

Test Methods Reviewed or Under Consideration by ICCVAM

Toxicity Area	No.	Test Method [No.]	Regulatory Application and ICCVAM Recommendations
Acute Systemic Toxicity	3	Up-and-Down Procedure (UDP)	In 2001, recommended as replacement alternative for OECD TG 401, the traditional <i>in vivo</i> rodent LD ₅₀ test for assessing acute oral systemic toxicity, and adopted by OECD as TG 425; in 2003 accepted by U.S. agencies.
		<i>In vitro</i> basal cytotoxicity methods [2]	In 2007, both <i>in vitro</i> test methods recommended as reduction alternatives to estimate the starting dose in the UDP and Fixed Dose Procedure (FDP) for assessing acute oral systemic toxicity.
Biologics Testing	23 ¹	<i>In vivo</i> alternatives <i>Ex vivo</i> alternatives <i>In vitro</i> cell-based methods <i>In vitro</i> enzymatic alternatives	In 2006, various reduction, refinement and replacement alternatives to the mouse LD ₅₀ assay for botulinum toxin detection and potency testing reviewed at an ICCVAM-NICEATM/ECVAM-sponsored workshop; future activities recommended.
Developmental Toxicity	1	Frog Embryo Teratogenesis Assay: <i>Xenopus</i> (FETAX)	In 2000, 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 identified, future activities recommended.
Endocrine Disruptors	138	<i>In vitro</i> androgen receptor (AR) binding [11] <i>In vitro</i> AR transcriptional activation (TA) [18]	In 2002, evaluated screens for identifying potential endocrine-disrupting chemicals, to be included in EPA's Endocrine Disruptor Screening Program; in 2003, report with guidance for protocol standardization and validation studies released; in 2006, reference substance list revised.
		<i>In vitro</i> estrogen receptor (ER) binding [14] <i>In vitro</i> ER TA [95]	Same as for <i>in vitro</i> AR assays.
Eye Corrosion/Irritation	7	<i>In vitro</i> 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 recommended as screening tests for identifying corrosives and severe irritants, with certain limitations; two other methods not recommended for regulatory hazard classification purposes until further developed and evaluated.
		<i>In vitro</i> 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.
Pyrogenicity	5	<i>In vitro</i> pyrogenicity	In 2007, <i>in vitro</i> pyrogenicity test methods measuring cytokine release from human cells recommended as replacements for the rabbit test, subject to product specific validation, to detect endotoxin contamination in parenteral drugs.
Skin Corrosion	4	Corrositex® EpiDerm™ EPISKIN™ Rat Transcutaneous Electrical Resistance (TER) Assay	In 1999, Corrositex® recommended 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 (EPISKIN™, EpiDerm™) recommended as part of a tiered testing strategy; in 2004, adopted by OECD as TG 430/431.

Toxicity Area	No.	Test Method [No.]	Regulatory Application and ICCVAM Recommendations
Skin Sensitization	7	Murine Local Lymph Node Assay (LLNA) <ul style="list-style-type: none"> - Limit dose approach - Use for potency determination - Applicability domain - Performance standards LLNA non-radiolabelled methods [3]	In 1999, LLNA recommended and accepted by regulatory agencies as alternative for guinea pig tests for allergic contact dermatitis; adopted in 2002 as TG 429 by OECD. Use of LLNA for potency determination, LLNA limit dose approach, the LLNA applicability domain and three non-radiolabeled LLNA methods are currently under review, in addition to revised LLNA performance standards.
Total	188		

No. = Number of methods reviewed in each toxicity area, OECD = Organisation for Economic Co-operation and Development

¹These 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, replace, and refine (less pain and distress) the use of mice for botulinum toxin testing (see: http://iccvam.niehs.nih.gov/methods/biologics/bot_workshop.htm)