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

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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
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OECD Adverse Outcome Pathway (AOP) for Skin Sensitization

- 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

Skin Sensitization Process

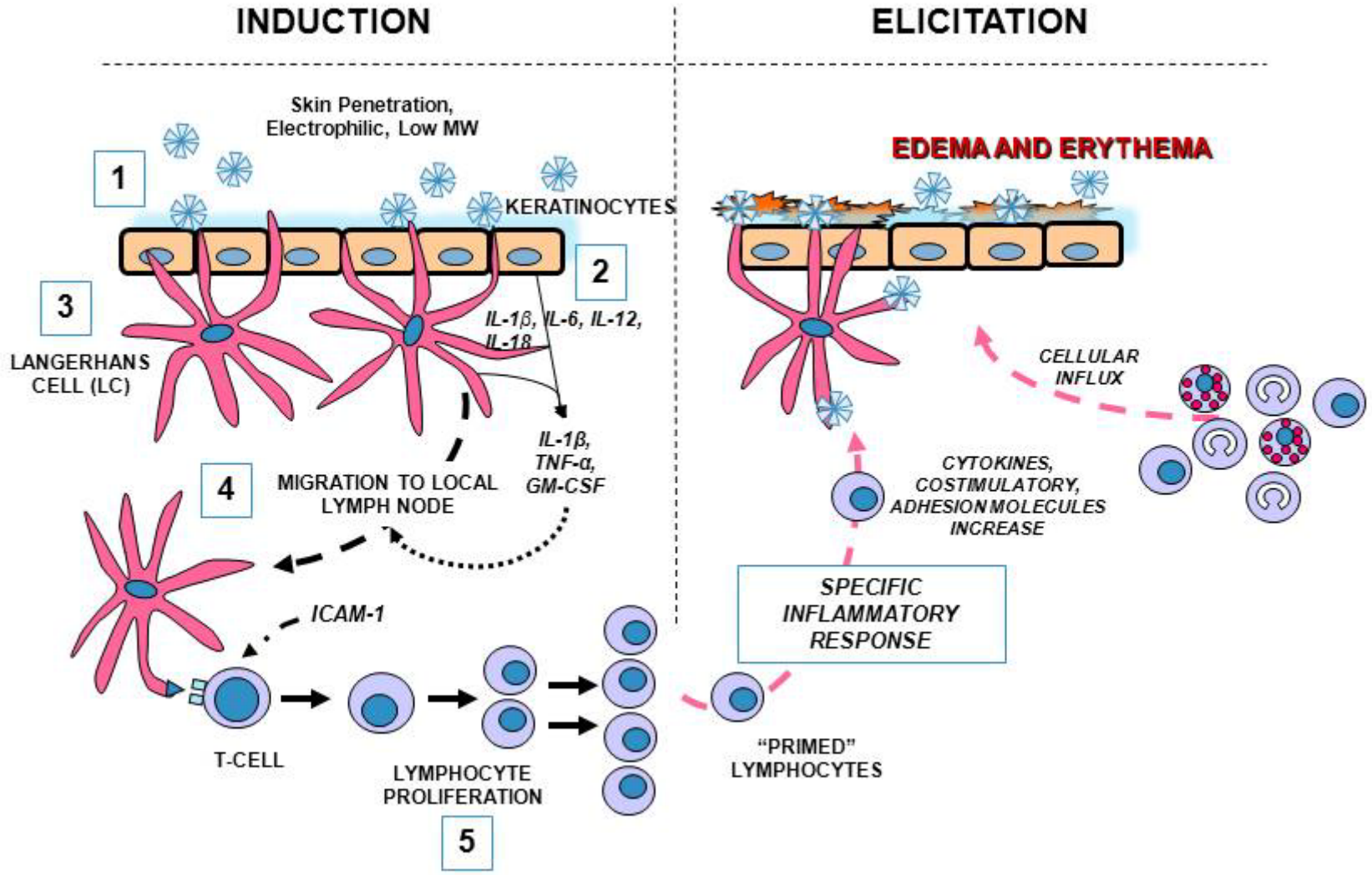
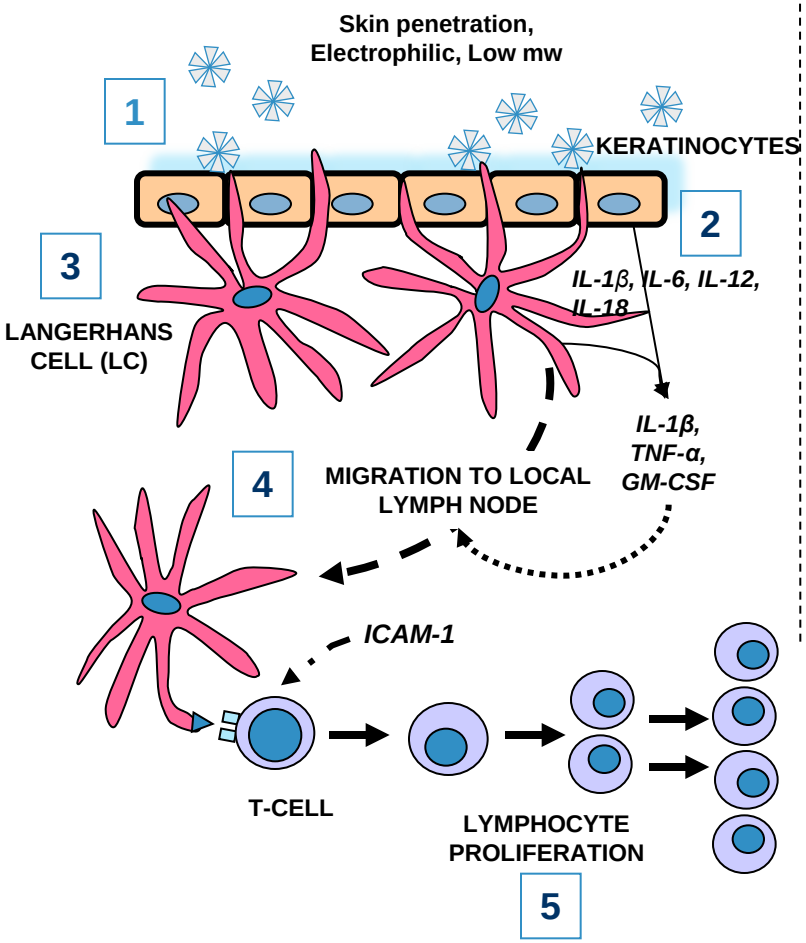


*Illustration by D. Sailstad

Key Events in the Skin Sensitization Process

INDUCTION



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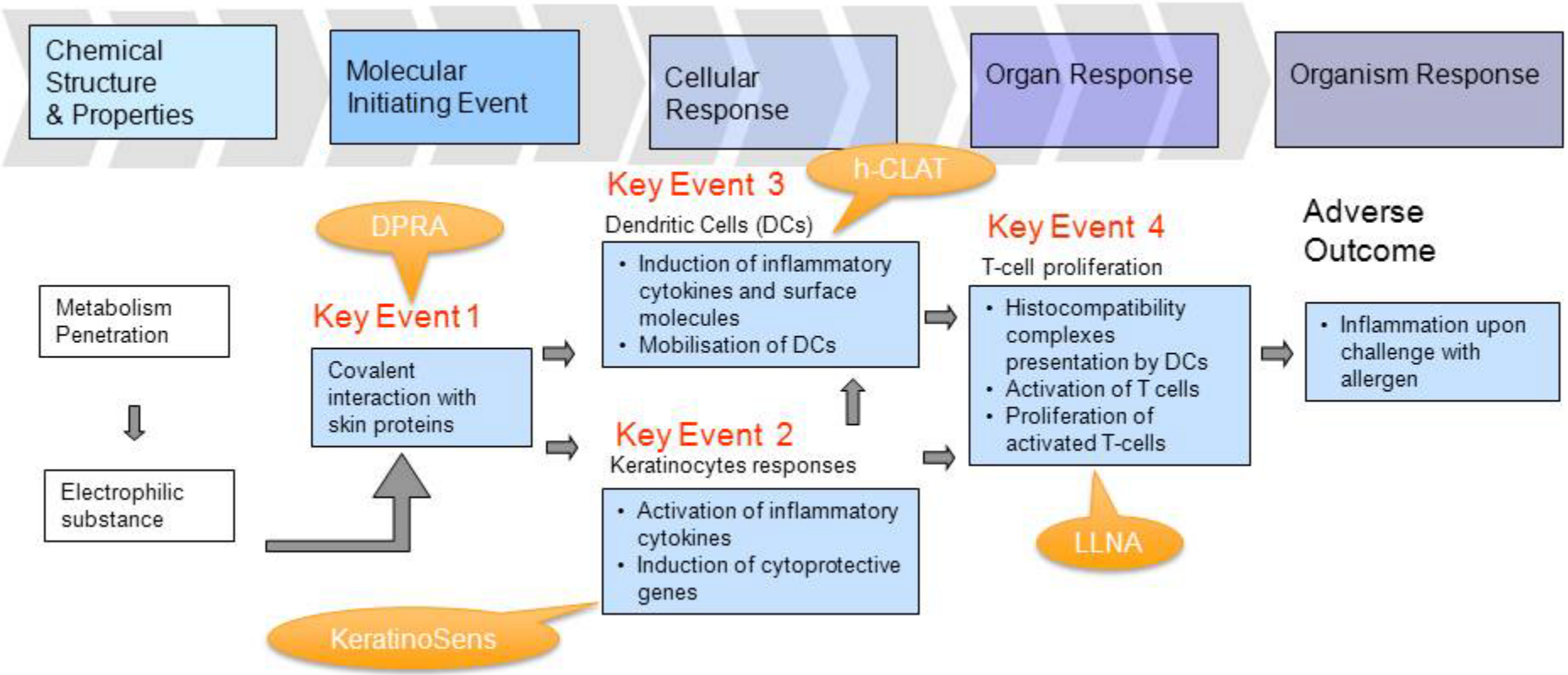
EVENTS AND ASSAYS

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 (KeratinoSens^{SM}, AREc32, LuSens, SENS-IS, NCTC, SenCeeTox)
3. Dendritic cell (DC) activation and maturation (h-CLAT, MUSST, PBMDC, 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)

OECD AOP for Skin Sensitization



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 Toxicological 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 *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
  - http://ntp.niehs.nih.gov/pubhealth/evalatm/integrated-testing-strategies/index.html
- 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
  - 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

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)

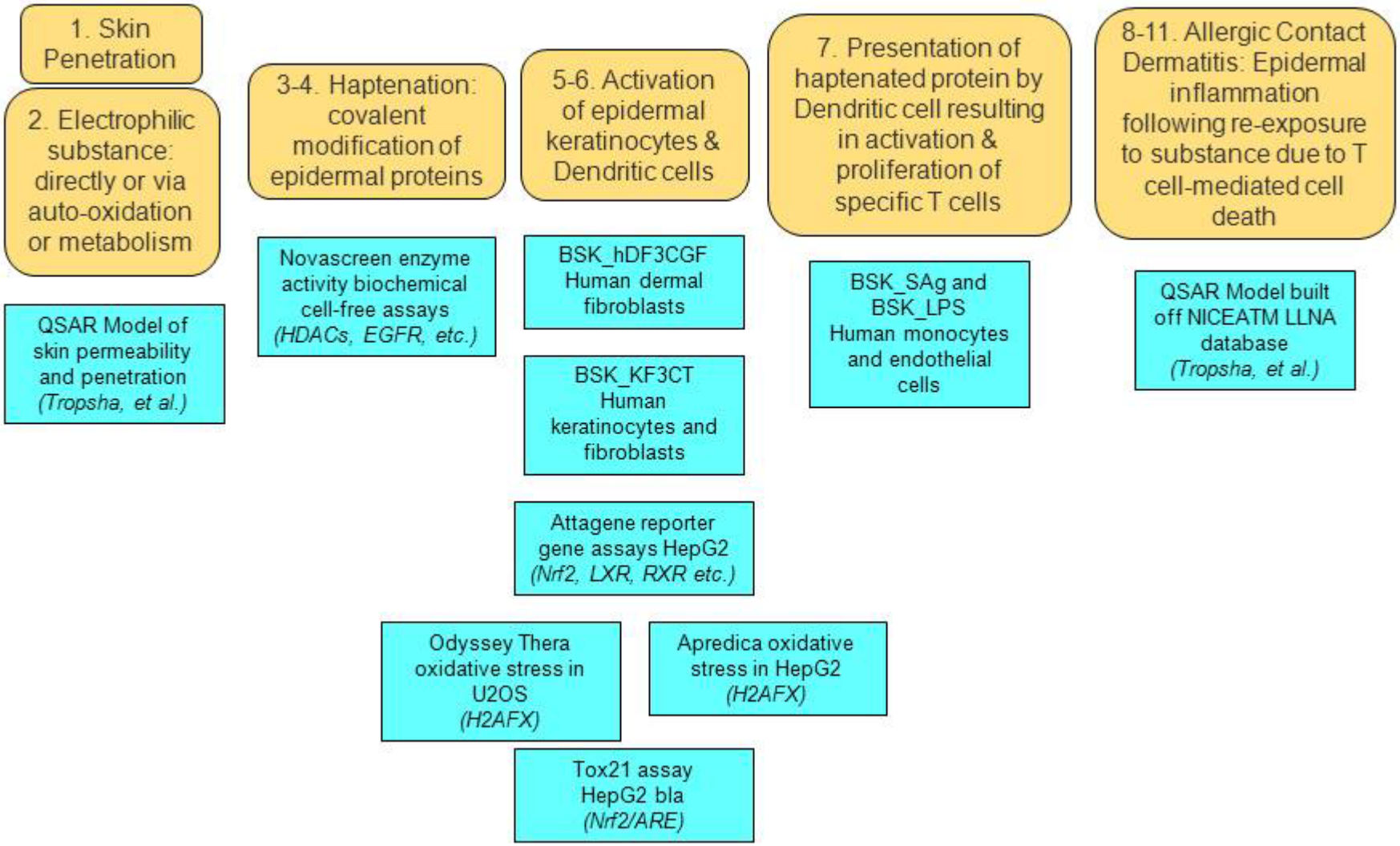
Proposed Statistical Methods

- 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



Comments and/or Questions?

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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
  - http://caat.jhsph.edu/programs/workshops/july13validation.html

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

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- Yuan 2009 Prediction of skin sensitization with a particle swarm optimized support vector machine *Int J Mol Sci* 10: 3237-3254
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