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 on testing and assessment (IATA) for serious eye damage and irritation		OECD guidance document (GD) 263, published in 2017	--
	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 test guideline (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 Co, Ltd, South Korea)		
	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 2018	ICCVAM report (2013); JaCVAM statement (2016); KoCVAM guideline (2017)
	Vitrigel-eye irritancy test (EIT) method		OECD TG 494, published in 2019	--
	In vitro macromolecular test method		OECD TG 496, published in 2019	--
	Bovine corneal opacity and permeability (BCOP) test method		OECD TG 437, revised in 2017	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 on 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 2019	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)		--

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			OECD	OTHER AUTHORITY
SKIN SENSITISATION	Adverse outcome pathway (AOP) for skin sensitisation		OECD GD 168 (Part 1, Part 2), published in 2012	--
	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 for 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 adverse outcome pathway (AOP) key event on covalent binding to proteins	Direct peptide reactivity assay (DPRA)	OECD TG 442C, revised in 2019	EURL ECVAM recommendation (2013); JaCVAM statement (2015); KoCVAM guideline (2016)
		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 adverse outcome pathway (AOP) key event on activation of dendritic cells	Human cell line activation test (h-CLAT)	OECD TG 442E, revised in 2018	EURL ECVAM recommendation (2015); JaCVAM statement (2017); KoCVAM guideline (2017)
		IL-8 Luc assay		--
		U937 skin sensitization test (U-SENS™)		
PHOTOTOXICITY	3T3 neutral red uptake (NRU) phototoxicity test		OECD TG 432, revised in 2019	ESAC statement (1997); ICH S10; KoCVAM guideline (2007)
	Reactive oxygen species (ROS) assay		OECD TG 495, published in 2019	JaCVAM statement (2015); ICH S10
	Reconstructed human skin model assays		--	ICH S10
SKIN ABSORPTION/ PENETRATION	*In vitro* diffusion method		OECD TG 428, published in 2004	JaCVAM statement (2014); KoCVAM guideline (2009)
ACUTE SYSTEMIC TOXICITY	Guidance for 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) (2019)
	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 S2(R1)
	Bacterial reverse mutation test		OECD TG 471, revised in 1997	ICH S2(R1)
	In vitro mammalian chromosome aberration test		OECD TG 473, revised in 2016	ICH 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 S2(R1)

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		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 2016	JaCVAM statement (2016)
	H295R steroidogenesis assay	OECD TG 456, published in 2011	--
	BG1Luc oestrogen receptor method	OECD TG 457, published in 2012	JaCVAM statement (2014)
	Stably transfected human androgen receptor transcriptional activation assay	OECD TG 458, published in 2016	--
	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 (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	--
	In vitro fish acute toxicity test using the permanent rainbow trout cell line RTgill-W1	--	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 for 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 (Q)SAR models	OECD GD 69, published in 2007	--
	OECD QSAR Toolbox: Guidance documents and training materials	OECD, revised in 2018	--
	QSAR Model Database	--	Maintained by the European Commission Joint Research Centre
	Various modelling programs	--	For example, programs from Lhasa Limited, Leadscope, 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 for 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

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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 reduction and refinement methods can be found here:

- NICEATM Accepted Alternative Methods
- EURL ECVAM Tracking System for Alternative Methods Towards Regulatory Acceptance

ADDITIONAL METHODS FOR REDUCING AND REPLACING ANIMAL USE ARE AVAILABLE IN THE FOLLOWING DATABASES:

- EURL ECVAM Database Service on Alternative Methods to Animal Experimentation
- EURL ECVAM Tracking System for Alternative Methods Towards Regulatory Acceptance

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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