

## Summary of ICCVAM Test Method Evaluations

Toxicity Endpoint	Test Method	Regulatory Application and ICCVAM Recommendations
Acute Systemic Toxicity	Up-and-down procedure (UDP) for acute oral toxicity	In 2001, ICCVAM recommended the revised UDP as a replacement alternative for the traditional <i>in vivo</i> rodent LD <sub>50</sub> test for assessing acute oral systemic toxicity. The UDP was adopted by the OECD as TG 425.
	<i>In vitro</i> basal cytotoxicity methods	In 2007, ICCVAM recommended both <i>in vitro</i> 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	NICEATM and ICCVAM participants are providing input and guidance to a EURL ECVAM validation study of a human hepatic biotransformation enzyme induction assay using cryopreserved HepaRG cells and human hepatocytes. The study was completed in 2013 and a report is being prepared.
Biologics Testing	Alternatives to the mouse LD <sub>50</sub> assay for botulinum toxin testing	In 2006, various reduction, refinement and replacement alternatives to the mouse LD <sub>50</sub> assay for botulinum toxin detection and potency testing were reviewed at an ICCVAM workshop and future activities recommended.
	<i>In vitro</i> ELISA replacement potency release tests for veterinary <i>Leptospira</i> vaccines	<i>In vitro</i> ELISA antigen quantification methods for potency determination of four veterinary <i>Leptospira</i> vaccines were reviewed at ICCVAM workshops in 2010 and 2012; participants made recommendations for future use and implementation. 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 ICCVAM workshop and future activities recommended.
Developmental Toxicity	Frog embryo teratogenesis assay: <i>Xenopus</i> (FETAX)	FETAX was reviewed at a May 2000 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	<i>In vitro</i> androgen receptor (AR) binding and transactivation (TA) assays	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.
	<i>In vitro</i> estrogen receptor (ER) binding and TA assays	These assays were also addressed in the 2003 report and 2006 addendum. NICEATM coordinated validation studies of two <i>in vitro</i> 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 <i>in vitro</i> estrogen receptor agonist and/or antagonist activity. The test method protocols for the CertiChem MCF-7 assay require additional development to improve interlaboratory reproducibility before this method can be considered validated.

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Eye Corrosion/ Irritation	<i>In vitro</i> test methods for detecting ocular corrosives and severe irritants	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 <i>in vivo</i> ocular safety testing	ICCVAM recommendations on routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during required <i>in vivo</i> ocular safety testing were accepted by Federal agencies in 2011.
	<i>In vitro</i> test methods for assessment of the eye irritation potential of antimicrobial cleaning products	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.
	<i>In vitro</i> tissue-based test methods for detecting mild to moderate irritants and nonirritants	ICCVAM recommended that these four <i>in vitro</i> 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.
	<i>In vitro</i> cell function-based test methods for detecting mild to moderate irritants and nonirritants	EURL ECVAM evaluations of four cell function-based <i>in vitro</i> methods (fluorescein leakage, neutral red release, CM and red blood cell haemolysis test methods) for classification of ocular hazards have been 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)	ICCVAM recommended to Federal agencies that the LVET should not be used for future regulatory testing due to poor predictivity when compared to the current 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	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, the Japanese Center for the Validation of Alternative Methods (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 prepared a summary review document on the validation status of the STE based on a BRD provided by the test method developer, provided this to peer reviewers, and subsequently provided a final report to JaCVAM and the test method developer.

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Genetic Toxicity	<i>In vitro</i> mammalian cell micronucleus test	NICEATM and ICCVAM scientists participated in development of OECD Test Guideline 487: <i>In Vitro</i> Mammalian Cell Micronucleus Test, published in 2010.
	<i>In vivo</i> rodent alkaline comet assay for detection of genotoxic carcinogens	NICEATM and ICCVAM scientists participated in development of the validation study plan, the proposed protocol, and proposed list of reference substances, and had representatives on the validation study management team.
	<i>In vitro</i> TK6 alkaline comet assay	NICEATM and ICCVAM scientists participated 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 ICCVAM scientists 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 EURL ECVAM-sponsored peer review panel; also provided comments to EURL ECVAM and U.S. National Coordinator on proposed OECD Test Guideline.
Pyrogenicity	<i>In vitro</i> pyrogenicity test methods (monocyte activation test [MAT] and four other test methods	In 2008, ICCVAM recommended five <i>in vitro</i> 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 2012, the Food and Drug Administration issued guidance on the use of alternatives to standard pyrogen tests that was consistent with the ICCVAM recommendations
Skin Corrosion	Corrositex® EpiDerm™ EPISKIN™ Rat Transcutaneous 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 or as part of a tiered testing strategy for other regulatory applications; these recommendations were accepted by U.S. agencies in 2000. Corrositex® was adopted by OECD in 2006 as TG 435. The TER and human skin models were adopted by OECD as TG 430 and TG 431 in 2004; NICEATM representatives served on the OECD Expert Group responsible for revisions to TG 430 and TG 431 adopted in 2013.
Skin Irritation	EpiDerm™ EPISKIN™ SkinEthic Assay	In 2008, OECD Test Guidelines were proposed based on three <i>in vitro</i> 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.

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Skin Sensitization	Murine local lymph node assay (LLNA) <ul style="list-style-type: none"> <li>- Reduced LLNA (rLLNA)</li> <li>- Performance standards</li> <li>- Applicability domain</li> <li>- Use for potency determination</li> <li>- Nonradioactive methods</li> </ul>	<ul style="list-style-type: none"> <li>- 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.</li> <li>- In 2009, ICCVAM made recommendations to Federal agencies on performance standards for the LLNA, an updated 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 TG 429 published in July 2010.</li> <li>- 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 (TG 442A and TG 442B) incorporate ICCVAM recommendations. ICCVAM deferred a formal recommendation on a third nonradioactive method pending the receipt of additional data.</li> <li>- 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.</li> </ul>
	<i>In vitro</i> approaches <ul style="list-style-type: none"> <li>- <i>In vitro</i> cell-based methods</li> <li>- Peptide reactivity assay</li> </ul>	NICEATM and ICCVAM scientists participated on the validation management teams for studies coordinated by EURL ECVAM and JaCVAM on <i>in vitro</i> methods to identify potential skin sensitizers. EURL ECVAM issued recommendations on the use of the direct peptide reactivity assay in 2014. JaCVAM-coordinated studies of the human cell line activation test and the IL-8 Luc assay are ongoing.

Abbreviations: BRD = background review document; EURL ECVAM = European Union Reference Laboratory for Alternatives to Animal Testing (formerly the European Centre for the Validation of Alternative Methods); ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; ICATM = International Cooperation on Alternative Test Methods; JaCVAM = Japanese Center for the Validation of Alternative Methods; LD<sub>50</sub> = Dose producing lethality in 50% of test animals; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; OECD = Organisation for Economic Co-operation and Development; TG = Test Guideline.

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