Direct peptide reactivity assay (DPRA): validation of the assay on ten chemicals

Introduction

The DPRA is a chemistry-based assay exploiting the fact that most chemical allergens have electrophilic properties and are therefore able to react with the nucleophilic side chains of amino acids to form covalent bonds.

The underlying rationale of the assay is that if a chemical is capable of reacting with proteins then it has the potential to act as a sensitizer. The endpoint measured in the assay is the percentage depletion over time of two synthetic peptides (containing respectively a cysteine and a lysine amino acid) from the peptide mixtures following an approximate 24 hour incubation with the test chemical.

The percentage of peptide depletion is calculated by High Performance Liquid Chromatography using ultra-violet detection.

The DPRA test allows for a yes/no answer in terms of sensitization potential and furthermore allows quantification of a chemical's reactivity.

Method

The DPRA measured the reaction of 10 reference chemicals of known sensitization potential with synthetic peptides containing Cystine (Ac-RFAACAA-COOH) and Lysine (Ac-RFAAKAA-COOH). Each analytical run contained a positive control (cinnamic aldehyde) and co-elution controls (reference standard and peptide buffer without the peptide). Prior to analysis the solubility of each reference chemical was assessed in acetonitrile at concentrations of 100 mM.

Test solutions of each reference chemical were prepared at 100 mM in acetonitrile and solutions of each peptide (cysteine at 0.667 mM in phosphate buffer pH 7.5 and Lysine at 0.667 mM in ammonium phosphate buffer pH 10.2) were spiked with the reference chemical at a ratio of 1:50 and 1:10 respectively. The test solutions were then incubated in the dark at 25°C for a minimum of 22 hours prior to injection on the HPLC along with a peptide standard curve, the positive control, co elution controls and a reagent blank.

crs.eu@envigo.com

© 2016 Envigo.

The amount of remaining unreacted peptide was measured and peptide depletion measured:

$$\% \text{ Peptide depletion} = 100 - \frac{\text{Peptide peak area in replicate depletion samples (x 100)}}{\text{Mean Peptide peak area of reference control samples}}$$

The reactivity category of each reference chemical was assessed based on the following prediction model:

Mean of cysteine and lysine % depletion	Reactivity class	DPRA prediction
0%≤mean%depletion≤6.38%	No or minimal activity	Negative
6.38%<mean%depletion≤22.62%	Low reactivity	
22.62%<mean%depletion≤42.47%	Moderate reactivity	
42.47%<mean%depletion≤100%	High reactivity	Positive

Results

Annex 2 of the OECD guidelines states that to demonstrate technical proficiency, laboratories should be able to replicate the DRPA predictions from 8 of 10 reference chemicals in the guidelines document (TG 442). At Envigo technical proficiency was achieved with all 10 results matching the predictive model. In addition, method robustness was confirmed and additional solvents and solvent mixtures were assessed.

Reference chemical	Cysteine peptide depletion (%)	Lysine peptide depletion (%)	Overall mean depletion (%)	Reactivity class	DRPA result	DRPA prediction
2,4-Dinitrochlorobenzene	100	25.1	62.6	High	Positive	Positive
Oxazolone	72.5	52.7	62.6	High	Positive	Positive
Formaldehyde	63.5	1.95	32.7	Moderate	Positive	Positive
Benzylideneacetone	93.9	3.85	48.9	High	Positive	Positive
Farnesal	16.9	7.14	12.0	Low	Positive	Positive
2,3-Butanedione	67.0	19.5	43.2	High	Positive	Positive
1-Butanol	-0.983	0.274	0.0	Minimal	Negative	Negative
6-Methylcoumarin	-0.638	-0.725	0.0	Minimal	Negative	Negative
Lactic Acid	-0.870	-0.717	0.0	Minimal	Negative	Negative
4-Methoxyacetophenone	-0.449	-0.165	0.0	Minimal	Negative	Negative

Conclusion

DPRA has been validated to GLP for use at Envigo in accordance with *In chemico* Skin Sensitization: Direct Peptide Reactivity Assay (DPRA), OECD/OCDE document TG 442C.

Contact us

crs.eu@envigo.com

ENVIGO

Envigo, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, UK

envigo.com/nonanimalalternatives

© 2016 Envigo.