

## List of Alternative Test Methods and Strategies (or New Approach Methodologies [NAMs])

June 22, 2018

Section 4(h)(2)(C) requires EPA develop a list, “which the Administrator shall update on a regular basis, of particular alternative test methods or strategies the Administrator has identified that do not require new vertebrate animal testing and are scientifically reliable, relevant, and capable of providing information of equivalent or better scientific reliability and quality to that which would be obtained from vertebrate animal testing.” Furthermore, Section 4(h)(2)(D) requires EPA “provide an opportunity for public notice and comment on the contents of the [Strategic] plan...including the criteria for considering scientific reliability and relevance” of the NAMs.

The lists presented here are not exhaustive lists of possible NAMs which could potentially be used by EPA for TSCA decisions in some contexts. Rather, they are representative lists for consideration by the EPA of what has been reviewed and established as acceptable NAMs by different organizations (i.e., OECD<sup>1</sup>, EURL-ECVAM and ICCVAM). The extensive and transparent process<sup>2</sup> developed by EURL-ECVAM, and accepted internationally as described in the OECD Guidance Document 34,<sup>3</sup> includes four steps: (1) assessment of proposed method; (2) planning and executing validation studies; (3) coordinating scientific peer review; and (4) developing an EURL-ECVAM recommendation on the validity status of test methods. This last step includes summarizing “mechanistic relevance, performance, limitations and applicability”.<sup>4</sup>

Tables 1 and 2 below identify OECD Guidelines that meet the criteria for NAMs under the principles identified above and the principles in Chapter 5 in the Strategic Plan, and thus Sections 4(h)(2)(C). Table 3 includes an EPA-specific NAM policy adopted by EPA’s Office of Chemical Safety and Pollution Prevention which demonstrates progress in implementing requirements of section 4(h). The policy explains OCSPP’s general approach to replace vertebrate tests for skin sensitization with non-animal tests. Each of the tests incorporated under the policy are existing OECD Test Guidelines (i.e., 442C, D & E). The policy organizes these tests into an approach/context for regulatory risk conclusions. In Table 4, EPA has also provided NAMs (e.g., computational toxicology tools, chemical category and tiered testing approaches, screening methods) that have been used by OPPT in the new chemicals program for several decades, many of which have been vetted through the OECD or incorporated into OECD predictive tools (e.g., OECD QSAR Toolbox). EPA notes that every test, model or assessment approach has an “applicability domain”<sup>5</sup>, i.e., a description of the assumptions underlying the

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<sup>1</sup> EPA has played a key role for many years in the review and validation/vetting process for OECD test guidelines program, including the new performance-based and defined approach methods identified in Chapter 5 of the Strategic Plan. Beginning in 2018, ICCVAM, is now a recognized, official partner in these OECD deliberations. The collaboration of NICEATM, ICCVAM and EPA is an important and strong presence in the international arena as new NAMs are being identified, developed and implemented for regulatory use.

<sup>2</sup> <https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/eurl-ecvams-validation-process>

<sup>3</sup> [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2005\)14&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2005)14&doclanguage=en)

<sup>4</sup> *Ibid* at 2

<sup>5</sup> Hartung *et al.* define the applicability domain as “the definition of chemical classes and/or ranges of test method end points for which the model makes reliable predictions.” Under regulatory acceptance criteria for alternative test

test, model or approach that enables a user or evaluator of the method to determine whether it is reliable and applicable for the intended purpose. Hence, the equivalency of scientific reliability, relevance, quality of any NAM need be considered within the context of each methods application (i.e., purpose).

Importantly, EPA will review any NAM information submitted by industry (or others) and make decisions based on the merits/relevance of the submission; as it has always done with conventional, *in vivo* studies. To this end, EPA encourages consultation with the Agency on the use of alternative test methods and strategies (NAMs) to determine how best to meet both information needs and the objective of TSCA section 4(h).

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methods, ICCVAM (2003) requires description of “classes of materials” and “species for which the test results are applicable and a description of the known limitations of the test.” [Hartung, T. et al. 2004. A modular approach to the ECVAM principles on test validity. *Altern Lab Anim*, 32:467-472; Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. National Institute of Environmental Health Sciences National Institutes of Health U.S. Public Health Service Department of Health and Human Services]

**Table 1. OECD Test Guidelines – Health Effects**

<b>Test Guideline (TG) No.</b>	<b>Title</b>	<b>Endpoint Assessed</b>
<a href="#">TG 428</a>	Skin Absorption: <i>in vitro</i> Method	Provide information on absorption of a test substance (can be from human or animal source)
<a href="#">TG 430</a>	<i>in vitro</i> Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)	Evaluate corrosivity (rat skin as source)
<a href="#">TG 431</a>	<i>in vitro</i> Skin Corrosion: Reconstructed Human Epidermis (RHE) Test	Evaluate corrosivity (human skin as source)
<a href="#">TG 432</a>	<i>in vitro</i> 3T3 NRU Phototoxicity Test	Evaluates Phototoxicity to mouse cells in culture
<a href="#">TG 435</a>	<i>in vitro</i> Membrane Barrier Test Method for Skin Corrosion	Evaluates corrosion using a synthetic membrane
<a href="#">TG 437</a>	Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants	Evaluates eye irritation/corrosivity in bovine eyes
<a href="#">TG 438</a>	Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants	Evaluates eye irritation/corrosivity in chick eyes
<a href="#">TG 439</a>	<i>in vitro</i> Skin Irritation: Reconstructed Human Epidermis Test Method	Evaluate irritation (human skin as source)
<a href="#">TG 442C</a>	<i>In chemico</i> Skin Sensitisation	No animal or human cells used, evaluates simple binding of a chemical to a receptor
<a href="#">TG 442D</a>	<i>In vitro</i> Skin Sensitisation: ARE-Nrf2 Luciferase Test Method	Skin sensitization evaluated – human cells used
<a href="#">TG 442E</a>	<i>In vitro</i> Skin Sensitisation: (h-CLAT)	Skin sensitization evaluated – human cells used
<a href="#">TG 455</a>	Performance-based Test Guideline for Stably Transfected Transactivation <i>in vitro</i> Assays to Detect Estrogen Receptor Agonists and Antagonists	Evaluates estrogenic effects – human cells used
<a href="#">TG 456</a>	H295R Steroidogenesis Assay	Evaluates possible endocrine effects – human cells used
<a href="#">TG 458</a>	Stably Transfected Human Androgen Receptor Transcriptional Activation Assay	Evaluates androgenic effects using chinese hamster ovary cells

**Table 1. OECD Test Guidelines – Health Effects**

<b>Test Guideline (TG) No.</b>	<b>Title</b>	<b>Endpoint Assessed</b>
	for Detection of Androgenic Agonist and Antagonist Activity of Chemicals	
<a href="#">TG 460</a>	Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants	Evaluates eye corrosivity/severe irritation with canine kidney cells
<a href="#">TG 471</a>	Bacterial Reverse Mutation Test	Evaluates mutagenicity in bacterial cells
<a href="#">TG 473</a>	<i>in vitro</i> Mammalian Chromosome Aberration Test	Evaluates chromosomal effects in either human or rodent cells
<a href="#">TG 476</a>	<i>in vitro</i> Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes	Evaluates gene mutations in either human or rodent cells
<a href="#">TG 487</a>	<i>in vitro</i> Mammalian Cell Micronucleus Test	Evaluates chromosomal effects in either human or rodent cells
<a href="#">TG 490</a>	<i>In vitro</i> Thymidine Kinase Mutation Test	Evaluates gene mutations in either human or rodent cells
<a href="#">TG 491</a>	Short-time Exposure for the Detection of Chemicals Causing Serious Eye Damage, and Chemicals Not Requiring Classification for Serious Eye Damage or Eye Irritation	Evaluates eye corrosivity/severe irritation with rabbit cornea cells
<a href="#">TG 492</a>	Reconstructed Human Cornea-like Epithelium for the Detection of Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage	Evaluates eye irritation with reconstructed human cells (either eye or skin)
<a href="#">TG 493</a>	Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) <i>in vitro</i> Assays	Evaluates estrogenicity in human cells

**Table 2. OECD Test Guidelines – Effects on Biotic Systems<sup>1</sup>**

<b>Test Guideline (TG) No.</b>	<b>Title</b>	<b>Endpoint Assessed</b>
<a href="#">TG 201</a>	Freshwater Alga and Cyanobacteria Test	Evaluates toxicity to algae
<a href="#">TG 202</a>	Daphnia Species Acute Immobilization test	Evaluates toxicity to freshwater invertebrates
<a href="#">TG 207</a>	Earthworm Acute Toxicity test	Evaluates toxicity to soil invertebrates
<a href="#">TG 211</a>	Daphnia magna Reproduction Test	Evaluates reproductive effects in freshwater invertebrates
<a href="#">TG 222</a>	Earthworm Reproduction Toxicity Test	Evaluates reproductive effects in soil invertebrates
<a href="#">TG 236</a>	Fish Embryo Acute Toxicity (FET)	Evaluates toxicity to fish using zebrafish embryos
<sup>1</sup> Does not include tests in terrestrial plant species.		

**Table 3. EPA Policies Related to NAMs Within the TSCA Program**

<b>Link to Policy</b>	<b>Type of NAM</b>	<b>Endpoint Assessed</b>
<a href="#">OCSPP Skin Sensitization Policy</a> (To be updated when finalized)	Choice of Two Defined Approaches (DAs)	Combination of NAMs to predict skin sensitization in humans

**Table 4. EPA-Specific NAMs (For TSCA<sup>1</sup> Unless Otherwise Noted)**

NAM	Parameter Assessed
Ecological Structure-Activity Relationships Program (ECOSAR)	Hazard <sup>2</sup> - <i>In silico</i> tool to predict aquatic hazard
OncoLogic	Hazard <sup>2</sup> - <i>In silico</i> tool to predict potential to cause cancer in humans
Analog Identification Methodology (AIM)	Hazard <sup>2</sup> - Database tool to facilitate identification of analogs for read-across
Chemical Assessment Clustering Engine (ChemACE)	Hazard <sup>2</sup> – Database tool to facilitate structural clustering
New Chemical Categories Document	Hazard <sup>2</sup> – Documentation of TSCA chemical categories
Estimation Programs Interface (EPISuite™)	Physical/chemical properties and environmental fate <sup>3</sup> – e.g., bioconcentration/bioaccumulation
Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER)	Exposure <sup>3</sup> – tools and models to estimate environmental releases and worker exposures
Exposure and Fate Assessment Screening Tool (E-FAST)	Exposure <sup>3</sup> - tools and models to estimate consumer, general public and environmental exposures to chemicals.
Approaches to Estimate Consumer Exposure	Exposure <sup>3</sup> – a variety of tools and models to estimate exposure to various consumer products and materials
<p><sup>1</sup> General Guidance on all approaches - <a href="https://www.epa.gov/tsca-screening-tools">https://www.epa.gov/tsca-screening-tools</a></p> <p><sup>2</sup> Hazard - <a href="https://www.epa.gov/tsca-screening-tools/using-predictive-methods-assess-hazard-under-tsca#models">https://www.epa.gov/tsca-screening-tools/using-predictive-methods-assess-hazard-under-tsca#models</a> ;</p> <p><sup>3</sup>Physical/Chemical Properties, Environmental Fate and Exposure - <a href="https://www.epa.gov/tsca-screening-tools/using-predictive-methods-assess-exposure-and-fate-under-tsca#fate">https://www.epa.gov/tsca-screening-tools/using-predictive-methods-assess-exposure-and-fate-under-tsca#fate</a></p>	