BASF’s Testing Strategy for the detection of potential skin sensitizers

A selection of test methods that represent key events of the sensitization process were evaluated to identify the combination delivering the best overall accuracy for detection of skin sensitizers. The most predictive and technically most feasible test combination identified based on the results obtained with 54 substances uses the DPRA, LuSens reporter gene assay and MUSST. This combination allows evaluations of the intrinsic reactivity of chemicals and also of their capacity to activate cells of the immune system (DCs). The decision criteria considers that any 2 of 3 tests must be positive to rate the substance as a skin sensitizer.

This combination was able to predict skin sensitizers with an overall accuracy of 94% compared to human data and could be considered to be a “weight of evidence approach”.

Further steps

1. The applicability of the assays and the prediction model to a wider set of substances will be explored and the individual assays as well as the prediction model may be adapted for specific substance classes.

2. The models presented here are not yet able to classify substances into weak, moderate, strong or extreme skin sensitizers; i.e. do not allow assessments of potency. Potency is, however, an important parameter used in risk and safety assessments. The LLNA still remains the gold standard for potency assessments.

In vitro skin sensitization testing strategy
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BASF SE Experimental Toxicology and Ecology offers a comprehensive testing battery for the identification of the sensitization potential of substances.

Identification of skin sensitization potential is required for chemicals registrations, e.g. under REACh and for safety assessment of cosmetic ingredients. Currently, only results from the in vivo tests described in OECD 406 (guinea pig tests) or OECD 429 and OECD 442 (murine local lymph node assay, LLNA) are accepted by regulators.

BASF SE Experimental Toxicology and Ecology has designed and implemented a strategy for hazard identification of skin sensitization without the use of animals. The test strategy is based on test methods that are in the validation process at ECVAM.

Based on the evaluation with 54 substances, BASF has selected two key events (protein reactivity and dendritic cell activation) to design a testing battery to predict the skin sensitization potential of substances. This testing battery offers an overall accuracy for predicting human sensitizers equivalent to that of the LLNA (> 94%).

**Methods available at BASF SE**

1. **Protein Reactivity:**
   - Direct peptide reactivity assay (DPRA): An in chemico method that detects the interaction of potential haptens with two model peptides containing either cysteine or lysine. Peptide depletion as a marker for protein reactivity is measured by HPLC-UV.
   - LuSens assay: A BASF validated cell-based test system based on intracellular cysteine reactivity of haptens. It uses a transgenic HaCaT cell line carrying the luciferase reporter gene under the control of an ARE-element from the rat NADPH quinone oxidoreductase 1 gene. A chemical is categorized to have an ARE activating potential, when luciferase activity is induced. The LuSens assay has a similar predictivity as the KeratoSens® assay.

2. **Activation of Dendritic cells:**
   - MUSST and h-CLAT: Dendritic cells process and present foreign antigens to the T cells located in the local lymph nodes to induce immune reactions. MUSST employs the human myeloid U937 cells and h-CLAT uses the human monocytic leukemia cell line THP-1, a dendritic cell-like cell line. The tests measure the up-regulation of the co-stimulatory surface molecule(s) CD86 or CD54 and CD86 as markers of dendritic cell activation, respectively.

**Test Endpoint Evaluation criteria Analytics**

<table>
<thead>
<tr>
<th>Test</th>
<th>Endpoint Evaluation criteria</th>
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<tbody>
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<td>DPRA</td>
<td>Peptide depletion: Positive if ≥ 6.38% mean peptide depletion</td>
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<td>Luciferase activity: Positive if ≥ 1.5x luciferase activity when viability is &gt;70% of the control</td>
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<td>MUSST</td>
<td>CD86 expression: Positive if ≥ 1.2x of CD86 when viability is &gt;70% of the control</td>
<td>Flow cytometry</td>
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<td>CD54 and CD86 expression: Positive if ≥2.0x of CD54 and/or ≥1.5 of CD86 when viability is &gt;70% of the control</td>
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**Figure 1:** Schematic representation of the sensitization process. After haptens penetrate the epidermis (1), they are able to react with proteins and/or cells on the epidermis (2), leading to the formation of hapten-protein complexes. Those complexes are then recognized by the dendritic cells (DCs), triggering their activation (4), thus activated DCs (aDC) migrate and express differential markers to present the hapten-protein complexes to the T cells (TC) (7). After repeated contact with the hapten (8), TC will proliferate and migrate the affected area (9), where they will lead to an inflammation process (10).

**References**

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Methods available at BASF SE

1. We use the combination of several methods addressing two major steps of the sensitization process: protein reactivity and activation of dendritic cells.
2. Protein Reactivity:
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![U937 cell line used to performed the MUSST assay](image1)

Microscopic quality control of in vitro culture cell lines

LuSens cells cultured in vitro
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