J R C T E C H N I C A L R E P O R T S

ICATM Alternative Test Method Validation and Regulatory Acceptance January 2014 Status Report for ICCR

Compiled by the European Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) for the International Cooperation on Cosmetics Regulation (ICCR)

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International Cooperation on Alternative Test Methods
High Level Update[1], January 2014

Prepared for the International Cooperation on Cosmetics Regulation - ICCR

International Acceptance since last update (June 2013)

The OECD WNT meeting takes place once a year in April, so there is no new element under this point.

On-going Validation Studies by ICATM Organizations

Ongoing validation studies by the ICATM Organizations include:

1. ***Allergic contact dermatitis/skin sensitization:*** Validation studies for four *in vitro* test methods have been finalized. EURL ECVAM has submitted three Standard Project Submission Form (SPSF) proposals for skin sensitization to the OECD that have all been approved. The JaCVAM validation study of an IL-8 reporter gene assay (IL-8 Luc assay) is on-going.
2. ***Toxicokinetics:*** The validation study for two *in vitro* hepatic biotransformation test methods based on CYP induction continues and an SPSF for a Performance-based Test Guideline (PBTG) has been approved by WNT25 in April 2013.
3. ***Endocrine disruptor activity screening:*** The ICCVAM validation study for the MCF-7 cell proliferation test method was completed. The protocols have to be revised for adequate reproducibility. The MELN validation study on a manual protocol led by EURL ECVAM is concluded. The validation of the automated protocol, on the other hand, is still on-going and peer review is anticipated for 2014**.** Similar ER-TA test methods are to be added to the OECD PBTG after peer review. JaCVAM is validating an antagonist STTA assay to be added to TG 455. A validation study on androgen receptor transcriptional activation assays (to identify agonists and antagonists) is on-going under the coordination of JaCVAM. Another study was started by EURL ECVAM in 2013.
4. ***Genetic toxicity:*** The validation of the *in vitro* Comet assay is ongoing. Validation of *in vitro* genotoxicity studies (Comet and Micronucleus) in 3D reconstructed human skin models are ongoing.
5. ***Serious eye damage / eye irritation:*** The validation study for two reconstructed human tissue models (EpiOcular™ EIT and SkinEthic™ HCE) finished in 2013. The EpiOcular™ EIT will enter peer review in 2014. An SPSF has been submitted to the OECD for the development of a new TG. Another SPSF has been submitted to increase the applicability of all RhT/MTT based test methods to colored chemicals interfering with the MTT assay. Validation studies on an *in vitro* cytotoxicity test using rabbit corneal cells (SIRC CVS assay) and on Vitrigel-EIT are on-going.
6. ***Reproductive toxicity:*** A validation study on the Hand-1 Luc assay is on-going.

[1] For a more detailed overview, see the annexed ICATM Alternative Test Method Validation and Regulatory Acceptance Status Report" or the EURL ECVAM Cosmetics Report: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-releases-2013-progress-report-development-validation-regulatory-acceptance-alternative-methods

Peer Reviews and Harmonized Recommendations

1. ***Acute toxicity:*** The ESAC peer review of the Zebrafish Embryo Toxicity Test (ZFET) for acute fish toxicity testing was finalized and the EURL ECVAM Recommendation has undergone restricted commenting (by PARERE[2], ESTAF[3] and ICATM). It is planned to be published during the first quarter of 2014. The OECD TG 236 (led by Germany) was approved in 2013.
2. ***Carcinogenicity:*** The peer-review of the Bhas42 cell transformation assay has been completed and an EURL ECVAM Recommendation was published in 2013.
3. ***Allergic contact dermatitis/skin sensitization:*** The ESAC peer review of the DPRA and the KeratinoSens™ methods were finalized and an EURL ECVAM Recommendation on the DPRA was published in 2013. The EURL ECVAM Recommendation on the KeratinoSens™ will be published in the first quarter of 2014. Draft OECD TGs on DPRA and KeratinoSens™ were circulated for commenting late 2013. The ESAC peer review of the Human Cell Line Activation Test (h-CLAT) is on-going.
4. ***Serious eye damage / eye irritation:*** The peer review of the STE test method was finalized in 2013 and a draft OECD TG was circulated for commenting late 2013. The peer review of the SIRC-CVS test method is planned in 2014.
5. ***Genetic toxicity:*** The peer review for the *in vivo* Comet assay by the OECD Comet Assay Expert Group has been finalized.
6. ***Phototoxicity:*** The peer review for an *in vitro* test based on reactive oxygen species and photostability was finalized in 2013.
7. ***Endocrine disruption:*** The peer review of the antagonist STTA assay is planned in 2014.

[2] Preliminary Assessment of Regulatory Relevance Network
[3] ECVAM Stakeholder Forum

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Note: This report is a document meant to inform about the recent activities of ICATM in the area of alternative test methods. The projects and completed Test Guidelines listed in the table represent test methods where one or more ICATM partners have been involved in the validation or peer review process. Whereas the report is drawn up for ICCR, the scope of activities of ICATM partners is not limited to application in the area of cosmetics, and so the list is not limited to replacement methods, but also includes reduction and refinement ones. This report and the table are a purely informative document and should not be intended in any way as a recommendation for cosmetics manufacturers or other industrial sectors. Furthermore, it should be noted that the list is not exhaustive, as there are additional alternative methods available that have been accepted for international regulatory use by OECD that are not listed in this table, as they were neither developed nor validated by ICATM partners. For more complete information on alternative methods for cosmetics testing please see the EURL ECVAM Report[4].

Method	Current Status	Lead Action Organization	International Acceptance
Dermal Corrosivity Test Methods			
CORROSITEX Skin Corrosivity Test	**Completed**		**OECD TG 435 (2006)**
EpiSkin™, EpiDerm™, SkinEthic™, epiCS™ Skin Corrosivity Tests	**Completed**		**OECD TG 431 (2004), updated version (sub-categorization, inclusion of performance standards, inclusion of SkinEthic™ RHE and epiCS™) adopted in 2013. Currently under revision for inclusion of sub-categorization with the epiCS™ test method**
Rat TER Skin Corrosivity Test	**Completed**		**OECD TG 430 (2004), updated version**

[4] http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-releases-2013-progress-report-development-validation-regulatory-acceptance-alternative-methods

Method	Current Status	Lead Action Organization	International Acceptance
			(inclusion of performance standards) adopted in 2013
Dermal Irritation Test Methods			
In vitro* reconstructed human epidermis (RhE) test methods: EpiDerm™, EpiSkin™, SkinEthic™ RHE and LabCyte EPI-MODEL24 *SIT	**Completed**		**OECD TG 439 (2010), updated version (inclusion of LabCyte™ EPI-model) adopted in 2013**
In vitro reconstructed human epidermis (RhE) test methods: Korean epidermis model	KoCVAM sponsored validation study is on-going	KoCVAM; EURL ECVAM, NICEATM-ICCVAM, Health Canada and JaCVAM VMT liaisons	
Phototoxicity Test Methods			
3T3 NRU Phototoxicity Test	**Completed**		**OECD TG 432 (2004)**
Test method battery to predict phototoxicity (yeast growth inhibition phototoxicity assay and red blood cell photohemolysis assay)	Japanese Regulatory Acceptance Board recommended additional work be performed	JaCVAM	
In vitro test method based on reactive oxygen species (ROS) and photostability	Peer review of the JaCVAM-sponsored validation study finalized in 2013.	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, Health Canada and KoCVAM VMT liaisons	ICH S10 including the ROS assay and the 3T3 NRU test method will be completed in 2014
Ocular Toxicity Test Methods			
Bovine Corneal Opacity and Permeability (BCOP) Test Method	**Completed**		**OECD TG 437 (2009), updated version (positive control, use in a bottom-up approach to identify non-classified chemicals) adopted in 2013**
Isolated Chicken Eye (ICE) Test Method	**Completed**		**OECD TG 438 (2009), updated version (use in a bottom-up approach to identify non-**

Method	Current Status	Lead Action Organization	International Acceptance
			classified chemicals) adopted at WNT in 2013
Use of Histopathology as an additional endpoint in Ocular Safety Testing	**Completed**		**OECD GD 160 (2011)**
Cytotoxicity test: SIRC CVS	JaCVAM-sponsored validation study is ongoing	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT	
Cytotoxicity test: three-dimensional dermal model (MATREX)	JaCVAM-sponsored validation study in the planning stage	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT	
Cytotoxicity test: Short Time Exposure (STE) test	Peer review coordinated by NICEATM-ICCVAM of the JaCVAM-sponsored validation study completed. The draft TG was submitted to OECD for comments.	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT liaisons	SPSF submitted and approved in 2012. Draft TG under discussion at the OECD. Adoption of draft TG by WNT 26 expected in 2014
Use of anesthetics, analgesics, and humane endpoints for routine use in TG 405	**Completed**		**OECD updated TG 405 (2012)**
Low volume eye test; recommendation for no future use.	Completed		
In vitro approach for categorization of anti-microbial cleaning products: recommendations for further studies	Completed. EPA/OPP has concluded from submission and review of alternative eye irritation tests conducted on antimicrobial pesticide products with cleaning claims (AMCPs) that the proposed testing approach is acceptable for determining the appropriate eye hazard classification and labeling for AMCPs (see http://www.epa.gov/pesticides/regulating/eye-policy.pdf for the details of the scope of the policy).	NICEATM-ICCVAM	
Cytosensor Microphysiometer® (CM) Test method	The draft TG was submitted to OECD for comments including a set of Performance Standards	EURL ECVAM; NICEATM-ICCVAM	
Fluorescein Leakage (FL) test method	**Completed**		**OECD TG 460 (2012)**
Human reconstructed tissue models for eye irritation	EURL ECVAM validation study finalized (experimental part started	EURL ECVAM; JaCVAM,	

Method	Current Status	Lead Action Organization	International Acceptance
EpiOcular™ EIT SkinEthic™ HCE	in 2010 and ended in April 2013; the validation of an optimized EpiOcular™ solids protocol was completed in June 2013). Peer review of EpiOcular™ EIT anticipated for 2014.	NICEATM-ICCVAM, and Health Canada VMT liaisons	
Vitrigel-EIT	MAFF-sponsored validation study is on-going	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT liaisons	
Immunotoxicity (Allergic Contact Dermatitis) Test Methods			
Murine local lymph node assay (LLNA) for skin sensitization	**Completed**		**OECD TG 429 (2002)** **ISO (2002)**
Updated Murine local lymph node assay (LLNA) for skin sensitization (20% reduction)	**Completed**		**Update to TG 429 OECD (2010)** **ISO (2010)**
Reduced LLNA (rLLNA)	**Completed**		**Update to TG 429 OECD (2010)**
Nonradioactive LLNA protocol (LLNA: BrdU-ELISA)	**Completed**		**OECD TG 442B OECD (2010)**
Nonradioactive LLNA protocol, LLNA:DA	**Completed**		**OECD TG 442A OECD (2010)**
Harmonized performance standards for the LLNA	**Completed**		**Update to TG 429 OECD (2010)**
Nonradioactive LLNA protocol (LLNA: BrdU-Flow Cytometry)	ICCVAM international peer review, 2009 Recommendations pending a review of lead laboratory data and inter-laboratory study. KoCVAM validation study is on-going	NICEATM-ICCVAM, KoCVAM	
In vitro skin sensitization assays (h-CLAT; DPRA; MUSST)	Multi-laboratory validation ended in August 2012 (h-CLAT and MUSST). DPRA peer review finalized. h-CLAT peer review on-going.. EURL ECVAM recommendation on DPRA published.	EURL ECVAM; JaCVAM and NICEATM-ICCVAM VMT liaison members	SPSFs for TGs on the DPRA, and hCLAT approved in 2012 and 2013, respectively. Draft TG on DPRA under discussion at OECD
In vitro skin sensitization assay KeratinoSens™	External Validation Study, peer review finalized. EURL ECVAM recommendation pending	EURL ECVAM	SPSF for a TG on the Keratinosens

Method	Current Status	Lead Action Organization	International Acceptance
	publication.		approved in 2012. Draft TG under discussion at OECD
In vitro skin sensitization assay IL-8 Luc assay	METI-sponsored validation study is on-going	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, KoCVAM and Health Canada VMT liaisons	
Acute Toxicity Test Methods			
Up and Down Procedure (UDP)	**Completed**		**OECD TG 425 (2008)**
***In vitro* cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity tests**	**Completed**		**OECD GD 129 (2010)**
In vitro cytotoxicity test (3T3 Neutral Red Uptake) for identifying substances with acute oral LD50 > 2000 mg/kg b.w.	EURL ECVAM ESAC peer review completed, and EURL ECVAM Recommendation published in 2013.	EURL ECVAM and ICATM organisations	
Zebrafish Embryo Toxicity test (ZFET)	ESAC peer review finalized. EURL ECVAM recommendation pending publication.	EURL ECVAM	Adoption of OECD TG 236 in April 2013
Toxicokinetic test methods			
In vitro hepatic biotransformation – CYP induction: Hepa RG and cryopreserved human hepatocytes	ESAC peer review foreseen in 2014	EURL ECVAM; NICEATM-ICCVAM, and JaCVAM VMT liaisons	SPSF for a PBTG approved in April 2013
Endocrine Disruptor Test Methods			
Stably transfected human estrogen receptor-α transcriptional activation assay for detection of estrogenic agonist-activity of chemicals	**Completed**		**OECD TG 455 (2009), updated 2012**
Stably transfected human estrogen receptor-α transcriptional activation assay for detection of estrogenic antagonist-activity of chemicals	International validation study in progress	JaCVAM and VMG NA liaisons	To be added to TG 455 when validated and peer reviewed
LUMI-CELL® human estrogen receptor transcriptional activation assay: agonist and antagonist protocols	**Completed**		**OECD TG 457 (2012)**

Method	Current Status	Lead Action Organization	International Acceptance
CertiChem MCF-7 cell proliferation assay for the detection of human estrogen receptor agonists and antagonists	International validation study completed. Protocol must be revised for adequate reproducibility	NICEATM-ICCVAM; EURL ECVAM, JaCVAM and KoCVAM VMT liaisons	
Stably transfected CHO Androgen receptor-α transcriptional activation assay for detection of androgenic agonist and antagonist activity of chemicals.	METI-sponsored validation is on-going	JaCVAM and VMG NA liaisons	
MELN® human estrogen receptor transcriptional activation assay: agonist and antagonist protocols	Validation study ongoing (EURL ECVAM). Peer review foreseen in 2014.	EURL ECVAM (lead), NICEATM-ICCVAM, JaCVAM	To be added to the PBTG when validated and peer reviewed
Stably Transfected Transactivation *in vitro* Assay to detect Androgen Receptor Agonists and Antagonists	Validation study starts in 2014	EURL ECVAM (lead), NICEATM-ICCVAM, JaCVAM	SPSF to develop a PBTG on ARTA approved in April 2013
Genetic Toxicity Test Methods			
***In vitro* micronucleus test**	**Completed**		**OECD TG 487 (2010)**
In vivo/in vitro comet assay	The peer review of the *in vivo* Comet assay by the OECD Comet assay expert group has been finalised. Validation of *in vitro* study ongoing	JaCVAM (lead); EURL ECVAM, NICEATM-ICCVAM, KoCVAM and Health Canada VMT liaisons	Adoption of draft OECD TG on the *in vivo* Comet assay expected in 2014
Genotoxicity assays (micronucleus and comet) in 3D skin models	Validation study ongoing	Cosmetics Europe (lead); EURL ECVAM support	
Transgenic rodent *in vivo* gene mutation assays. OECD TG 488 (2011)	Draft TG to be updated.	Health Canada	SPSF approved in April 2013.
Carcinogenicity Test Methods			
Bhas cell transformation assay	Peer review coordinated by EURL ECVAM completed EURL ECVAM Recommendation published in 2013	JaCVAM (lead); EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT liaisons	SPSF approved in 2010
SHE pH 6.7, SHE pH 7 and Balb/c 3T3 cell transformation assays	Pre-validation study and ESAC peer review completed Feb 2011; EURL ECVAM recommendation published.	EURL ECVAM	Adoption of draft TG expected by WNT 26 in 2014.

Method	Current Status	Lead Action Organization	International Acceptance
Reproductive Test Methods			
Hand-1 Luc assay	METI-sponsored validation is on-going	JaCVAM (lead); EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT liaisons	

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Abstract
The European Union Reference Laboratory for Alternatives to Animal Testing supports the post-validation regulatory acceptance process at both the European Union and international levels such as the Organisation for Economic Co-operation and Development (OECD). International cooperation in developing alternative test methods for cosmetics takes place under ICATM – the Framework for International Cooperation on Alternative Test Methods. Under the auspices of the International Cooperation of Cosmetics Regulators (ICCR), the JRC compiles periodically an overview of the validation and/or international acceptance status of test methods worked upon within ICATM.

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