

**U.S. and International Acceptance of Alternative Methods 1998-2012
(Listed by Test Method Evaluation Area)**

Acute Oral Systemic Toxicity

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Up-and-down procedure for acute oral toxicity	ICCVAM peer review and report; recommended in 2001	Accepted by U.S. agencies in 2003; EPA OPPTS 870.1100 (2002)	OECD TG 425 (2001)	Via OECD
Fixed dose procedure for acute oral toxicity	ICCVAM working group contributed to test guideline development	Accepted by U.S. via OECD TG 420	OECD TG 420 (2001)	Via OECD
Acute toxic class method for acute oral toxicity	ICCVAM working group contributed to test guideline development	Accepted by U.S. via OECD TG 423	OECD TG 423 (2001)	Via OECD
Acute toxicity <i>in vitro</i> starting dose procedure, 3T3 cells	ICCVAM 2001 workshop report; ICCVAM 2006 peer review and report; recommended in 2008	Accepted by U.S. agencies in 2008	OECD GD 129 (2010)	Via OECD
Acute toxicity <i>in vitro</i> starting dose procedure, NHK cells	ICCVAM 2001 workshop report; ICCVAM 2006 peer review and report; recommended in 2008	Accepted by U.S. agencies in 2008	OECD GD 129 (2010)	Via OECD
Avian acute oral toxicity test (reduction of animal use)	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 223	OECD TG 223 (2010)	Via OECD
Harmonized guidance for nonclinical safety studies for pharmaceuticals (reduction of animal use)	ICCVAM agency initiative	Accepted by FDA via ICH in 2010	ICH Guidance Document M3(R2)	
Waiving or bridging of mammalian acute toxicity tests for pesticides and pesticide products (reduction of animal use)	ICCVAM agency initiative	EPA guidance (2012) provides for authorizing exemptions from standard requirements under 40 CFR 158.500, 40 CFR 161.340, other regulations and notices	NA	
<i>In vitro</i> post-column oxidation test method for paralytic shellfish toxin detection	ICATM activity	<i>(may be used on an interim basis while awaiting implementation)</i>	Association of Analytical Communities official method (2011); Canadian Food Inspection Agency (2011)	

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Acute Oral Systemic Toxicity (cont'd)

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/Endorsement
Harmonized guidance for preclinical safety evaluation of biotechnology-derived pharmaceuticals (reduction of animal use)	ICCVAM agency initiative	Accepted by FDA via ICH in 2012.	ICH Guidance Document S6(R1)	

Acute Dermal Systemic Toxicity

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/Endorsement
<i>In vitro</i> dermal absorption methods	ICCVAM contributed to U.S. OECD test guideline review, expert consultation meetings	Accepted by U.S. via OECD TG 428	OECD TG 428 (2004)	Via OECD

Acute Inhalation Toxicity

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/Endorsement
Inhalation toxicity—acute toxic class method	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 436	OECD TG 436 (2009)	Via OECD

Dermal Phototoxicity

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/Endorsement
3T3 NRU phototoxicity test for skin photo-irritation	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 432	OECD TG 432 (2004)	Via OECD
3T3 NRU phototoxicity test: application to UV filter chemicals	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 432	OECD TG 432 (2004)	Via OECD

Dermal Corrosivity and Irritation

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Corrositex® <i>in vitro</i> membrane barrier skin corrosivity test	ICCVAM peer review and report; recommended in 1999	Accepted by U.S. agencies in 1999; 49 CFR 173.137 (2011)	OECD TG 435 (2006)	Via OECD
EpiSkin™ <i>in vitro</i> human skin model skin corrosivity test	ICCVAM review and report; recommended in 2002	Accepted by U.S. via OECD TG 431; 49 CFR 173.137 (2011)	OECD TG 431 (2004)	Via OECD
EpiDerm™ <i>in vitro</i> human skin model skin corrosivity test	ICCVAM review and report; recommended in 2002	Accepted by U.S. via OECD TG 431; 49 CFR 173.137 (2011)	OECD TG 431 (2004)	Via OECD
SkinEthic™ <i>in vitro</i> human skin model skin corrosivity test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 431 (meets performance standards 2006)	OECD TG 431 (2004)	Via OECD
Rat TER <i>in vitro</i> skin corrosivity test	ICCVAM review and report; recommended in 2002	Accepted by U.S. via OECD TG 430	OECD TG 430 (2004)	Via OECD
EST-1000 <i>in vitro</i> test method for skin corrosivity testing	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 431 (meets performance standards 2009)	OECD TG 431 (2004)	Via OECD
EpiSkin™ <i>in vitro</i> human skin model skin irritation test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 439	OECD TG 439 (2010)	Via OECD
EpiDerm™ <i>in vitro</i> human skin model skin irritation test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 439	OECD TG 439 (2010)	Via OECD
SkinEthic™ <i>in vitro</i> human skin model skin irritation test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 439	OECD TG 439 (2010)	Via OECD

Genetic Toxicity

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
<i>In vitro</i> mammalian cell micronucleus test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 487	OECD TG 487 (2010); included in 2011 ICH harmonized guideline for testing human pharmaceuticals	Via OECD

Immunotoxicity: Allergic Contact Dermatitis

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Murine local lymph node assay (LLNA) for skin sensitization	ICCVAM peer review and report; recommended in 1999	Accepted by U.S. agencies in 1999; EPA OPPTS 870.2600 (2003) and FDA Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs (2002)	OECD TG 429 (2002) ISO (2002)	Via OECD
Updated LLNA protocol (requires 20% fewer animals)	ICCVAM peer review and report; recommended in 2009	Accepted by U.S. agencies in 2010; EPA updated policy on the use of the LLNA for end-use pesticide products in 2011	OECD TG 429 (2010)	Via OECD
Reduced LLNA protocol (requires 40% fewer animals by using only the high dose group)	ICCVAM peer review and report; recommended in 2009	Accepted by U.S. agencies in 2010; EPA adopted the rLLNA in 2011	OECD TG 429 (2010)	Via OECD
LLNA: DA for skin sensitization testing (a nonradioisotopic LLNA test method)	ICCVAM peer review and report; recommended in 2010	Accepted by U.S. agencies in 2010	OECD TG 442A (2010)	Via OECD
LLNA: BrdU-ELISA for skin sensitization testing (a nonradioisotopic LLNA test method)	ICCVAM peer review and report; recommended in 2010	Accepted by U.S. agencies in 2010	OECD TG 442B (2010)	Via OECD
LLNA for potency categorization of skin sensitizers (refinement and reduction of animal use)	ICCVAM peer review and report; recommendations in 2011	Accepted by U.S. agencies in 2012	GHS (2009)	Via GHS

Reproductive and Developmental Toxicity

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Extended one-generation reproductive toxicity study (reduction of animal use)	ICCVAM agencies contributed to OECD test guideline review	Accepted by U.S. agencies via OECD TG 443	OECD TG 443 (2012)	Via OECD

Ocular Corrosivity and Irritation

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Bovine corneal opacity and permeability <i>in vitro</i> test method to identify severe eye irritants/corrosives	ICCVAM review and report; recommended in 2007	Accepted by U.S. agencies in 2008	OECD TG 437 (2009)	Via OECD
Isolated chicken eye <i>in vitro</i> test method to identify severe eye irritants/corrosives	ICCVAM review and report; recommended in 2007	Accepted by U.S. agencies in 2008	OECD TG 438 (2009)	Via OECD
Cytosensor microphysiometer <i>in vitro</i> test method for eye safety testing	ICCVAM peer review and report; recommended in 2010	Accepted by U.S. agencies in 2011	<i>New OECD test guideline under consideration</i>	
Use of anesthetics, analgesics, and humane endpoints for <i>in vivo</i> eye safety testing (refinement of animal use)	ICCVAM peer review and report; recommended in 2010	Accepted by U.S. agencies in 2011	Updated OECD TG 405 (2012)	Via OECD
<i>In vitro</i> fluorescein leakage test method for identifying ocular corrosives and severe irritants	ICCVAM contributed to OECD test guideline review	Accepted by U.S. agencies via OECD TG 457	OECD TG 460 (2012)	Via OECD

Endocrine Disruptors

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Uterotrophic bioassay in rodents: a short-term screening test for estrogenic properties	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 440 in 2007; EPA 890.1600 (2009)	OECD TG 440 (2007)	Via OECD
Hershberger bioassay in rats: a short-term screening assay for (anti) androgenic properties	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 441; EPA OPPTS 890.1400 (2009)	OECD TG 441 (2009)	Via OECD
Stably transfected human estrogen receptor- α transcriptional activation assay for the detection of estrogenic agonist-activity of chemicals	ICCVAM contributed to U.S. OECD test guideline review, expert consultation meetings	Accepted by U.S. via OECD TG 455; EPA OPPTS 890.1300 (2009)	OECD TG 455 (2009); updated 2012 to include performance standards	Via OECD
<i>In vitro</i> H295R steroidogenesis assay	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. agencies via OECD TG 456	OECD TG 456 (2011)	Via OECD
<i>In vitro</i> BG1Luc ER TA agonist assay to identify substances that induce human ER activity	ICCVAM peer review and report; recommendations in 2012	Accepted by U.S. agencies in 2012	OECD TG 457 (2011)	Via OECD
<i>In vitro</i> BG1Luc ER TA antagonist assay to identify substances that inhibit human ER activity	ICCVAM peer review and report; recommendations in 2012	Accepted by U.S. agencies in 2012	OECD TG 457 (2011)	Via OECD

Pyrogen Testing

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Human whole blood/interleukin-1 β <i>in vitro</i> pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing	NA	Published in European Pharmacopoeia
Human whole blood/interleukin-1 β <i>in vitro</i> pyrogen test: application of cryopreserved human whole blood	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing	NA	Published in European Pharmacopoeia
Human whole blood/interleukin-6 <i>in vitro</i> pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing	NA	Published in European Pharmacopoeia
Human peripheral blood mononuclear cell/interleukin-6 <i>in vitro</i> pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing	NA	Published in European Pharmacopoeia
Monocytoid cell line Mono Mac 6/interleukin-6 <i>in vitro</i> pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing	NA	Published in European Pharmacopoeia
<i>In vitro</i> monocyte activation type pyrogen test	ICCVAM agency initiative	FDA guidance (2012) provides for use in place of USP methods	NA	

Biologics Testing

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/Endorsement
ELISA test for batch potency testing of human tetanus vaccines (refinement: antibody quantification)	ICCVAM agency consideration	27 CFR 610.10; use reviewed on a case-by-case basis	NA	Published in European Pharmacopoeia (2003)
ToBI test for batch potency testing of human tetanus vaccines (refinement: antibody quantification)	ICCVAM agency consideration	27 CFR 610.10; use reviewed on a case-by-case basis	NA	Published in European Pharmacopoeia (2003)
Use of humane endpoints in animal testing of biological products	ICCVAM agency initiative	Addressed in 9 CFR 117.4e, CVB Notice No. 04-09 (2004)	NA	
Rabies vaccine, humane endpoints	ICCVAM agency initiative	Addressed in 9 CFR 117.4e, CVB Notice No. 04-09 (2004)	NA	
Relevance of the target animal safety test for batch safety testing of vaccines for veterinary use	ICCVAM agency consideration	9 CFR 113.4 provides for authorizing exemptions from standard requirements	NA	Published in European Pharmacopoeia (2004)
ELISA test for batch potency testing of <i>Leptospira interrogans</i> serovar <i>pomona</i> (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 624 (2008)	NA	
ELISA test for batch potency testing of <i>Leptospira interrogans</i> serovar <i>canicola</i> (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 625 (2008)	NA	
ELISA test for batch potency testing of <i>Leptospira interrogans</i> serovar <i>icterohaemorrhagiae</i> (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 627 (2008)	NA	
ELISA test for batch potency testing of erysipelas vaccines (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 613 (2008)	NA	Published in European Pharmacopoeia
ELISA test for batch potency testing of <i>Leptospira kirschneri</i> serovar <i>grippotyphosa</i> (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 626 (2009)	NA	

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Biologics Testing (cont'd)

Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
Cell-based potency assay for stability and potency of botulinum neurotoxin type A products (replacement and reduction of animal use)	ICCVAM workshop in 2006	Allergan, Inc., method accepted by FDA in 2011	NA	
USDA guidelines on master reference qualification and requalification for vaccine potency assays (reduction of animal use)	ICCVAM agency initiative	Addressed in 9 CFR 113.8(d)(2), Veterinary Services Memorandum 800.211 (2011)	NA	
USDA guidelines on use of humane endpoints and methods in animal testing of biological products (refinement of animal use)	ICCVAM agency initiative	Addressed in 9 CFR 117.4(e), CVB Notice No. 12-12 (2012)	NA	
Serum neutralization test for potency testing of inactivated veterinary rabies vaccines (reduction and refinement of animal use)	ICCVAM workshop in 2011	NA	NA	Published in European Pharmacopoeia Monograph 0451 (2012)
Alternative test procedure for tuberculin, PPD Bovis, intradermic (reduces animal use by 65%)	ICCVAM agency initiative	Applies to 9 CFR 113.409(c), described in Veterinary Services Memorandum 800.114 (2012)	NA	
Histamine sensitization test for acellular pertussis vaccines based on temperature measurement (refinement of animal use)	ICCVAM workshop in 2010	NA	WHO Technical Service Report 878 (update adopted 2012)	

Abbreviations: CFR = U.S. Code of Federal Regulations; CVB = Center for Veterinary Biologics (USDA); ELISA = enzyme-linked immunosorbent assay; EPA = U.S. Environmental Protection Agency; EU = European Union; FDA = U.S. Food and Drug Administration; GD = guidance document; GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; ICH = International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; ISO = International Organization for Standardization; LLNA = murine local lymph node assay; NA = not applicable; NHK = normal human keratinocyte; NRU = neutral red uptake; OECD = Organisation for Economic Co-operation and Development; OPPTS = Office of Prevention, Pesticides, and Toxic Substances (EPA); rLLNA = reduced LLNA; SAM = Supplemental Assay Method; TER = transcutaneous electrical resistance; TG = Test Guideline; WHO = World Health Organization; USDA = U.S. Department of Agriculture; UV = ultraviolet.

¹Updated June 24, 2013