Background Information

• The induction of allergic contact dermatitis typically involves an initial exposure of the chemical or hapten to the skin and then binding of the chemical to a protein carrier in a process known as haptenisation.

• The majority of chemical allergens possess electrophilic centres or can be metabolically converted to electrophiles (reactive metabolites).

• DPRA was first developed by Frank Gerberick and colleagues in 2004 and was further refined in 2007 (Gerberick et al., 2007).

• The direct peptide reactivity assay (DPRA) is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centres in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine.

• In 2013, ECVAM recommended the DPRA for use in skin sensitisation testing, and draft OECD guidelines are currently in review. Due to the complex nature of skin sensitisation, the guidelines suggest that DPRA should be used in combination with other mechanistic assays for the prediction of skin sensitisation.

• DPRA results generated during ECVAM validation and in other published studies suggest accuracy of DPRA in discriminating between non-sensitisers and sensitisers is 80% with a sensitivity of 80% and a specificity of 77% when compared with the local lymph node assay (LLNA).

Protocol

Test System
Test article incubated with synthetic peptides containing either cysteine (10:1 ratio) or lysine (50:1 ratio)

Analysis Platform
LC-MS/MS or HPLC-UV

Test Compound Concentrations
Single concentration in triplicate

Time Points
Single 24 hour exposure

Data Delivery
% Depletion of cysteine and lysine relative to vehicle control
Reactivity class
Prediction of sensitising potential

Related Services

Skin irritation
SenCeeTox® skin sensitisation
Skin corrosion
Ocular irritation
Phototoxicity
Skin absorption

To find out more contact enquiries@cyprotex.com
The ability of a chemical to react with skin proteins is thought to play a key role in the development of skin sensitization.\(^7\)

### Table 1

<table>
<thead>
<tr>
<th>Mean of cysteine and lysine depletion</th>
<th>Reactivity class</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean % depletion between 0% and 6.38%</td>
<td>Minimal reactivity</td>
<td>Non-sensitiser</td>
</tr>
<tr>
<td>Mean % depletion between 6.38% and 22.62%</td>
<td>Low reactivity</td>
<td>Sensitiser</td>
</tr>
<tr>
<td>Mean % depletion between 22.62% and 42.47%</td>
<td>Moderate reactivity</td>
<td>Sensitiser</td>
</tr>
<tr>
<td>Mean % depletion between 42.47% and 100%</td>
<td>High reactivity</td>
<td>Sensitiser</td>
</tr>
</tbody>
</table>

Depletion of the DPRA peptides can be used to classify chemicals into four different categories of reactivity allowing discrimination between non-sensitising and sensitising chemicals.

**Figure 1**

Mean cysteine and lysine depletion in the DPRA for non sensitisers (lactic acid, 4-hydroxybenzoic acid, 6-methylcoumarin) and sensitisers (p-benzoquinone, dinitrochlorobenzene and phthalic anhydride).

The graph illustrates good comparison of the DPRA data generated at Cyprotex with DPRA data generated by Gerberick et al., (2007).

---

**References**

2. Gerberick GF et al., (2007) Quantification of Chemical Peptide Reactivity for Screening Contact Allergens: A Classification Tree Model Approach Toxicol Sci 97(2); 417-427
3. EC EURL ECVAM (2012) Direct peptide reactivity assay (DPRA) validation study report pp 1-74
7. Troutman JA et al., (2011) The incorporation of lysine into the peroxidase peptide reactivity assay for skin sensitization assessment Toxicol Sci 122(2); 422-436