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	5	Up-and-Down Procedure (UDP)	In 2001, ICCVAM recommended the UDP as a replacement alternative for OECD Test Guideline (TG) 401, the traditional *in vivo* rodent LD_{50} test for assessing acute oral systemic toxicity. The UDP was adopted by OECD as TG 425 in 2003.
		In vitro basal cytotoxicity methods [2]	In 2007, ICCVAM recommended both *in vitro* test methods as reduction alternatives to estimate the starting dose in the UDP and Fixed Dose Procedure for assessing acute oral systemic toxicity. Recommendations were accepted by U.S. Federal agencies.
		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 HepaRG cells and cryopreserved human hepatocytes.
Biologics Testing	24	*In vivo* alternatives *Ex vivo* alternatives *In vitro* cell-based methods *In vitro* enzymatic alternatives [23 total[1]]	In 2006, various reduction, refinement and replacement alternatives to the mouse LD_{50} assay for botulinum toxin detection and potency testing were reviewed at an ICCVAM-NICEATM/ECVAM-sponsored workshop and future activities recommended.
		In vitro potency test for Leptospirosis	One ICCVAM Agency, USDA, has an ongoing validation study in conjunction with Michigan State University on an *in vitro* potency test for a Leptospirosis vaccine.
Developmental Toxicity	1	Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX)	In 2000, FETAX was reviewed at a 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.
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, to be included in EPA's Endocrine Disruptor Screening Program. In 2003, a report with guidance for protocol standardization and validation studies was released; in 2006, reference substance list was revised.
		In vitro estrogen receptor (ER) binding [14] *In vitro* ER TA [95]	Same as for *in vitro* AR assays. NICEATM-ICCVAM are sponsoring an ongoing international validation study of an *in vitro* ER TA assay, and is working with the test method developer to develop and implement validation study protocols for a second *in vitro* ER TA assay.

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Eye Corrosion/ Irritation	17	*In vitro* test methods for detecting ocular corrosives and severe irritants [4]	In 2007, the bovine corneal opacity and permeability (BCOP) and the isolated chicken eye test methods were recommended as screening tests for identifying corrosives and severe irritants, with certain limitations. Recommendations were accepted by U.S. Federal agencies. Two other methods were not recommended for regulatory hazard classification purposes until further developed and evaluated.
		In vitro test methods for assessment of the eye irritation potential of antimicrobial cleaning products [3]	An approach using the BCOP, the EpiOcular and the Cytosensor Microphysiometer test methods for evaluating the eye irritation potential of certain antimicrobial cleaning products is currently under review.
		In vitro tissue-based test methods for detecting mild to moderate irritants and nonirritants [4]	The four tissue-based *in vitro* methods evaluated by ICCVAM for detection of ocular corrosives (described above) are currently being evaluated by ICCVAM for their utility for identification of mild to moderate irritants and substances not labeled as ocular irritants.
		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, cytosensor microphysiometer and red blood cell haemolysis test methods) for identification of mild to moderate irritants and substances not labeled as ocular irritants are currently being reviewed by ICCVAM for U.S. regulatory applicability.
		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.
Genetic Toxicity	4	*In vitro* mammalian cell micronucleus test	NICEATM and the ICCVAM Genetic Toxicity Working Group (GTWG) are involved in development of a draft OECD Test Guideline and have provided comments on a study to determine the most appropriate measure of cytotoxicity for inclusion in the Test Guideline.
		In vivo rodent alkaline comet assay for detection of genotoxic carcinogens	NICEATM and the GTWG are 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 will be 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.
		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; also provided nominations of independent experts to serve on an ESAC peer review panel.
Pyrogenicity	5	*In vitro* pyrogenicity	In October 2008, ICCVAM recommended five *in vitro* pyrogenicity test methods measuring cytokine release from human cells as replacements for the rabbit test, subject to product-specific validation, to detect endotoxin contamination in parenteral drugs. All agencies have accepted or endorsed the 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 DOT; 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 based on three *in vitro* tests. An expert consultation hosted by U.S. took place in June 2009.
Skin Sensitization	10	Murine Local Lymph Node Assay (LLNA) - Reduced LLNA (rLLNA) - Use for potency determination - Applicability domain - Performance standards	In 1999, the LLNA was recommended by ICCVAM and accepted by regulatory agencies as alternative for guinea pig tests for allergic contact dermatitis; adopted in 2002 as TG 429 by OECD. In 2009, ICCVAM recommended the rLLNA to regulatory agencies and finalized performance standards for the LLNA. Recommendations included an updated protocol that uses fewer animals. ICCVAM has determined that the LLNA may be useful in determining the relative potency of sensitizers as part of a weight-of-evidence approach. ICCVAM recommendations for the LLNA applicability domain and three non-radiolabeled LLNA methods are currently being finalized.
		LLNA non-radiolabeled methods [3]	
		In vitro approaches - *In vitro* cell-based methods [2] - Peptide reactivity assay	The human cell line activation test (h-CLAT), the myeloid U937 skin sensitization test (MUSST), and the dipeptide reactivity assay are under consideration for further validation studies. ICCVAM is participating in the Validation Management Group with ECVAM and JaCVAM.
Total	**212**		

Abbreviations: ECVAM = European Centre for the Validation of Alternative Methods; EPA = U.S. Environmental Protection Agency; 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; USDA = U.S. Department of Agriculture.

[1]These methods were reviewed and discussed at an ICCVAM-NICEATM/ECVAM sponsored workshop to review the state-of-the-science and current knowledge of alternatives that may reduce, refine (reduce or eliminate pain and distress), and replace the use of mice for botulinum toxin testing (see: http://iccvam.niehs.nih.gov/methods/biologics/bot_workshop.htm)

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