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
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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
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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>
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*Strickland et al. 2017 in prep*
# U.S. Agency Requirements/Considerations

<table>
<thead>
<tr>
<th>Reference Animal Method</th>
<th>Classification Criteria</th>
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</thead>
<tbody>
<tr>
<td><strong>Pesticides</strong></td>
<td><strong>NS</strong></td>
</tr>
<tr>
<td>Industrial chem</td>
<td>LLNA</td>
</tr>
<tr>
<td><strong>Household Products</strong></td>
<td><strong>NS</strong></td>
</tr>
<tr>
<td>LLNA</td>
<td></td>
</tr>
<tr>
<td><strong>Dermatological Products</strong></td>
<td><strong>Potency</strong>*</td>
</tr>
<tr>
<td>LLNA</td>
<td></td>
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*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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Accuracy Against Human Clinical Data (~150 chems)

**LLNA**

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

**GPMT / Buehler**

<table>
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<th>Hazard</th>
<th>Potency</th>
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<tr>
<td>~72%</td>
<td>~60%</td>
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Reproducibility of Multiple Tests (~100 chems)

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

ICCVM. 1999. NIH Publication No. 99-4494
ICCVM. 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
- Electrophilic substance
- Metabolism

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

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

LLNA TG429, 442A/B

DPRA TG 442C

http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmentpublicationsbnumber.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

![Diagram of modeling approaches]

- Meta models
  - Regression equations
  - Ordinary differential equation
  - Bayesian Networks
  - Artificial Neural Networks
  - Support vector machine
  - Consensus model
  - 2 out of 3 WoE
  - Sequential testing strategy with defined decision criteria after each step

**Prediction**
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

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