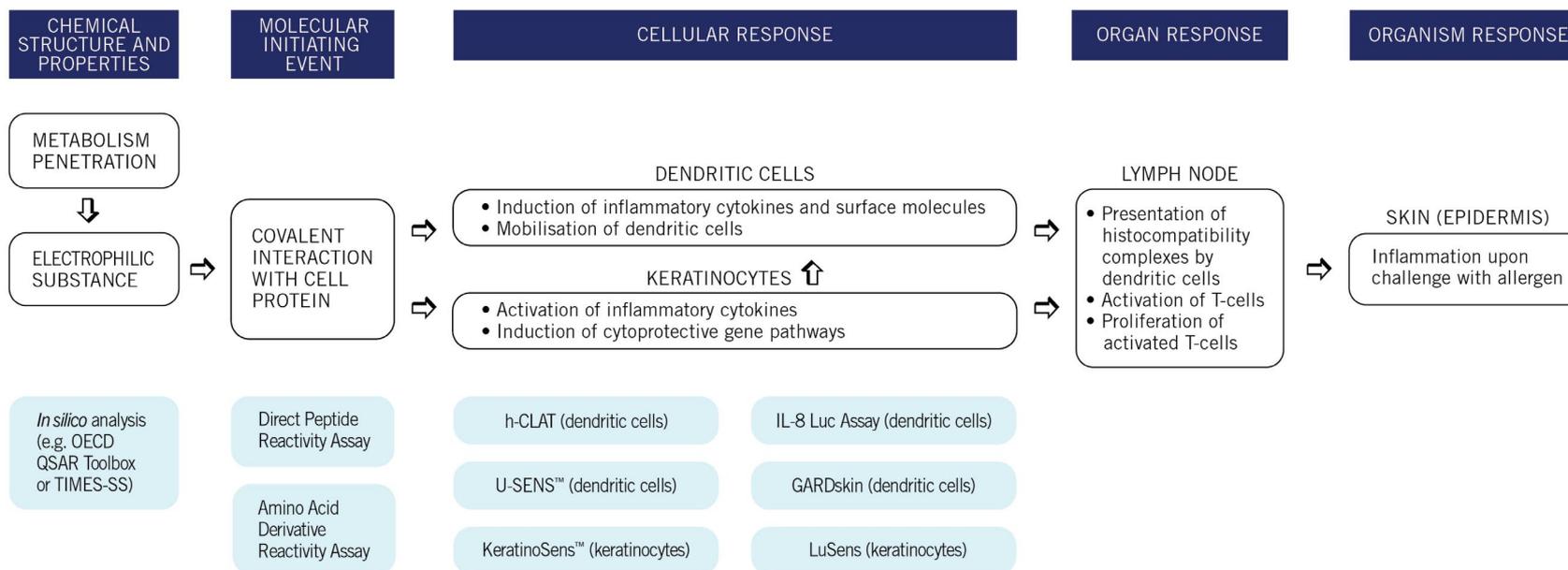


# IN VITRO AND IN CHEMICO METHODS FOR PREDICTING SKIN SENSITISATION

## The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins



METHOD	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN	GHS CATEGORISATION
<b>OECD Guideline 497: Defined Approaches on Skin Sensitisation</b>	This guideline describes approaches to combining methods for evaluating skin sensitisation hazard potential based on the skin sensitisation adverse outcome pathway (AOP). The “2 out of 3” defined approach combines results from the direct peptide reactivity assay (DPRA), KeratinoSens™, and/or h-CLAT and provides sufficient information for hazard identification. The integrated testing strategies (ITSv1 and ITSv2) collate information from the DPRA and human cell line activation test (h-CLAT), along with <i>in silico</i> predictions, to predict hazard and potency.	The applicability domain is defined by the assays used in the testing approach.	The “2 out of 3” defined approach supports discrimination between skin sensitisers (GHS Cat 1) and non-sensitisers. ITSv1 and ITSv2 support discrimination between GHS Cat 1A, GHS Cat 1B, and non-sensitisers.
<b>OECD Test Guideline 442c: In Chemico Skin Sensitisation – Assays Addressing the AOP Key Event on Covalent Binding to Proteins</b>	The DPRA and the amino acid derivative reactivity assay (ADRA) are <i>in chemico</i> methods that address the molecular initiating event of the skin sensitisation AOP by quantifying the depletion of synthetic cysteine- or lysine-containing peptides, in the case of the DPRA, and the cysteine derivative N-(2-(1-naphthyl)acetyl)-L-cysteine (NAC) or the lysine derivative α-N-(2-(1-naphthyl)acetyl)-L-lysine (NAL), in the case of the ADRA, following 24-hour exposure to the test chemical using high-performance liquid chromatography coupled with an ultraviolet detector. Cysteine and lysine peptide or derivative percentage depletion values are calculated and used in prediction models, which are used to discriminate between skin sensitisers and non-sensitisers.	These tests are applicable to chemicals that are soluble in a suitable solvent, e.g. acetonitrile, water, isopropanol, and acetone. They can be used for mixtures of known substances but not of unknown substances. They cannot be used for metals, because they are known to react with proteins via mechanisms other than covalent binding, and they cannot be used for complex reaction products or biological materials. Chemicals that require enzymatic bioactivation to exert their skin sensitisation potential also cannot be detected.	To support discrimination between skin sensitisers (GHS Cat 1) and non-sensitisers in the context of integrated approaches to testing and assessment (IATA)
<b>OECD Test Guideline 442d: In Vitro Skin Sensitisation – Assays Addressing the AOP Key Event on Keratinocyte Activation</b>	The KeratinoSens™ and LuSens methods address the second key event in the skin sensitisation AOP using an immortalised adherent cell line derived from human keratinocytes stably transfected with a selectable plasmid. The cell line contains the luciferase gene under the transcriptional control of a constitutive promoter fused with an antioxidant/electrophile response element from a gene that is known to be upregulated by contact sensitisers. This allows for quantitative measurement (by luminescence detection) of luciferase gene induction.	These tests are applicable to chemicals that are soluble or form a stable dispersion in water or dimethyl sulfoxide (DMSO). They can be used for testing multi-constituent substances and mixtures. Highly cytotoxic test chemicals or test chemicals that interfere with the luciferase enzyme cannot always be reliably assessed.	To support discrimination between skin sensitisers (GHS Cat 1) and non-sensitisers in the context of IATA

METHOD	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN	GHS CATEGORISATION
<p><b>OECD Test Guideline 442e: <i>In Vitro</i> Skin Sensitisation – Assays Addressing the AOP Key Event on Activation of Dendritic Cells</b></p>	<p>The h-CLAT addresses the third key event in the skin sensitisation AOP, and measures expression of CD86 and CD54 in THP-1 cells (human monocytic leukaemia cell line) following 24-hour exposure to the test chemical, via flow cytometry following cell staining with fluorescently labelled antibodies.</p> <p>The U-SENS™ method addresses the third key event in the skin sensitisation AOP by quantifying changes in CD86 expression in the human histiocytic lymphoma cell line, U937. CD86 is a cell surface marker associated with the activation of monocytes and dendritic cells in U937 cells following exposure to skin sensitisers. After a 45-hour exposure to the test chemical, CD86 expression is measured via flow cytometry following cell staining with fluorescently labelled antibodies.</p> <p>The IL-8 Luc assay addresses the third key event in the skin sensitisation AOP using the THP-G8 cell line. THP-G8 cells are derived from the human acute monocytic leukaemia cell line, THP-1, and contain a luciferase gene under the control of the IL-8 promoter. IL-8 is a cytokine associated with the activation of dendritic cells, and this assay allows for the quantitative measurement of luciferase gene induction as an indicator of IL-8 activity.</p> <p>The Genomic Allergen Rapid Detection for assessment of skin sensitisers (GARDskin) assay addresses the third key event in the skin sensitisation AOP by evaluating genomic biomarker signatures in the SenzaCell™ cell line. The SenzaCell™ cell line, a subclone of the myeloid leukaemia cell line MUTZ-3, is used as a model for dendritic cells.</p>	<p>The h-CLAT is applicable to chemicals that are soluble or form a stable dispersion in saline, medium, or DMSO. It can be used for testing multi-constituent substances and mixtures. In cases in which a strongly fluorescent test chemical emits at the same wavelength as fluorescein isothiocyanate (FITC) or as propidium iodide (PI), another fluorochrome-tagged antibody should be used.</p> <p>The U-SENS™ method is applicable to chemicals that are soluble or form a stable dispersion in an appropriate solvent. It can be used for testing multi-constituent substances and mixtures. Positive results for surfactants should be considered with caution. In cases in which a strongly fluorescent test chemical emits at the same wavelength as FITC or as PI, another fluorochrome-tagged antibody should be used.</p> <p>The IL-8 Luc assay is applicable to chemicals that are soluble in X-VIVO™ 15 (Lonza) or other solvents, provided there is sufficient scientific rationale. It can be used for testing multi-constituent substances and mixtures. Negative results for respiratory sensitisers should be interpreted with caution. It cannot be used for detergents.</p> <p>No specific chemistries are excluded from the applicability domain of the GARDskin assay. Although issues may arise regarding the solubility of the test substances or the compatibility of test substances with vehicles and the aqueous cell system, circumventions for these issues are included in the test guideline. Note that autofluorescent test substances may interfere with flow cytometry-based cytotoxicity assessments.</p>	<p>To support discrimination between skin sensitisers (GHS Cat 1) and non-sensitisers in the context of IATA</p>

#### Additional Reading

- Organisation for Economic Co-operation and Development. 2016. Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to Be Used Within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation. No 256. Series on Testing and Assessment. See also Annex 1 and Annex 2.
- Organisation for Economic Co-operation and Development. 2012. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. No 168. Series on Testing and Assessment. Part 1, Part 2.
- US Environmental Protection Agency. 2018. Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing.
- European Chemicals Agency. 2017. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint Specific Guidance. Version 6.0. See R.7.3.4–R.7.3.7.