Test Methods Reviewed or Under Consideration by ICCVAM by Toxicity Endpoint

Toxicity Endpoint	No.	Test Method [No.]	Regulatory Application and ICCVAM Recommendations
Acute Systemic Toxicity	6	Up-and-down procedure (UDP) for acute oral toxicity	In 2001, ICCVAM recommended the revised UDP as a replacement alternative for the traditional *in vivo* rodent LD_{50} test for assessing acute oral systemic toxicity. The updated UDP was adopted by OECD as TG 425 in 2003.
		In vitro basal cytotoxicity methods [2]	In 2007, ICCVAM recommended two *in vitro* test methods as reduction alternatives to estimate the starting dose in the UDP, the acute toxic class method, and the fixed dose procedure for assessing acute oral systemic toxicity. Recommendations were accepted by U.S. Federal agencies. OECD Guidance Document 129 for implementation of the test methods was published in 2010.
		Biotransformation enzyme induction assays [2]	NICEATM and ICCVAM participants are providing input and guidance to an ECVAM Validation Study of a human hepatic biotransformation enzyme induction assay using cryopreserved HepaRG cells and cryopreserved human hepatocytes. This project is ongoing.
		UDP for acute dermal toxicity	NICEATM and the ICCVAM Interagency Acute Toxicity Working Group are developing the dermal UDP as a potential replacement alternative for OECD TG 402, the traditional *in vivo* rodent LD_{50} test for assessing acute dermal systemic toxicity. This project is ongoing.
Biologics Testing	28	*In vivo* alternatives *Ex vivo* alternatives *In vitro* cell-based methods *In vitro* enzymatic alternatives [23 total]	In 2006, various reduction, refinement and replacement alternatives to the mouse LD_{50} assay for botulinum toxin detection and potency testing were reviewed at a NICEATM-ICCVAM workshop and future activities recommended.
		In vitro ELISA replacement potency release tests for veterinary *Leptospira* vaccines [4]	*In vitro* ELISA antigen quantification methods for potency determination of four veterinary *Leptospira* vaccines were reviewed at a 2010 NICEATM-ICCVAM workshop and future activities recommended. These tests replace the vaccination-challenge test previously performed in hamsters.
		Serology potency test for veterinary rabies vaccines	The serum neutralization test approved by the European Pharmacopoeia Commission to replace the vaccination-challenge test in mice was reviewed at a 2011 NICEATM-ICCVAM workshop and future activities recommended.
Developmental Toxicity	1	Frog embryo teratogenesis assay: *Xenopus* (FETAX)	FETAX was reviewed at a 2000 NICEATM-ICCVAM-sponsored workshop as a reduction or replacement alternative to assess the developmental toxicity of chemicals and mixtures. Data gaps and inadequacies were identified and future activities recommended.

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Eye Corrosion/ Irritation	20	*In vitro* test methods for detecting ocular corrosives and severe irritants [4]	ICCVAM recommended the bovine corneal opacity and permeability (BCOP) and the isolated chicken eye (ICE) test methods as screening tests for identifying corrosives and severe irritants, with certain limitations. Recommendations were accepted by U.S. Federal agencies in 2008. Two other methods were not recommended for regulatory hazard classification purposes until further developed and evaluated. OECD Test Guidelines for BCOP (TG 437) and ICE (TG 438) are now available.
		Use of topical anesthetics, systemic analgesics, and humane endpoints in *in vivo* ocular safety testing [1]	ICCVAM recommendations on routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during required *in vivo* ocular safety testing were accepted by Federal agencies in 2011.
		In vitro test methods for assessment of the eye irritation potential of antimicrobial cleaning products [3]	ICCVAM evaluated an approach using the BCOP, the EpiOcular and the Cytosensor microphysiometer (CM) test methods to assess the eye irritation potential of certain antimicrobial cleaning products. ICCVAM recommendations for future studies were accepted by Federal agencies in 2011.
		In vitro tissue-based test methods for detecting mild to moderate irritants and nonirritants [4]	ICCVAM recommended that these four *in vitro* test methods must be improved before they can be used in regulatory safety testing to classify substances as having the potential to cause reversible, nonsevere eye injuries or as not requiring hazard labeling for eye irritation. The ICCVAM recommendations were accepted by U.S. Federal agencies in March 2011.
		In vitro cell function-based test methods for detecting mild to moderate irritants and nonirritants [4]	ECVAM evaluations of four cell function-based *in vitro* methods (fluorescein leakage, neutral red release, CM and red blood cell haemolysis test methods) for classification of ocular hazards were reviewed by ICCVAM for U.S. regulatory applicability. ICCVAM recommendations on use of the CM test method for classification of ocular hazards were accepted by U.S. Federal agencies in March 2011.
		Low volume eye test (LVET) [1]	ICCVAM recommended to Federal agencies that the LVET should not be used for future regulatory testing due to poor predictivity when compared to the standard rabbit eye test. However, data from past LVET studies may be considered in a weight-of-evidence approach to classify ocular hazards. The ICCVAM recommendations were accepted by U.S. Federal agencies in March 2011.
		Recombinant human tissue models [2]	NICEATM and ICCVAM representatives are serving on the Validation Management Group for a prospective validation of reconstructed human tissue models (EpiOcular and SkinEthic HCE) for identification of mild to moderate irritants and substances not labeled as ocular irritants.
		Short time exposure (STE) test method	As part of the ICATM collaboration, JaCVAM requested that ICCVAM conduct an international peer review of the STE test method, which assesses eye irritation potential by measuring cytotoxicity in rabbit corneal epithelial cells. NICEATM will prepare a summary review document on the validation status of the STE to be considered by the ICCVAM peer review based on a BRD provided by the test method developer.

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Endocrine Disruptors	138	*In vitro* androgen receptor (AR) binding [11] *In vitro* AR transcriptional activation (TA) [18]	In 2002, ICCVAM evaluated screens for identifying potential endocrine-disrupting chemicals. A 2003 report based on that evaluation provided guidance for protocol standardization and validation studies. A 2006 addendum to the report provided a revised reference substance list.
		In vitro estrogen receptor (ER) binding [14] *In vitro* ER TA [95]	These assays were also addressed in the 2003 report and 2006 addendum. NICEATM-ICCVAM coordinated validation studies of two *in vitro* test methods used to detect estrogenic and anti-estrogenic activities: the BG1Luc ER TA test method (also known as the LUMI-CELL ER assay) developed by XDS, Inc., and the MCF-7 cell proliferation assay developed by CertiChem, Inc. In 2012, Federal agencies accepted ICCVAM recommendations that the BG1Luc ER TA test method could be used as a screening test to identify substances with *in vitro* estrogen receptor agonist and/or antagonist activity. Data from the validation study of the CertiChem MCF-7 assay is currently being evaluated.
Genetic Toxicity	4	*In vitro* mammalian cell micronucleus test	NICEATM and the ICCVAM Interagency Genetic Toxicity Working Group (GTWG) were involved in development of OECD Test Guideline 487: *In Vitro* Mammalian Cell Micronucleus Test, published in 2010.
		In vivo rodent alkaline comet assay for detection of genotoxic carcinogens	NICEATM and the GTWG were involved in development of the validation study plan, the proposed protocol, and proposed list of reference substances, and have representatives on the Validation Study Management Team.
		In vitro TK6 alkaline comet assay	NICEATM and the GTWG were involved in development of the validation study plan, the proposed protocol, and proposed list of reference substances, and have representatives on the Validation Study Management Team.
		Syrian hamster embryo cell transformation assay	NICEATM and the GTWG provided comments to JaCVAM on their validation study plan and protocol for their validation study, as well as providing liaison members to the Validation Study Management Team; provided nominations of independent experts to serve on an ESAC peer review panel; also provided comments to ECVAM and U.S. National Coordinator on proposed OECD Test Guideline.
Pyrogenicity	5	*In vitro* pyrogenicity test methods (monocyte activation test [MAT] and four other test methods)	In 2008, ICCVAM recommended five *in vitro* pyrogenicity test methods measuring cytokine release from human cells as replacements for the rabbit pyrogen test to detect endotoxin contamination in parenteral drugs, subject to product-specific validation. All applicable Federal agencies accepted or endorsed the ICCVAM recommendations in May 2009. BiotestAG is preparing a comprehensive BRD for NICEATM to consider the validation status of the MAT for identifying nonendotoxin pyrogens. The MAT was one of the five test methods accepted by Federal agencies in 2009; the BiotestAG BRD will include results of studies performed in response to ICCVAM recommendations.

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Skin Corrosion	5	Corrositex® EpiDerm™ EPISKIN™ Rat trancutaneous electrical resistance (TER) assay SkinEthic assay	In 1999, ICCVAM recommended Corrositex® as a stand-alone assay for evaluating acids, bases and acid derivatives for the U.S. Department of Transportation; otherwise, recommended as part of a tiered testing strategy; in 2000, accepted by U.S. agencies; in 2006, adopted by OECD as TG 435. In 2002, TER and human skin models recommended as part of a tiered testing strategy; in 2004, adopted by OECD as TG 430/431.
Skin Irritation	3	EpiDerm™ EPISKIN™ SkinEthic assay	In 2008, OECD Test Guidelines were proposed for three *in vitro* tests. An expert consultation hosted by U.S. took place in 2009, and the test methods were adopted by OECD as TG 439 in 2010.
Skin Sensitization	10	Murine local lymph node assay (LLNA) - Reduced LLNA (rLLNA) - Performance standards - Applicability domain - Use for potency categorization	- In 1999, the LLNA was recommended by ICCVAM and accepted by regulatory agencies as an alternative for guinea pig tests for allergic contact dermatitis hazard testing. The LLNA was adopted in 2002 as TG 429 by OECD. - In 2009, ICCVAM made recommendations to Federal agencies on performance standards for the LLNA, an updated LLNA protocol that uses fewer animals, and use of the rLLNA to regulatory agencies. Federal agencies accepted the ICCVAM recommendations in March 2010. ICCVAM recommendations were incorporated into an updated OECD Test Guideline 429 published in July 2010. - In 2010, ICCVAM made recommendations to Federal agencies for the LLNA applicability domain and two nonradioactive LLNA methods. Federal agencies accepted the ICCVAM recommendations in February 2011. OECD test guidelines for the two nonradioactive methods (Test Guidelines 442A and 442B) incorporate ICCVAM recommendations. ICCVAM deferred a formal recommendation on a third nonradioactive method pending the receipt of additional data. - In 2011, ICCVAM recommended to Federal agencies that the LLNA may be used to categorize substances as strong sensitizers. Federal agencies accepted the ICCVAM recommendations in February 2012.
		LLNA nonradioactive methods [3]	
		In vitro approaches - *In vitro* cell-based methods [4] - Peptide reactivity assay	The KeratinoSens assay and the dipeptide reactivity assay (DPRA) are undergoing peer review at ECVAM. Validation activities are underway for the human cell line activation test (h-CLAT), the myeloid U937 skin sensitization test (MUSST), and the IL-8 Luc assay. ICCVAM is participating on the Validation Management Teams with ECVAM and JaCVAM.
Total	**220**		

Abbreviations not defined in table: ECVAM = European Centre for the Validation of Alternative Methods (now EURL ECVAM: European Union Reference Laboratory for alternatives to animal testing); EPA = U.S. Environmental Protection Agency; ESAC = ECVAM Scientific Advisory Committee; ICATM = International Cooperation on Alternative Test Methods; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; JaCVAM = Japanese Center for the Validation of Alternative

Methods; LD_{50} = Dose producing lethality in 50% of test animals; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; No. = Number of methods reviewed in each toxicity area; OECD = Organisation for Economic Co-operation and Development; TG = Test Guideline (OECD); USDA = U.S. Department of Agriculture; XDS, Inc. = Xenobiotic Detection Systems, Inc.

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