



# Interagency Coordinating Committee on the Validation of Alternative Methods

## ICCVAM Skin Sensitization Working Group Activities

September 16, 2014

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

# SSWG Scope and Charge

- Fostering the evaluation and promotion of alternative test methods for regulatory use in skin sensitization hazard assessment has been one of ICCVAM's long-standing priorities. ICCVAM is committed toward continued work in this area and believes it has promise for the near-term development of testing strategies that do not require the use of animals.
- As ICCVAM's goal is to advance the state of the science for alternative test methods and testing strategies for skin sensitization, the ICCVAM skin sensitization working group has been established to provide expertise in the evaluation of the following activities:

# Work Group Activities

1. The consideration of criteria for ICCVAM acceptance of ECVAM individually validated methods in the area of skin sensitization.
2. The design and examination of the predictive value of a battery of EVCAM validated methods and of *in silico* methods (e.g., QSAR predictions) based on statistical methods.

# Work Group Activities

3. A review of the Cosmetics Europe recommended battery for dermal sensitization, based on Cosmetics Europe's review of 16 non-animal methods.
4. Disposition of the NIOSH Electrophilic Allergen Screening Assay (EASA) nomination.

# EURL ECVAM

## Validation/Recommendations

- Direct Peptide Reactivity Assay (DPRA, Procter & Gamble)
  - Uses HPLC to monitor chemical depletion of nucleophile-containing synthetic peptides
  - EURL ECVAM recommendations published Nov 2013
- Myeloid U937 Skin Sensitization Test (MUSST; L'Oréal)
  - Flow cytometry detection of induced surface protein marker in human monocytic cell line
  - Interlaboratory testing Phase B1 completed (9 coded substances)
  - VMG recommended further protocol development due to interlaboratory variability
- Human Cell Line Activation Test (h-CLAT; Kao, and Shiseido)
  - Flow cytometry detection of 2 induced surface protein markers in human monocytic leukemia cell line
  - Interlaboratory reproducibility testing completed (24 coded substances, 4 labs)
  - EURL ECVAM report released to ICCVAM Jul 2014
- KeratinoSens™
  - Is a reporter gene assay measuring activation of the Keap1-Nrf2-ARE signaling pathway. Measures luciferase activity via luminescence
  - EURL ECVAM recommendations published Feb 2014

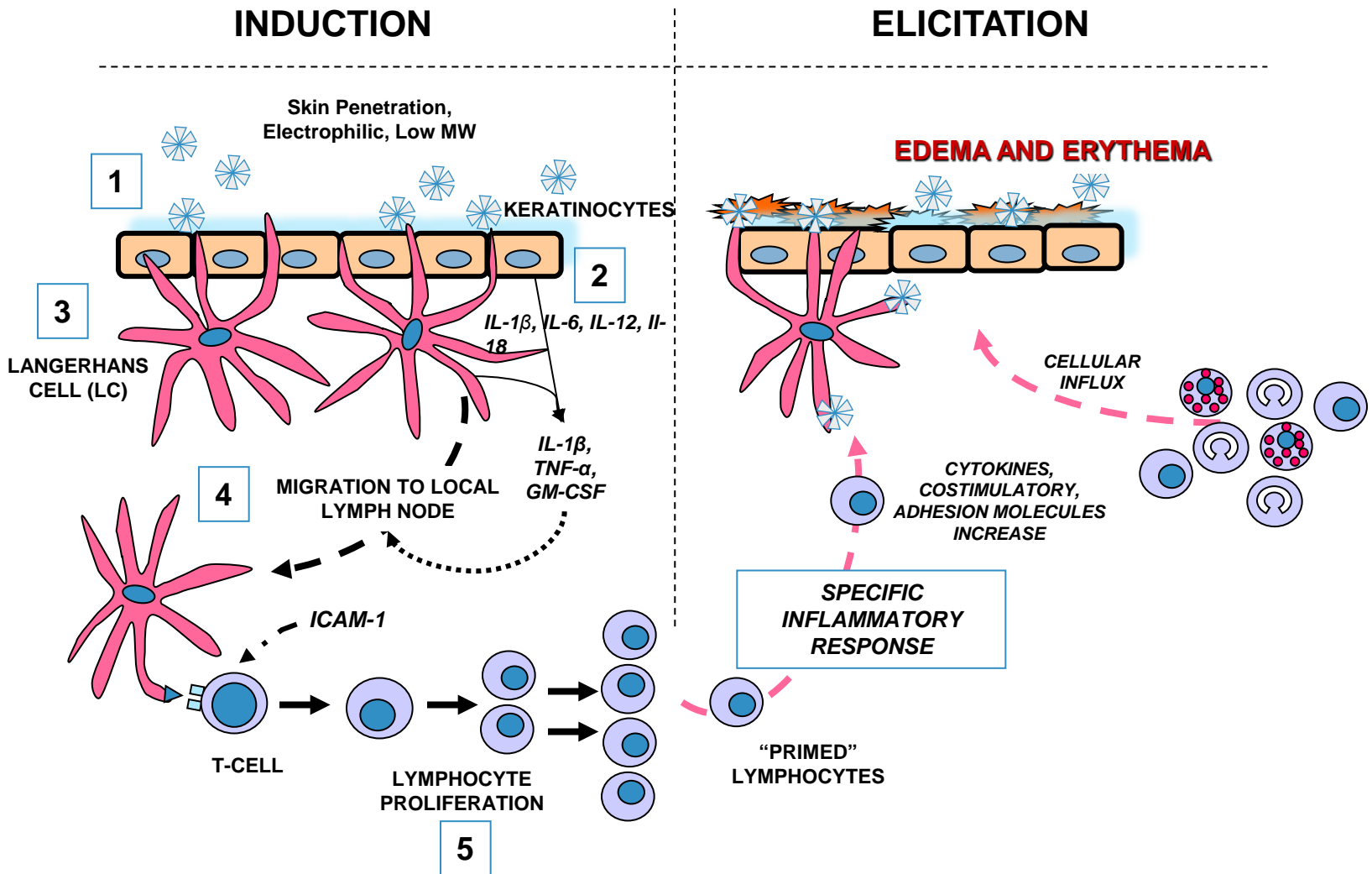
# Acceptance Criteria

- Is the test method intended for use as stand-alone or as part of a test battery? The tolerance for misclassifications is higher for individual tests that are part of a battery provided that the other tests in the battery ultimately yield the correct result.
- Data and analysis present in the evaluation report:
  - Human & animal data; characteristics of the data (# test subjects, sensitized, number of positive responses, type of test [HRIPT, HMT], dose-response curve, raw data [individual results], dose-response curve; vehicle used
  - False negative and false positive rates
  - Applicability domain explained, strengths and weaknesses
  - Inter- and intra-laboratory reproducibility:  $\geq 80\%$  or not
  - Reference chemical list: known reactive moieties/structural alerts (Schiff Base Formation, Michael Addition); what tested (e.g., single substance, formulation, purity), CAS# for each substance
  - Potential test method technical limitations: ease of use, proprietary or non-proprietary, cost, availability of the test components (cells, tissues)

# Integrated Testing Strategy

Because the adverse outcome pathway is well-characterized, and a number of non-animal test methods have been developed, it has promise for the near-term development of testing strategies that do not require the use of animals

# Skin Sensitization Process



\*Illustration by D. Sailstad



# ICCVAM Proposal

- Produce and test an integrated decision strategy for skin sensitization using
  - Physicochemical parameters
  - An *in silico* method
  - The three *in chemico* or *in vitro* assays validated by EURL ECVAM
- Design the integrated decision strategy to predict skin sensitization (yes/no) based on LLNA results

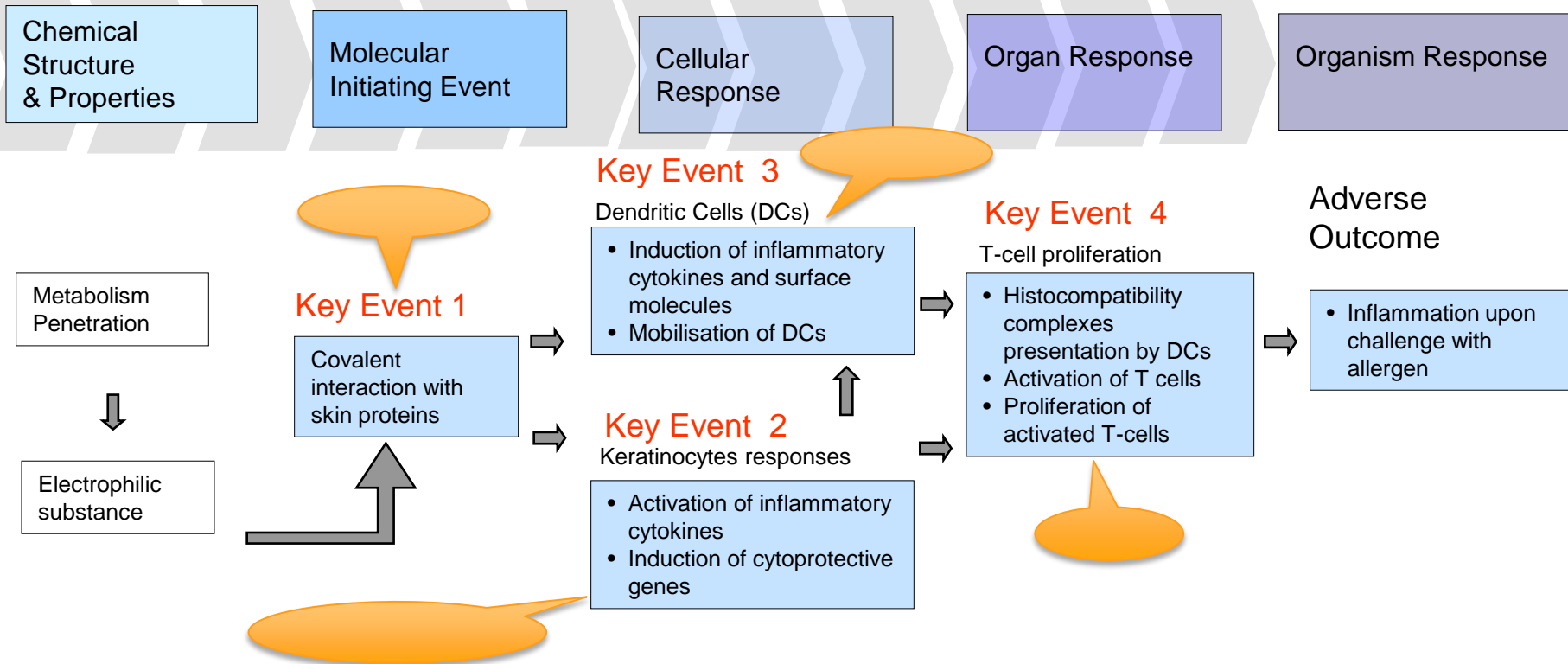
# Outline

- Physicochemical Parameters
  - Log Kow – octanol:water coefficient
  - Rationale: related to the ability to penetrate the skin; used in a number of bioavailability models and skin sensitization models
- *In Silico* Method
  - OECD QSAR Toolbox <http://www.qsartoolbox.org/>;
  - Recommended by the European Chemicals Agency for making chemical categories for read-across predictions (filling data gaps) to support chemical registrations
  - Can simulate metabolites
  - Uses mechanistic and structural features to group chemicals into categories

# Proposed *In Chemico* and *In Vitro* Methods

- Direct peptide reactivity assay (DPRA)
- Human cell line activation test (h-CLAT)
- KeratinoSens™
- Rationale
  - Completed or nearly completed pre-validation and peer review process at EURL ECVAM
  - OECD test guidelines for DPRA and KeratinoSens™ will be finalized in 2014; h-CLAT will follow
  - Covers 3 key events of the AOP

# OECD Adverse Outcome Pathway (AOP) for Skin Sensitization<sup>1</sup>



<sup>1</sup> For sensitization that is initiated by covalent binding to proteins.

OECD 2012. Guidance Document No. 168: The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins: Part 1, Part 2.

<http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmentpublicationsbynumber.htm>

# Outline

- Selection of chemicals
  - NICEATM has identified 120 substances with DPRA, h-CLAT, KeratinoSens, and LLNA data; QA/QC
  - Characterize by: physicochemical characteristics, such as structure, LogKow; range of LLNA potency; skin penetration coefficient
  - Evaluate relevance to applicability domain of the *in chemico/in vitro* assays
  - Split database into training set to build models and a test set to test the models (80/20 split)

# Statistical Methods

- Bayesian networks have been used to predict LLNA potency category
- Artificial neural network, a computational model that can compute values from inputs by feeding information through the network. Has been used to predict LLNA thresholds using h-CLAT and measurement of cell surface thiols.
- Support vector machine analyzes data and recognizes patterns. Is non-probabilistic. Has been used to build QSAR models to predict LLNA and guinea pig results; also used to predict LLNA sensitizer/nonsensitizers using gene expression results from the Genomic Allergen Rapid Detection (GARD) assay.
- Logistic regression, linear discrimination analysis, simple battery approach

# Cosmetics Europe Activity

- Developing data integration strategy
  - Predictive compared to LLNA and/or humans
  - Relevant for 5-6 potency classes
- Prioritized 8 methods for testing 100 chemicals
  - DPRA, PPRA, KeratinoSens, Lu-Sens, SENS-IS (Immunosearch), MUSST, h-CLAT, and VITO-SENS
  - Additional methods will be evaluated as funding is acquired
- Collaboration Agreement with NICEATM to explore non-animal integrated approaches for skin sensitization safety assessment of cosmetic ingredients.

# EASA

- Nominated by Dr. Paul Siegel, NIOSH, in 2012
  - Mechanistically similar to the DPRA
  - Identifies electrophilic allergens that react with nucleophilic amino acids to form stable covalent bond
  - Binding to protein is the molecular initiating event in the Adverse Outcome Pathway (AOP) leading to skin sensitization response
- A preliminary evaluation was performed by NICEATM
- The Immunotoxicity Working Group and SACATM gave the test method a high priority for a validation study
- ICCVAM gave the EASA a conditional high priority pending feedback that the work is not duplicating efforts in Europe



# Skin Sensitization Working Group

Dave Allen-ILS NICEATM

Paul Brown-FDA

Silvia Casati-EURL ECVAM

Warren Casey-NIEHS

Wei Ding-FDA

Dori Germolec-NIEHS

Abby Jacobs-FDA

Brett Jones-ILS NICEATM

Nicole Kleinstreuer-ILS NICEATM

David Lehmann-EPA

Anna Lowit-EPA

Vasant Malshet-FDA

Joanna Matheson-CPSC

Tim McMahon-EPA

Moiz Mumtaz-ATSDR

Michael Paris-ILS NICEATM

Diego Rua-FDA

Judy Strickland-ILS NICEATM

Jeffrey Toy-FDA

Jeffrey Yourick-FDA

Dan Zang-ILS NICEATM



# Interagency Coordinating Committee on the Validation of Alternative Methods

## Comments and/or Questions?

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



# Interagency Coordinating Committee on the Validation of Alternative Methods

## Charge Questions:

Please comment on ICCVAM's progress, to date, in carrying out the priority areas of work described in the ICCVAM vision and strategy document.

Please comment on whether there might be additional short to intermediate term (1-5 years) scientific areas that ICCVAM and NICEATM should consider pursuing.

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