ECVAM update

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Joint Research Centre (JRC)
European Commission

http://ecvam.jrc.it/

Overview

- EU actions in the area of 3Rs
- Timelines
- ECVAM key areas
- International Cooperation on Alternative Test Methods (ICATM)

EU actions in the area of 3Rs

- **Cosmetics Directive 76/768/EEC and its amendments**
- **REACH regulation 2006/1907/EC**
  European Chemicals Agency (ECHA)
- **Directive 86/609/EEC (Protection of animals for laboratory use) currently under revision**
- **ECVAM within the Joint Research Centre (JRC) since 1991**
- **EU funded R&D Projects (DG RTD)**
- **Community Action Plan on protection and welfare of animals**
- **European Partnership for Alternative Approaches to animal testing (EPAA)**

DG RTD

DG JRC ECVAM

Policy-DGs (DG JRC)

Collaboration with academia & industry:

research, *in vivo* data, reference chemicals and in-house methods

The 7th amendment of the Cosmetics Directive

- **Phasing out ingredient testing with test and marketing bans in 2009 and 2013**
- **Urgent need for alternative methods especially for complex toxicity endpoints**

Toxicological endpoints for which methods are ready before 2009 and 2013

Toxicological endpoint	ESAC statement	Accepted in the Guidelines
Skin corrosion	Already validated ECAC 1998	Reference number: -B.40 in Annex V of Council Directive 67/548/EEC, TG 430, 431 OECD Guidelines
Phototoxicity	Already validated ESAC 1997	B.41 in Annex V of Council Directive, 67/548/EEC; -TG 432 in OECD Guidelines
Skin irritation	ESAC 2007	Regulatory acceptance pending
Eye irritation for severe irritant	ESAC 2007	Regulatory process started
*Eye irritation (mild irritants)**	*ESAC 2009-2010 (to be expected)*	
Skin absorption/penetration	ESAC 2008-2009 (ongoing validation of skin models)	OECD TG 428
Photogenotoxicity	ESAC likely in 2010	
Acute toxicity, A-Cute-tox	ESAC likely in 2012	
Skin sensitisation	ESAC likely in 2012	
Genotoxicity and mutagenicity (micronucleus and COMET test)	ESAC likely in 2009-2011	

Toxicological endpoints for which methods are not ready before 2009 and 2013

Toxicological end points	Estimated ESAC statement	Acceptance in the Guidelines
Subacute and subchronic toxicity, repeated-dose toxicity	No tests developed yet Not before 2013	
Toxicokinetics and metabolism	Not before 2012	
Carcinogenicity	ESAC, 2009 Needs more efforts, as partial replacement only	
Reproductive and developmental toxicity -embryotoxicity -strategies from ReProTect project	 ESAC 2002, does not fully cover the animal test Not before 2013, probability for full replacement very little (about 10 % only)	

- **Publication of SIVS (Skin Irritation Validation Study) and Test Chemicals Selection (*ATLA* 35, 559-601, 2007 and *ATLA* 35, 603-619, 2007)**
- **Drafting of EU Test Method/OECD Test Guideline and submission to EU/OECD**
- **Reply to comments of national coordinators**
- **Re-activation of ECVAM task force on skin irritation for dealing with comments at OECD level planned**
- **Evaluation of reproducibility of IL-1 alpha endpoint planned**
- **Evaluation of SkinEthic assay, currently in peer review**
- **Submission of validation study on optimised EpiDerm assay received on 23 April 2008, currently in peer review**

Key area topical toxicity: Eye Irritation

Retrospective validation of Cytotoxicity- / cell function- based assays: NRR, RBC, FL, CM

Timelines

June '08	**5th VMG meeting (if all documents available),**
July-October '08	**preparation of dossier for ESAC peer review (if outcome is appropriate), publication of study**

Eye Irritation

1) Human reconstituted tissue models: SkinEthic and EpiOcular

Submission of the COLIPA reports on SkinEthic and EpiOcular optimisation-prevalidation studies (March 2008)

Meeting with members of COLIPA to present/discuss SkinEthic submission (ECVAM; 5 May 2008)

Draft validation study plan prepared and submitted to COLIPA

2) Organotypic assays: follow-up activities

OECD Test Guidelines (ICCVAM), EU Test Guidelines (ECVAM)
Further improvements and analyses as recommended by ESAC (ECVAM)
Evaluation for mild ranges of irritancy (ICCVAM-NICEATM)

3) Biostatistical support (collaboration agreement with COLIPA)

Second meeting of the Biostat Steering Committee (ECVAM/COLIPA): 19-20 May 2008
Work programme priorities: HRT models: planning of validation study
Analyses of *in vivo* data supporting evaluation of *in vitro* methods
Organotypic assays analyses as recommended by ESAC
Test strategies conceptual frame

Key Area Sensitization

Workshop Reports	Evaluation of Chemical Reactivity Methodologies for Screening Skin Sensitisation Potential	*ATLA 36, 2008*
	An Evaluation of Performance Standards and Non-Radioactive Endpoints for the Local Lymph Node Assay	*ATLA 36, 2008*
Expert Meeting:	Progress in the Development of New Approaches to the Identification of Respiratory Allergens *(2-3 April 2008)*	*manuscript in preparation*

ECVAM-Colipa Collaboration

Integrated approaches for skin sensitisation testing	*Workshop planned Autumn 2008*
Reference chemicals for method development/evaluation	*manuscript in preparation*
Representations on respective Task Forces	

Key Area Sensitization

Forthcoming prevalidation/validation studies

- Peptide-binding assay — draft Study Plan prepared
- Dendritic Cells based assays (U937 CD86, hCLAT) — and submitted to Colipa
- Vitosens

Reduced LLNA

- Follow up regulatory implementation
- Retrospective evaluation of data in the NCD (new chemicals database)

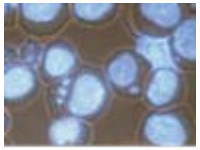
LLNA Performance Standards

Revised version distributed to the ESAC

Evaluation of a non-RI LLNA

Background Review Documents in preparation

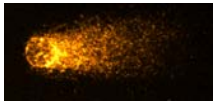
Key area Genotoxicity



MNT *in vitro* validation study

- published in *Corvi et al., Mutagenesis*, 2008
- Regulatory acceptance: follow-up with OECD and UK working group

COMET Assay



- VMT meeting on the validation of the comet assay *in vivo* and *in vitro* coordinated by JaCVAM (March 2008).

3D-skin model

- Inter-laboratory study on genotoxicity assays applied to 3D-skin models coordinated by COLIPA.

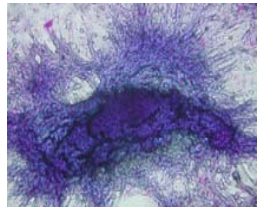
False positives in genotox testing

- Chemical selection for genotoxicity testing (*Kirkland et al., Mut. Res., in press*)

ECVAM Workshop on "Reduction in Regulatory Genotoxicity Testing" (June 2008)

Key area Carcinogenicity

Prevalidation of cell transformation assays (CTA)



- **Initiation of prevalidation of CTA standard pH (Jan 2008)**
- **Complete results of prevalidation (foreseen September 2008)**
- **Advisory members in the prevalidation of CTA BHAS42 coordinated by Hatano Institute (Japan)**

Kinetics

Ongoing studies

- Metabolic stability & metabolites of 72 substances
- Establishment of a kinetics database
- In vitro blood-brain barrier models for predicting absorption/uptake into the brain
- In vitro models for the prediction of gastrointestinal absorption

Workshop/publication

- Workshop report 63: Physiologically based Kinetic Modelling (PBK modelling): Meeting the 3Rs Agenda. Bouvier d'Yvoire et al. (2007); ATLA 35, 661-671

International collaboration

- COST B25 and IPCS PBPK initiatives

Strategic Developments

Robotic platform

- **Validation of 3T3 NRU for acute toxicity of non-toxic class chemicals**

Emerging technologies, "omics" & profiling methods

- **van Vliet et al (2008) A novel in vitro metabolomics approach for neurotoxicity testing, proof of principle for methyl mercury chloride and caffeine, Neurotoxicology 29, 1-12**
- **van Vliet et al (2007) Electrophysiological recording of re-aggregating brain cell cultures on multi-electrode arrays to detect acute neurotoxic effects, Neurotoxicology, 28, 1136-46**

Developmental Neurotoxicity Testing (DNT)

- **Expert meeting in March 2008 to prepare upcoming 2nd DNT in USA 2009 (ECVAM in steering committee)**

Biologicals & Food

- Workshop report on *The consistency approach for the quality control of vaccines* (Hendriksen et al, 2008; Biologicals, 36, 73-78)
- Workshop on 3Rs Approaches in QC of fish vaccines, January 2008
- Collaboration with EMEA/VICH on harmonisation of target animal safety test for batch release of veterinary vaccines
- Member of EFSA working group on the welfare of experimental animals and contribution to report “Overview of the test requirements in the area of food and feed safety” (EFSA/SC/WEA/465 rev 5)
- ECVAM on management team of CRLMB (Community Reference Laboratory on Marine Biotoxins) validation study Toxiline-DSP Test for detection of okadaic acid and dinophysistoxins in shellfish - CRLMB intends to submit the study for ESAC peer review

Ecotoxicology

Bioaccumulation

- Validation (Module 1-3) of in vitro trout S9 fraction assay started in January 2008 – 6 months interim report due in June 2008

Acute aquatic toxicity

- OECD Fish Embryo Toxicity ad hoc Expert Group; Meeting in May 2008

ECVAM on Steering Committee of HESI Subcommittee on animal alternative needs in environmental risk assessment

- Workshop on the use of fish embryos in ERA in March 2008

Endocrine Disrupters
(Miriam Jacobs/Patricia Pazos)

- **OPTIMISATION:** "ReProTect"
  MELN, PALM, ER-CALUX, AR-CALUX "PANVERA" AR-binding test and *"PANVERA"* ER-binding test (now in validation)

- **VALIDATION**: Hosting the 5th OECD VMG-NA meeting in Ispra; 13-15 November.
  Outsourced validation studies in progress :
  -"PANVERA" -ER binding Test (CCR.IHCP.C432905.XO):
  Chaired by US-EPA - ECVAM in the MT
  -"HeLa" ER antagonist (OJ ref. 2006/S 46-047741 of 08/03/2006)
  Co-Chaired by ECVAM
  -"LUMICELL" ER Agonist and Antagonist,
  Participation of ECVAM laboratory

3 **REGULATORY ACCEPTANCE**: 2nd draft of OECD test guideline on the "HeLa" agonist ER validation study submitted by Japan to the OECD VMG NA, ECVAM's chair of various committees to progress the 1st in vitro ED test guideline for final regulatory acceptance in June 2008

Reduction/Refinement: Extended F1 generation study

Susanne Bremer

- **EXPERT GROUPS**: Participation in the OECD expert groups, EPAA working group 2, ECETOC taskforce

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- PUBLICATION: **Co-author on the ECETOC Technical document No 45 *"Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study"* No. 45; March 2008**

ecetoc

- WORKSHOP: **Co-organizing a workshop on "Triggering / Waiving Criteria for the Extended One Generation Reproduction Toxicity Study" external participants 41 Barza d'Ispra; 14-15April**

LRI The Long-range Research initiative

- **OCED TG 43** Neither ext.F1 generation study nor in vitro tests that were included in the 1st draft of OECD TGD 43 as part of a conceptual framework are part of the 2nd draft. As consequence In vitro tests that are currently developed within the IP "ReProTect" are not part of the OECD TGD anymore.
  EC-Testing Methods Coordinator who has submitted our comments to the OECD as a COM position, but the WNT has approved the 2nd version

DB-ALM Projects Progress* (November '07 - April '08)

- User satisfaction & quality of the service:
  - Public announcements of the DB-ALM **continued in 2007 by European, National and International Institutions, Centres and Universities, *e.g.* the EU Commissioner for Research, EU-Japan-USA trilateral programme VICH, US Animal Welfare Information Centre etc.**
  - **Total n°:** 1151 registrations **from 64 countries**
  - **74% out of the total registrations are** active users **(during first 3 months of 2008, already 53%)**
  - **Download of medially** 309 documents / month
- Updated online information content**: 11 % of all method descriptions reviewed and updated, 5 new method descriptions,**
  308 new test results, 304 new references (see Annex 1)

see also DB-ALM progress report n°13 (ESAC 27.08)

DB ALM

Follow-up of ICCR (International Cooperation on Cosmetics Regulation) recommendations of September 2007 meeting:

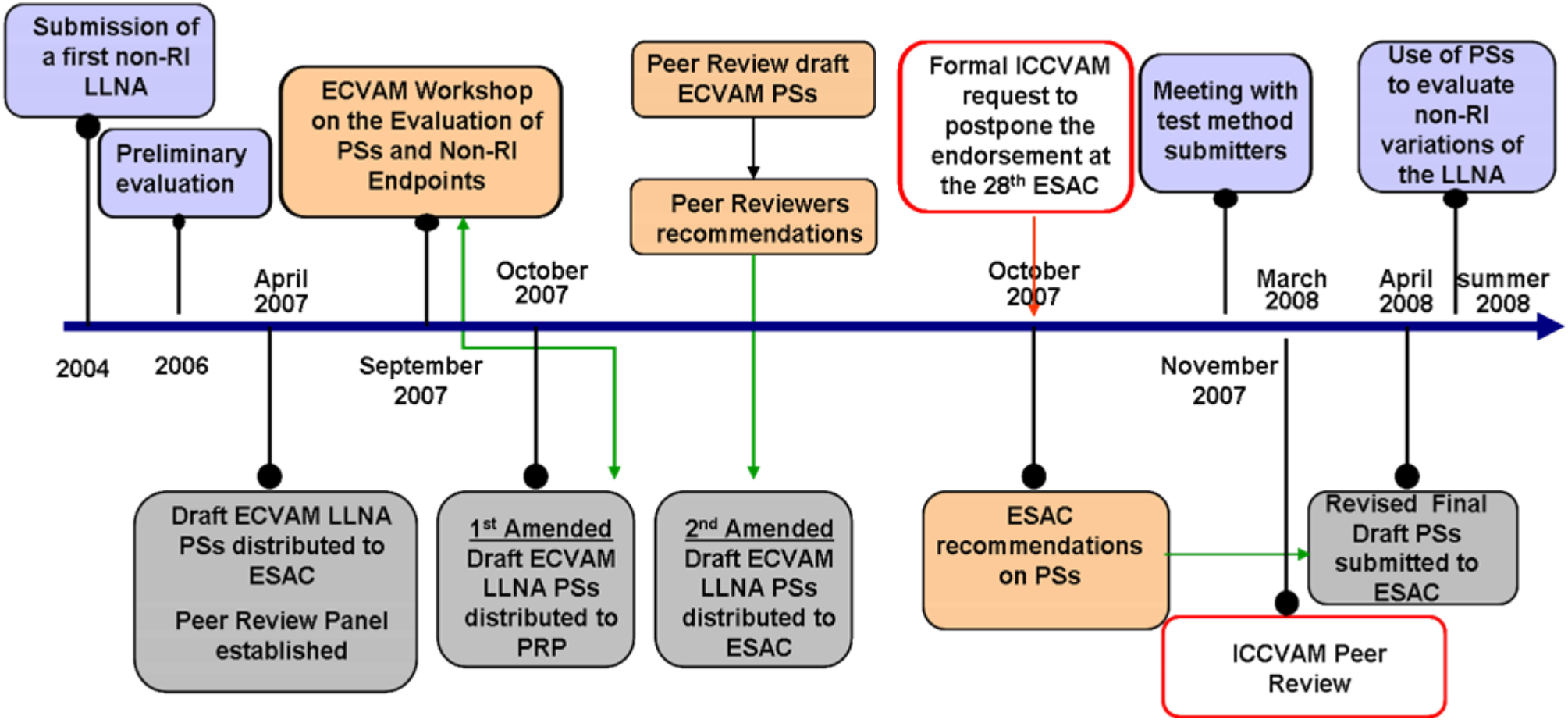
Proposal for an

International Cooperation on Alternative Test Methods (ICATM)

Purpose: to promote international cooperation, collaboration and communication among national/European validation organisations

NICEATM-ICCVAM, ECVAM-ESAC, JaCVAM, and Health Canada

ECVAM LLNA Performance Standards



ECVAM / ICCVAM LLNA Performance Standards

ECVAM Performance Standards	ICCVAM Performance Standards
Procedural Standards **Adherence to OECD TG 429**	**Procedural Standards** **Adherence to ICCVAM recommended protocol**
Concurrent positive control may be omitted if the test laboratory have available historic positive control data	*Concurrent positive control mandatory*
A minimum of four animals used per dose group	*Five animals per dose group*
Lymph node cells can be obtained either from pooled treatment groups or from individual animals	*Lymph node cells from individual animals only*

Acknowledgments:
Elke Anklam
Michel Bouvier d'Yvoire
Susanne Bremer
Silvia Casati
Sandra Coecke
Raffaella Corvi
Salvador Fontaner
Laura Gribaldo
Claudius Griesinger
Marlies Halder
Sebastian Hoffmann
Miriam Jacobs
Agnieszka Kinsner
Siegfried Morath
Maria Pilar Prieto
Anna Price
Annett Roi
Valérie Zuang
et al.

Thank you for your attention!
Further information:
http://ecvam.jrc.it