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, revised in 2024	--
	Guideline on defined approaches to eye irritation		OECD test guideline (TG) 467, revised in 2024	--
	Chemical toxicity assessment strategy		--	European Chemicals Agency guidance Chapter R.7a., R.7.2.8–R.7.2.11 (2017)
	Framework to assess eye irritation or corrosion in new chemicals		--	US Environmental Protection Agency policy (2024)
	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 2024	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, revised in 2024	--
	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, revised in 2024	--
	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); ISO 10993-23:2021-biological evaluation of medical devices, Part 23: Tests for irritation; JaCVAM statement (2013); KoCVAM guideline (2017)
		SkinEthic™ (L'Oréal, France)		ESAC statement (2008); ISO 10993-23:2021-biological evaluation of medical devices, Part 23: Tests for irritation; 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, 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 2024	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 2024	EURL ECVAM recommendation (2014); JaCVAM statement (2015); KoCVAM guideline (2017)
		LuSens		--
		Epidermal sensitisation assay (EpiSensA)		
	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 2024	EURL ECVAM recommendation (2015); JaCVAM statement (2017); KoCVAM guideline (2017)
		IL-8 Luc assay		--
		U937 skin sensitization test (U-SENS™)		--
		GARDskin assay		--
PHOTOTOXICITY	Integrated approaches to testing and assessment (IATA) for phototoxicity testing		OECD GD 397, published in 2024	--
	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)

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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)
	Case study on the use of integrated approaches for testing and assessment (IATA) for chronic toxicity and carcinogenicity of agrichemicals with exemplar case studies		OECD GD 402	--
DEVELOPMENTAL NEUROTOXICITY	Initial recommendations on evaluation of data from the developmental neurotoxicity (DNT) *in vitro* testing battery		OECD GD 377, published in 2023	--
IMMUNOTOXICITY	*In vitro* immunotoxicity	IL-2 Luc Assay	OECD TG 444A, published in 2023	--
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); *Indian Pharmacopeia* chapter 2.2.25
	Suppression of the rabbit pyrogen test			EDQM statement (2024); *European Pharmacopoeia* general chapter 5.1.13 Pyrogenicity
	Recombinant Factor C (rFC) tests			*European Pharmacopoeia* general chapter 2.6.32
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, revised in 2024	--
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	--
	Fish cell line acute toxicity, the RTgill-W1 cell line assay		OECD TG 249, published in 2021	ISO 21115 standard (2019)
BIOACCUMULATION	*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	

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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	--
	Guidance on the validation, qualification, and regulatory acceptance of new approach methodologies	--	ICCVAM report (2024)
	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 (2023)
	In vitro erysipelas vaccine potency assay	USDA SAM 612 (2015) and 613 (2022)
	In vitro clostridial vaccine potency assay	USDA draft SAM 220
	In vitro tetanus toxoid potency assay	USDA SAM 217 (2022)
	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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