EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
European Union Reference Laboratory for Alternatives to animal testing (EURL ECVAM)

Joint Meeting of the EURL ECVAM advisory body on Preliminary Assessment of Regulatory Relevance (PARERE) and the EURL ECVAM Stakeholder Forum (ESTAF)

held on the 6th June 2013 at the Institute for Health and Consumer Protection (IHCP), European Commission Joint Research Centre, Ispra, Italy.

Summary Record

This was the 2nd joint meeting of PARERE and ESTAF organised by EURL ECVAM. The meeting was primarily dedicated to updates on EURL ECVAM activities regarding test method submissions, validation studies, peer reviews, Recommendations and the establishment of the new ESAC, in addition to updates on dissemination and communication activities and the presentation of a new draft EURL ECVAM strategy for genotoxicity. The meeting agenda is included in Annex I. Slide presentations during the meeting are available at the CIRCABC website.

Test method submissions, validation studies and regulatory acceptance

As a reminder to meeting participants, EURL ECVAM summarised its validation process, as published on its website: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvams-validation-process/eurl-ecvams-validation-process.

Briefly, the process comprises four main steps: i) assessment of submitted test methods and priority setting; ii) planning and execution of validation studies; iii) scientific peer review of validation studies and reports, and iv) development of EURL ECVAM Recommendations on the validity and potential use for regulatory or other purposes. During this process test method submitters, regulators, stakeholders and international ICATM partners are involved at key stages where their experience and expertise can be exploited to ensure the best possible outcome. Successful validation of an alternative method may trigger the development of an international Test Guideline by the OECD which EURL ECVAM, on behalf of the Commission, may contribute to - see http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm

EURL ECVAM has recently compiled a comprehensive review of the development, validation and regulatory acceptance of alternative methods which is available at: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-releases-2013-progress-report-development-validation-regulatory-acceptance-alternative-methods).

There has been a considerable increase in the number of test methods submitted to ECVAM since 2008 (http://tsar.jrc.ec.europa.eu/documents/TSAR_test_method_submissions_2013-06-03.pdf). This increase is mainly due to the fact that: i) the EURL ECVAM submission process became more standardised/streamlined; ii) the submission of new *in vitro* methods developed by the cosmetics industry (in view of the 2009 animal testing ban and 2009/2013 marketing bans of cosmetics tested on animals stipulated in the EU Cosmetics Directive); iii) the adoption of other relevant EU legislation such as REACH (where the 3Rs play a role) triggered the development of alternative methods and; iv) some EU FP6/FP7 projects in

which *in vitro* methods had been developed (*e.g.* ReProTect and Sens-it-iv) came to an end and some of the developed methods became available.

Validation studies coordinated by EURL ECVAM are currently on-going in the areas of toxicokinetics, eye irritation and endocrine disruption. It is expected that these studies will end during 2013 and that the ESAC peer review process will start in Q4 of 2013 (or early 2014). EURL ECVAM has recently launched a new validation project aimed at establishing performance standards for Androgen Receptor Transactivation Assays (ARTA) which will hopefully lead to the development of a performance-based Test Guideline at OECD.

In the future, EURL ECVAM-coordinated validation studies which involve a ring trial will select test facilities from the newly established EU Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL see - http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eu-netval). EU-NETVAL has been set up by EURL ECVAM in the framework of Directive 2010/63/EU which requires that Member States identify suitable laboratories to support validation projects.

With regard to the recent OECD projects either led by EC/EURL ECVAM or for which EURL ECVAM has carried out validation studies on behalf of the OECD, they were all adopted by the WNT apart from the Syrian Hamster Cell Transformation Assay (SHE CTA) and the Cytosensor Microphysiometer (CM) which are still under discussion within the OECD WNT. All this information and more can be found in the recent EURL ECVAM progress report on the development, validation and regulatory acceptance of alternative methods (2010-2013) available for download at: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-releases-2013-progress-report-development-validation-regulatory-acceptance-alternative-methods

EURL ECVAM Recommendations, ESAC peer reviews and establishment of the new ESAC

EURL ECVAM presented an update on its Recommendation, upcoming ESAC (ECVAM Scientific Advisory Committee) peer reviews and the renewal of the ESAC

EURL ECVAM Recommendations

Formal EURL ECVAM Recommendations on test methods are a relatively new product of EURL ECVAM and constitute the formal endpoint of ECVAM's validation process. EURL ECVAM Recommendations communicate the validation status of test methods evaluated by EURL ECVAM by outlining; i) the scientific design of test methods and the biological/toxicological information they generate (which may be useful also in a variety of contexts, *i.e.* beyond the health effect the method is primarily intended to address); ii) key performance characteristics (reliability, limitations, biological/predictive relevance), iii) potential use for standardised applications in a regulatory context and for research applications (including reference to the protocol published in EURL ECVAM's DB-ALM database). Recommendations also suggest possible follow-up activities in view of closing knowledge gaps.

EURL ECVAM Recommendations are initially developed on the basis of ESAC opinions (resulting from ESAC scientific peer reviews) and in-house scientific expertise. Importantly however, the development of the Recommendations involves consultation rounds with EU regulators (MS level, Commission DGs and agencies (ECHA, EFSA, EMA)) via PARERE, with stakeholders via ESTAF and with international validation partner organisations and regulators via the ICATM collaboration. EURL ECVAM stressed that the involvement of

regulators and stakeholders is intended to lead to a consolidated view on the usability of test methods before the actual formal acceptance process starts. This is expected to facilitate and expedite the acceptance and recognition of the EURL ECVAM recommended test methods for regulatory and other purposes - both on EU and international level.

EURL ECVAM Recommendations are published as JRC Scientific and Policy Reports online at the "EU bookshop" (publications from the European institutions: https://bookshop.europa.eu/en/home/) and EURL ECVAM's website (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations).

Currently, two EURL ECVAM Recommendations are undergoing consultation with PARERE, ESTAF and ICATM:

- on the Direct Peptide Reactivity (DPRA) Assay to support the assessment of skin sensitisation potential of chemicals
- on the BHAS 42 Cell Transformation Assay (CTA) to support carcinogenicity assessment of chemicals

Recommendations on the KeratinoSens™ test method (skin sensitisation) and the Zebrafish Embryo Toxicity Test (acute fish toxicity) will be forwarded for comments by PARERE, ESTAF and ICATM in the coming months.

ESAC peer reviews

The publication of four further EURL ECVAM Recommendations is foreseen for 2014, summarising test methods for skin sensitisation, endocrine disruption, toxicokinetics/biotransformation and eye irritation. The validation studies are currently being finalised and the ESAC peer review will take place in autumn 2013 (sensitisation method) and first two quarters of 2014 (the remaining three methods). In this context, EURL ECVAM also provided a short overview of the ESAC peer review process and the nature of advice requested from ESAC. This concerns advice on; i) validation studies, ii) on EURL ECVAM strategies, and iii) on the assessment of the similarity of putative similar methods seeking validation on the basis of performance standards.

Renewal of ESAC (ECVAM Scientific Advisory Committee)

The ESAC Rules of procedure foresee a three-year term for ESAC members. As the last ESAC had been formed in 2009, renewal of the Committee was due in 2012/2013. Following an open call for applications published from November 2012 to January 2013, 85 applications of experts from 21 countries (EU and outside EU) had been received. These were evaluated by a Selection Committee including two external neutral reviewers with regard to the eligibility and aptitude of the candidates. 15 experts will be appointed at the first ESAC meeting on 18/19 June following completion of the necessary administrative requirements (*i.e.* furnishing of declarations of interests, confidentiality and commitment). [Note: the new committee is now published on the EURL ECVAM website (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/scientific-advice-stakeholders-networks/ecvam-scientific-advisory-committee-esac)]

EURL ECVAM strategy for genotoxicity

EURL ECVAM presented for the first time a preliminary EURL ECVAM strategy for advancing the field of genotoxicity that either avoids or minimises the number of animals used. This strategic document is based on an EURL ECVAM assessment of the regulatory needs for this endpoint within different pieces of EU legislation where the generation of

genotoxicity information is a standard requirement. EURL ECVAM also reviewed the state-of-the-science and current international efforts in the field.

Although several *in vitro* tests are available at different stages of development and acceptance, they cannot at the moment be considered to fully replace animal tests needed to evaluate the safety of substances for genotoxicity. In light of this, EURL ECVAM proposes a pragmatic approach to improve the traditional genotoxicity testing paradigm that offers solutions in the short- and medium-term and that draws on the considerable experience of 40 years of regulatory toxicology testing in this area. In addition, it aims at providing a framework for prioritising the development and validation of new in vitro test methods. Completely new testing strategies may arise in the future, but this will depend on extensive investment in research and development in the longer term.

EURL ECVAM considers therefore that immediate efforts should be directed towards the overall improvement of the current testing strategy for better hazard and risk assessment approaches which uses fewer animals but which satisfies regulatory information requirements. Opportunities for improvement have been identified which aim to i) enhance the performance of the in vitro testing battery so that fewer in vivo follow up tests are necessary and ii) guide more intelligent *in vivo* follow-up testing to reduce unnecessary use of animals. The implementation of this approach will rely on the cooperation of EURL ECVAM with other initiatives and the coordinated contribution from various stakeholders.

In general, the proposed strategy was well received by PARERE and ESTAF members. EURL ECVAM invited members to provide further comments (in writing) on the draft document by the end of July 2013 in order to support its revision. The following specific feedback was noted:

- Better describe the possible role of new test methods within the proposed strategy.

- Consider site of contact in the development of new *in vitro* test models. For these assays, it will be crucial to characterise their metabolic capacity.

- Mention should be made of the fact that in the pharmaceutical sector, integration of endpoints in a single in vivo study has shown to lead to a reduction of up to two thirds in the number of animals used.

- For the assessment of pharmaceuticals, the CTA was not considered bringing any added value.

- Take advantage of the ICHS2 Genotoxicity Guideline revision to inform the EURL ECVAM strategy.

- Develop intelligent follow-up tests that target organs of interest. In particular, the development of an *in vivo* micronucleus test in intestinal cells was considered important by some workshop participants.

- ECHA is developing a revised guidance document on the use of transgenic animal tests and the *in vivo* comet assay.

- Training on data interpretation for regulatory purposes is needed.

- QSAR activities should be described that build on recent and current efforts.

- Prioritise the aims and objectives outlined in the strategy, if possible.

ICATM

The meeting participants were informed of the on-going collaborations within the International Collaboration on Alternative Test Methods (ICATM) and the next meeting that will be held in Seoul, Korea on 1-4 July 2013. At the meeting, preliminary discussions will be held on the Memorandum of Cooperation and how well it describes the actual on-going collaborations under the ICATM framework. A table that ICATM provides to the International Committee on Cosmetics Regulation (ICCR) in relation to the availability and regulatory acceptance of alternative methods for the assessment of cosmetic ingredients will be shared with PARERE and ESTAF (via the CIRCA BC site).

Dissemination activities of EURL ECVAM: Databases on Alternative Methods

The main activities regarding the dissemination of information on advanced and alternative techniques via the operation of specialised databases included:

- *Data Base service on advanced and ALternative Methods (DB-ALM)* that provides comprehensive test method descriptions, including those that are linked to EURL ECVAM Recommendations. DB-ALM also provides an ideal repository for methods developed within EU integrated projects. It was pointed out that the service is continually gaining in popularity and current usage has exceeded twice the level recorded during the same period last year.

- *(Q)SAR Model Inventory* providing peer-reviewed and standardised QSAR model descriptions by using an internationally recognised reporting format that summarises their main characteristics. The system will be further improved during 2013 with a new search interface.

- *Tracking System for Alternative methods towards Regulatory acceptance (TSAR).* A first version is publicly available to provide summary details on methods undergoing validation and acceptance. EURL ECVAM is currently in the progress of refining and expanding TSAR to provide more comprehensive information and also to include details on methods being progressed by other validation bodies in ICATM.

- *ECVAM Search Guide*. Encouraged by the success of the first publication in 2012, the JRC will re-publish an updated version this July. Meeting participants were invited to send addresses of relevant contacts in their countries, such as ethical committees, authorities and university contacts to receive a copy directly from the EU Bookshop.

Communication, website and news items

The newly revamped EURL ECVAM website was presented (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam). Regular news items inform the stakeholders and the general public about the most important activities and outcomes of ECVAM's work. In addition, since the beginning of the year EURL ECVAM posts its news on a social network platform (Twitter). Meeting participants were encouraged to get into the habit of visiting the EURL ECVAM website on a frequent basis to get the latest information. EURL ECVAM intends to refrain from sending emails to communicate such information. A new EURL ECVAM Web Manager has been recently appointed and thus PARERE and ESTAF members can expect further improvements and add-ons to the EURL ECVAM website. Suggestions on how PARERE and ESTAF information needs could be better addressed are always welcome.

Work plan, AOB

The work plan for the coming 12-month period was presented (see Annex II) and updated versions will be posted regularly on the CIRCA BC website.

Some further clarification was sought regarding the handling of comments received by EURL ECVAM during consultation with PARERE and ESTAF. EURL ECVAM confirmed that since the last joint meeting in September 2012 it had made the following provisions, i) any comments received by members are immediately acknowledged by return (email), ii) a summary of important changes made to the draft Recommendation after the consultation round with ESTAF and PARERE is sent to all members at the time that the revised version is posted for public commenting, and iii) if any member of PARERE or ESTAF requires further clarification (in addition to the summary of changes already received) on handling of specific comments then they should contact EURL ECVAM so that the enquiry can be managed on a bilateral basis. Meeting participants agreed to go ahead with this approach for now with the option of revisiting the issue again at a later date if need be.

Annex I

PARERE & ESTAF

Meeting of the Preliminary Assessment of Regulatory Relevance (PARERE) and the ECVAM Stakeholder Forum (ESTAF) Networks
Thursday, 6 June 2013
EC JRC, Ispra, Italy, Building 36/02

Agenda PARERE & ESTAF Meeting 6 June 2013

(Version 17 May 2013)

Time	Thursday, 6 June Joint Meeting PARERE & ESTAF	
09:00 – 09:15	Welcome and Introduction (M. Whelan)	
09:15 – 10:00	Test method submissions, validation studies and regulatory acceptance (V. Zuang)	Presentation | Discussion
10:00 – 10:30	ESAC peer reviews & EURL ECVAM Recommendations. Establishment of the new ESAC (C. Griesinger)	Presentation | Discussion
10:30 – 11:00	Coffee Break	
11:00 – 11:45	Genotox Strategy Document (R. Corvi)	Presentation | Discussion
11:45 – 12:00	ICATM updates (P. Amcoff)	Presentation | Discussion
12:00 – 12:30	Dissemination: Databases on alternative methods (TSAR, DB-ALM, QSAR) & Search Guide (A. Janusch Roi)	Presentation | Discussion
12:30 – 12:45	Communication, website, news items (S. Belz)	Presentation | Discussion
12:45 – 13:00	PARERE/ESTAF Work plan, AOB	Presentation | Discussion
13:00 – 14:00	Buffet Lunch	

Annex II

Preliminary rolling work plan for PARERE and ESTAF consultations

Consultation	**Timelines**
Recommendation on the BHAS Cell Transformation Assay (CTA) to support carcinogenicity assessment of chemicals.	Commenting round until 25 June 2013
Recommendation on the Direct Peptide Reactivity (DPRA) Assay to support the assessment of skin sensitisation potential of chemicals.	Commenting round until 25 June 2013
Strategy document on Genetic toxicity testing	1st draft available for commenting until 31 July 2013
Recommendation on the KeratinoSens™ assay to support the assessment of skin sensitisation potential of chemicals.	Commenting round until 20 September 2013.
Recommendation on the Zebrafish embryo toxicity test (ZFET) to support the assessment of acute aquatic toxicity of chemicals.	1st draft for commenting expected in late 2013
Strategy document for Ecotoxicity toxicity testing	1st draft for commenting expected in 4th quarter 2013
Recommendation on the hCLAT assay to support the assessment of skin sensitisation potential of chemicals.	ESAC peer review expected autumn 2013 and 1st draft for commenting expected 1-2nd quarter 2014
Strategy for Acute systemic toxicity testing	1st draft for commenting expected for 2nd quarter 2014
Recommendation on the EpiOcular assay to support the assessment eye irritation testing of chemicals.	ESAC peer review planned for 1st-2nd quarter 2014 and 1st draft for commenting expected latter part of 2014
Recommendation on 2 CYP-induction assays to support assessment of toxicokinetics/biotransformation of chemicals.	ESAC peer review planned for 1st-2nd quarter 2014 and 1st draft for commenting expected latter part of 2014
Recommendation on the MELN estrogen receptor transactivation assay to support the assessment of chemicals with endocrine disruption potency.	ESAC peer review planned for 1st-2nd quarter 2014 and 1st draft for commenting expected latter part of 2014

Note: Consultations on test method submissions will be performed on an *ad hoc* basis.