EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Health, Consumers and Reference Materials
Chemical Safety and Alternative Methods Unit
EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

Summary Record

Joint PARERE-ESTAF Meeting

8th November 2016, Ispra, Italy

The joint PARERE-ESTAF meeting started in the afternoon of 8th November 2016 and was followed by a 1.5 day workshop to explore the possible regulatory use of alternative approaches to systemic toxicity for the safety assessment of chemicals used in a variety of sectors. (Agendas included in Annex 1). All presentations and documents are available on CIRCABC.

Welcome and highlights since the last meeting

EURL ECVAM opened the meeting with a selection of highlights of activities since the last meeting, including the 25 year celebration of the establishment of (EURL) ECVAM.

There have been significant achievements in the area of **skin sensitisation** (see EURL ECVAM Strategy for Replacement of Animal Testing for Skin Sensitisation). Following up on the workshop on Integrated Approaches to Testing and Assessment (IATA) in 2015 and the combined efforts from a range of contributors, progress is moving swiftly in the area.

- Various approaches for integrating data within Defined Approaches (DAs), in particular data generated with the regulatory adopted *in vitro* methods, have been proposed as components of Integrated Approaches to Testing and Assessment (IATA) and documented in OECD GD 256 using harmonised templates for their reporting (provided in OECD GD 255). The drafting of these two documents were led by EURL ECVAM within the OECD Working Party on Hazard Assessment
- Three of the Adverse Outcome Pathways (AOPs) published during 2016 by the OECD have been developed by EURL ECVAM. These AOPs relate to chemical-induced liver fibrosis, aspects of neurotoxicity in adults, and certain neurotoxicological effects that can be caused during human development.
- EURL ECVAM has provided major contributions to the updating of **REACH annexes VII and VIII** to reflect scientific progress in the areas of skin corrosion/irritation; serious eye damage/eye irritation; skin sensitisation and acute systemic toxicity. In the area of skin sensitisation, these revised provisions makes information from the newly adopted non-animal methods (Direct Peptide Reactivity Assay (**DPRA**), OECD, 2015a; **KeratinoSens™**, OECD, 2015b and the human Cell Line Activation Test (**h-CLAT**), OECD, 2016c) the default requirement. This has also prompted an update of the European Chemical Agency's (ECHA) guidance for industry, which EURL ECVAM is supporting.

Since the last meeting, there have been **six test submissions** which include 3 pre-submissions and 3 full submissions. The ESAC peer reviews which were completed in 2016 covering the endpoints of eye damage/irritation, skin irritation and skin sensitisation were described. EURL ECVAM is also preparing Recommendations on reconstructed human cornea-like based test methods (EpiOcular EIT and SkinEthic HCE), human-based CYP induction assays, and on the use of non-animal approaches for skin sensitisation assessment. Full details of all of these are available in the EURL ECVAM Status Report which was published in October 2016.

The EC launched the **Endocrine Active Substances Information System** (EASIS) in September 2016. This web-based application, developed by the JRC, allows searching and collecting results from different scientific studies on chemicals related to endocrine activity.

EURL ECVAM reported on the **meetings and workshops** which it has hosted during 2016 with stakeholders and international partners. This included a successful training workshop with the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL), in which the members participated in practical training sessions on DPRA, KeratinoSens™/LuSens and h-CLAT OECD TG methods, as well as a highly productive workshop with the International Cooperation on Alternative Test Methods (ICATM) and international regulators. This workshop brought together regulators and alternative method experts from a variety of regions and sectors, to facilitate a common understanding of the non-animal approaches that are available in the area of skin sensitisation and their current proposed use. The outcome of the workshop will be captured in two peer-reviewed journal papers, to be submitted.

EURL ECVAM is working with other Commission services and EU agencies to develop guidance for the assessment of human and environmental health risks from chemical mixtures. This is a cross-sectorial issue which will require the input from many actors to devise a coherent strategy for mixtures.

Dissemination and promotional activities on the use of alternative methods this year have included:

- TSAR (Tracking System on Alternative Methods towards Regulatory acceptance) – launched December 2016.
- DB-ALM (EURL ECVAM DataBase service on ALternative Methods) – enhanced version completed and released in September 2016 with access to over 300 method descriptions.
- EURL ECVAM Search Guide – main use in national training programmes, particularly related to FELASA courses and outside Europe; interactive website development started.

EURL ECVAM continues to provide the following databases and information systems: ChemAgora portal, Chemical Lists Information System (CheLIST), EURL ECVAM Genotoxicity and Carcinogenicity Consolidated Database of Ames Positive Chemicals and JRC QSAR Model Database.

The Good *In Vitro* Method Practice (GIVIMP) technical Guidance Document is being developed within a project of the OECD Test Guidelines Programme (joint activity with the OECD GLP Working Group) and describes best practice for the development and reliable implementation of *in vitro* methods intended for regulatory use in human safety assessment. EURL ECVAM is leading this document and there has been very active engagement from many experts, including EU-NETVAL.

EURL ECVAM highlighted the publication of the new book, "Validation of Alternative Methods for Toxicity Testing", which provides an overview of the state of the art in method validation. Validation strategies are discussed for methods employing the latest technologies, such as tissue-on-a-chip systems, stem cells and transcriptomics, and for methods derived from pathway-based concepts in toxicology.

The JRC report, "Replacement, reduction and refinement of animal testing in the quality control of human vaccines", provides brief descriptions of projects developing or validating 3Rs methods for quality control of human vaccines, in which EURL ECVAM is involved. The new project "Vaccine batch to vaccine batch comparison by consistency testing" - VAC2VAC - started in March and is a private-public consortium involving 20 partners which aims to develop non-animal methods and apply them within the consistency approach (in other words integrated testing strategy) for the quality control of human and veterinary vaccines.

EURL ECVAM detailed its significant contribution to the **OECD Test Guideline (TG) Programme**. It is currently co-leading an OECD project aiming at the reduction of control fish, and contributing to the revision of TG203 on acute fish toxicity testing and GD126 on threshold approach to include the fish embryo acute toxicity test. In collaboration with the US, EURL ECVAM is working on the development of two OECD test guidelines using trout S9 and hepatocytes to determine *in vitro* hepatic clearance. EURL ECVAM is also leading the development of a Test Guideline for the establishment of a human-derived hepatic system to investigate biotransformation and toxicity of compounds by evaluation of CYP450 induction competence, a Performance-based Test Guideline on androgen receptor transactivation assays and co-leading a Guidance Document on an Integrated Approach to Testing and Assessment for Serious Eye Damage and Eye Irritation. It also submitted two new project proposals to the OECD. One proposal relates to the development of a Performance-based Test Guideline on defined approaches and test methods for skin sensitisation and the other to a Guidance Document for the characterisation and description of *in vitro* hepatic metabolic clearance methods. EURL ECVAM published its report exploring possibilities for avoiding chronic fish tests by extrapolating from existing acute fish test data in May 2016.

As a member of the European Partnership on Alternative Approaches to Animal Testing (EPAA), EURL ECVAM is involved in three projects; two are dealing with replacement of severe animal tests and the third with harmonisation of 3Rs approaches. EURL ECVAM is also a topic leader on behalf of the European Medicines Agency (EMA) at VICH[1] working towards harmonisation of criteria to waive the target animal batch safety test (TABST) and laboratory animal safety test (LABST) for veterinary vaccines.

In the context of the European Commission's communication in reply to the European Citizens' Initiative - Stop Vivisection, EURL ECVAM has identified opportunities to encourage knowledge sharing across disciplines and sectors aimed at more efficient development and use of alternative approaches. The report will be published shortly.

Discussion

[1] International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

Regarding the submissions of new test methods, the question was raised about the regularity of consultation with ESTAF. EURL ECVAM responded that PARERE are consulted in the first instance to give their opinion on the regulatory relevance of a proposed test method. EURL ECVAM may also consult ESTAF on a case by case basis, depending on the relevance of the consultation to individual stakeholders. ESTAF has a broad agenda for engagement and is also consulted at the beginning on draft EURL ECVAM Strategies and EURL ECVAM Recommendations. ESTAF are also consulted on priority topics, such as on the development of IATA (2015 workshop) and in the scope of this year's meeting, on ESTAF's activities in the field of basic and applied research and education and training, EURL ECVAM reminded ESTAF members that they are also invited to contact EURL ECVAM in case there is a specific topic which they wish to discuss in order that these issues might be prioritised at future meetings.

Review of Directive 2010/63/EU: uptake of alternative approaches in basic, applied and translational research and education and training.

Directorate General for Environment described the review of Directive 2010/63, which will be completed by the end of 2017. However, Member States (MS) and the user community still have limited experience of working with the Directive and so the review report will be informed by targeted consultations with users, breeders and suppliers, stakeholders and MS authorities. It will also be informed by an updated scientific opinion on non-human primate use by the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER), and by alternatives by the EURL ECVAM Status Report. Additional information on the effectiveness of the Directive in promoting development and uptake of alternatives in the areas of education, training and basic and applied research will be obtained through more focused questions to relevant members of ESTAF, Three Rs Centres and specific organisations in the area of education and training.

Identifying promising *in vitro* methods

EURL ECVAM outlined the current test method submission process, which follows two mandatory steps, (presubmission and complete submission). This approach mainly fits test methods which have a regulatory application. However, with this approach it is becoming increasingly difficult to assess regulatory relevance of mechanistic methods one by one in the absence of an overarching framework and/or of a toolbox of methods to be assessed simultaneously. The current process is also not particularly well suited to identify promising regulatory methods at an early stage of development/standardisation, and test systems/methods for use in basic, applied and translational research (e.g. disease models). Therefore, several ideas have been suggested and these include more effective direct engagement with external laboratories which may be developing or working with improved or innovative methods, better engagement with research consortia as well as strengthening the established network with 3Rs centres. Systematic surveys of the scientific literature could also assist in the identification of promising new methods. A new submission portal is being considered which could be designed to fit both regulatory test methods and test methods/systems relevant for basic, applied and translational research and could be used to survey identified priority areas.

Discussion

ESTAF raised the question of how to harvest the many methods potentially lost in the research stages which do not proceed any further. The general agreement was that funding is the key to providing an incentive, particularly to academic researchers. By mapping the regulatory needs and engaging with the funding bodies to communicate these needs within the funding criteria could be a way of identifying promising new methods at earlier stages of development.

Disease models in biomedical research (BMR)

During 2011 (see Commission report), more than 60% of the animals used in Europe for scientific experiments were used for research and development in the fields of human medicine, veterinary medicine, dentistry and in biological studies of fundamental nature. Areas of research include neurological, infectious, digestive, genetic, connective tissue, and chronic diseases. Ethical concerns, as well as the cost, maintenance and relative inefficiency of animal research, have encouraged the development of alternative methods for the study of disease. Major efforts are needed to identify and promote the use of non-animal tools and methods in biomedical research, particularly in developing ambitious education and training programmes, as well as improving communication and dissemination.

EURL ECVAM outlined what is currently being done, and what can be done, to promote the use of non-animal tools and methods in biomedical research. EURL ECVAM, for instance, has provided DB-ALM and the EURL ECVAM Search Guide to promote the use of non-animal methods whilst Science Europe and the NC3Rs organised a joint workshop in 2015 to explore some of the key issues relating to research involving animals in biomedical research and development. During the meeting of European 3Rs Centres in April 2015, the following priorities were proposed to reduce animal use in biomedical research:

- More systematic and effective critical assessment of animal-based studies.
- Improve communication about all aspects of the 3Rs including the development of ambitious education and training programmes.
- Understand and describe organisational and institutional obstacles to the 3Rs.
- Facilitate more extensive input from 3Rs centres in the project evaluation process.

ESTAF and PARERE members were asked to contribute their ideas and initiatives for this area. Taking the priorities listed above as a basis to establish common goals, strategic aims can be identified for the network to collaborate on.

Highlights on alternatives used for basic, applied and/or translational research purposes and/or for education & training

During the organisational phase of the joint PARERE-ESTAF meeting, some selected ESTAF members (i.e. those involved in research, education and training activities) had been asked to give 10-minutes flash presentations on their activities related to alternatives used for basic, applied and/or translational research purposes and/or education and training during the meeting. The following was presented:

ESTIV

The European Society of Toxicology In Vitro (ESTIV) works to promote *in vitro* and *in silico* toxicology scientifically and educationally worldwide. ESTIV's membership represents 31 countries and several sectors, including academia, government, non-profit organisations, industry, industry associations and consultancy, with about half its membership being public and non-for-profit and half representing the private sector. Training and dissemination of information on 3Rs across sectors is a priority. As such, ESTIV holds regular applied and practical training courses and workshops (both in Europe and abroad) as well as the international biennial congress (19 congresses organised since 1980) to encourage research and the use of 3Rs methodologies, and issues a newsletter as means to distribute information on *in vitro* and *in silico* toxicology across its membership. ESTIV also financially supports several conferences every year organised by similar organisations and societies. Another priority in ESTIV's strategy is to attract and integrate young scientists, offering free membership to students and giving awards for best poster and oral presentations at ESTIV and EUROTOX meetings. ESTIV is an affiliated society to EUROTOX and is an accredited stakeholder within organisations such as EURL ECVAM (member of ESTAF), ECHA and ILSI-HESI, working as a communication channel between its membership and these organisations. Working with a range of affiliated and partner societies, such as SSCT, Celltox, INVITROM, CAAT, CAAT-Academy, IVTIP, EUSAAT, SFT, SPTC and ESACT, ESTIV enables research through cross-sectorial communication and networks.

EUROTOX

EUROTOX is the federation of national societies of toxicology in Europe, which together have approximately 7,000 members. Members come from academia, industry, contract laboratories and regulatory authorities. Historically, EUROTOX has its roots in the European Society for the Study of Drug Toxicity, which was founded in 1962 in Zürich. The first annual scientific meeting was held in 1963 in Paris; in September 2017, the 53rd Congress of the European Societies of Toxicology will be held in Bratislava, Slovakia (http://www.eurotox2017.com/).

The mission of EUROTOX is to advance human, environmental and animal health by being the leading voice of toxicology in Europe. EUROTOX is interested to be in touch with European authorities and institutions to help create awareness of the demand of alternative methods, especially in the field of toxicology. EUROTOX is organising congresses, workshops and modular training programs and is able to pick up new trends and disseminate knowledge on alternative methods within the scientific community. The organisation is providing a platform to discuss regulatory expectations and technical feasibility of alternative methods as it has already demonstrated in the past.

ECOPA

The European consensus-platform for alternatives (ecopa, www.ecopa.eu/) is a European not-for-profit organisation. Ecopa is an umbrella organisation for national platforms. It was established in 2001. Ecopa and the national platforms strive for consensus between the four stakeholders of government and regulatory authorities, academia, industry and animal protection and welfare organisations. All four stakeholders have to be represented in both ecopa´s and the national governing bodies. Associate members such as individuals, academic institutions, professional associations, companies, other European or international networks and any other organisation

which supports ecopa´s aim but fail to qualify for membership as a National Contact Point (NCP) can also be approved to be members of ecopa but without voting rights.

The primary aim of ecopa is to promote "the three Rs" (Replacement, Reduction and Refinement, 3Rs) in use of animals in research, testing, education and training in Europe. This is done by facilitating the exchange of scientific information, expertise and experience between the national platforms, academia, animal welfare, industry and government and the EU, promote further development and implementation of 3R-methods in the EU and worldwide and raise public, governmental and scientific awareness for a better acceptance of alternative methods. The main forums for promotion of 3Rs are via yearly organised ecopa workshops, through the ecopa web page and newsletter, by being a member of stakeholder forums such as in ESTAF and by participating in expert group meetings such as those of ECHA´s PEG. Ecopa´s special focus is to get young scientists involved in the 3Rs. Ecopa also provides funding to participate in ecopa workshops.

CAAT Europe

CAAT (Centre for Alternatives to Animal Test), was founded in 1981. At that time the main reason to look at alternative methods was mainly ethical, while nowadays it is clear that the so called alternative methods constitute advanced techniques to get to a more efficient and eventually better way towards human response prediction. Today CAAT is an international organisation with offices in Konstanz, hosting CAAT-Europe, and in Brussels with the CAAT Policy Program. In the EU, CAAT-Europe has no research laboratory, rather it serves as a forum to foster discussion among diverse groups, such as academia, industry and regulators, leading to creative approaches to facilitate acceptance and implementation of alternatives. It also aims at the dissemination of reliable and scientific information to relevant stakeholders, including the general public. Another branch is CAAT Academy which is dedicated to educating and training scientists and students in the application of these non-animals techniques. CAAT-Europe is mainly dedicated to the organisation of specific workshops and symposia, plus more general conferences or congress sessions. It is also a partner in the EU-ToxRisk project (www.eu-toxrisk.eu), where it is leading the work package on dissemination and training and participates in the work package for quality control and validation of the new methods. Collaboration with EU institutions such as EURL ECVAM, but also ECHA (European Chemical Agency) or EFSA (European Food Safety Authority) represents a fundamental point for CAAT-Europe's mission.

The CAAT newsletter is distributed among 8500 recipients, and CAAT-Europe members are authors in 51 publications over 6 years, with 351 co-authors. The main journal is Altex (www.altex.ch), which is open access and has gained an Impact Factor (IF) of 5.8. This is not the arrival, but the starting point for intensive work. Success of alternative methods may come only through the commitment of the actors involved and, more importantly, from the collaboration of stakeholders.

AFABILITY

The global antibody industry produces an indispensable resource but that is generated using millions of unaccounted-for animals. Yet despite the irrefutable maturation and availability of Animal Friendly Affinity Reagents (AFAs), typically produced by phage display, animal immunization continues to be authorized for antibody generation. AFAs are structurally equivalent, functional in a wide range of applications, have equal or greater specificity and affinity to a huge repertoire of

antigens and offer greater control over their properties, generation time and cost. They are available commercially and are reproducible in the laboratory. Remarkably, even though the impact on animal numbers is enormous owing to the reliance on antibodies by scientists, healthcare professionals and consumers, in all areas of research, safety testing, health and the environment, the opportunity for replacement has been overlooked. Misconceptions exist regarding the replacement technology including their efficiency and range of technical applications. There is a lack of expertise or motivation by users of traditional methods and a propensity toward familiar methods. There is also lack of awareness and therefore enforcement at the level of the Member State. AFABILITY is a non-commercial scientific organisation striving to replace the use of animals in antibody production by using AFAs, thereby significantly reducing the numbers and suffering of animals in the biomedical sciences. AFABILITY challenges the *enforcement* of Directive 2010/63/EU on the use of animal-derived antibodies as a scientific procedure where alternatives exist, creates *awareness* of the use of animal derived antibody production methods by all scientific disciplines, and improves the *availability* of animal derived antibody replacement methods. For more information, visit www.afability.com

PISC

The PETA International Science Consortium promotes the best non-animal research methods and coordinates the scientific and regulatory expertise of 8 PETA affiliates. The Consortium is involved in the development, validation, global implementation and harmonisation of alternatives to testing on animals, and adopts a broad approach to reducing and replacing tests on animals through education and training. The Consortium is a member of the International Council on Animal Protection in OECD Programmes and ESTAF, and is an accredited stakeholder at the European Chemicals Agency.

With regard to education and training, the Consortium hosts workshops and webinars, organises in-person training sessions and develops educational resources. For example, the Consortium presented a webinar series promoting the use of alternative methods for REACH, co-hosted workshops and webinars on alternative approaches for identifying acute systemic toxicity and acute and subchronic inhalation toxicity. Furthermore, the Consortium cooperates with organisations such as the Institute for In Vitro Sciences to offer training to regulators and develops fact sheets on the use of alternative methods, publishes in peer-reviewed journals and presents at conferences.

Actions:

With regard to the field of biomedical research, some priorities were identified during the 3Rs Centres meeting in 2015:

- More systematic and effective critical assessment of animal-based studies.
- Improve communication about all aspects of the 3Rs including the development of ambitious education and training programmes.
- Understand and describe organisational and institutional obstacles to the 3Rs.
- Facilitate more extensive input from 3Rs centres in the project evaluation process.

PARERE and ESTAF members are invited to identify one or more of these areas for future collaboration, or to provide further suggestions for collaboration.

Annex I – Agenda

PARERE-ESTAF Meeting

JRC Ispra, 8th November 2016

Building 58, Auditorium (Room 11)

14:00-14:30	Welcome and highlights since the last meeting *Maurice Whelan, EURL ECVAM* *Valérie Zuang, EURL ECVAM*
14:30-16:00	Review of Directive 2010/63/EU: uptake of alternative approaches in basic, applied and translational research and education and training. *Susanna Louhimies, DG Environment* Identifying promising *in vitro* methods *João Barroso, EURL ECVAM* Disease models in biomedical research *Laura Gribaldo, EURL ECVAM*
16:00-16:30	*Coffee Break*
16:30-18:00	Highlights on alternatives used for basic, applied and/or translational research purposes and/or for education & training Flash (10 minutes) presentations from: **ESTIV** **EUROTOX** **ECOPA** **CAAT Europe** **AFABILITY** **PISC**
18:00	Meeting closure
18:30 Bus transfer to Hotel Hilton for social dinner	