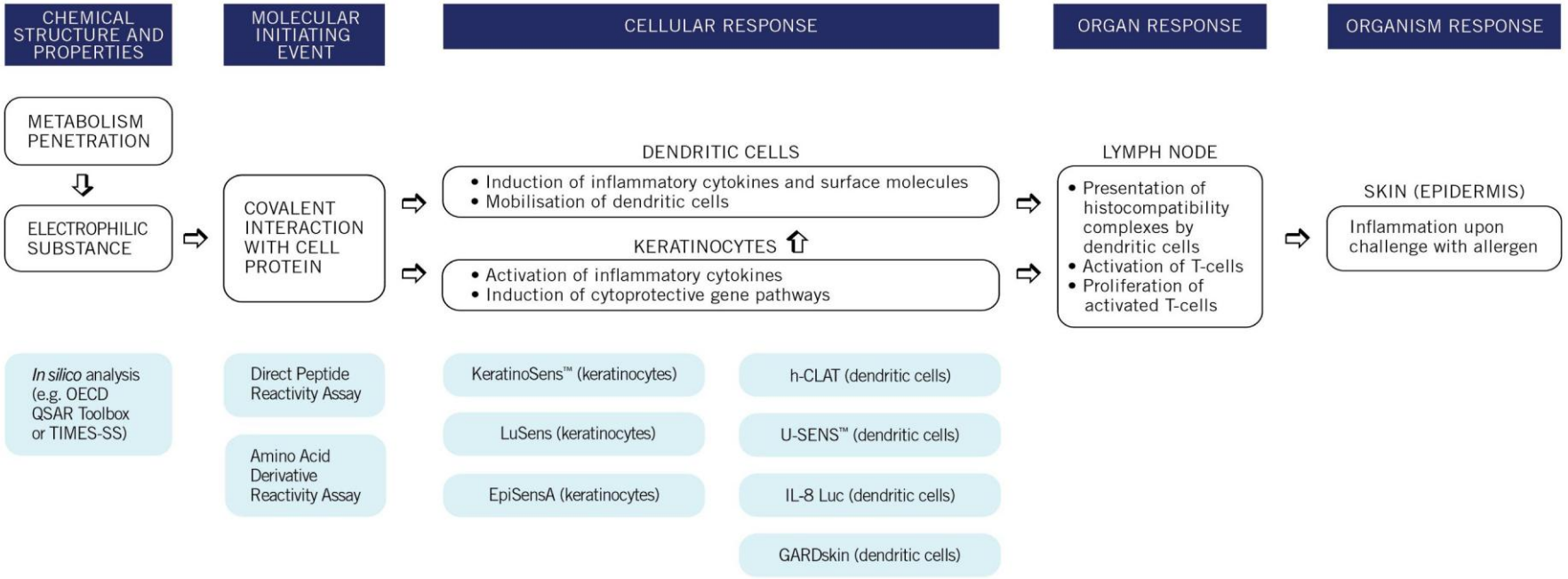
METHODS FOR PREDICTING SKIN SENSITISATION

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



METHOD	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN	GHS CATEGORISATION
OECD Guideline 497: Defined Approaches on Skin Sensitisation	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 test methods described in OECD TG 442C, 442D, and 442E and provides sufficient information for hazard identification. The integrated testing strategies (ITSv1 and ITSv2) collate information from OECD TG 442C and OECD TG 442E assays, along with *in silico* predictions, to predict hazard *and* potency. In addition, a quantitative point of departure can be predicted based on existing and novel *in vitro* data using the *in silico* defined approach, SARA-ICE, which uses Bayesian modeling.	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. SARA-ICE: Quantitative point of departure
OECD Test Guideline 442c: *In Chemico* Skin Sensitisation – Assays Addressing the AOP Key Event on Covalent Binding to Proteins	The DPRA and the amino acid derivative reactivity assay (ADRA) are *in chemico* 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)

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OECD Test Guideline 442d: *In Vitro* Skin Sensitisation – Assays Addressing the AOP Key Event on Keratinocyte Activation	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. EpiSensA uses a three-dimensional reconstructed human epidermis model, LabCyte EPI-MODEL24, to address the second key event by assessing the expression of four marker genes known to correlate with sensitisation: 1) ATF3, which modulates inflammatory cytokines; 2) GCLM, an Nrf2-ARE–dependent GHS regulator; 3) DNAJB4, an Nrf2-ARE–dependent gene which prevents protein misfolding; and 4) IL-8, a chemotactic peptide for neutrophils.	KeratinoSens™ and LuSens are applicable to chemicals that are soluble or that 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. No specific chemistries are excluded from the applicability domain of EpiSensA. It is broadly applicable across substances with low solubility and lipophilic chemistries, and it can detect chemicals that require metabolic activation (pro-haptens) as well as pre-haptens.	To support discrimination between skin sensitisers (GHS Cat 1) and non-sensitisers in the context of IATA
OECD Test Guideline 442e: *In Vitro* Skin Sensitisation – Assays Addressing the AOP Key Event on Activation of Dendritic Cells	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. 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. The IL-8 Luc assay addresses the third key event in the skin sensitisation AOP using the THP-G8 cell line. THP-G8 cells, derived from the human acute monocytic leukaemia cell line, THP-1, 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. 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.	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. 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. 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. 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.	To support discrimination between skin sensitisers (GHS Cat 1) and non-sensitisers in the context of IATA

Additional Reading

- Organisation for Economic Co-operation and Development. 2017. 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.
- Organisation for Economic Co-operation and Development. 2014. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. No 168. Series on Testing and Assessment.
- 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.

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IN SILICO TOOLS FOR PREDICTING SKIN SENSITISATION

Chemicals are routinely assessed for their ability to cause skin sensitisation, the regulatory endpoint preceding Allergic Contact Dermatitis (ACD). Current *in silico* strategies for predicting skin sensitisation are anchored in the OECD's Adverse Outcome Pathway (AOP) for skin sensitisation, a conceptual framework that maps the progression from chemical exposure to an allergic response. *In silico* models are particularly well suited to predict bioavailability (e.g., EPI Suite), skin metabolism (e.g., OASIS TIMES-SS module in the OECD QSAR Toolbox), and binding of the test chemical to skin cell proteins (key event 1).

In silico predictions are integrated with *in chemico* (e.g., DPRA) and *in vitro* (e.g., KeratinoSens™ or h-CLAT) data using formal algorithms known as Defined Approaches (DAs) (Figure 1). These DAs, such as the Integrated Testing Strategy (ITS) or SARA-ICE, weigh computational predictions alongside *in chemico* and *in vitro* assay results to generate a regulatory classification (e.g., a UN GHS Category) or a quantitative Point of Departure (PoD) for risk assessment.

A non-exhaustive list of *in silico* tools for predicting skin sensitisation is provided below. Please contact Kyle Martin at kmartin@thepsci.eu to include additional resources on this list or with any questions.

TOOL	DEVELOPER	METHOD/APPROACH	AVAILABILITY
ACD/Percepta	ACD Labs	Statistical	Commercial
ADMET Predictor	Simulations Plus	Statistical	Commercial
CASE Ultra	MultiCASE Inc.	Statistical	Commercial
Danish EPA (Q)SAR Database	Danish EPA	Statistical	Open-source
Derek Nexus	Lhasa Limited	Expert rule-based	Commercial
EPI Suite	US EPA	Expert rule-based	Open-source
iSafeRat	KREATiS	Statistical	Commercial
OECD QSAR Toolbox	OECD/Laboratory of Mathematical Chemistry	Statistical (read-across/QSAR)	Open-source
SARA-ICE	US NIH	Statistical	Open-source
Toxtree	IDEAconsult Ltd./European Commission	Expert rule-based	Open-source

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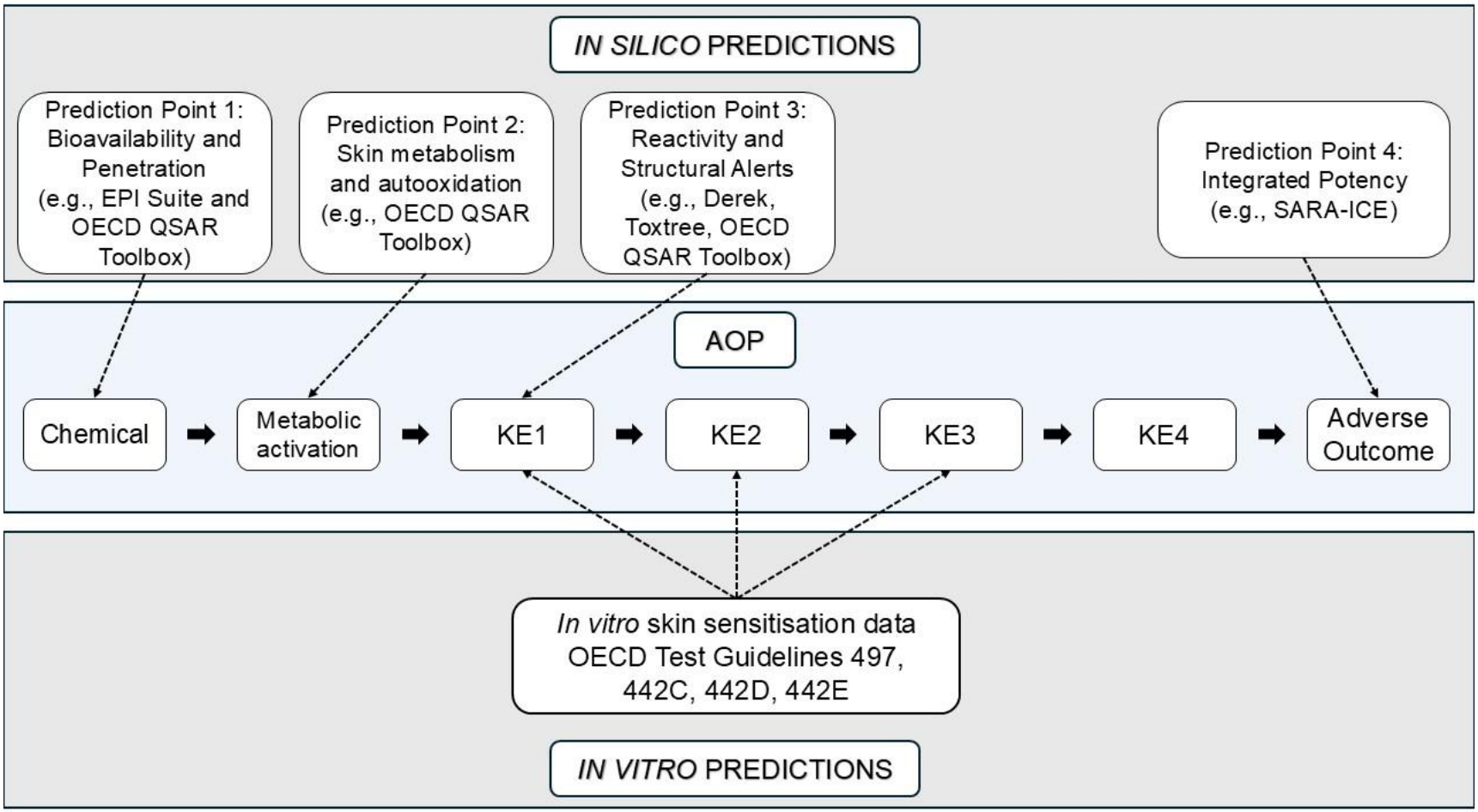

Figure 1. *In silico* predictions and *in chemico* and *in vitro* test data can be integrated to provide human-relevant predictions of skin sensitisation hazard and potency. Abbreviations: AOP = adverse outcome pathway; KE = key event.

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