currently Used Corneal Holder

- Conventional holder has a circular opening of 17 mm but the bovine cornea is oval shaped and has dimensions of approximately 24 mm high and 30 mm wide
- Conventional holder has flat inner surfaces but the bovine cornea is convex
- These elements of the currently used corneal holder could
  - force the bovine cornea into an unnatural shape when mounted
  - lead to damage of the cornea since the cornea could come in contact with the holder opening

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## Ubels-type Corneal Holder

- New holder was initially described by Ubels et al. (2002)
- Dimensions of holder are designed to better fit the bovine cornea and maintain the shape of the cornea during the assay
- Holder was designed to contact the sclera rather than the cornea
  - Therefore, it minimizes mechanical damage to the cornea by the holder
- Limited studies suggest that the limitations associated with the original corneal holder are resolved with the newer holder

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## ICCVAM Nomination

- NICEATM and ICCVAM, working in partnership with interested stakeholders, will manage a study, using the ICCVAM recommended reference substances, to evaluate the performance (relevance and reliability) of the BCOP test method:
  - using an alternative corneal holder (e.g., the holder developed by Ubels et al. 2002)
  - using alternative vehicles for the test substance diluent (e.g., 0.9% NaCl rather than distilled water)

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## Recommended Priority is “High”

1. The proposed test method is:
  - Applicable to regulatory testing needs
  - Applicable to all regulatory agencies that require ocular toxicity testing
2. The extent of expected use or application and impact on human, animal, or ecological health
  - Ocular toxicity testing is required to protect human health
3. The potential for the method, compared to current methods, to refine, reduce, and replace animal use
  - Will reduce and refine the use of animals in ocular toxicity testing
4. The potential for the method to provide improved prediction of an adverse health or environmental effect, compared to current methods
  - Not known but likely given the ability to obtain more mechanistic data
5. The extent to which the test method provides other advantages, such as reduced cost and time to perform, compared to current methods
  - Should result in reduced cost and reduced time to perform

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## Discussion Questions for SACATM

(Lead Discussants: Drs. Barile, Bradlaw, Charles, Dong)

- Do you have any comments on the proposed activities?
- *What priority would you assign the MCF-7 cell proliferation assay for estrogenicity?*
- Do you agree with the draft recommended priorities assigned to the ICCVAM nominations?