An Example of Cross-Sector Dialogue at a Global Scale: The Case of Skin Sensitization

An *in vitro* Test Strategy for Assessment of the Skin Sensitization Hazard

S.N. Kolle¹, C. Bauch¹, T. Ramirez¹, T. Eltze¹, E. Fabian¹, A. Mehling², W. Teubner³, B. van Ravenzwaay¹, R. Landsiedel¹

¹BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany
²BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany
³BASF Schweiz AG, Basel, Switzerland
Skin Sensitization

Skin sensitization is a complex process leading to contact allergies; it is estimated that 15–20% of the population suffers from contact allergies.

Skin sensitization must be assessed for all substances registered under REACH.

The 7th Amendment of the EU Cosmetics Directive (now Cosmetics Regulation) foresees the phasing out of animal tests for safety assessments, including skin sensitization.
Identification of Skin Sensitizers

- Identification and evaluation of potentially sensitizing chemicals relies on animal models
- In rare cases, confirmatory human repeated insult patch tests are conducted

Non-human animal tests
- Bühler and guinea pig maximization test (OECD TG 406)
- Local lymph node assay (OECD TG 429)

Human test
- Human patch test

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Adverse Outcome Pathway (AOP)

Chemical Properties

Molecular initiating events

Cellular Response

1st Exposure
- dermal penetration
- interactions with proteins
- interaction with KC
- interactions with DC
- DC activation
- DC migration
- T-cell interaction
- T-cell proliferation
- T-cell migration

2nd Exposure
- inflammation
- T-cell migration
- T-cell interaction

SKIN

LYMPH NODE

Organ Response

Organism Response

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**In Vitro Test Strategy along AOP**

**Dermal Penetration**

**Protein Reactivity**

Direct Peptide Reactivity Assay

**Keratinocyte Activation**

LuSens or KeratinoSens

ROS electrophiles

Nucleus

Luminescence

Nrf-2

P

P

Modified from M.B. Sporn, K.T. Libby, 2005, Nat Clin Pract Oncol

X = Transcription of antioxidative and cytoprotective genes

**Dendritic Cell Activation**

MUSST or h-CLAT

**MUSST or h-CLAT**


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Test Substance Selection for Validation

- 59 test substances including LLNA performance standards
  - Additives/ stabilizers/ detergents 30%
  - Fragrances 24%
  - Cosmetic preservatives 22%
  - Cosmetic solvents 11%
  - Cosmetic dyes 7%

- 5/59 substances initially selected turned out not to be applicable due to technical reasons

- 54 substances with available LLNA and human skin sensitization information were evaluated in 5 in vitro/ in chemico assays
## Predictivity of assays and their Combinations

<table>
<thead>
<tr>
<th>Compared to human</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vivo standard</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLNA</td>
<td>86 %</td>
<td>94 %</td>
<td>89 %</td>
</tr>
<tr>
<td><strong>Individual assays</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPRA</td>
<td>88 %</td>
<td>86 %</td>
<td>87 %</td>
</tr>
<tr>
<td>LuSens</td>
<td>85 %</td>
<td>81 %</td>
<td>83 %</td>
</tr>
<tr>
<td>MUSST</td>
<td>100 %</td>
<td>73 %</td>
<td>85 %</td>
</tr>
<tr>
<td>h-CLAT</td>
<td>83 %</td>
<td>71 %</td>
<td>78 %</td>
</tr>
<tr>
<td><strong>Combinations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPRA and LuSens</td>
<td>80 %</td>
<td>100 %</td>
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</tr>
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<td>80 %</td>
</tr>
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<td>LuSens and h-CLAT</td>
<td>88 %</td>
<td>66 %</td>
<td>76 %</td>
</tr>
<tr>
<td><strong>Prediction model</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPRA, LuSens and MUSST</td>
<td>97 %</td>
<td>91 %</td>
<td>94 %</td>
</tr>
</tbody>
</table>
Proposed Testing Strategy

Adverse outcome pathway

Protein reactivity
Keratinocyte activation
DC activation

DPRA
LuSens or KeratinoSens
MUSST (or h-CLAT)

If both results are negative:
NON-SENSITIZER
(High Sensitivity, 100%)

If positive:
SENSITIZER
(High Specificity, 100%)

Weight of evidence
High Overall Accuracy (94%)

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Conclusions

- Combination of assays addressing **key events of AOP** rather than single assays (all assays under ECVAM “review”)

- **Accuracy of 94%** (LLNA 89%) with options for high sensitivity and for high specificity

- This *in vitro* test **strategy** for qualitative **hazard assessment** has been submitted to **ECVAM**

- Ongoing work (postvalidation): define **applicability domain** (acrylates, surfactants, isocyanates, plant extracts, polyethyleneimines, agrochemical formulations)

- Future challenge: **potency** (risk assessment!)
Thank you!

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  - Lab for Biokinetics

BASF References


- Bauch et al. (2012) Putting the parts together: Combining in vitro methods to test for skin sensitizing potentials. Reg Tox Pharmacol 63(3):489-504

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