Identifying Integrated *In Vitro/In Silico* Testing Strategies by Mapping to the Skin Sensitization AOP

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OECD Adverse Outcome Pathway (AOP) for Skin Sensitization

• For sensitization that is initiated by covalent binding to proteins.

Skin Sensitization Process

**INDUCTION**

1. Skin Penetration, Electrophilic, Low MW

2. Keratinocytes
   - IL-1β, IL-6, IL-12, IL-18
   - IL-1β, TNF-α, GM-CSF

3. Langerhans Cell (LC)

4. Migration to Local Lymph Node
   - ICAM-1

5. T-Cell
   - Lymphocyte Proliferation

**ELICITATION**

- Edema and Erythema
- Cellular Influx
- Cytokines, Costimulatory, Adhesion Molecules Increase

- "Primed" Lymphocytes

*Illustration by D. Sailstad*
Key Events in the Skin Sensitization Process

**INDUCTION**

1. Skin penetration, Electrophilic, Low mw

**EVENTS AND ASSAYS**

In *silico* toxicokinetic model, QSARs, permeability methods

1. Haptenation: attachment of allergen to skin protein (DPRA, PPRA, EASA)

2. Epidermal inflammation: release of pro-inflammatory signals by epidermal keratinocytes (KeratinoSensSM, AREc32, LuSens, SENS-IS, NCTC, SenCeeTox)

3. Dendritic cell (DC) activation and maturation (h-CLAT, MUSST, PBMD, VITOSens, GARD, Sensi-Derm)

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 (LLNA, hTCPA)

*Illustration by D. Sailstad*
OECD AOP for Skin Sensitization

Key Event 1
- Metabolism Penetration
- Covalent interaction with skin proteins

Key Event 2
- Keratinocytes responses
  - Activation of inflammatory cytokines
  - Induction of cytoprotective genes

Key Event 3
- Dendritic Cells (DCs)
  - Induction of inflammatory cytokines and surface molecules
  - Mobilisation of DCs
  - h-CLAT

Key Event 4
- T-cell proliferation
  - Histocompatibility complexes
  - Presentation by DCs
  - Activation of T cells
  - Proliferation of activated T-cells

Adverse Outcome
- Inflammation upon challenge with allergen
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
International Groups
Skin Sensitization IATA/ITS/Battery

• ICCVAM: 15 Federal regulatory and research agencies
  – NICEATM: NTP Interagency Center for the Evaluation of Alternative Toxicalogical Methods
  – U.S. regulatory agencies that have needs and/or requirements for sensitization testing: EPA, FDA, OSHA, CPSC

• EURL-ECVAM: European Union Reference Laboratory for Alternatives to Animal Testing
  – ICATM (International Cooperation on Alternative Test Methods): ICCVAM, EURL-ECVAM, JaCVAM, KoCVAM, Health Canada

• OECD drafting Group on the IATA for Skin Sensitization
• Cosmetics Europe (COLIPA)
ICCVAM Skin Sensitization Working Group (SSWG)

- 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.

- Because the AOP 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.

- The design and examination of the predictive value of a battery of EVCAM validated methods and of \textit{in silico} methods (e.g., QSAR predictions) based on statistical methods.
NICEATM Activities

• NICEATM collaboration to develop and evaluate chemical structure-activity relationship (SAR) models to predict skin sensitization

• NICEATM collaboration with industry scientists to develop an open-source Bayesian network as an operational framework for an ITS

• NICEATM evaluation of various high-throughput screening assays in coordination with NIEHS Tox21 activities
ICCVAM Skin Sensitization Battery 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 of ICCVAM Proposal

• 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
  – 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
ICCVAM SSWG Current Work 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)
Proposed Statistical Methods

• Bayesian networks: 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; to predict LLNA sensitizer/nonsensitizers using gene expression results from the GARD assay.

• Logistic regression, linear discrimination analysis, simple battery approach
NICEATM High Throughput Activities

• Relevant assays which may predict skin sensitizing activity
  
  – EPA’s ToxCast:
    
    • Evaluating activity signatures across the 700+ assays to determine the ability to predict reference immunotoxicity endpoints
    
    • 52 substances nominated by the NTP based on immunological relevance and correspondence to the AOP
  
  – NTP’s High Throughput Screening program with the National Human Genome Research Institute’s NIH Chemical Genomics Center (NCGC), with a library of 10,000+ compounds
Tox21 Assays aligned to AOP key events

1. Skin Penetration
2. Electrophilic substance: directly or via auto-oxidation or metabolism

3-4. Haptenation: covalent modification of epidermal proteins
   - Novascreen enzyme activity biochemical cell-free assays (HDACs, EGFR, etc.)

4. QSAR Model of skin permeability and penetration (Tropsha, et al.)

5-6. Activation of epidermal keratinocytes & Dendritic cells
   - BSK_hDF3CGF Human dermal fibroblasts
   - BSK_KF3CT Human keratinocytes and fibroblasts

6. Odyssey Thera oxidative stress in U2OS (H2AFX)

7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells
   - BSK_SAg and BSK_LPS Human monocytes and endothelial cells

8. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death
   - QSAR Model built off NICEATM LLNA database (Tropsha, et al.)

- Aprenda oxidative stress in HepG2 (H2AFX)
- Tox21 assay HepG2 bla (Nrf2/ARE)
Comments and/or Questions?
Skin Sensitization AOP References

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• Hirota 2013 Artificial neural network analysis of data from multiple in vitro assays for prediction of skin sensitization potency of chemicals. *Toxicol In Vitro* 27: 1233-1246
• Jaworska 2013 Bayesian integrated testing strategy to assess skin sensitization potency: from theory to practice. *J Appl Toxicol* 33: 1353-1364
• Jaworska 2011 Integrating non-animal test information into an adaptive testing strategy – skin sensitization proof of concept case *ALTEx* 28(3): 211-225

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- MacKay 2013 From pathways to people: applying the adverse outcome pathway (AOP) for skin sensitization to risk assessment *ALTEx* 30(4): 473-486

- Maxwell 2014 Applying the skin sensitisation adverse outcome pathway (AOP) to quantitative risk assessment *Toxicol In Vitro* 28(1): 8-12
Skin Sensitization AOP References (cont.)

- Pirone 2014 Open source software implementation of an integrated testing strategy for skin sensitization potency based on a Bayesian network *ALTEX* 31(3): 336-340