In Vitro Skin Sensitization Testing – Direct Peptide Reactivity Assay (DPRA)

Need

Assessing the risk of dermal sensitization is a primary consideration in determining the safety of a dermatological drug or cosmetic. It is also of concern in the case of accidental exposures to chemicals, formulations, and other products. Skin sensitization is a complex chemical and biological mechanism which has been summarized as an Adverse Outcome Pathway (AOP; OECD 168). The first key event in skin sensitization is the molecular initiating event.

For new dermatological products and substances that may come in contact with human skin, the cost and time necessary to assess dermal sensitization in vivo is prohibitory to efficient product development and safety assessment. Assessment of sensitization potential has historically been performed using animal models, however, the anatomy and barrier physiology of research animal model skin, including hair follicle density and stratum corneum and viable epidermis thickness, do not accurately mimic that of human skin.

Solution

The effect of dermatological product formulation on dermal sensitization should be determined in early in vitro efficacy studies. Dermal sensitization can be determined by using non-animal in vitro methods, more specifically, using three separate assays, each corresponding to one of the first three key events in the AOP. The first assay is the Direct Peptide Reactivity Assay, or DPRA, which assesses the first key event, covalent binding of electrophilic substances to nucleophilic centers in skin proteins (OECD 442C).

Solubility assessments are conducted with the test article to determine the appropriate solvent for the assay. Once an appropriate solvent is identified, the test article is then incubated with peptides containing cysteine and lysine residues at precise molar ratios. Samples are then analyzed by HPLC coupled with a UV detector (220 nm) and compared to standard curves of the peptides. The % depletion of free peptide is then calculated according to Figure 1 below. Based on the mean percent depletion, a Reactivity Class and subsequent DPRA Prediction can be determined according to Table 1 below. It is important to note that the full sensitization potential of a test article cannot be determined using the DRPA assay alone. The results must be taken in context of other in vitro assays (OECD 442D and OECD 442E).

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Standard Protocol

ASSAY PARAMETERS	PROTOCOL
Model	Two synthetic peptides one containing cysteine (Ac-RFAA-CAA-COOH) and one containing lysine (Ac-RFAAKAA-COOH)
Replicates	3
Test Article Formulation	100 mM in appropriate solvent as determined by solubility assessment
Reference Controls	Samples containing only the peptides dissolved in the appropriate solvent
Positive Control	Cinnamic aldehyde (CAS 104-55-2)
Exposure Time	24 ± 2 hours
Peptide Depletion Assessment	HPLC coupled with an UV detector
Time to Complete	3-4 weeks from test article receipt
Deliverables	Full Report including: Sensitization potential (positive or negative) and reactivity class (No or Minimal Reactivity, Low Reactivity, Moderate Reactivity, High Reactivity)

Key References

UN (2013), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth Revised Edition, UN New York and Geneva.

OECD (2014), The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence, Series on Testing and Assessment No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

FDA (2023) Guidance Document: Nonclinical Evaluation of the Immunotoxic Potential of Pharmaceuticals, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, FDA-2019-D-5607.

OECD (2024), Test No. 442C: In chemico skin sensitisation assays addressing the Adverse Outcome Pathway Key Event on Covalent Binding to Proteins, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264229709-en.

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OECD (2024), Test No. 442E: In Vitro Skin Sensitisation Assays Addressing the Adverse Outcome Pathway Key Event on Activation of Dendritic Cells, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264359-en.

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Figure 1. Calculation of % peptide depletion.

$$\text{Percent peptide depletion} = \left[1 - \left(\frac{\text{Peptide peak area in replicate injection}}{\text{Mean peptide peak area in reference controls C}}\right)\right] \times 100$$

Table 1. Sensitization prediction model

MEAN OF CYSTEINE AND LYSINE % DEPLETION	REACTIVITY CLASS	DPRA PREDICTION*
0% ≤ mean % depletion ≤ 6.38%	No or Minimal Reactivity	Negative
6.38% < mean % depletion ≤ 22.62%	Low Reactivity	Positive
22.62% < mean % depletion ≤ 42.47%	Moderate Reactivity	Positive
42.47% < mean % depletion ≤ 100%	High Reactivity	Positive

* A DPRA prediction should be considered in the framework of a DA (defined approach) or an IATA (Integrated Approach to Testing and Assessment) and in accordance with the provisions of OECD 442C.

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