NON-ANIMAL TOXICITY TEST METHODS AND GUIDANCE

PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.

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VALIDATED NON-ANIMAL TOXICITY TEST METHODS AND GUIDANCE

TOXICITY ENDPOINT	TEST METHODS AND APPROACHES		RECOMMENDATIONS AND STANDARD METHODS	
			OECD	OTHER AUTHORITY
SERIOUS EYE DAMAGE AND IRRITATION	Integrated approach to testing and assessment (IATA) for serious eye damage and irritation		OECD guidance document (GD) 263, published in 2017	--
	Guideline on defined approaches to eye irritation		OECD test guideline (TG) 467, published in 2022	--
	Chemical toxicity assessment strategy		--	European Chemicals Agency guidance Chapter R.7a., R.7.2.8–R.7.2.11 (2017)
	Use of a testing framework employing cytosensor microphysiometer (CM), BCOP, and the EpiOcular™ model for classification of pesticide products		--	US Environmental Protection Agency policy (2015)
	Reconstructed human cornea-like epithelium (RhCE) test method	EpiOcular™ (MatTek, US)	OECD TG 492, revised in 2019	ESAC statement (2014); JaCVAM statements (2017 and 2018); KoCVAM guideline (2016)
		SkinEthic™ (L'Oréal, France)		
		LabCyte (J-TEC, Japan)		
		MCTT HCE™ (Biosolution, South Korea)		
	Reconstructed human cornea-like epithelium (RhCE) test method (SkinEthic™)		OECD TG 492B, published in 2022	--
	Fluorescein leakage (FL) test method		OECD TG 460, revised in 2017	ESAC statement (2009); JaCVAM statement (2013)
	Short time exposure (STE) *in vitro* test method		OECD TG 491, revised in 2020	ICCVAM report (2013); JaCVAM statement (2016); KoCVAM guideline (2017)
	Vitrigel-eye irritancy test (EIT) method		OECD TG 494, revised in 2021	--
	In vitro macromolecular test method		OECD TG 496, published in 2019	--
	Bovine corneal opacity and permeability (BCOP) test method		OECD TG 437, revised in 2020	ICCVAM report (2006); ESAC statement (2007); JaCVAM statements (2009 and 2014); KoCVAM guideline (2011)
	Isolated chicken eye (ICE) test method		OECD TG 438, revised in 2018	ICCVAM report (2006); ESAC statement (2007); JaCVAM statement (2009)
	Cytosensor microphysiometer (CM) assay		--	ESAC statement (2009); ICCVAM report (2010)
SKIN CORROSION AND IRRITATION	Integrated approach to testing and assessment (IATA) for skin corrosion and irritation		OECD GD 203, published in 2014	--
	Chemical toxicity assessment strategy for skin corrosion and irritation		--	European Chemicals Agency guidance Chapter R.7a., R.7.2 (2017)
	In vitro membrane barrier test Corrositex for skin corrosion		OECD TG 435, revised in 2015	ICCVAM report (1999); ESAC statement (2000); JaCVAM statement (2017)
	In vitro skin corrosion: Reconstructed human epidermis (RhE) test	EpiSkin™ (L'Oréal, France)	OECD TG 431, revised in 2019	ICCVAM report (2002); ESAC statement (1998); JaCVAM statement (2017)
		EpiDerm™ (MatTek, US)		ICCVAM report (2002); ESAC statement (2000); JaCVAM statement (2017)
		SkinEthic™ (L'Oréal, France)		ESAC statement (2006); JaCVAM statement (2017)
		epiCS® (Phenion, Germany)		ESAC statement (2009); JaCVAM statement (2017)
		LabCyte EPI-MODEL24 SCT (J-TEC, Japan)		--
		Vitrolife-Skin™	--	JaCVAM statement (2008)
	In vitro skin irritation: Reconstructed human epidermis (RhE) test	EpiSkin™ (L'Oréal, France)	OECD TG 439, revised in 2021	ESAC statement (2007); JaCVAM statement (2010); KoCVAM guideline (2014)
		EpiDerm™ (MatTek, US)		ESAC statement (2008); JaCVAM statement (2013); KoCVAM guideline (2017)
		SkinEthic™ (L'Oréal, France)		ESAC statement (2008); JaCVAM statement (2013); KoCVAM guideline (2017)
		LabCyte EPI-MODEL24 SIT (J-TEC, Japan)		JaCVAM statement (2013); KoCVAM guideline (2017)
		Skin+® (Sterlab, France)		--
		epiCS® (Phenion, Germany)		--
		KeraSkin™ (Biosolution, South Korea)		--

TOXICITY ENDPOINT	TEST METHODS AND APPROACHES		RECOMMENDATIONS AND STANDARD METHODS	
			OECD	OTHER AUTHORITY
SKIN SENSITISATION	Adverse outcome pathway (AOP) for skin sensitisation		OECD GD 168 (Part 1, Part 2), published in 2012	--
	Guideline on defined approaches to skin sensitisation		OECD Guideline 497, published in 2021	--
	Guidance on reporting of defined approaches and individual information sources to be used within integrated approaches to testing and assessment (IATA)		OECD GD 256 (Annex I, Annex II), published in 2016	--
	Use of alternative approaches to skin sensitisation as a replacement for animal testing		--	US Environmental Protection Agency policy (2018)
	Chemical toxicity assessment strategy		--	European Chemicals Agency guidance Chapter R.7a., R.7.3.4–R.7.3.7 (2017)
	OECD QSAR Toolbox	Implementing AOP workflow for skin sensitisation	OECD training manual, released in 2017	--
		Example for predicting skin sensitisation of a mixture		
		Example of how to predict the skin sensitisation potential of a chemical by read-across based on an analogue approach		
	In chemico assays addressing the AOP key event on covalent binding to proteins	Direct peptide reactivity assay (DPRA)	OECD TG 442C, revised in 2022	EURL ECVAM recommendation (2013); JaCVAM statement (2015); KoCVAM guideline (2016)
		Kinetic DPRA		--
		Amino acid derivative reactivity assay (ADRA)		--
	ARE-Nrf2 luciferase test method	KeratinoSens™	OECD TG 442D, revised in 2018	EURL ECVAM recommendation (2014); JaCVAM statement (2015); KoCVAM guideline (2017)
		LuSens		--
	In vitro assays addressing the AOP key event on activation of dendritic cells	Human cell line activation test (h-CLAT)	OECD TG 442E, revised in 2022	EURL ECVAM recommendation (2015); JaCVAM statement (2017); KoCVAM guideline (2017)
		IL-8 Luc assay		--
		U937 skin sensitization test (U-SENS™)		--
		GARDskin assay		--
PHOTOTOXICITY	3T3 neutral red uptake (NRU) phototoxicity test		OECD TG 432, revised in 2019	ESAC statement (1997); ICH safety guideline S10; KoCVAM guideline (2007)
	Reactive oxygen species (ROS) assay		OECD TG 495, published in 2019	JaCVAM statement (2015); ICH safety guideline S10
	Reconstructed human epidermis phototoxicity test method		OECD TG 498, published in 2021	ICH safety guideline S10
SKIN ABSORPTION/ PENETRATION	*In vitro* diffusion method		OECD TG 428, published in 2004	JaCVAM statement (2014); KoCVAM guideline (2009)
ACUTE SYSTEMIC TOXICITY	Guidance on waiving tests for pesticide formulations		--	Canada Pest Management Regulatory Agency guidance (2013); US Environmental Protection Agency guidance for acute dermal toxicity tests (2016)
	Strategy to replace, reduce, and refine the use of animals in the assessment of acute mammalian systemic toxicity		--	EURL ECVAM guidance (2014)
	Collaborative Acute Toxicity Modeling Suite (CATMoS)		--	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) (2021)
	3T3 neutral red uptake (NRU) cytotoxicity test to identify substances not requiring classification		--	EURL ECVAM recommendation (2013)
GENOTOXICITY/ MUTAGENICITY	OECD QSAR Toolbox: Example for predicting Ames mutagenicity using read-across		OECD training manual, released in 2017	--
	In vitro micronucleus test		OECD TG 487, revised in 2016	ESAC statement (2006); ICH safety guideline S2(R1)
	Bacterial reverse mutation test		OECD TG 471, revised in 2020	ICH safety guideline S2(R1)
	In vitro mammalian chromosome aberration test		OECD TG 473, revised in 2016	ICH safety guideline S2(R1)
	In vitro mammalian cell gene mutation test		OECD TG 476, revised in 2016	--
	In vitro mammalian cell gene mutation tests using the thymidine kinase gene		OECD TG 490, revised in 2016	ICH safety guideline S2(R1)

TOXICITY ENDPOINT	TEST METHODS AND APPROACHES	RECOMMENDATIONS AND STANDARD METHODS	
		OECD	OTHER AUTHORITY
CARCINOGENICITY	*In vitro* cell transformation assays (CTA)	OECD GD 214, published in 2015; OECD GD 231, published in 2016	EURL ECVAM recommendations (2012 and 2013)
PYROGENICITY	*In vitro* monocyte activation tests (MAT)	--	ICCVAM report (2008); ESAC statement (2006); *European Pharmacopoeia* general chapter 2.6.30; US Food and Drug Administration guidance (2012)
HAEMATOTOXICITY	CFU-GM assay	--	ESAC statement (2006)
REPRODUCTIVE TOXICITY	Embryonic stem cell test (EST)	--	ESAC statement (2001)
	Micromass embryotoxicity assay (*Note*: Animal embryos are used, therefore this test should be used only if replacing a regulatory requirement for a live-animal test using later life stages.)		
	Whole rat embryotoxicity assay (*Note*: Animal embryos are used, therefore this test should be used only if replacing a regulatory requirement for a live-animal test using later life stages.)		
ENDOCRINE DISRUPTOR SCREENING	Stably transfected transactivation *in vitro* assays to detect oestrogen receptor agonists and antagonists	OECD TG 455, revised in 2021	JaCVAM statement (2016)
	H295R steroidogenesis assay	OECD TG 456, published in 2011	--
	Stably transfected human androgen receptor transcriptional activation assay	OECD TG 458, revised in 2020	--
	Human recombinant oestrogen receptor (hrER) *in vitro* assays to detect chemicals with ER binding affinity	OECD TG 493, published in 2015	--
	Xenopus eleutheroembryonic thyroid assay (XETA) (*Note*: Animal embryos are used, therefore this test should be used only if replacing a regulatory requirement for a live-animal test using later life stages.)	OECD TG 248, published in 2019	--
AQUATIC TOXICITY	OECD QSAR Toolbox: Example for predicting acute aquatic toxicity to fish of mixture with known components	OECD training manual, released in 2017	--
	EnviroTox database to calculate threshold values	--	Health and Environmental Sciences Institute database (2018)
	Freshwater alga and cyanobacteria growth inhibition test	OECD TG 201, published in 2011	--
	Daphnia sp acute immobilisation test	OECD TG 202, published in 2004	--
	Fish cell line acute toxicity, the RTgill-W1 cell line assay	OECD TG 249, published in 2021	ISO 21115 standard (2019)
	In vitro intrinsic clearance test using cryopreserved rainbow trout hepatocytes (*Note*: Animal primary cells are used, therefore this test should be used only if replacing a regulatory requirement for a live-animal test.)	OECD TG 319A, published in 2018; OECD GD 280, published in 2018	--
	In vitro intrinsic clearance test using rainbow trout liver S9 sub-cellular fraction (*Note*: Animal primary cells are used, therefore this test should be used only if replacing a regulatory requirement for a live-animal test.)	OECD TG 319B, published in 2018; OECD GD 280, published in 2018	--
	Fish embryo acute toxicity test (FET) (*Note*: Animal embryos are used, therefore this test should be used only if replacing a regulatory requirement for a live-animal test using later life stages.)	OECD TG 236, published in 2013	EURL ECVAM recommendation (2014)
ALL ENDPOINTS	Guidance on considerations for waiving or bridging of mammalian acute toxicity tests	OECD GD 237, published in 2016	--
	Guidance on waiving or bridging of acute toxicity tests for pesticides	--	Health Canada guidance (2013)
	Guidance on the reporting of defined approaches to be used within integrated approaches to testing and assessment	OECD GD 255, published in 2016	--
	Guidance on the validation of QSAR models	OECD GD 69, published in 2007	--
	OECD QSAR Toolbox: Guidance documents and training materials	OECD, revised in 2018	--
	QSAR Model Database	--	Database maintained by the European Commission Joint Research Centre
	Various modelling programs	--	For example, programs from Lhasa Limited, Instem, ScitoVation, and Simulations Plus
	Guidance on the grouping of chemicals	OECD GD 194, published in 2014	--
	Read-across assessment framework	--	European Chemicals Agency guidance (2017)
	Guidance on good *in vitro* method practices	OECD GD 286, published in 2018	--
	Guidance on describing non-guideline *in vitro* test methods	OECD GD 211, published in 2014	--
	Classification of mixtures based on the toxicity of ingredients	--	United Nations "Globally Harmonized System of Classification and Labelling of Chemicals" guidance (2015); US Environmental Protection Agency pilot program

ENDPOINT	REPLACEMENT METHOD OR STRATEGY	REGULATORY ACCEPTANCE
BIOLOGICS TESTING	*In vitro* leptospirosis vaccine potency assay	USDA supplemental assay methods (SAM) 624, 625, 626, and 627
	In vitro erysipelas vaccine potency assay	USDA SAM 612 and 613
	In vitro clostridial vaccine potency assay	USDA draft SAM 220
	In vitro tetanus toxoid potency assay	USDA SAM 217
	In vitro recombinant antibody production methods	See ThePSCI.eu/antibodies.
	Veterinary target animal batch safety test (TABST)	Can be waived following demonstration of compliance; USDA CVB memorandum 800.116
	Revocation of general safety tests (GST)/abnormal toxicity tests (ATT)	FDA amended biologics regulations to revoke GST (2015); all *European Pharmacopoeia* monographs revised to revoke the ATT (2017)

FOR ALL ENDPOINTS, *IN VITRO* METHODS DEVELOPED IN HOUSE SHOULD ALWAYS BE USED.

Researchers should make every effort to use available non-animal methods. If these methods are not accepted by regulatory agencies, information on additional replacement, reduction, and refinement methods can be found here:

- NICEATM Accepted Alternative Methods
- EURL ECVAM Tracking System for Alternative Methods Towards Regulatory Acceptance
- EURL ECVAM Dataset on Alternative Methods to Animal Experimentation

DETAILED INFORMATION ON THE GUIDANCE DOCUMENTS AND TEST METHODS DESCRIBED IN THIS DOCUMENT CAN BE FOUND AT THE FOLLOWING SITES:

- OECD Guidelines for the Testing of Chemicals
- OECD Adopted Guidance and Review Documents, Series on Testing and Assessment
- EURL ECVAM Validated Test Methods
- NICEATM Accepted Alternative Methods
- ICCVAM Test Method Evaluations
- USDA Listing of Supplemental Assay Methods

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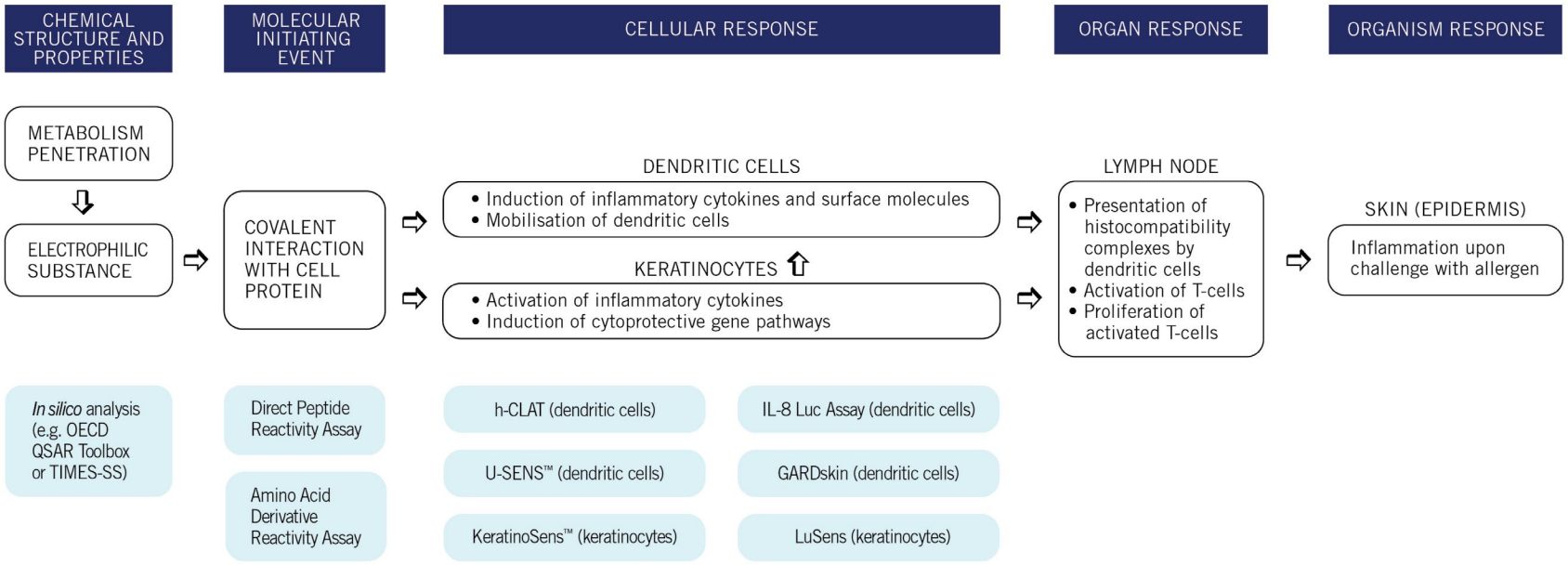
IN VITRO AND *IN CHEMICO* METHODS FOR PREDICTING SKIN SENSITISATION

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



METHOD	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN	GHS CATEGORISATION
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 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 *in silico* 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.
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)
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.	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
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 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. 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. 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.

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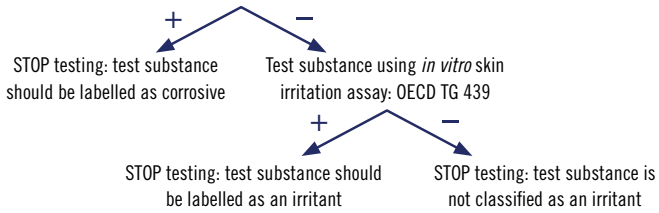
IN VITRO METHODS FOR PREDICTING SKIN IRRITATION AND CORROSION

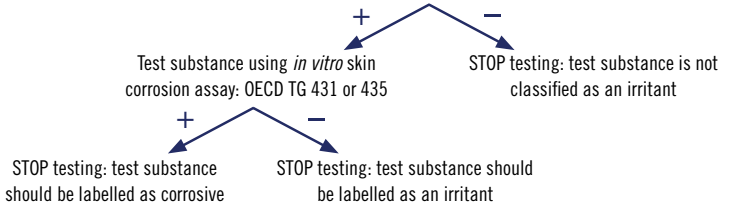
START here if you suspect your test substance is corrosive

TOP-DOWN APPROACH



START here if you suspect your test substance is not corrosive

BOTTOM-UP APPROACH



METHOD	PRINCIPLE OF THE TEST	RHE MODEL (IF APPLICABLE)	APPLICABILITY DOMAIN	GHS CATEGORISATION
OECD TG 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method	The test substance is applied to a three-dimensional reconstructed human epidermis (RhE) model. Following exposure and a post-exposure incubation period, the vital dye MTT is added and cell viability determined. Optional histology may also be conducted to gain further information.	• EpiSkin™ (L'Oréal, France) • EpiDerm™ (MatTek, US) • SkinEthic™ (L'Oréal, France) • LabCyte EPI-MODEL24 (J-TEC, Japan) • epiCS® (Phenion, Germany) • Skin+™ (Sterlab, France) • KeraSkin™ SIT (Biosolution Co, Republic of Korea)	Applicable to solids, semi-solids, liquids, waxes, and mixtures	Discriminates skin irritants (Cat 2) from substances not classified for skin irritation (No Cat). Materials that test positive should be tested for skin corrosion (bottom-up approach).
OECD TG 431: *In Vitro* Skin Corrosion: Reconstructed Human Epidermis Test Method	The test substance is applied topically to a three-dimensional RhE model. Corrosive chemicals are able to penetrate the tissue and are cytotoxic to cells in the underlying layers. Cell viability is measured using the vital dye MTT.	• EpiSkin™ (L'Oréal, France) • EpiDerm™ (MatTek, US) • SkinEthic™ (L'Oréal, France) • epiCS® (Phenion, Germany) • LabCyte EPI-MODEL24 (J-TEC, Japan)	Applicable to solids, semi-solids, liquids, waxes, and mixtures	Discriminates non-corrosive substances from corrosive substances (Cat 1) and allows subcategorisation into 1A or 1B and 1C together. Materials that test negative should be tested for skin irritation (top-down approach).
OECD TG 435: *In Vitro* Membrane Barrier Test Method for Skin Corrosion	The test substance is applied to the surface of an artificial membrane barrier designed to respond in a manner similar to skin *in vivo*. The time taken for the test substance to penetrate the barrier predicts corrosivity.	N/A	Applicable to solids, liquids, and emulsions. Aqueous chemicals with a pH in the range of 4.5 to 8.5 may not qualify for testing.	Discriminates non-corrosive substances from corrosive substances and allows full subcategorisation into 1A, 1B, and 1C.

Additional Reading

• Organisation for Economic Co-operation and Development. 2014. New guidance document on an integrated approach on testing and assessment (IATA) for skin corrosion and irritation. Series on Testing and Assessment, No 203.
• European Chemicals Agency. 2017. Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance. Version 6.0. See R.7.2.
• PETA Science Consortium International. In vitro methods for skin irritation and corrosion testing. Training tool.

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METHODS FOR PREDICTING SERIOUS EYE DAMAGE AND IRRITATION

METHOD	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN	GHS CATEGORISATION
DEFINED APPROACHES			
OECD Test Guideline 467: Defined Approaches for Serious Eye Damage and Eye Irritation, comprising two defined approaches for liquids (DAL1 and DAL2)	DAL1 combines information from three sources: (1) physiochemical properties of the test substance, (2) results from testing in OECD TG 437 BCOP assay using the laser light–based opacitometer, and (3) results from testing in OECD TG 492 (either the EpiOcular Eye Irritation Test or the SkinEthic Human Corneal Epithelium Eye Irritation Test). DAL2 combines information from two sources: (1) results from testing in OECD TG 437 BCOP assay using the laser light–based opacitometer and (2) OECD TG 491 Short Time Exposure assay.	DAL1 is applicable to neat non-surfactant liquids, and DAL2 is applicable to neat and diluted non-surfactant liquids. For both approaches, the applicability domain of each test guideline used within the defined approach should also be considered.	Both approaches can be used for the identification of substances causing serious eye damage (GHS Cat 1), causing eye irritation (GHS Cat 2), and not requiring classification for eye irritation or serious eye damage (GHS No Cat). Each approach can be used as a full replacement for the Draize rabbit eye irritation test.
RECONSTRUCTED THREE-DIMENSIONAL HUMAN TISSUE ASSAYS			
OECD Test Guideline 492: Reconstructed Human Cornea-like Epithelium Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage (e.g. EpiOcular™, SkinEthic™, LabCyte, and MCTT HCE™)	The test substance is applied to reconstructed tissue from human cells, which have been cultured to form a stratified, highly differentiated squamous epithelium that is morphologically similar to that found in the human cornea. Cell viability (MTT or WST-8 assay) is used to predict toxicity.	This assay is applicable to substances and mixtures and to solids, liquids, semi-solids, and waxes.	This assay can be used for the identification of substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).
OECD Test Guideline 492B: Reconstructed Human Cornea-like Epithelium Test Method for Eye Hazard Identification (SkinEthic™)	The test substance is applied to reconstructed tissue from human cells (as in OECD TG 492). Depending on whether the test substance is a solid or a liquid, cell viability is assessed at two or three exposure times, respectively.	This assay is applicable to substances and mixtures and to solids, liquids, semi-solids, and waxes.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1), causing eye irritation (GHS Cat 2), and not requiring classification for eye irritation or serious eye damage (GHS No Cat). This method can be used as a full replacement for the Draize rabbit eye irritation test.
OECD Test Guideline 494: Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage	Human corneal epithelium models fabricated in a collagen vitrigel membrane are exposed to a test substance. Damage to the barrier function of the models is assessed by analysing time-dependent changes in transepithelial electrical resistance values.	This assay is applicable to substances and mixtures with a pH > 5 (based on 2.5% weight/volume preparation). It is not applicable to solids.	This assay can be used for the identification of substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).
ORGANOTYPIC *EX VIVO* ASSAYS			
OECD Test Guideline 437: Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	The test substance is directly applied to cows' eyes obtained as by-products from abattoirs. Corneal opacity (measured quantitatively as the amount of light transmission through the cornea) and permeability (measured quantitatively as the amount of sodium fluorescein dye that passes across the full thickness of the cornea) are measured. Optional histopathology can be conducted for additional information.	This assay is applicable to solids, liquids (including semi-solids, creams, and waxes), and mixtures.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).
OECD Test Guideline 438: Isolated Chicken Eye (ICE) Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	The test substance is directly applied to chickens' eyes obtained as by-products from abattoirs. Corneal swelling, opacity, and fluorescein retention are assessed.	This assay is applicable to solids (which may be soluble or insoluble in water), liquids, emulsions, and gels.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).
CYTOTOXICITY AND CELL FUNCTION BASED *IN VITRO* ASSAYS			
OECD Test Guideline 460: Fluorescein Leakage (FL) Test Method for Identifying Ocular Corrosives and Severe Irritants	Epithelial monolayer Madin-Darby canine kidney cells are cultured on permeable inserts. The test chemical is applied for 1 minute and then removed. Next, the non-toxic, highly fluorescent sodium fluorescein dye is added, and the amount of dye that passes through the cell layer is measured spectrofluorometrically and used to predict toxicity.	This assay is applicable to water-soluble chemicals or mixtures. There are limitations for coloured or highly viscous substances. (However, predictivity is improved by increasing the number of wash steps.) It is not applicable to strong acids and bases, cell fixatives, or highly volatile substances.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1).
OECD Test Guideline 491: Short Time Exposure (STE) *In Vitro* Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	This test measures cell viability (via the MTT assay) of Statens Seruminstitut Rabbit Cornea (SIRC) corneal epithelial cells in 96 well plates. As compounds are generally cleared from human eyes in 1 to 2 minutes and from rabbit eyes in 3 to 4 minutes, this test requires a 5-minute exposure.	This assay is applicable to test chemicals that are soluble in saline, DMSO, or mineral oil.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).

METHOD	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN	GHS CATEGORISATION
MACROMOLECULAR MATRIX ASSAYS			
OECD Test Guideline 496: *In Vitro* Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	The test substance is directly applied to an *in chemico* macromolecular matrix model composed of lipids, proteins, glycoproteins, carbohydrates, and low molecular weight substances that model the cellular structure of the human corneal epithelium. An increase in optical density is used to predict the ocular hazard effects of chemicals.	This assay is applicable to solids (which may be soluble or insoluble in water) and liquids (which may be viscous or non-viscous) whose 10% solution/dispersion has a pH in the range $4 \leq pH \leq 9$. There are some limitations for intensely coloured chemicals, chemicals that cause salting-out precipitation, high concentrations of some surfactants, and highly volatile chemicals. It is also applicable to mixtures.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).

For more information on these methods, please see our publication, Clippinger AJ et al. Human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations. *Cutan Ocul Toxicol.* 2021;40(2):145-167.

For more information on tiered testing strategies for serious eye damage and eye irritation, please see the following publications:

- Organisation for Economic Co-operation and Development. 2019. Guidance document on integrated approaches to testing and assessment (IATA) for serious eye damage and eye irritation. No 263. Series on Testing and Assessment.
- US Environmental Protection Agency. 2015. Use of an alternate testing framework for classification of eye irritation potential of EPA pesticide products.
- European Chemicals Agency. 2017. Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance. Version 6.0. See R.7.2.8–R.7.2.11.

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