Direct Peptide Reactivity Assay (DPRA)

In Chemico Skin Sensitisation Assay

The *in chemico* direct peptide reactivity assay (DPRA) addresses the first molecular key event of the adverse outcome pathway (AOP) of skin sensitisation [1]. The UN GHS (United Nations Globally Harmonized System of Classification and Labelling of Chemicals) defines a skin sensitiser as a substance that will cause an allergic response after skin contact [2].

The DPRA is validated by the EURL ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing) and is performed in accordance with the OECD guidance OECD 442C at Eurofins BioPharma Product Testing Munich GmbH [1, 3] with chemicals, cosmetics or personal care products and pharmaceuticals.

The DPRA is one of three test methods (KeratinoSens™ and h-CLAT (Link)) for the assessment of skin sensitisation potential.

Assessment of Skin Sensitisation Potential with the DPRA

- The molecular initiating event of skin sensitisation addresses epidermal protein binding and reactivity towards proteins, by mimicking the reaction with artificial peptides.
- A cysteine and a lysine containing peptide are mixed with the test substance and after 24 hours the sample is analyzed by high-performance liquid chromatography (HPLC) with UV detection at 220 nm.
- If a depletion or a diminishment of the peptides is detected it can be used for classification of reactivity of chemicals and to discriminate between sensitisers and non-sensitisers.

"The molecular initiating event is the covalent binding of electrophilic substances to nucleophilic centers in skin proteins. The ability of a chemical to react with skin proteins is thought to play a key role in the development of skin sensitisation." [1, 4]

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Procedure

Principle of the DPRA

Protocol	
Peptides	Ac-RFAACAA-COOH (Cysteine) Ac-RFAAKAA-COOH (Lysine)
Analysis	Quantification of cysteine and lysine peptide concentration by high-performance liquid chromatography (HPLC) using UV detection at 220 nm
Concentrations	Test chemical = 100 mM Cysteine peptide = 10:1 ratio to test chemical Lysine peptide = 50:1 ratio to test chemical
Exposure time	24 h
Quality controls	Positive control: cinnamic aldehyde (100 mM) Different reference controls Co-elution controls of test chemical and positive control
Solvents of test chemical	Acetonitrile, water, acetonitrile / water 1:1, isopropanol, methanol, ethanol, 1,4-butandiol, N,N-dimethyl formamide, tert. butanol
Data delivery	Depletion of cysteine and lysine peptide (%) Reactivity category Prediction of sensitising potential
Positive prediction	Depletion of cysteine and lysine peptide is >6.38% Depletion of cysteine is >13.89% (Cysteine-only)

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Based on the peptide depletion of cysteine and lysine, chemicals can be classified with the DPRA into four reactivity categories and can be discriminated between sensitisers and non-sensitisers.

Mean of cysteine and lysine depletion (%)	Reactivity class	Prediction
mean % depletion between 0 and 6.38	no or minimal reactivity	negative
mean % depletion between 6.39 and 22.62	low reactivity	positive
mean % depletion between 22.63 and 42.47	moderate reactivity	
mean % depletion between 42.48 and 100	high reactivity	

Table 1: Classification of reactivity and prediction of sensitising potential dependent on cysteine and lysine peptide depletion (cysteine 1:10 / lysine 1:50 prediction model).[2]

If lysine has a co-elution or it cannot be evaluated and it is negative, we can use the cysteine for evaluation on its own:

Mean of cysteine depletion (%)	Reactivity class	Prediction
mean % depletion between 0 and 13.89	no or minimal reactivity	negative
mean % depletion between 13.90 and 23.09	low reactivity	positive
mean % depletion between 23.10 and 98.24	moderate reactivity	
mean % depletion between 98.25 and 100	high reactivity	

Table 2: Classification of reactivity and prediction of sensitising potential dependent on cysteine peptide depletion (cysteine 1:10 prediction model).[2]

If cysteine has a co-elution or it cannot be evaluated and it is negative, then the result remains inconclusive. A positive result can still be used.

Data

Eurofins data for demonstrating technical proficiency of the DPRA

Chemical	Cysteine PPD (OECD)	Lysine PPD (OECD)	Prediction (OECD)	Cysteine PPD (EF)	Lysine PPD (EF)	Prediction (EF)
Non-sensitising Chemicals						
1-Butanol	0-7	0-5.5	**Negative**	0.00	0.72	**Negative**
6-Methylcoumarin	0-7	0-5.5	**Negative**	0.00	0.25	**Negative**
Lactic Acid	0-7	0-5.5	**Negative**	0.05	0.25	**Negative**
4-Methoxyacetophenone	0-7	0-5.5	**Negative**	1.60	1.43	**Negative**
Sensitising Chemicals						
2,4-Dinitrochlorbenzene	90-100	15-45	**Positive**	100.00	40.18	**Positive**
Oxazolone	60-80	10-55	**Positive**	72.24	36.85	**Positive**
Formaldehyde	30-60	0-24	**Positive**	49.91	2.42	**Positive**
Benzylidene acetone	80-100	0-7	**Positive**	92.75	3.10	**Positive**
Farnesal	15-55	0-25	**Positive**	35.78	8.93	**Positive**
2,3-Butandione	60-100	10-45	**Positive**	71.87	19.58	**Positive**

PPD: Percent peptide depletion EF = Eurofins Munich GmbH

Table 3: Eurofins data of the DPRA – ten tested proficiency chemicals compared to the data of the OECD guideline.[1]

In Table 3 the obtained data from the DPRA of four non-sensitising and six sensitising chemicals are shown. The prediction of all tested chemicals was correct in comparison to the classification of the OECD guideline.

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References

1) OECD Guidelines for Testing of Chemicals, number 442C "*In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)" (adopted: February 04, 2015).

2) UN (2015), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Sixth revised edition, UN New York and Geneva.

3) EC EURL-ECVAM (2013). Recommendation on the Direct Peptide Reactivity Assay (DPRA) for skin sensitisation testing.

4) 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.