



## Nomination of the Electrophilic Allergen Screening Assay

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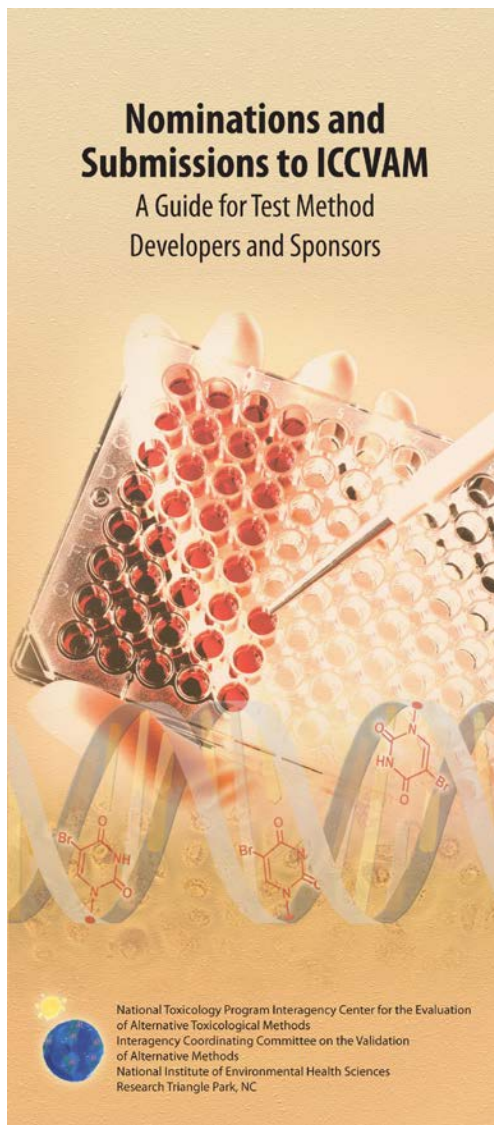
SACATM Meeting

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National Institute of Environmental Health Sciences  
Research Triangle Park, NC



# Nominations to NICEATM and ICCVAM



## ■ Nominations include

- Test methods proposed for validation studies that appear promising based on available data
- Test methods recommended for a workshop or other activity
- Test methods proposed for ICCVAM evaluation that have completed validation studies, but lack a complete submission package and background review documents

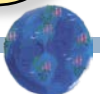
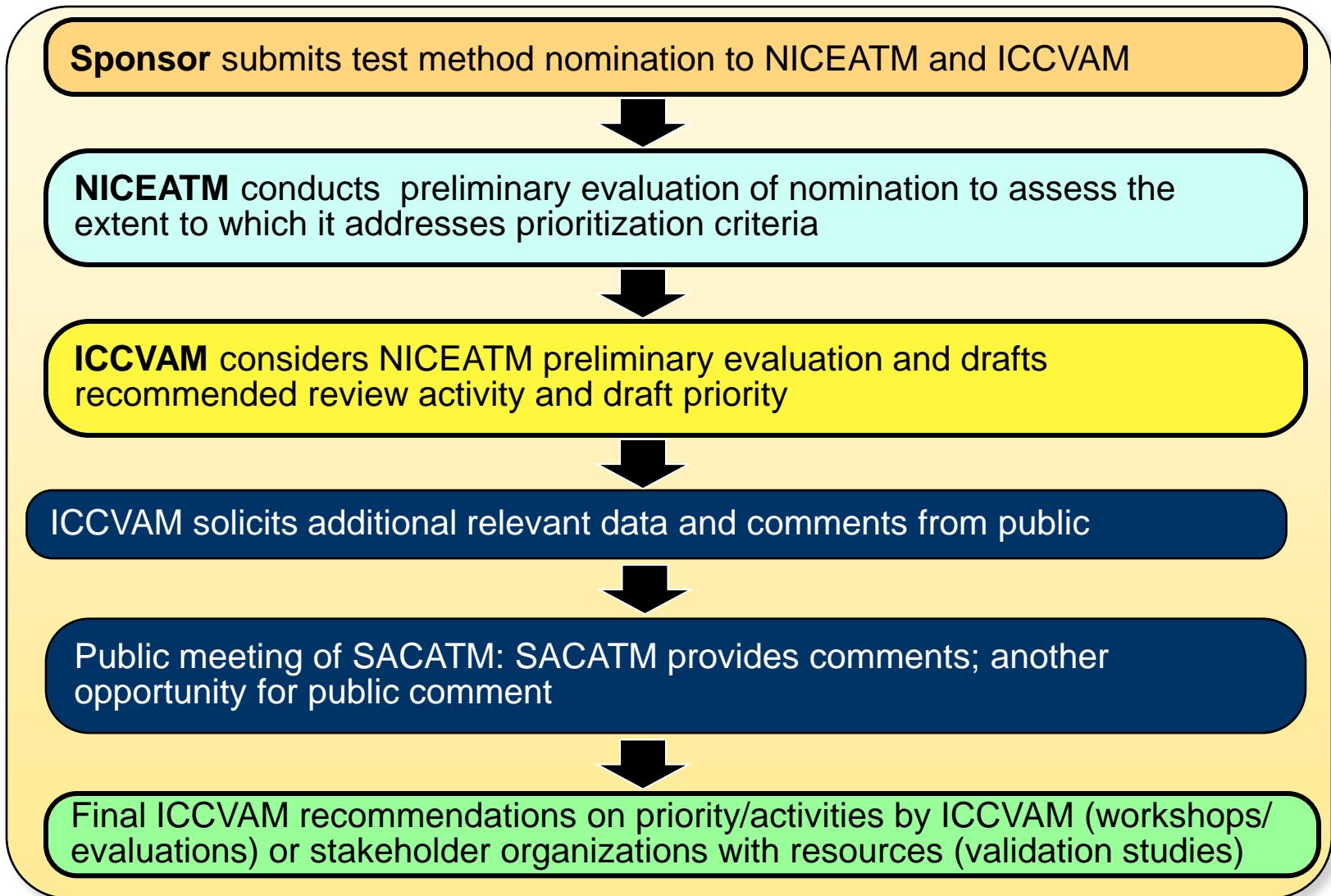
More information and instructions on submitting a nomination or submission are available at <http://iccvam.niehs.nih.gov/SuppDocs/submission.htm>

ICCVAM

NICEATM

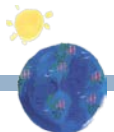


# Process for Nominations of Test Methods to ICCVAM



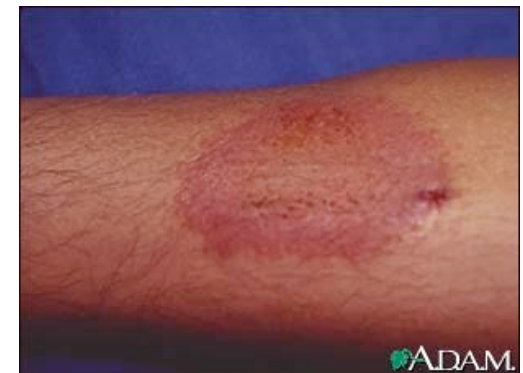
# ICCVAM Criteria for Prioritization

1. The extent to which the proposed test method is applicable to regulatory testing needs and agency programs
2. The potential for the proposed test method, compared to current test methods accepted by regulatory agencies, to reduce, refine, and replace animal use
3. The extent to which the proposed test method is warranted, based on the extent of expected use or application and impact on human, animal, or ecological health
4. The 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.
5. The extent to which the proposed test method provides other advantages (e.g., reduced cost and time to perform) compared to current test methods



# Why Evaluate the Skin Sensitization Potential of Chemicals and Products?

- Allergic contact dermatitis (ACD) is a significant public health problem
  - Skin diseases comprise at least 15% of all reported occupational diseases<sup>1</sup>
    - ACD is the most common type
  - 12% (8.9 million) of children in the United States had ACD in 2009<sup>2</sup>
  - More than 3700 substances have been identified as contact allergens<sup>3</sup>

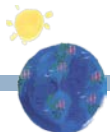


ACD photo – Medline Plus<sup>4</sup>

<sup>1</sup> BLS. 2010. Injuries, Illnesses, and Fatalities. Figure 3. Available: <http://www.bls.gov/iif/oshwc/osh/os/charts2009/charts.htm> - Figure3

<sup>2</sup> National Center for Health Statistics. 2010. FastStats: Allergies and Hay Fever. Available: <http://www.cdc.gov/nchs/fastats/allergies.htm>

<sup>3</sup> Beltrani et al. 2006. Contact dermatitis: A practice parameter. *Ann Allergy Asthma Immunol* 97(3):S1-S38.



# Why Regulate Products That Cause ACD?

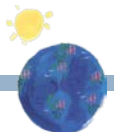
- ACD can significantly diminish quality of life<sup>1,2</sup>
- Prognosis may be poor<sup>3</sup>
  - Thus, prevention of ACD is crucial
- Regulatory authorities worldwide require testing for ACD potential and appropriate hazard labeling to prevent exposure



<sup>1</sup> Hutchings et al.. 2001. Occupational contact dermatitis has an appreciable impact on quality of life. *Contact Dermatitis* 45: 17-20.

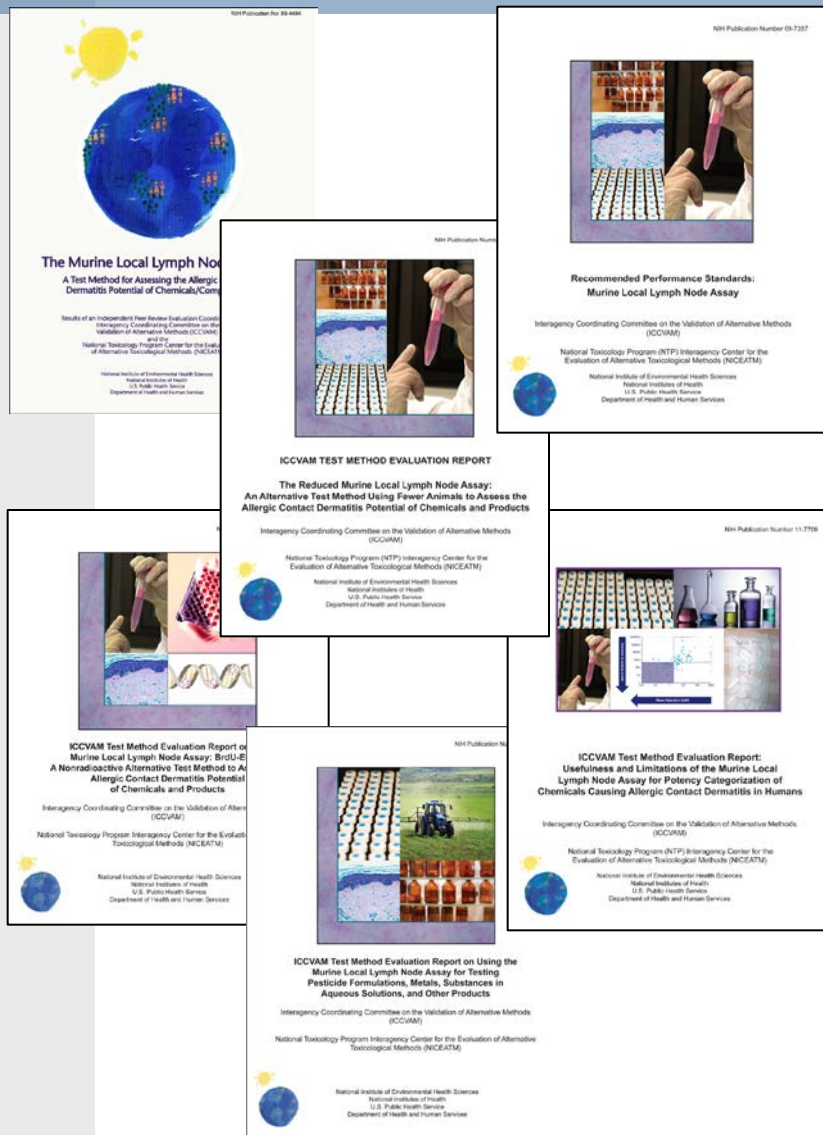
<sup>2</sup> Skoet et al. 2003. Contact dermatitis and quality of life: a structured review of the literature. *Br J Dermatol* 149: 452-456.

<sup>3</sup> Hogan et al. 1990. The prognosis of contact dermatitis. *J Am Acad Dermatol* 23: 300-307.

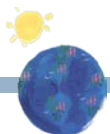




# NICEATM-ICCVAM ACD Activities (1)

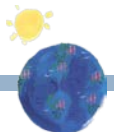


- 1999: Murine local lymph node assay (LLNA) evaluation
  - First ICCVAM test method evaluation
  - Reduces animal use by 33% compared with the standard guinea pig test methods
  - Avoids the use of irritating adjuvants
  - Eliminates the pain and distress associated with a positive response to an allergen



# NICEATM-ICCVAM ACD Activities (2)

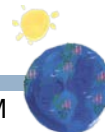
- 2007: Consumer Product Safety Commission nomination
  - 2009: LLNA performance standards
    - Updated ICCVAM protocol reduced animal use by an additional 20% (reduction from guinea pig test = 47%)
  - 2009: Reduced LLNA
    - Further reduces animal use by 40% (reduction from guinea pig test = 60%)
  - 2010: Expanded applicability domain of the LLNA
  - 2010: Two nonradiolabeled versions of the LLNA
  - 2011: Use of the LLNA for potency categorization



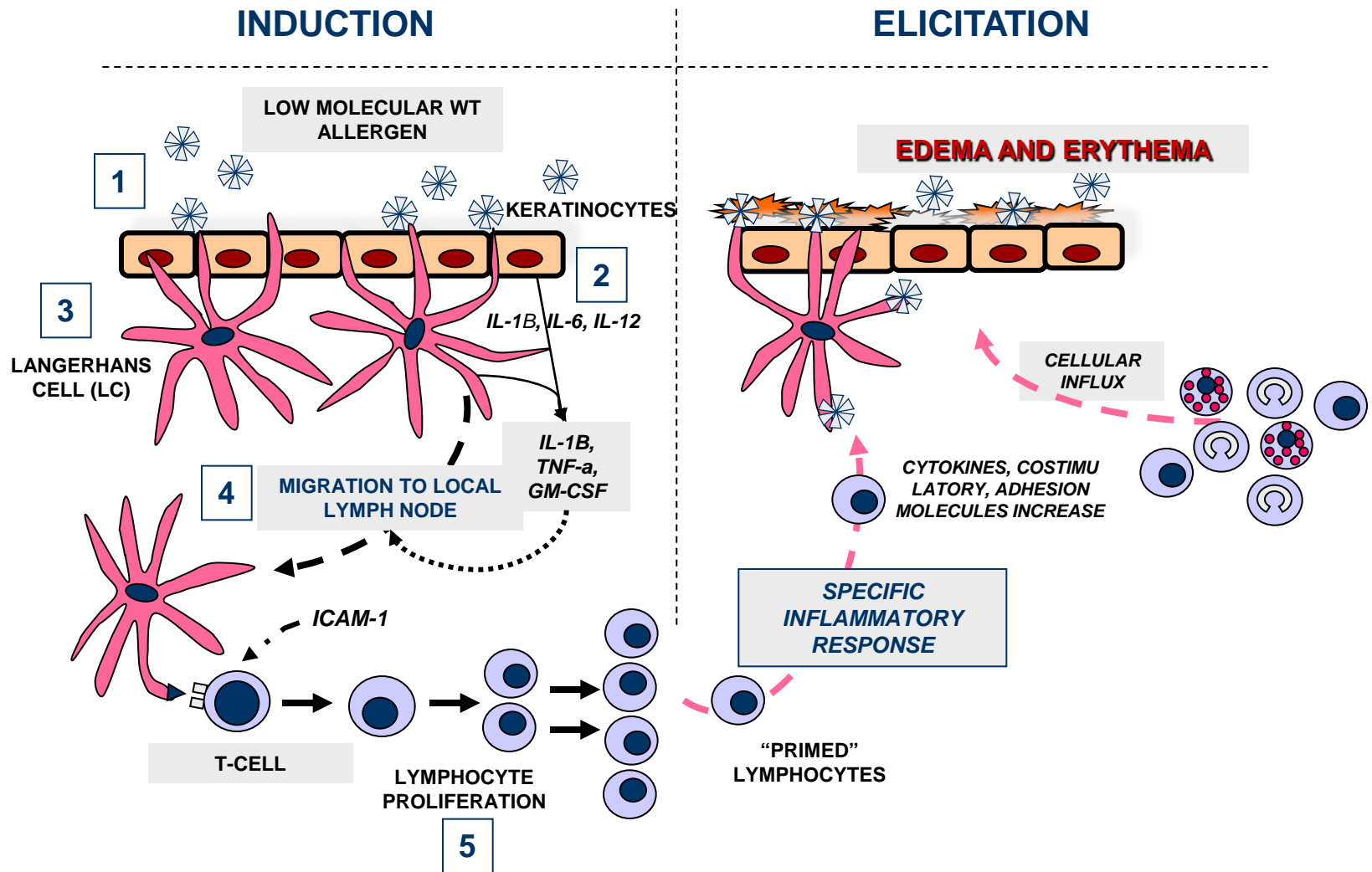


# Nomination from Dr. Paul Siegel, NIOSH: Electrophilic Allergen Screening Assay

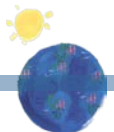
- *In chemico* test method for screening ACD hazards
  - Identifies electrophilic substances, which react with proteins
    - Electrophilic allergens believed to react with nucleophilic amino acids to form a stable covalent bond, which is critical to initiate a skin sensitization response
  - Mechanistically similar to the direct peptide reactivity assay
- Nominated for
  - Evaluation as a screening assay to identify contact allergens
  - Interlaboratory validation studies to determine the most appropriate decision criteria to maximize sensitivity and specificity of the assay



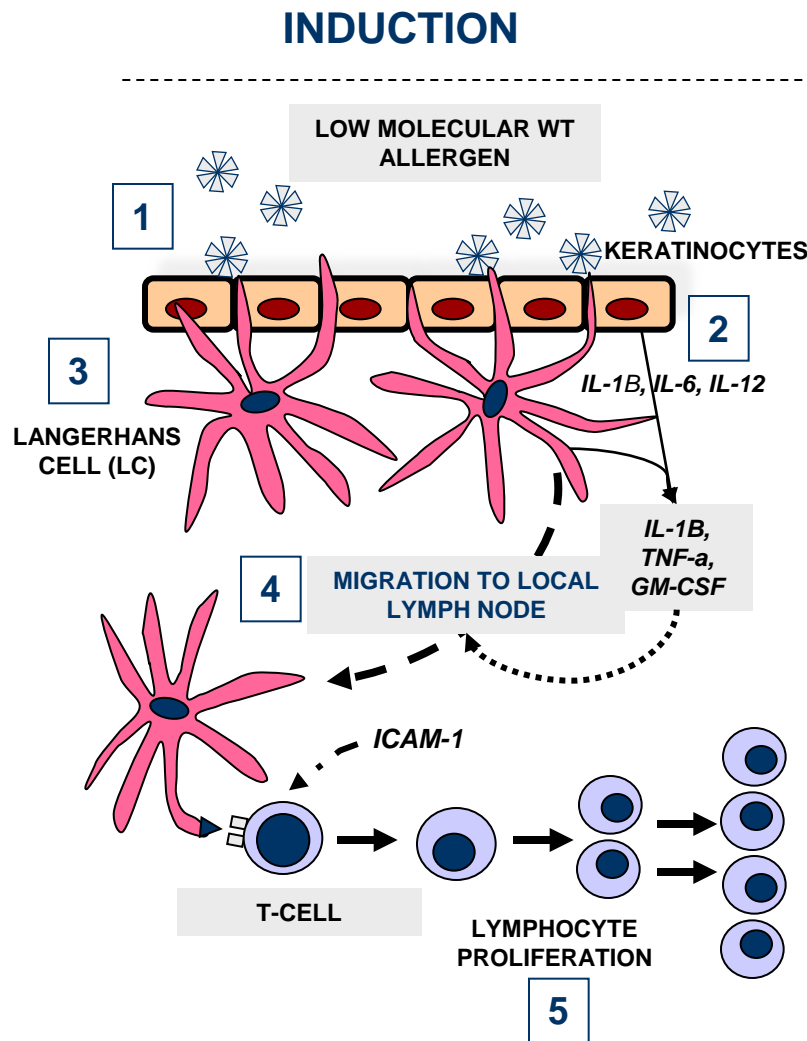
# Key Events in the ACD Adverse Outcome Pathway (1)



\*Illustration by D. Sailstad



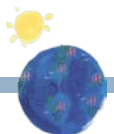
# Key Events in the ACD Adverse Outcome Pathway (2)



## EVENTS AND ASSAYS

1. Haptentation: attachment of allergen to skin protein (Direct Peptide Reactivity Assay [DPRA])
2. Epidermal inflammation: release of pro-inflammatory signals by epidermal keratinocytes (KeratinoSens<sup>SM</sup> assay)
3. Dendritic cell (DC) activation and maturation (Human cell line activation test [h-CLAT] or Myeloid U937 Skin Sensitization Test [MUSST])
4. DC migration: movement of DC bearing hapten-protein complex from skin to draining local lymph node
5. T-cell proliferation: clonal expansion of hapten-peptide specific T-cells (Murine local lymph node assay [LLNA])

\*Illustration by D. Sailstad

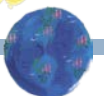


# 1. Applicable to Regulatory Testing Needs and Agency Programs

- U.S. regulatory agencies that have needs and/or requirements for ACD testing
  - FDA
  - CPSC
  - EPA
  - OSHA
- Dermal toxicity testing, including ACD testing, is a high priority area for ICCVAM<sup>1,2</sup>

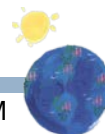
<sup>1</sup> ICCVAM. 2008. The NICEATM–ICCVAM Five-Year Plan (2008-2012): A Plan to Advance Alternative Test Methods of High Scientific Quality to Protect and Advance the Health of People, Animals, and the Environment. NIH Publication No. 08-6410. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: <http://iccvam.niehs.nih.gov/docs/5yearplan.htm>.

<sup>2</sup> ICCVAM 2012. The NICEATM-ICCVAM Five-Year Plan (2013-2017): A Plan to Advance Innovative Test Methods of High Scientific Quality to Protect and Improve the Health of People, Animals, and the Environment. Draft, May 14, 2012. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: <http://iccvam.niehs.nih.gov/docs/5yearplan.htm>.



## 2. Potential to Reduce, Refine, Replace Animal Use

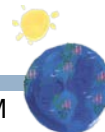
- The updated LLNA uses 20 mice; the reduced LLNA uses 12 mice
- The Electrophilic Allergen Screening Assay is an *in chemico* method that uses no animals
  - Could provide information for integrated testing and decision strategies for ACD hazard
  - Positive results may potentially be accepted without further testing



### 3. Extent of Expected Use or Application and Impact on Human, Animal, or Ecological Health

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- The Electrophilic Allergen Screening Assay could be used as an *in chemico* alternative to the LLNA to test substances for human ACD hazard
  - It identifies electrophiles, which produce ACD
- The Electrophilic Allergen Screening Assay can also provide potency information

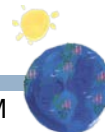




## 4. Potential to Provide Improved Prediction of Adverse Health or Environmental Effects

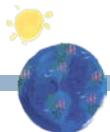
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- Good sensitivity, specificity, and accuracy within the applicability domain (electrophiles)
- Could provide essential information (i.e., protein reactivity) to improve integrated testing and decision strategies for ACD hazard identification
- May potentially provide information for human health risk assessment



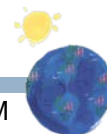
## 5. Other Advantages Provided by the Electrophilic Allergen Screening Assay

- Easy to perform
  - No specialized training
  - Mix chemicals and read the result
- Rapid results (2 hours or less)
- Low cost
  - Necessary equipment includes a spectrophotometer and spectrofluorometer, both with temperature control
    - No high performance liquid chromatography or mass spectrometry systems necessary
  - Small amounts of test substance and protein surrogate are used
  - No animals
  - No radioactive reagents
- Amenable to automation to increase throughput



# Draft ICCVAM Prioritization and Draft Recommended Activities

- Nominated activity is of sufficient interest and applicability to warrant validation studies to characterize its usefulness and limitations for predicting ACD potential
- Nomination should have a high priority
  - The ICCVAM Interagency Immunotoxicity Working Group and NICEATM will contribute by providing advice on
    - Optimization and standardization of the test method protocol
    - Validation study design to assess intra- and interlaboratory reproducibility and accuracy for the classification of ACD hazard
    - Selection of reference chemicals for the validation study



# ICCVAM Interagency Immunotoxicity Working Group

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