NTP Approaches to Assessment of Dermal Hypersensitivity

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Board of Scientific Counselors Meeting
December 7-8, 2017
Skin Sensitization

“Allergic Contact Dermatitis”

Accounts for 10-15% of all occupational disease (Anderson et al. 2010)

Major testing requirement for cosmetics, pesticides, industrial chemicals, etc.
**In Vivo Tests for Assessment of Dermal Sensitization**

**Guinea Pig Maximization Test**
- Intradermal and topical sensitization
- Topical challenge
- Measure erythema response 24 - 48 hours post challenge

**Buehler**
- Topical sensitization with closed patch
- Topical challenge distal to sensitization with closed patch
- Measure erythema response following removal of patch

**Local Lymph Node Assay**
- Topical treatment on dorsal surface of the ear
- Inject with radiolabel or fluorochrome
- Measure cell proliferation in the lymph nodes associated with the site of application
Daniel et al. 2017 in preparation

*preference
### Accuracy of Animal Tests Against Human Data

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

ICCVAM. 2010. NIH Publication No. 11-7709
Urbisch et al. 2015. Reg Tox Pharm 71:337-351.
Hoffmann et al. 2017 in preparation
Reproducibility of LLNA Data

Analysis of the Local Lymph Node Assay (LLNA) variability for assessing the prediction of skin sensitisation potential and potency of chemicals with non-animal approaches

Coralie Dumont, João Barroso, Izabela Matys, Andrew Worth, Silvia Casati *

Joint Research Centre, European Commission, Ispra, Italy

How concordant are multiple LLNA outcomes for a single chemical?

- ~78% for hazard
- ~62% for potency classification
LLNA Variability

[From: S. Hoffmann, Altex 32(4), 2015]
Comparison of LLNA and Human Data

Accuracy:

- **75%** for Hazard (NS/S)
- **60%** for Potency 3-class (NS, Weak/ Moderate, Strong/ Extreme)
- **47%** for Potency 5-class (NS, Weak, Moderate, Strong, Extreme)

Provides a benchmark for comparison with new approaches

Basketter et al. 2014
Key Events in the Skin Sensitization Process

1. Skin penetration, Electrophilic, Low mw
2. KERATINOCYTES
   - IL-1β, IL-5, IL-12, IL-18
   - IL-1β, TNF-α, GM-CSF
3. LANGERHANS CELL (LC)
4. MIGRATION TO LOCAL LYMPH NODE
   - ICAM-1
5. T-CELL
   - LYMPHOCYTE PROLIFERATION

*Illustration by D. Saiistad*
OECD AOP for Skin Sensitization

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
  - Histo compatibility complexes presentation by DCs
  - Activation of T cells
  - Proliferation of activated T-cells

Adverse Outcome
- Inflammation upon challenge with allergen
OECD AOP for Skin Sensitization

Key Event 1
- DPRA
- Covalent interaction with skin proteins
- Metabolism, Penetration
- Electrophilic substance
- In vitro skin penetration
- In silico toxicokinetic models

Key Event 2
- Keratinocytes responses
- Activation of inflammatory cytokines
- Induction of cytoprotective genes
- KeratinoSens

Key Event 3
- Dendritic Cells (DCs)
- Induction of inflammatory cytokines and surface molecules
- Mobilization of DCs
- h-CLAT

Key Event 4
- T-cell proliferation
- Histiocompatibility complexes presentation by DCs
- Activation of T cells
- Proliferation of activated T-cells
- LLNA

Adverse Outcome
- Inflammation upon challenge with allergen
Global Skin Sensitization Project

• Objective: analysis of available non-animal approaches
  – OECD submitted case studies

• Collaboration with Cosmetics Europe
  – 128 substance dataset
  – LLNA and human data
  – Curation/generation of *in vitro* data
    • DPRA, KeratinoSens, hCLAT, U-SENS
    • PPRA, SENS-IS (underway)

• Analyze five OECD-submitted defined approaches (i.e., code packages); open source and transparent (R, Python)

• Evaluate performance against the LLNA and human hazard/potency categories
In Vitro Models for Assessing Dermal Sensitization

- **In vitro assays**
  - **Direct peptide reactivity assay**
    - Assesses the ability of a substance to form a hapten-protein complex
  - **KeratinoSens**
    - Assesses the ability of a substance to activate cytokines and induce cytoprotective genes in keratinocytes
  - **h-CLAT**
    - Assesses the ability of a substance to activate and mobilize dendritic cells in the skin
- Assesses protein reactivity of a test substance
- Uses two heptapeptides
  - One with cysteine (Cys) and one with lysine (Lys) as the reactive center
  - Incubate with test substance and measure disappearance of peptides with HPLC
  - Average depletion (Ave. Lys. Cys) > 6.38% = sensitizer
- OECD Test Guideline 442C (2015)
• Assesses the activation of the AKR1C2-ARE element, an indication of keratinocyte activation, in KeratinoSens cells (derived from HaCaT keratinocytes)
  
  - Caused by electrophilic agents, which tend to be skin sensitizers
  
  - Measures fold-induction of luciferase activity; induction >1.5-fold in 2/3 experiments = sensitizer
  

**Measurements**

Fold induction

EC1.5

% viability

• Measures 2 cell surface markers, CD86 and CD54, on dendritic cell surrogates (THP-1 cells)
  
  - Assesses the maturation process of dendritic cells as they transform from antigen processing cells to antigen presenting cells
  
  - CD86 relative fluorescence intensity (RFI) ≥ 150% and/or CD54 RFI ≥ 200% at any dose, in at least 2/3 experiments, then substance is a sensitizer
  
  - OECD Test Guideline 442E (2016)

[Diagram showing THP-1 cells, chemical exposure, 24h, measurements, FcR blocking, Flow cytometric analysis, Cell staining (CD86 & CD54), Graphic from Dr. Sakaguchi, Kao Corp.]
<table>
<thead>
<tr>
<th>Assay</th>
<th>hCLAT vs LLNA</th>
<th>DPRA vs LLNA</th>
<th>Keratino vs LLNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity %</td>
<td>84.0</td>
<td>66.0</td>
<td>67.4</td>
</tr>
<tr>
<td>Specificity %</td>
<td>48.5</td>
<td>66.7</td>
<td>66.7</td>
</tr>
<tr>
<td>Accuracy %</td>
<td>74.8</td>
<td>66.1</td>
<td>67.2</td>
</tr>
</tbody>
</table>

n=127          n=127          n=128

Hoffman et al 2017, in preparation
## Individual Assays Compared to Human

<table>
<thead>
<tr>
<th></th>
<th>hCLAT vs Human</th>
<th>DPRA vs Human</th>
<th>Keratino vs Human</th>
</tr>
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<tbody>
<tr>
<td><strong>NEG</strong></td>
<td>20</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td><strong>POS</strong></td>
<td>19</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td><strong>Sensitivity %</strong>:</td>
<td>87.5</td>
<td>71.6</td>
<td>75.3</td>
</tr>
<tr>
<td><strong>Specificity %</strong>:</td>
<td>51.3</td>
<td>74.4</td>
<td>79.5</td>
</tr>
<tr>
<td><strong>Accuracy %</strong>:</td>
<td>76.4</td>
<td>72.4</td>
<td>76.6</td>
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≥ LLNA

n=127           n=127          n=128

Hoffman et al 2017, in preparation
Defined Approach Evaluation

- Most non-animal testing strategies evaluated so far perform better than the LLNA at predicting human skin sensitization hazard and potency.
- Combining multiple in vitro assays and in silico methods or physico chemical properties increases the ability to predict sensitizers.
Combining in vitro assays and other approaches increases the ability to predict sensitzers

<table>
<thead>
<tr>
<th>No.</th>
<th>Model (Accuracy)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>DPRA + KeratinoSens + h-CLAT + Toolbox + Lys + Cys + Avg.Lys.Cys + 6 properties (95%)</td>
<td>89</td>
<td>91</td>
<td>89</td>
</tr>
<tr>
<td>5</td>
<td>KeratinoSens + h-CLAT + Toolbox + Avg.Lys.Cys + 6 properties (95%)</td>
<td>92</td>
<td>79</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>h-CLAT + Toolbox + 6 properties (97%)</td>
<td>85 (84)</td>
<td>94 (48)</td>
<td>88 (75)</td>
</tr>
<tr>
<td>8</td>
<td>KeratinoSens + Toolbox + Avg.Lys.Cys + 6 properties (94%)</td>
<td>84 (87)</td>
<td>91 (67)</td>
<td>86 (67)</td>
</tr>
<tr>
<td>9</td>
<td>KeratinoSens + h-CLAT + Avg.Lys.Cys + 6 properties (92%)</td>
<td>89</td>
<td>73</td>
<td>84</td>
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<td>10</td>
<td>h-CLAT + Toolbox + Avg.Lys.Cys + 6 properties (92%)</td>
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<td>89</td>
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<td>11</td>
<td>KeratinoSens + h-CLAT + Toolbox + 6 properties (92%)</td>
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<td>79</td>
<td>86</td>
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Avg.Lys.Cys, average depletion for lysine and cysteine; Cys, average % cysteine; DPRA, direct peptide reactivity assay; h-CLAT, human cell line activation test; LOOCV, leave-one-out cross-validation; Lys, average % lysine depletion; Toolbox, read-across using QSAR Toolbox; SVM, support vector machine.

*Average accuracy of the training and test sets for predicting the reference LLNA outcomes.

(Individual assay compared to the LLNA)

Expanding Coverage of Chemical Space

- Most chemicals used in the validation of non-animal test methods have been cosmetics ingredients

- NTP is supporting testing of other types of chemicals in three alternative test methods: DPRA, LuSens, hCLAT
  - Expanded chemical space includes: pesticides, agrochemical formulations, dermal excipients, personal care product ingredients, “challenge” chemicals

- Have compiled chemical nominations from multiple ICCVAM agencies
  - EPA: Office of Pesticides, Office of Pollution Prevention and Toxics, Office of Research and Development
  - Consumer Product Safety Commission
  - Food and Drug Administration
  - NTP
Expanding Coverage of Chemical Space

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

- Combine with \textit{in silico} data and physico chemical properties when available
- Evaluate the dataset using methods previously developed by NICEATM (Strickland et al 2016)
- Evaluate predictive performance of non-animal defined approaches submitted to OECD (Kleinstreuer et al. 2018) in comparison to LLNA data
- Characterize applicability domain of in vitro test methods and non-animal defined approaches
- Work with ICCVAM agencies to adopt non-animal defined approaches where appropriate
Acknowledgments

- Nicole Kleinstreuer, Warren Casey (NICEATM)
- Victor Johnson, Michelle Miller (Burleson Research Technologies)
- Bradley Collins (NTP Program Operations)
- David Allen, Judy Strickland, Dan Zang, Mike Paris, Eileen Phillips (ILS)
- Evisabel Craig, Anna Lowit (EPA/OPP)
- Joanna Matheson (CPSC)