Implementation: Alternatives for Skin Sensitization Testing

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Consumer Product Safety Commission
Co-Chair, ICCVAM Skin Sensitization Workgroup

SACATM Meeting
September 18-19, 2017
Skin Sensitization Implementation Plan:

- Coordinate activities via the ICCVAM Skin Sensitization Workgroup (SSWG)
- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for skin sensitization data
- Coordinate efforts with stakeholders
- Identify, acquire, and curate high quality data from reference test methods
- Identify and evaluate non-animal alternative approaches to skin sensitization testing
- Gain regulatory acceptance and facilitate use of non-animal approaches
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Current ICCVAM SSWG Roster

- Moiz Mumtaz (ATSDR)
- Patricia Ruiz (ATSDR)
- John Gordon (CPSC)
- Joanna Matheson (CPSC)
- Emily N. Reinke (DOD)
- Evisabel Craig (EPA)
- David Lehmann (EPA)
- Anna Lowit (EPA)
- Timothy McMahon (EPA)
- Mamta Naidu (EPA)
- Todd Stedeford (EPA)
- Simona Bancos (FDA)
- Paul C. Brown (FDA)
- Rakhi M. Dalal-Panguluri (FDA)
- Wei Ding (FDA)
- Robert Heflich (FDA)
- Abigail C. Jacobs (FDA)
- Diego Rua (FDA)
- Nakissa Sadrieh (FDA)
- Stanislav Vukmanovic (FDA)
- Jeffrey Yourick (FDA)
- Warren Casey (NIEHS)
- Dori Germolec (NIEHS)
- Nicole Kleinstreuer (NIEHS)

ICATM Liaison Members
- Silvia Casati (EURL ECVAM)

NICEATM Support Staff (ILS)
- Michael Paris
- Judy Strickland
- David Allen
**ICCVAM Skin Sensitization Models**

### Research article

**Prediction of skin sensitization potency using machine learning approaches**

Qingda Zang, Michael Paris, David M. Lehmann, Shannon Bell, Nicole Kleinstreuer, and Warren Casey

**Abstract:** The replacement of animal models that use data from such as not using animal data has been classified into potency categories. This model suggests that no single test is a sufficient predictor of skin sensitization potency.

**Research article**

**Multivariate models for prediction of human skin sensitization hazard**

Judy Strickland, Qingda Zang, Michael Paris, David M. Lehmann, David Allen, Neena Choksi, Joanna Matheson, Abigail Jacobs, Warren Casey, and Nicole Kleinstreuer

**Abstract:** One of the challenges of predicting human skin sensitization is the development and evaluation of non-animal approaches to identify hazard. ICCVAM is developing an integrated approach to testing and integrating data from various sources.

**Research article**

**Integrated decision strategies for skin sensitization hazard**

Judy Strickland, Qingda Zang, Nicole Kleinstreuer, Michael Paris, David M. Lehmann, Neena Choksi, Joanna Matheson, Abigail Jacobs, Anna Lowit, David Allen, and Warren Casey
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## U.S. Statutes and Regulations

<table>
<thead>
<tr>
<th>US Statute/Regulations</th>
<th>Agency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Federal Hazardous Substances Act (FHSA) (1964): 16 CFR 1500.3: <strong>Consumer Products</strong></td>
<td>CPSC</td>
</tr>
<tr>
<td>Federal Food, Drug, and Cosmetic Act (1938): <strong>Cosmetics</strong></td>
<td>FDA</td>
</tr>
<tr>
<td>Federal Food, Drug, and Cosmetic Act (1938): <strong>Pharmaceuticals</strong></td>
<td>FDA</td>
</tr>
</tbody>
</table>

*Strickland et al. 2017 in prep*
<table>
<thead>
<tr>
<th>Reference Animal Method</th>
<th>Classification Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticides: Industrial chem</td>
<td>NS</td>
</tr>
<tr>
<td>Household Products</td>
<td>NS</td>
</tr>
<tr>
<td>Dermatological Products</td>
<td></td>
</tr>
</tbody>
</table>

*human data preferred
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International Cooperation on Alternative Test Methods (ICATM)

- First ever ICATM Workshop: “International regulatory applicability and acceptance of alternative non-animal approaches to skin sensitization assessment of chemicals used in a variety of sectors”

- Convened by EURL ECVAM on October 4-5, 2016, in Ispra, Italy
ICATM Workshop Outcomes

- White paper characterizing international regulatory requirements for skin sensitization testing (final draft)

- Position paper authored by ICATM partners covering workshop outcomes and ICATM recommendations (final draft)
  - Including proposed acceptance criteria for defined approaches to testing and assessment of skin sensitization

- OECD SPSF for “development of a performance based test guideline for defined approaches to testing and assessment of skin sensitization” (submitted)
  - Develop framework detailing performance standards and acceptance criteria for the assessment of defined approaches as replacements for the LLNA
  - Apply performance standards and acceptance criteria to OECD case studies

- Annual ICATM workshop (e.g. performance standards, validation approaches, respiratory sensitization, computational approaches...
Skin Sensitization Data Collection: Ongoing Efforts

- Multiple conventional & antimicrobial registrants have kindly provided data to support our skin sensitization efforts.

- We continue to collect additional, voluntary data submissions to expand current datasets:
  - Paired *in vitro* & LLNA data that could increase coverage of various defined approaches.
  - Other LLNA studies to help assess variability.
  - Additional human data to assist in evaluating defined approaches.
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Accurancy Against Human Clinical Data (~150 chems)

**LLNA**

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>72%-82%</td>
<td>54% - 60%</td>
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</table>

**GPMT / Buehler**

<table>
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<tr>
<th>Hazard</th>
<th>Potency</th>
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<tbody>
<tr>
<td>~72%</td>
<td>~60%</td>
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</table>

Reproducibility of Multiple Tests (~100 chems)

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>~78%</td>
<td>~62%</td>
</tr>
</tbody>
</table>

ICCVAM. 1999. NIH Publication No. 99-4494
ICCVAM. 2010. NIH Publication No. 11-7709
Urbisch et al. 2015. Reg Tox Pharm 71:337-351.
Dumont et al. 2016. Tox In Vitro 34: 220-228
Hoffmann et al. 2017 submitted
95 chemicals with multiple LLNA results (541 total tests)
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AOP for Skin Sensitization: Available Methods

For sensitization that is initiated by covalent binding to proteins.

Key Event 1: Covalent interaction with skin proteins
- Metabolism
- Penetration
- Electrophilic substance

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
- Mobilization of DCs

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

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http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmentpublicationsbynumber.htm
Global Skin Sensitization Project

- Collaboration with Cosmetics Europe – Analyze OECD-submitted modeling approaches
  - 128 substance dataset
  - Evaluate performance against mouse and human hazard/potency categories
Non-Animal Approach Evaluation

Most non-animal testing strategies evaluated so far perform better than the LLNA at predicting human skin sensitization hazard and potency.

(And when compared to the LLNA, are equivalent in performance to the LLNA at predicting itself.)
Validation Study: Electrophilic Allergen Screening Assay (EASA)

- To characterize the usefulness and limitations of a non-animal *in chemico* test method (EASA) to classify the allergic contact dermatitis (ACD) hazard of products and chemicals
  - Optimize and standardize the test method protocol
  - Assess intra- and inter-laboratory reproducibility
  - Assess accuracy for the classification of ACD hazard
EASA Validation Study Organization

Validation Management Team

NTP Chemical Acquisition and Distribution

NICEATM

NIOSH

CFSAN

CPSC/NIST
NIST/CPSC collaboration with EASA assay

- Participate as one of the three laboratories (i.e. instrument sharing) in an interlaboratory comparison

- Collaboration provides the opportunity to assess the robustness and reproducibility of the assay and potential protocol modifications to provide evidence for measurement assurance

- Evaluate usage of this assay with challenging test compounds such as nanomaterials
Some key preliminary findings

- Hazard assessment of laboratory and protocol is critical due to skin sensitization reagents! Resulted in approximately 10 pages Hazard Review.

- NBT assay is light sensitive and steps need to be taken to minimize decrease in signal of negative control

- There are substantial variations among suppliers for the NBT reagent with regards to reproducibility of negative control readings

- Discussions about dosing concentrations to use for positive controls in the assay

- Key impact of cuvette design with some cuvettes potentially leading to cross-contamination among samples or risk of exposure to skin sensitizers on gloves
NIST/CPSC laboratory setup to minimize hazard and improve measurement precision within laboratory
Future efforts

- Calibration standard for absorbance and fluorescence instruments between laboratories
- Identification of intermediate process measurements to provide troubleshooting and in-line controls for measurement assurance
- Translation of assay to 96-well plate format with a quartz microplate
- Evaluation and protocol modifications for use with challenging substances (i.e. nanomaterials)
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International Harmonization

• OECD proposal (SPSF) submitted November 2016
  – Co-led by U.S., EU, and Canada
  – Create an international performance based test guideline for non-animal defined approaches to skin sensitization testing
  – Achieve widespread replacement of mouse test

• Comments from OECD member countries received January 2017, revised SPSF submitted March 2017

• National coordinators from 35 member countries voted unanimously to approve the project on April 27, 2017
Scientific and Non-scientific Challenges

- Animal methods currently provide the reference data for evaluating alternatives
  - Results are variable
  - Many testing strategies outperform the LLNA in predicting human outcomes

- Data requirements vary across U.S. and global regulatory authorities, and are often ambiguous/subjective

- Coverage of chemical space

- Limited commercial availability of alternatives

- Overcoming regulatory and institutional inertia
  - Education and training
Expanding Chemical Space Coverage

- Prospective *in vitro* testing supported by NTP (D. Germolec)
- Chemicals with existing LLNA data nominated by ICCVAM agencies
  - NTP, EPA (OPP, OPPT, ORD), CPSC, FDA
  - Pesticides, formulations, excipients, industrial chemicals, etc.
- NTP Contractor (BRT) running:
  - LuSens (me-too method under OECD TG442D)
  - DPRA (OECD TG442C)
  - h-CLAT (OECD TG442E)
- Screening of 47 chemicals underway
- Procurement of ~150 additional test chemicals is ongoing
- Results will expand defined approach evaluations