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Non-animal Methods for Toxicity Testing

Table of Validated and Accepted Alternative Methods

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Validation & Regulatory Acceptance Status of Alternative Test Methods & Testing Strategies

Endpoint	Method Name	Test Type[1]	Endorsement of Scientific Validity		Regulatory Acceptance	
			Lead Authority	Subsequent Endorsement(s)	International (click here for approved OECD TGs, here for Draft TGs, and here for GDs)	National/ Regional (for methods not yet accepted internationally)
Acute aquatic toxicity	Upper threshold concentration step-down approach	*In vivo*	ESAC 17 (2006)		OECD GD 126 (2010)	
Acute mammalian toxicity (oral)	Acute toxic class method	*In vivo*		ESAC 33 (2007)	OECD TG 423 (2001)	
	Fixed dose procedure	*In vivo*		ESAC 32 (2007)	OECD TG 420 (2001)	
	Up-and-down procedure	*In vivo*	ICCVAM (2001)	ESAC 34 (2007)	OECD TG 425 (2006)	
	Normal human keratinocyte neutral red uptake (NHK NRU) assay	*In vitro*[2]	ICCVAM (2006)		OECD GD 129 (2010)	US agencies (2008)
	Balb/c 3T3 neutral red uptake assay	*In vitro*[2]	ICCVAM (2006)		OECD GD 129 (2010)	US agencies (2008)
Acute mammalian toxicity (inhalation)	Acute toxic class method	*In vivo*			OECD TG 436 (2009)	
	Fixed concentration procedure	*In vivo*			Draft TG OECD 433	
Biologics & Vaccines	ELISA for erysipelas vaccines batch potency testing	*In vitro*	ESAC 14 (2002)			European Pharmacopeia
	ELISA for human tetanus vaccines batch potency testing	*In vitro*	ESAC 9 (2000)			European Pharmacopeia
	Toxin binding inhibition test for	*In vitro*	ESAC 10			European

	human tetanus vaccines batch potency testing		(2000)			Pharmacopeia
Chronic toxicity	*Ending* 1-year dog studies of pesticides	*In vivo*	ESAC 26 (2006)			Revised US EPA Pesticide Data Requirements
Dermal penetration	*In vitro* skin absorption methods	*In vitro*	OECD Expert Group (2002)		OECD TG 428 (2004) OECD GD 28 (2004) Draft OECD Guidance Notes (2010)	
Endocrine active substances[3]	Androgen receptor binding assay (rat prostate cytosol)	*Ex vivo*				OPPTS TG 890.1150 (EPA, 2009)
	Aromatase inhibition assay (human recombinant)	*In vitro*				OPPTS TG 890.1200 (EPA, 2009)
	Estrogen receptor (ER)-alpha transcriptional activation assay for estrogen agonists (STTA)	*In vitro*	OECD/EPA		OECD TG 455 (2009)	OPPTS TG 890.1300
	Estrogen receptor binding assay rat uterine cytosol (ER-RUC)	*Ex vivo*				OPPTS TG 890.1250 (EPA, 2009)
	H295R steroidogenesis assay	*In vitro*	OECD/EPA		OECD TG 456 (2011)	OPPTS TG 890.1550 (EPA, 2009)
	US EPA Tier 1 Screening Battery	*In vitro*/*In vivo*				US EPA (2009)
	BG1Luc ER TA test method for estrogen agonists and antagonists	*In vitro*	ICCVAM (expected 2011)			
Eye corrosion	Bovine corneal opacity permeability (BCOP) test	*In vitro*	ICCVAM (2007)	ESAC 27 (2007) INVITTOX Protocol 127 JaCVAM (2009) JaCVAM Regulatory Acceptance	OECD TG 437 (2009) OECD Proficiency Standards Draft OECD GD on Supplement to TG 437 and	

				Board (2009)	438 (histopathology) (2009)	
	Cytosensor Microphysiometer modified (cytotoxicity/cell-based assay)	*In vitro*	ESAC 40 (2009)[4] INVITTOX Protocol 102 modified	ICCVAM (2010)[5]	Draft OECD TG (2010)	
	Fluorescein Leakage (cytotoxicity/cell-based assay)	*In vitro*	ESAC 40 (2009)[6] INVITTOX Protocol 71		Draft OECD TG (2010)	
	Hen's egg test-chorioallantoic membrane (HET-CAM)	*In vitro*				EU Competent Authorities for Dangerous Substances Directive
	Isolated chicken eye (ICE) test	*In vitro* or *Ex vivo*	ICCVAM (2007)	ESAC 28 (2007) INVITTOX Protocol 80 JaCVAM (2009) JaCVAM Regulatory Acceptance Board (2009)	OECD TG 438 (2009) OECD Proficiency Standards Draft OECD GD on Supplement to TG 437 and 438 (histopathology) (2009)	
	Isolated rabbit eye test	*In vitro* or *Ex vivo*				EU Competent Authorities for Dangerous Substances Directive
	Routine use of topical anesthetics, systemic analgesics, and humane endpoints	*In vivo*	ICCVAM (2009)		Expected 2012	
Eye irritation	Cytosensor Microphysiometer modified (cytotoxicity/cell-function based *in vitro* assay)	*In vitro*	ESAC 40 (2009)[4] INVITTOX Protocol 102 modified	ICCVAM (2010)[5]	Draft OECD TG (2010)	
	Rabbit low-volume eye test (LVET)	*In vivo*	ESAC 41 (2009)[7]	ICCVAM (2009)[8]		
	Routine use of topical anesthetics, systemic analgesics, and	*In vivo*	ICCVAM (2009)		Expected 2012	

	humane endpoints					
Genotoxicity	Bacterial reverse mutation (Ames) test	*In vitro*			OECD TG 471 (1997)	
	In vitro cell gene mutation test	*In vitro*			OECD TG 476 (1997)	
	In vitro chromosomal aberration test	*In vitro*			OECD TG 473 (1997)	
	In vitro mammalian cell micronucleus test	*In vitro*	ESAC 24 (2006)	ICCVAM comments to OECD	OECD TG 487 (2010)	
	In vitro sister chromatid exchange test	*In vitro*			OECD TG 479 (1986)	
	In vitro unscheduled DNA synthesis test	*In vitro*			OECD TG 482 (1986)	
	Saccharomyces cerevisiae gene mutation assay	*In vitro*			OECD TG 480 (1986)	
	Saacharomyces cerevisiae mitotic recombination assay	*In vitro*			OECD TG 481 (1986)	
Hematotoxicity: acute neutropenia	Colony forming unit granulocyte macrophage (CFU-GM) assay	*In vitro*	ESAC 18 (2006)			Submitted to EMEA
Immunotoxicity/Skin Sensitization	Local lymph node assay (LLNA)	*In vivo*	ICCVAM (1999) ICCVAM Protocol and Performance Standards (2009)	ESAC 6 (2000)	OECD TG 429 (2002) Updated OECD TG 429 (2010)	
	Reduced LLNA: rLLNA[9]	*In vivo*	ESAC 29 (2007) Performance Standards: ESAC 37 (2008)	ICCVAM (2009) Performance Standards (2009)	Updated OECD TG 429 (2010)	
	Nonradiolabelled LLNA: DA	*In vivo*	JaCVAM (2008) JaCVAM Regulatory Acceptance Board (2008)	ICCVAM (2009)[10]	OECD TG 442A (2010)	

	Nonradiolabelled LLNA: BrdU-ELISA	*In vivo*	ICCVAM (2009)[10]	JaCVAM (2010) JaCVAM Regulatory Acceptance Board (2010)	OECD TG 442B (2010)	
	LLNA for Potency Categorization	*In vivo*	ICCVAM (2009)		UN GHS (2009)	US agencies (anticipated 2011)
Phototoxicity	3T3 Neutral Red Uptake Phototoxicity Test	*In vitro*	ESAC 1 (1997)		OECD TG 432 (2004)	
	3T3 NRU Phototoxicity Test: Application to UV filter chemicals	*In vitro*	ESAC 4 (1998)		OECD TG 432 (2004)	
Pyrogenicity	Human whole blood IL-1	*In vitro*	ESAC 19 (2006)	ICCVAM (2008)[11]		EMEA; European Pharmacopeia; US agencies
	Human whole blood IL-6	*In vitro*	ESAC 20 (2006)	ICCVAM (2008)[11]		EMEA; European Pharmacopeia; US agencies
	Human cryopreserved whole blood IL-1	*In vitro*	ESAC 23 (2006)	ICCVAM (2008)[11]		EMEA; European Pharmacopeia; US agencies
	PBMC IL-6	*In vitro*	ESAC 21 (2006)	ICCVAM (2008)[11]		EMEA; European Pharmacopeia; US agencies
	MM6 IL-6	*In vitro*	ESAC 22 (2006)	ICCVAM (2008)[11]		EMEA; European Pharmacopeia; US agencies
	Limulus amebocyte lysate (LAL) test	*In vitro*				European Pharmacopeia (5.0); US Pharmacopeia (85)
Reproductive & developmental toxicity	Embryonic stem cell test for embryotoxicity	*In vitro*	ESAC 13 (2002)		See Draft OECD GD 43, page 18	
	Micromass embryotoxicity assay	*Ex vivo*	ESAC 11 (2002)			

	Whole rat embryotoxicity assay	*Ex vivo*	ESAC 12 (2002)			
	Extended one-generation reproductive toxicity study	*In vivo*			OECD TG 443 (2011)	
Skin corrosion	EST-1000 human reconstructed epidermis	*In vitro*	ESAC 39 (2009) ESAC 43 (2009)[12]		OECD TG 431 (2004) Draft Updated TG 431 (2009)	
	Corrositex® noncellular membrane	*In vitro*	ICCVAM (1999)	ESAC 8 (2000)	OECD TG 435 (2006)	
	EpiSkin® human skin model	*In vitro*	ESAC 2 (1998) ESAC 43 (2009)[12]	ICCVAM (2002) Performance Standards (2004)	OECD TG 431 (2004) Draft Updated TG 431 (2009)	
	EpiDerm™ human skin model	*In vitro*	ESAC 7 (2000) ESAC 43 (2009)[12]	ICCVAM (2002) Performance Standards (2004)	OECD TG 431 (2004) Draft Updated TG 431 (2009)	
	Rat skin transcutaneous electrical resistance (TER)assay	*Ex vivo*	ESAC 3 (1998)	ICCVAM (2002) Performance Standards (2004)	OECD TG 430 (2004) Draft Updated TG 430 (2009)	
	SkinEthic™ human skin model	*In vitro*	ESAC 25 (2006) ESAC 43 (2009)[12]	ICCVAM	OECD TG 431 (2004) Draft Updated TG 431 (2009)	
	Vitrolife-Skin human reconstructed epidermis	*In vitro*	JaCVAM (2008) JaCVAM Regulatory Acceptance Board (2008)	ESAC 43 (2009)[12]	OECD TG 431 (2004) Draft Updated TG 431 (2009)	
Skin irritation	EpiSkin® skin irritation test (with MTT reduction)	*In vitro*	ESAC 31 (2007) ESAC 38 (2009) (Performance under UN GHS; Reference Chemicals; Performance	JaCVAM (2010) JaCVAM Regulatory Acceptance Board (2010)	OECD TG 439 (2010)	

			Standards) ESAC 42 (2009) (Updated Performance Standards)			
	EpiDerm™ skin irritation test (with MTT reduction)	*In vitro*	ESAC 30 (2007)[13]			EU test method B.46 in COM regulation 440/2008/EC
	EpiDerm™ SIT model (EPI-200)	*In vitro*	ESAC 36 (2008) ESAC 38 (2009) (Performance under UN GHS; Reference Chemicals; Performance Standards) ESAC 42 (2009) (Updated Performance Standards)		OECD TG 439 (2010)	
	SkinEthic RHE model	*In vitro*	ESAC 36 (2008) ESAC 38 (2009) (Performance under UN GHS; Reference Chemicals; Performance Standards) ESAC 42 (2009) (Updated Performance Standards)		OECD TG 439 (2010)	

[1] All *in vitro* and *ex vivo* methods listed; *in vivo* methods proposed to reduce or refine animal use also listed

[2] Replaces animal use for initial dose setting, but *in vivo* test required to complete assessment

[3] Screening assays to be used as part of a broader testing strategy

[4] Recommended for use as initial step within a Top-Down Approach to identify ocular corrosives and severe irritants (EU R41, GHS Category 1, and EPA Category I) for water-soluble chemicals and/or as initial step within a Bottom-Up Approach to identify non-irritants (EU:NC; GHS: NC; EPA: cat IV) for water-soluble surfactants and water-soluble surfactant-containing mixtures; does NOT correctly identify moderate and mild ocular irritants (EU: R36; GHS: Cat 2A/B; EPA: Cat II/III) so can be used for only two of the three EU and GHS classification categories for ocular irritation; cannot be used for default categorization; additional limitations on equipment availability

[5] Can be used as screening test to distinguish water-soluble surfactant chemicals and certain types of surfactant-containing formulations that are not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (i.e., EPA Category I, II, III; EU R41, R36; FHSA Irritant) for hazard classification and labeling under EPA, EU and FHSA classification systems; high false negative rate (24%-40%) for non-surfactant substances and formulations; high false positive rate (50% to 69%) for substances not labeled as irritants. Can be used as a screening test to identify water soluble substances as ocular corrosives and severe irritants (i.e., EPA Category I, EU R41, GHS Category 1) in tiered-testing strategy as part of weight-of-evidence approach; negative results need to be tested in another test method

[6] Recommended for use as initial step within a Top-Down Approach to identify ocular corrosives and severe irritants (EU R41, GSH Category 1, and EPA Category I) for water-soluble chemicals; further refinement with respect to variability and applicability domain recommended

[7] Restrictions include use of existing data only

[8] Retrospective LVET data can be used in a weight-of-evidence approach to identify potential ocular irritants

[9] rLLNA can be used for hazard classification when dose-response information is not needed

[10] ICCVAM recommendations on use of LLNA for skin sensitization potency categorization anticipated in 2011

[11] Subject to product-specific validation to demonstrate equivalence to the rabbit pyrogen test (RPT)

[12] Statement on 2 reference chemicals for in vitro skin corrosion testing

[13] Recommended as screening test or as part of sequential testing strategy; only positive test results accepted in the 2007 endorsement

Supplemental Information

A. ECVAM: Summary of test methods evaluated by ECVAM and accepted by EU regulatory authorities; ESAC statements; TSAR (Tracking System for Alternative test methods Review, Validation and Approval in the Context of EU Regulations on Chemicals)

B. ICCVAM: Summary of test methods evaluated by ICCVAM and accepted by US regulatory authorities; table showing validation and acceptance status of all methods reviewed

C. JaCVAM: Summary of test methods evaluated and accepted by Japan's regulatory authorities

D. OECD Test Guidelines (TGs), Guidance Documents, Draft Test Guidelines

E. ICH test guidelines

F. VICH test guidelines

Acronyms

EMEA: European Medicines Agency
EPA: US Environmental Protection Agency
ESAC: ECVAM Scientific Advisory Committee
GD: OECD Guidance Document
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
ICCVAM: US Interagency Coordinating Committee on the Validation of Alternative Methods
JaCVAM: Japanese Center for the Validation of Alternative Methods
OECD: Organisation for Economic Cooperation and Development
OPPTS: US EPA's Office of Prevention, Pesticides and Toxic Substances
TG: OECD Test Guideline