Skin Sensitization Implementation Plan

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Implementation Plan Outline

- Coordinate activities via ICCVAM Workgroups
- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts
- Coordinate efforts with stakeholders
- Identify, acquire, and curate high quality data from reference test methods
- Identify and evaluate non-animal alternative approaches
- Gain regulatory acceptance and facilitate use of non-animal approaches
Skin Sensitization Implementation Plan:

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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
Prediction of skin sensitization potency using machine learning approaches

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

ABSTRACT: The replacement of animal tests using data from such tests using animal data have been classified into potency categories. A machine learning approach, LNA, was used to classify these categories. The results showed that the approach was effective in predicting skin sensitization potency. Further research is needed to improve the accuracy of predictions.

Multivariate models for prediction of human skin sensitization hazard

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

ABSTRACT: One of the priorities of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) is the identification and evaluation of non-animal alternatives for skin sensitization testing. Although skin sensitization is a complex process, the key biological events of the process have been well characterized in an adverse outcome pathway (AOP). Accordingly, ICCVAM is working to develop integrated decision strategies for skin sensitization hazard.

Integrated decision strategies for skin sensitization hazard

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

ABSTRACT: One of the top priorities of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) is the identification and evaluation of non-animal alternatives for skin sensitization testing. Although skin sensitization is a complex process, the key biological events of the process have been well characterized in an adverse outcome pathway (AOP). Accordingly, ICCVAM is working to develop integrated decision strategies for skin sensitization hazard.
Human Data Project

• Analyze existing human data and primary study references to understand uncertainty and sources of variability
  – Current database: 420 chemicals, 919 records

• Develop/apply transparent, reproducible system for human skin sensitization potency categorization

• Work with industry consortia to encourage data sharing of human skin sensitization data
  – Semi-automated extraction of data from Cosmetics Ingredient Review Reports
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U.S. Agency Requirements/Needs

<table>
<thead>
<tr>
<th>Accepted Animal Method</th>
<th>Evaluation Needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrial chemicals</td>
<td>Not required</td>
</tr>
<tr>
<td>Pesticides</td>
<td>LLNA</td>
</tr>
<tr>
<td>Workplace chemicals</td>
<td>LLNA, GPMT, Buehler</td>
</tr>
</tbody>
</table>

Hazard, risk
- NS  S

Hazard
- NS  1B  1A

Potency
- NS  1B  1A

Non-animal alternatives considered on a case-by-case basis
U.S. Agency Requirements/Needs

- **FDA**
  - Dermatologic products: Unspecified
  - Medical devices: GPMT
  - Household products: LLNA, GPMT, Buehler

**Accepted Animal Method**

- **Evaluation Needs**
  - Potency*: NS S
  - Hazard: NS S SS
  - Potency: NS S SS

Non-animal alternatives considered on a case-by-case basis, except for medical devices.
International regulatory requirements for skin sensitization testing


• US regulatory requirements paper (Strickland et al.) in final agency clearance
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Expanding Substance Space Coverage

- NTP (Tox Branch/D. Germolec) is testing additional substances in three alternative test methods:
  - DPRA, KeratinoSens, hCLAT

- Expanded substance space includes:
  - pesticide/agrochemical formulations, dermal excipients, personal care product products, “challenge” chemicals

- Compiled nominations from multiple ICCVAM agencies/partners
  - EPA: Office of Pesticides, Office of Pollution Prevention and Toxics, Office of Research and Development
  - Consumer Product Safety Commission
  - Food and Drug Administration
  - National Toxicology Program
  - ICATM partners
Expanding Substance Space Coverage

- Total of 266 substances nominated
- NTP has procured 135 substances for initial testing phase (mostly nominations from the EPA)
- Testing began in late 2017
- Additional testing (~100 substances) to follow in mid-2018
- Coordinating with Dow Agro to test formulations already assessed in DPRA and KeratinoSens™ in the hCLAT assay
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Skin Sensitization Data Collection

• 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

• NICEATM sent letters of request to industry consortia; data will be published in ICE
### Accuracy Against Human Clinical Data (~150 chems)

<table>
<thead>
<tr>
<th>Method</th>
<th>Hazard</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLNA</td>
<td>72%-82%</td>
<td>54% - 60%</td>
</tr>
<tr>
<td>GPMT / Buehler</td>
<td>~72%</td>
<td>~60%</td>
</tr>
</tbody>
</table>

### Reproducibility of Multiple Tests (~100 chems)

<table>
<thead>
<tr>
<th>Method</th>
<th>Hazard</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>~78%</td>
<td>~62%</td>
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Test Methods Mapped to AOP

- **Chemical Structure & Properties**
  - Metabolism Penetration
  - Electrophilic substance

- **Molecular Initiating Event**
  - TG442D
  - Covalent interaction with skin proteins

- **Cellular Response**
  - Key Event 1: Dendritic Cells (DCs)
    - Induction of inflammatory cytokines and surface molecules
    - Mobilisation of DCs
  - Key Event 2: Keratinocytes responses
    - Activation of inflammatory cytokines
    - Induction of cytoprotective genes

- **Organ Response**
  - Key Event 3
    - Histocompatibility complexes presentation by DCs
    - Activation of T cells
    - Proliferation of activated T-cells
  - TG442E

- **Organism Response**
  - Adverse Outcome
    - Inflammation upon challenge with allergen

- **In Vitro**
  - KeratinoSens
  - LuSens
  - TG442C

- **In Vivo**
  - GPMT
  - LLNA
  - DPRA
  - TG442E
  - hCLAT, USENS, IL-8
Global Skin Sensitization Project

- Objective: analysis of available non-animal defined approaches (DAs)
- Collaboration with Cosmetics Europe
  - Curation/generation of
    - \textit{in vivo} LLNA and human data
    - \textit{in vitro} cell-based data that maps to AOP
    - \textit{in silico} computer predictions, chemical structural features & properties
- Qualitative and quantitative evaluation of OECD-submitted DAs
- Fully transparent approach (i.e., build open-source code packages)
- Evaluate performance against LLNA and human hazard/potency categories

Hoffmann et al. 2018 Crit Rev Tox
Kleinstreuer et al. 2018 Crit Rev Tox
Compilation of a Reference Database: Substance Selection

- LLNA data available

128 substances

Hoffmann et al. 2018 Crit Rev Tox
CE Chemical Use Space Coverage

U.S. EPA ACToR UseDB Categories

- Chemical warfare
- Petrochemical
- Antimicrobial
- Colorant
- Pesticide
- Fragrance
- Pharmaceutical
- Food additive
- Inert Ingredient
- Consumer use
- Personal care
- Industrial

Number of Chemicals

Number of Use Categories

Average of 4.3 use cases per substance
Types of Defined Approaches

- Meta models
- Regression Model
- Integrated Testing Strategy
- Bayesian Networks
- Artificial Neural Networks
- Support vector machine
- 2 out of 3 Consensus
- Sequential Testing Strategy

Prediction

Interagency Coordinating Committee on the Validation of Alternative Methods

- Advancing Alternatives to Animal Testing
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.)

Hoffmann et al. 2018 Crit Rev Tox
Kleinstreuer et al. 2018 Crit Rev Tox
Validation Study: Electrophilic Allergen Screening Assay (EASA)
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EPA Draft Science Policy

- EPA/OPP and OPPT now accept two non-animal defined approaches as alternatives to the LLNA

- Covers pesticide actives ingredients, inerts, and mono-constituent industrial chemicals regulated under TSCA
Defined Approaches: KE-based Hazard Prediction

AOP WoE: 2 out of 3 KE

- No differential weighting of individual test methods, or defined sequential order of testing
- Usually KE1 (e.g. DPRA) and KE2 (e.g. KeratinoSens) performed first since less expensive
- Third test is KE3 (e.g. hCLAT, U-SENS)
Defined Approaches: KE-based Hazard Prediction

**KE 1 & 3 STS**

- Prediction can be derived after first tier
- Depends on KE 3 (e.g. h-CLAT) and KE 1 (e.g. DPRA)
Cross-Partner Project Concept:

Development of High Throughput Screening Assays to Detect Chemicals that may Induce Skin Sensitization, and Skin or Eye irritation

- HTS versions of DPRA (OECD 442C), KeratinoSens (OECD 442D)
- HTS assays for irritation (~OECD TG491) using human primary keratinocytes and corneal epithelial cells
Acknowledgments

- Sebastian Hoffmann & Cosmetics Europe STTF
- Dori Germolec & NTP colleagues
- ICCVAM SSWG
- ILS/NICEATM
- US EPA/OPP & OPPT
- EURL ECVAM/JRC
- Health Canada
- ICATM partners