Skin Sensitization:
In Vitro Methods and Risk Assessment

Donald L. Bjerke, Cindy A. Ryan, G. Frank Gerberick
The Procter & Gamble Company
Identification of Allergens: Endpoint Evolution

- Guinea Pig Test Methods
- Local Lymph Node Assay
- Molecular Modeling
- Peptide Reactivity
- DC Gene Activation

1970s <2010 1990s
What do we know about Skin Sensitization?

Skin Penetration
Protein Binding
DC Activation
Antigen Presentation
T Cell Proliferation

Toxicological Sciences, 2011; 120(S1):S238-S268
Adverse Outcome Pathway and Predictive Testing

Chemical Structure & Properties → Molecular Initiating Event → Cellular Response → Organ Response → Organism Response

Key Event 1: Skin Penetration

Key Event 2 + 3: 3-4. Haptenation: covalent modification of epidermal proteins

Key Event 4: 5-6. Activation of epidermal keratinocytes & Dendritic cells

Adverse Outcome: 7-8. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells

9-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death

In silico models SAR/QSAR

In chemico models

In vitro cell-based models (keratinocytes, dendritic cells)

Reduced animal model LLNA

Animal models GPMT, Buehler HRIPT

SAR “read-across”

Modified version of flow diagram from ‘The Adverse Outcome Pathway for Skin Sensitisation,’ OECD report 2012
1. Skin Penetration
2. Electrophilic substance: directly or via auto-oxidation or metabolism
3-4. Haptenation: covalent modification of epidermal proteins
5-6. Activation of epidermal keratinocytes & Dendritic cells
7-8. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells
9-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death

Key Event 1
- KeratinoSens (Givaudan)
- DPRA (P&G)
- PPRA (P&G)
- ARE c32 (CXR Bio.)

Key Event 4
- LuSens (BASF)
- NCTC 2544 IL-18 (Univ. Milan)
- EE Potency assay (VUMC)

Adverse Outcome
- Human T cell priming assay (Univ. Lyon, Univ. Freiburg)
- LLNA (OECD 429)

Guidance on information requirements and Chemical Safety Assessment

Chapter R.7a: Endpoint specific guidance

Draft Version 5.0

June 2016

https://echa.europa.eu/view-article/-/journal_content/title/registrants-to-use-alternative-test-methods-for-skin-sensitisation
## Validation and adoption status of in chemico/in vitro methods for skin sensitization

<table>
<thead>
<tr>
<th>AOP Key Event</th>
<th>Test Method</th>
<th>Validation Status, Regulatory Acceptance</th>
<th>EU Test Methods/OECD TG</th>
<th>Outcome according to the test method</th>
<th>EURL ECVAM DB-ALM protocol no.</th>
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</thead>
<tbody>
<tr>
<td>Skin Sensitization</td>
<td>DPRA</td>
<td>Validated, regulatory acceptance</td>
<td>B.59/TG 442C</td>
<td>SS or NS with complementary information</td>
<td>154</td>
</tr>
<tr>
<td>Key Event 1 Peptide/protein binding</td>
<td>KeratinoSens™</td>
<td>Validated, Regulatory acceptance</td>
<td>B.60/TG 442D</td>
<td>SS or NS with complementary information</td>
<td>155</td>
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<td>Key Event 2 Keratinocyte response</td>
<td>LuSens</td>
<td>Under validation assessment</td>
<td></td>
<td>SS or NS with complementary information</td>
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<td></td>
<td>SENS-IS</td>
<td>Under validation assessment</td>
<td></td>
<td>SS or NS with complementary information</td>
<td></td>
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<tr>
<td>AOP Key Event</td>
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<tr>
<td>Skin Sensitization</td>
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<tr>
<td><strong>Key Event 3</strong> Monocytic/Dendritic cell response</td>
<td>h-CLAT</td>
<td>Validated, regulatory acceptance</td>
<td>TG 442E</td>
<td>SS or NS with complementary information</td>
<td>158</td>
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<tr>
<td></td>
<td>U-SENS™</td>
<td>Under validation assessment</td>
<td></td>
<td>SS or NS with complementary information</td>
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<tr>
<td></td>
<td>IL-8 Luc Assay</td>
<td>Under validation assessment</td>
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<td>SS or NS with complementary information</td>
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<td></td>
<td>SENS-IS</td>
<td>Under validation assessment</td>
<td></td>
<td>SS or NS with complementary information</td>
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<tr>
<td><strong>Key Event 4</strong> T-cell response</td>
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</tbody>
</table>
**in chemico DPRA Method**

**Cysteine** (Ac-RFAACAA-COOH)

**Incubation** for 24 h at 25°C (dark)

**HPLC/PDA**

\[
\text{Percent Peptide Depletion} = \left[ 1 - \left( \frac{\text{Peptide Peak Area in Replicate Injection}}{\text{Mean Peptide Peak Area in Reference Controls}} \right) \right] \times 100
\]


Alternatives to animal testing: EURL ECVAM publishes its Recommendation on the Direct Peptide Reactivity Assay for skin sensitisation testing

In its Recommendation, EURL ECVAM summarises key performance parameters of this in vitro method and its use within integrated approaches to assessing the skin sensitisation potential of chemicals.

On 12 December 2013 the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM, part of the European Commission Joint Research Centre) published its Recommendation on the Direct Peptide Reactivity Assay (DPRA) for skin sensitisation testing.

Information on the skin sensitisation potential of chemicals is a key requirement for chemical safety: under the EU Chemicals legislation (REACH), skin sensitisation is one of two health effects still requiring animal testing for chemicals produced or marketed at the lowest tonnage level requiring substance registration. Currently, assessment of the skin sensitisation potential of chemicals for regulatory purposes relies on animal testing, but scientific progress on understanding mechanisms of skin sensitisation has led to the development of alternative methods, amongst these the Direct Peptide Reactivity Assay (DPRA).
OECD GUIDELINE APPROVED!!!

**Test No. 442C: In Chemico Skin Sensitization - Direct Peptide Reactivity Assay (DPRA):** This Test Guideline provides an *in chemico* procedure (Direct Peptide Reactivity Assay – DPRA) used for supporting the discrimination between skin sensitizers and non-sensitizers in accordance with the UN GHS.

Cell-Based In Vitro Test Methods

- **KeratinoSens™**
  - Protocol developed by A. Natsch (Givaudan)

The Keap1 – Nrf2 – ARE signaling pathway of cells specifically responds to electrophiles
Alternatives to animal testing: EURL ECVAM publishes its Recommendation on the KeratinoSens™ assay for skin sensitisation testing

— filed under: ESIS, systems toxicology, in-vitro methods, ECVAM, alternatives to animal test, ESAC, EURL ECVAM

KeratinoSens™ may prove a useful component of integrated approaches for skin sensitisation hazard assessment, and may also be able to contribute to the assessment of sensitising potency.

On 17 February 2014 the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of the European Commission’s Joint Research Centre published its Recommendation on the KeratinoSens™ assay for skin sensitisation testing.

Chemicals can cause the immunological sensitisation of skin tissue (skin sensitisation) leading, upon repeated exposure, to an allergic reaction of the skin (Allergic Contact Dermatitis, ACD). Due the biological complexity of this effect, the intrinsic hazard and risk of chemicals to sensibilise skin has been assessed by animal experiments. However, there is increasing knowledge on the key biological and physiological mechanisms underlying skin sensitisation, supporting the development of mechanism-based in vitro assays. For example, the Keap1-Nrf2-antioxidant/electrophile response element (ARE)-dependent pathway is a key signalling pathway regulating the response of skin cells (keratinocytes) to chemical stressors including sensitisers.

The KeratinoSens™ assay uses activation of this pathway as a measure for the skin sensitisation potential of chemicals. The test method has been developed by Givaudan S.A. who also organised a validation study addressing mainly the test method’s transferability and within- and between-laboratory reproducibility. Following independent evaluation by EURL ECVAM and scientific peer review by EURL ECVAM’s Scientific Advisory Committee (ESAC), EURL ECVAM has now summarised the present validation status of the KeratinoSens™ and makes recommendations regarding further work and the assay’s potential use within integrated approaches.

As all EURL ECVAM Recommendations, also the present one has undergone extensive commenting by EU regulators (PARERE network), by stakeholders from industry, animal welfare and academia (ESTAF), by international partners and regulators (ICATM framework) as well as the general public. As a consequence, it is expected that this Recommendation, like EURL ECVAM’s recent Recommendation on the Direct Peptide Reactivity Assay (DPRA) for skin sensitisation testing, will facilitate scientific discussions at OECD in view of developing OECD Test Guidelines for skin sensitisation.
Test No. 442D: In Vitro Skin Sensitization - ARE-Nrf2 Luciferase Test

Method: This Test Guideline (TG) provides an in vitro procedure (the ARE-Nrf2 luciferase test method) used for supporting the discrimination between skin sensitizers and non-sensitizers in accordance with the UN GHS.

**In Vitro Cell-Based: Human Cell Line Activation Test (hCLAT)**

Pre-culture cells for 48-72 hours (0.2-0.4 x 10^6 cells/mL).

Plate (1x10^6 cells/well) in 24-well plate, treat with test chemical for 24 hours.

Harvest cells, wash and block FcR (0.01% Globulins) for 15 min.

Divide cells into 3 aliquots, stain with FITC-conjugated monoclonal antibodies (isotype control, CD86, CD54) for 30 min.

Analyze by flow cytometry - mean fluorescence intensity of CD86 and CD54, cell viability by propidium iodide exclusion.
EURL ECVAM Recommendation on the human Cell Line Activation Test (h-CLAT) for Skin Sensitisation testing

1. EURL ECVAM Recommendation, ESAC Opinion and ESAC WG Report

On 02.03.2015 EURL ECVAM published its Recommendation on the human Cell Line Activation Test (h-CLAT Sensitisation testing):

- ESAC Working group Peer Review Consensus Report

2. Relevant validation study reports

- h-CLAT validation study report
- h-CLAT validation study report - appendices
- Post-hoc analysis of the validation study data performed by EURL ECVAM

3. Summary of the changes made in response to public comments received

The recommendation was open for public comments from 28.11.2014 to 31.12.2014. In total three parties comments to EURL ECVAM. In response to these comments two paragraphs in section 4 on the limitations o
OECD GUIDELINE APPROVED!

Test No. 442E: In Vitro Skin Sensitization – human Cell line Activation Test (h-CLAT): This Test Guideline (TG) provides an in vitro procedure (the human cell line activation test h-CLAT method) used for supporting the discrimination between skin sensitizers and non-sensitizers in accordance with the UN GHS.
Alternatives for Skin Sensitization: The Challenge - Data Integration

- Bioavailability
- Peptide Reactivity
- T cell Activation
- Metabolism
- DC Activation
- SAR

Hazard ID and Potency (NESIL) for QRA

Data Integration / ITS / WoE / IATA

- Bayesian Network (P&G)
- Artificial Neural Network (Shiseido)
- Weight of Evidence (BASF)
Registrants to use alternative test methods for skin sensitisation

The REACH requirements for skin sensitisation are changing, making non-animal testing the default requirement. Registrants are encouraged to consider their testing strategies now for the 2018 registration deadline.

Helsinki, 5 July 2016 - The amended REACH annexes concerning skin sensitisation are expected to enter into force in autumn 2016. The information needed for the classification or risk assessment of a substance will then be obtained through non-animal methods as a first step. In vivo methods can only be used if the in chemico or in vitro test methods are not adequate for the substance or cannot be used for classification and risk assessment.

With the amended requirements, if a substance is predicted to be a skin sensitiser based on the available data, skin sensitisation potency should also be assessed. There is currently no standardised way to assess potency with the in vitro methods and therefore the in vivo test may still be necessary.

However, estimating potency is not necessary if an existing in vivo study does not allow potency estimation and the study has been performed according to internationally-adopted test methods and good laboratory practice.

The amended requirements will be implemented in the completeness check of IUCLID and REACH-IT in the autumn.

ECHA's draft guidance takes the amended information requirements into account and gives advice to registrants. Some minor changes might still occur in the final consultation process. The final guidance will be published in the autumn after the Annex amendment has been published in the Official Journal.
## Elements of IATA for skin sensitisation

<table>
<thead>
<tr>
<th>IATA Elements</th>
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<tbody>
<tr>
<td>Exposure consideration</td>
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<tr>
<td>Chemical descriptors</td>
</tr>
</tbody>
</table>
| Dermal bioavailability | Skin penetration  
                         | Skin metabolism |
| **AOP Key event 1**: covalent interaction with cellular proteins |
| **AOP Key event 2**: events in keratinocytes | Activation of biochemical pathways  
                                                  | Pathways-associated gene expression  
                                                  | Release of pro-inflammatory mediators |
| **AOP key event 3**: events in dendritic cells | Expression of co-stimulatory and adhesion molecules  
                                                   | Pathways-associated gene expression  
                                                   | Pathways-associated protein expression |
| **AOP key event 4**: Events in lymphocytes |
| Adverse Outcome        |
| Others                 |
## IATA GD - reported case studies

<table>
<thead>
<tr>
<th>Case Study</th>
<th>Bioavailability</th>
<th>Physico-chemical properties</th>
<th>In silico</th>
<th>Protein binding/reactivity</th>
<th>Events in Keratinocytes</th>
<th>Events in DC</th>
<th>Events in T cells</th>
<th>Adverse effect</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Sensitiser potency prediction Key event 1+2 (Givaudan)</td>
<td>X</td>
<td>X</td>
<td>TIMES SS</td>
<td>Cor1C420-assay</td>
<td>TG 442D</td>
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<tr>
<td>2 The artificial neural network model for predicting LLNA EC3 (Shiseido)</td>
<td>X</td>
<td></td>
<td>SH Test</td>
<td>AREc32 assay</td>
<td>h-CLAT</td>
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<tr>
<td>3 ITS/DS for hazard and potency identification of skin sensitisers (P&amp;G)</td>
<td>X</td>
<td></td>
<td>TIMES SS</td>
<td>TG 442C</td>
<td>h-CLAT U937 test TG 429</td>
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<tr>
<td>4 Tiered system for predicting sensitising potential and potency of a</td>
<td>X</td>
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<td>DEREK Nexus</td>
<td>TG 442C</td>
<td>h-CLAT</td>
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<td>substance (STS) – (Kao Corporation)</td>
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<td>5 Score-based battery system for predicting sensitising potential and</td>
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<td>potency of a substance (ITS)- (Kao Corporation)</td>
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<td>6 IATA for skin sensitisation risk assessment (Unilever)</td>
<td>X</td>
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<td>X</td>
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<td>7 Weight of evidence in vitro ITS for skin hazard identification (BASF)</td>
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<td>TG 442C</td>
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<td>8 STS for hazard identification of skin sensitisers (RIVM)</td>
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<td>.Varia</td>
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<tr>
<td>9 IATA-(Dupont)</td>
<td>X</td>
<td></td>
<td>Various</td>
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<tr>
<td>10 Decision strategy (L‘Oréal)</td>
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<td>Various</td>
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<tr>
<td>11 Integrated decision strategy for skin sensitisation hazard (ICCVAM)</td>
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<td>TIMES SS</td>
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<td>12 Consensus model for hazard identification (EC-JRC)</td>
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<td>TIMES SS</td>
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</tbody>
</table>
Bayesian Network Integrated Testing Strategy (BN-ITS3)

- **Graphical Model**
  - Integrate Data
  - Decision Tool

- **Target Variable**
  - Potency class
    - Non
    - Weak
    - Moderate
    - Strong
  - derived from the LLNA

Data set n=207: training set n=147; test set n=60

Jaworska et al. J. Archives of Toxicology. 2015, 89(12): 2355–2383
**BN-ITS3 Data Inputs**

<table>
<thead>
<tr>
<th>Input Type</th>
<th>Endpoint</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability</td>
<td>$W_s$ – Water solubility at pH = 7&lt;br&gt;Log D – Distribution coefficient at pH = 7&lt;br&gt;PB – Plasma protein binding fraction&lt;br&gt;Fraction ionized at pH = 7</td>
<td>$M$&lt;br&gt;[-]&lt;br&gt;[-]&lt;br&gt;[-]</td>
</tr>
<tr>
<td>In silico prediction of potency in vivo: TIMES</td>
<td>1. Mechanistic alert for direct reactivity (including direct Michael acceptor) and auto-oxidation&lt;br&gt;2. Prediction of 3 classes (non-sensitizer, weak, moderate/strong) based on the most potent among parent and metabolites.</td>
<td>Classes (NS, W, S)</td>
</tr>
<tr>
<td>Key Event 1: DPRACys, DPRALys</td>
<td>% of the cysteine (Cys) and lysine (Lys) peptide remaining in the DPRA</td>
<td>% peptide remaining</td>
</tr>
<tr>
<td>Key Event 2: KeratinoSens™ KEC1.5, KEC3, IC50</td>
<td>Concentration yielding 1.5-fold (KEC1.5); 3-fold (KEC3) induction of Nrf2-dependent luciferase activity; concentration resulting in 50% reduction of cell viability</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>Key Event 3: h-CLAT EC150, EC200, CV75</td>
<td>Concentrations yielding 150% induction of CD86 cell surface marker; 200% induction in CD54; 75% cell viability</td>
<td>$\mu$M</td>
</tr>
</tbody>
</table>
BN-ITS3 Processing and Predictions

- A post prediction processing step accounts for Michael Acceptor electrophiles because these are known to have anti-inflammatory activity.
- Conversion of probability distribution to Bayes Factors for final interpretation and decision.
  - remove prediction bias introduced by the training set class distribution
  - express prediction uncertainty, which allows transparent and consistent criteria for accepting the prediction
  - BF can be thought of a ‘likelihood ratio’

| BF > 3 | substantial evidence |
| BF 1 to 3 | minimal evidence |
| BF > 3 is required to conclude Non-Sensitizer |
Integrated Approaches to Testing and Assessment

Most traditional toxicity testing methods involve treating laboratory animals with a test substance and observing adverse effects. This approach is expensive and time-consuming, and the use of animals raises ethical concerns and issues of interspecies differences. While it usually takes several methods in combination to adequately account for the multiple mechanisms associated with toxicity, using cell-based, biochemical, and/or computational methods to predict chemical toxicity could overcome some of the drawbacks of traditional testing.

Integrated approaches to testing and assessment (IATAs) provide a means for combining the data from different methods. IATAs:

- Consider all available relevant information about a chemical in a weight-of-evidence assessment
- Inform regulatory hazard or risk decisions or the need for additional tests
- Rely on non-animal approaches to determine chemical hazard or risk

An integrated testing strategy is a limited type of IATA that that relies on:

- Input data generated from identified methods
- A computational model or other evaluation protocol through which the input data is run

Input data may be derived from in vitro test methods or from computational approaches such as "read-across," in which toxicity data from a known chemical is used to predict toxicity for another, similar chemical. The input data is run through the computational model or other evaluation protocol to generate a hazard prediction.

Please note that the terms "integrated testing strategy" and "integrated approach to testing and assessment" represent evolving concepts. NICEATM has adapted these definitions from a preliminary draft guidance document being prepared by the Organisation for Economic Co-operation and Development.

Integrated Testing Strategies to Identify Potential Skin Sensitizers

Integrated testing strategies have been created to identify potential skin sensitizers (substances with the potential to cause allergic contact dermatitis).

- NICEATM and other NTP scientists collaborated with Procter & Gamble scientists to develop an open-source version of a previously published proprietary integrated testing strategy.
- NICEATM and ICCVAM scientists developed an integrated testing strategy that uses data from three non-animal tests, read-across predictions of skin sensitization hazard, and physical properties such as partition coefficient to predict skin sensitization hazard.

Integrated Testing Strategy to Identify Potential Endocrine Disruptors

NICEATM and scientists with the U.S. Environmental Protection Agency (EPA) developed an integrated testing strategy that combines data from 13 high-throughput screening assays with a mathematical model to identify chemicals with the potential to interact with the estrogen receptor. Use of this integrated testing strategy has been accepted by the EPA as an alternative to three assays currently used in its Endocrine Disruptor Screening Program 2nd Tier I battery.

Integrated Testing Strategy Developed by ICCVAM

The evaluation and promotion of alternative approaches to replace, reduce, or refine animal use for potential skin sensitizer identification is an ICCVAM priority. ICCVAM scientists, in collaboration with NICEATM, developed integrated testing strategies that use non-animal data to predict skin sensitization hazard. These integrated testing strategies combine inputs from several sources (data from the direct peptide reactivity assay, the KeratoSens assay, and the human cell line activation test; a read across prediction of skin sensitization hazard generated by the QSAR Toolbox software package; and physical property data such as partition coefficients) to run through their model.

ICCVAM developed three versions of the skin sensitization integrated testing strategy:

- The first version uses computer algorithms to integrate data to predict murine local lymph node assay outcomes.
- The second version uses data from human exposures to predict human skin sensitization hazard (manuscript submitted).
- Further development of this approach aims to predict human or animal skin sensitization potency, enabling the classification of skin sensitizers as "weak" or "strong" without animal tests (manuscript in preparation).

NICEATM Collaboration With P&G to Develop an Open-source Integrated Testing Strategy

NICEATM and other NTP scientists collaborated with scientists at Procter & Gamble (P&G) to develop an integrated testing strategy to identify potential skin sensitizers without conducting animal tests. Using data from non-animal tests and other information such as solubility and computational predictions of skin sensitizer activity, the strategy produces a numerical probability that a chemical should be placed in a particular skin sensitization hazard class: strong, moderate, weak, or nonsensitizer. This probability could potentially be used to determine if a substance requires hazard labeling without conducting animal tests.

P&G and NTP scientists collaborated to develop this integrated testing strategy using free, publicly available software. The goal is to encourage organizations worldwide to use this approach for identifying potential skin sensitizers and support the elimination of animal testing in this area.

Reference: Prone et al. 2014. Open source software implementation of an integrated testing strategy for skin sensitization potency based on a Bayesian network. ALTEx 31:336-349.

P&G updated the integrated testing strategy in 2015. The updated strategy uses only validated non-animal tests, simplifies the bioavailability inputs, and nearly doubles the size of the database used to derive the previous network.

Reference: Jaworska et al. 2015. Bayesian integrated testing strategy (ITS) for skin sensitization potency assessment: A decision support system for quantitative weight of evidence and adaptive testing strategy. Arch Toxicol 89: 2355-2363.

Files for Running the Open-source Integrated Testing Strategy Analysis

Files to run the analysis described in Prone et al., including a script that uses the R programming language, are available below. To encourage collaboration and sharing of best practices, NICEATM has established a user community for the integrated test strategy via an NIH listserv. We strongly encourage all users of the R script to join the listserv.

- Prone et al.: Reproducing the ITS-2 Model Using R (updated March 31, 2014: read this document first)
  - Supplemental Information on Changes from ITS-2 to ITS-2 Lipid and Moving Toward Open-Source (updated March 31, 2014: save to your hard drive). Folder contains:
    - R code necessary for conducting the analysis (ITS2_R_version.R)
    - Document compiled by Sweave to produce the LaTeX file (ITS2_R_version.Rnw)
    - Sweave style file needed by LaTeX (Sweave sty)
    - File produced by running Sweave on "ITS2_R_version.Rnw" (ITS2_R_version.tex)
    - Data files (tab-delimited text format)
      - Training data (ITS2_Lipid_Train_102313.txt)
      - Test data (ITS2_Lipid_Test_102313.txt)
    - References in BibTeX format (ITS2_References.txt)

Additional Resources

A bioavailability calculator is available from the National Institute for Occupational Safety and Health (NIOSH) website. Please note that Java must be enabled in your web browser in order to run the calculator.

The software for running R can be obtained from the R Project website. Refer to the FAQs on the R Project website for system requirements and installation instructions.

The OECD QSAR Toolbox can be obtained from the OECD website. Installation instructions and user documentation are available on this page.

NICEATM Murine Local Lymph Node Assay (LLNA) Database

On behalf of ICCVAM, NICEATM conducted a number of analyses to evaluate the usefulness of the LLNA to identify potential skin sensitizers. Data from these analyses are available to interested stakeholders as a reference for developing and evaluating alternative approaches to testing and assessment that replace, reduce, or refine the use of animals for identification of potential skin sensitizers.
Skin Sensitization Quantitative Risk Assessment (QRA)

1. Threshold Determination
   - NESIL
   - Potency

2. Safety Assessment Factor Determination (SAF)

3. Calculate the Acceptable Exposure Level (AEL)

4. Determine the Consumer Exposure Level (CEL)

5. Risk Characterization
   - AEL ≥ CEL

6. Post-Market Surveillance Program
Thank you!