EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials
Chemical Safety and Alternative Methods
European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM)

Summary Record

Joint PARERE-ESTAF Meeting

28-29 November 2018, Ispra, Italy

The joint meeting of PARERE (EURL ECVAM Network for Preliminary Assessment of Regulatory Relevance) and ESTAF (EURL ECVAM Stakeholder Forum) was hosted by the Chemical Safety and Alternative Methods unit incorporating the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) of the European Commission's Joint Research Centre. It started in the afternoon of 28 November 2018 and was followed by a half day breakout group discussion on how to develop more purpose-driven methods and apply successfully the existing ones.
The agenda is included in Annex I.

Welcome and EURL ECVAM highlights

EURL ECVAM opened the meeting with a selection of highlights on its recent activities since the last meeting. These included:

- updates on the outcome of the 30th meeting of the Working Group of National Coordinators of the OECD Test Guidelines Programme;
- the publication of the OECD guidance document no. 286 on Good *In Vitro* Method Practices (GIVIMP);
- some preliminary results of the EURL ECVAM survey on issues influencing end-user confidence in complex *in vitro* models (e.g. 3D tissue cultures and Organ-on-Chip);
- recent activities and updates on endocrine disruptors (EDs), including the ECHA-EFSA guidance document for the identification of EDs drafted with the support of the JRC, the EC communication COM/2018/734 'Towards a comprehensive European Union framework on endocrine disruptors', the large scale validation study of *in vitro* methods for the detection of thyroid disruptors, and the status of the AR-CALUX test method;
- the current status and the expected progress of the Adverse Outcome Pathway (AOP) framework and related OECD programme on AOP development;
- the introduction of non-animal test methods in the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), and the expected revision of the United Nations Transport of Dangerous Goods Model Regulations to include the updated OECD Test Guideline no. 431 to avoid animal testing for safe transport of corrosive chemicals;

- the newly appointed EURL ECVAM Scientific Advisory Committee (ESAC) core members and the upcoming ESAC reviews;
- updates on EURL ECVAM projects such as: education and training initiatives; review of non-animal methods in use in basic and applied research; the BeAMS initiative aiming to increase connectivity and transdisciplinarity across biosciences; and indicators for monitoring the uptake of alternative methods for scientific purposes.

More details on EURL ECVAM activities are available in the EURL ECVAM 2018 Status Report [1].

Breakout group discussion on multi-stakeholder initiatives for purpose-driven development and application of non-animal methods

A multitude of non-animal methods are currently available while others are under development. However, many of these methods do not have a defined use for chemical safety assessment. In this context and to inform the break out group discussion, invited speakers gave plenary presentations touching on different aspects of validation.

Members of PARERE, composed of national regulators and representatives from EU regulatory agencies, and ESTAF, which comprises stakeholders from industry, scientific and civil society organisations, were divided in three breakout groups and discussed the following question:

"What multi-stakeholder initiatives can you propose that would lead to a more purpose-driven development of new methods and the successful application of existing methods?"

The aim was to collect proposals from the participants for collaborative actions that could contribute to the development of new methods or the application of existing ones.

Initially the three breakout groups discussed how to interpret the question, including what was meant by 'purpose-driven' in the framework of risk assessment. Non-animal methods for safety assessments were agreed to include both in-house methods used within industry and test guideline methods requested by regulators.

The outcome of the breakout group discussions is summarised below.

- **Better dialogue and coordination between regulators and test method developers**

Non-animal methods must be better tailored for regulatory purposes and regulators must make a more informed use of non-animal methods. While non-animal methods should be developed for use in the decision-making process, they often cannot be used in a regulatory context because fundamental pieces of information are missing, e.g. lack of metabolism in the methods, or the dose used cannot be related to the exposure level. To ensure that relevant tests are developed, developers need to be advised and informed on the regulatory framework in which their methods could eventually be used. Improving the dialogue between regulators and test method developers for example by means of advisory groups (including both scientists and regulators) would result in a better understanding of the information required for regulatory purpose.

Regulators could be involved in the different steps of method development to provide advice on regulatory applicability as well as be trained on how to apply methods once they become OECD test

guidelines. A greater involvement of regulators could be achieved by engaging them in projects from an early stage, in data interpretation from *in vitro* methods and in the preparation of mock dossiers, similar to what is already being done in EU-ToxRisk[1], the European collaborative project funded under Horizon 2020.

The development of new *in vitro* methods should be purpose-driven and for example prioritised with respect to specific data requirements. A screening phase could be based on alternative approaches, including *in vitro* methods anchored to key events defined in the existing Adverse Outcome Pathways (AOPs). Indeed, AOPs could help prioritise testing by identifying critical key events to be further investigated. It could also be more efficient to consider validation according to a specific purpose, i.e. research, prioritisation, hazard or risk assessment. The advantages and limitations of both *in vitro* and *in vivo* methods should be made clear and be captured in guidance documents, e.g. the ECHA Guidance on Information Requirements and Chemical Safety Assessment [2] contains a lot of knowledge on the relevance of observations in animal tests for human health.

Rethinking the regulatory requirements for toxicological property (endpoint) information is also key to developing more purpose-driven methods. In this regard, it should be considered whether to adapt the current requirements (e.g. to be based more on mechanistic data) and to start elaborating new frameworks.

Formal fora dealing with specific questions regarding human, environmental and veterinary health offer effective means to discuss these concepts. The fora might be led by the JRC or other internationally recognised institutions and involve all main stakeholders dealing with chemicals, medicines, medical devices, etc. with the aim of building on activities already existing at e.g. the OECD or EPAA, or developed in the context of FP7 and Horizon 2020 projects, like the *ab initio* approach [3]. To reach a large audience, the outcome of such discussions should be made publicly available. Developing and validating test methods to answer specific questions and fill identified gaps has already been followed for example in the area of topical toxicity (skin irritation/corrosion [4] and serious eye damage/eye irritation [5,6]), phototoxicity [7], the *in vitro* thyroid validation study [1], and the *in vitro* DNT project [8,9].

Another suggested action was training courses targeting EU agencies as well as regulators at Member States level. In this process the roles of networks such as PARERE, which comprises regulators from the Members States, and the National Contact Points of the Member States, who are responsible for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes, are pivotal. A success story was indeed the training course on AOPs provided by the Joint Research Centre / EURL ECVAM to EFSA experts (both staff and members of advisory/expert committees [1]).

The AOP framework is a useful tool in regulatory decision making, however, many researchers are not aware of its existence or trained in the development of AOPs. A possible way to overcome this can be to strongly encourage researchers to first look at the existing AOPs in the AOPwiki[2] before performing experimental work. It should also be considered that no reward systems or other incentives have been set up for the development of AOPs, which represents an additional barrier to the development and use of this tool. At the moment the AOPs are toxicology-centred, making them

[1] http://www.eu-toxrisk.eu/
[2] https://aopwiki.org/

relevant and intelligible for regulatory purposes. Widening the context to other areas (e.g. biomedical research) was discussed, but it could make the AOPs too complex for regulatory use as the foreseen use will determine the level of detail required.

Proposed initiatives:

- Experts in a specific field, e.g. heart disease, to explain to regulators what the key events are in a specific pathway and what methods may provide that type of information.

- Training in regulatory toxicology for academic scientists and training on the interpretation of data from non-animal approaches for regulators is needed. For example, the JRC might provide dedicated training for regulators, as was done for the AOP training at EFSA. JRC has also been asked to provide training to the new EURION cluster[3] developing methods for endocrine disruption.

- Organise open days on non-animal approaches involving several actors, such as test developers, industry, academia, and regulators.

- **Harmonisation of regulations across different sectors**

Several EU regulations which include data requirements for (eco)toxicity testing have been in place for many years and some of these are currently being updated. On the other hand, there are areas outside of these regulated sectors where non-animal methods could be introduced (e.g. the area of contaminants or mixtures). However, regulations of different sectors are based on different principles and this creates a barrier to harmonisation across sectors. For example, the latest update of REACH standard information requirements was carried out in 2017, while the information requirements for the biocide and pesticide regulations are currently under discussion. It is hoped that a greater harmonisation will be ensured between these different pieces of EU legislation. There are also differences between regulations in different countries. For instance, EU regulations on biocides, pesticides and REACH already include non-animal methods but this is not consistent with regulations from other non-EU countries. Consideration should also be given to the new regulation for medical devices that are in need of an independent authorisation process. Currently, the authorisation of medical devices in the EU is not under the responsibility of a specific European agency.

A clear overview of the non-animal approaches available would be highly relevant as a first step and this can be followed by a collective effort to evaluate their applicability under the existing regulations. Several databases on alternative methods are currently in place, such as the EURL ECVAM Tracking System for Alternative methods towards Regulatory acceptance (TSAR)[4] and the Belgian RE-Place project[5], which collects New Approach Methodologies (NAMs) from the Flemish

[3] http://www.uef.fi/en/web/edcmet/eurion
[4] https://tsar.jrc.ec.europa.eu/
[5] https://www.re-place.be/

and Brussels regions and also acts as platform to connect researchers and possible partners. The French institute INERIS has set up a public-private platform for the pre-validation of methods for endocrine disruption testing to help start-up companies, laboratories and consortia design and conduct the first phases of a pre-validation study of new methods before submitting them to a validation body. In this context it might be useful to explore the creation of a database where *in vitro* methods are described according to key events of AOPs.

Proposed initiatives:

- Identify the alternative approaches available and evaluate their applicability under the existing regulations.
- Develop databases of *in vitro* methods anchored to key events of existing AOPs.

- **Translation of mechanistic knowledge to the regulatory domain**

A common need to exploit all information that is available, e.g. read-across, toxicokinetics, threshold of toxicological concern (TTC), and human data, has been identified. In this context, a case study is based on an ontology project [10] by Cosmetics Europe, which aims at translating the knowledge from a mechanistic point of view into regulatory applications, as in the case of liver toxicity where many data are available, and in which some toxicity pathways and the cause-effect relationship are known. There is also a need to understand if and how transcriptomics data could be used.

Toxicology is not specific to a single organ. At the mechanistic level many phenomena are common to multiple organs. It is therefore necessary to move away from the concept of organ specific cytotoxicity.

It is time to think of a revolutionary approach and design a new framework for safety assessment that uses the information we really need, including human exposure data. These data are available from companies or from biomonitoring (e.g. occupational health) studies, but are sometimes not sufficiently exploited because of difficulties faced in the interpretation of results (i.e. confounding factors, uncertainties in external exposure data, high variability). In this regard, sharing databases of peer-reviewed information becomes highly relevant. An approach might be to first identify what data are missing and then correlate biological activity through transcriptomics pattern (genomic platforms) combined with toxicokinetics/physiologically based kinetic (PBK) modelling. Such data come primarily from *in vitro* studies and not from animal studies.

Crucial for the proper use of alternative approaches is the need to characterise the test system in terms of its kinetics. This is in order to adjust *in vitro* concentrations to real life exposure. The use of human-relevant *in vitro* methods in combination with *in vitro* to *in vivo* extrapolation (IVIVE), QSAR, PBK modelling, read across and other non-testing methods promises better toxicity prediction than any animal study. Metabolically competent systems should be included in testing strategies. Complex *in vitro* systems might be used such as co-cultures of a cell type with hepatocytes or add a standardised S9 microsomal fraction.

Proposed initiatives:

- Involve pre-clinical and clinical partners in projects as they have human data after chemical exposure that could give more insight in the mechanisms of action and aid development of tools for regulatory decision making.
- Better knowledge and characterisation of test systems from both developers and users.
- Better use of all information available, e.g. read-across, toxicokinetics, TTC, and human data whenever available.
- Move away from organ specific pathways of toxicity towards common multi-organ pathways.
- Develop case studies since they represent a relevant tool to build confidence in alternative methods and to demonstrate their applicability within testing strategies.

- **Calls for funding, publishers and open access**

Research proposals should be more focused on increasing knowledge in a specific field of non-animal approaches. For such research projects rewards and incentives (e.g. publications in high impact journals and continued grant funding) should play a greater role. Peer-reviewers can clearly contribute to moving forward in this area since the decision to accept and publish those studies is up to journals based on the recommendations of peer-reviewers.

It is recognised that research funds at the European level, e.g. EU framework programme Horizon 2020, have a great impact and more could be funded also at the national level if driven e.g. by regulators. Regulators could attend more regularly scientific conferences to stay informed about progress and new developments in the field.

The launch of open calls for input on a specific Three Rs or regulatory needs has proven very useful to move forward non-animal methods in a specific area. Successful examples are the EFSA (developmental neurotoxicity[6]) or EPA (acute oral toxicity[7]) initiatives. Consideration should be given to increase the number of such calls. This, in turn, will allow test method developers to align their research with regulatory needs, as scientists are not always aware that their methods could be used in a regulatory context. Another aspect of interest is requiring that the results from funded research are published in peer-reviewed journals as open access with availability of raw data and this could be included in funding agreements, as is currently done for the EU framework programme Horizon 2020. Automated searches could be used to harvest information provided in the papers.

[6] https://www.einnews.com/pr_news/350423533/developmental-neurotoxicity-oecd-efsa-experts-discuss-non-animal-test-methods
[7] https://www.epa.gov/toxics-release-inventory-tri-program/acute-toxicity-data-tri-listed-chemicals

Proposed initiatives:

- Open access for publications with, ideally, availability of raw data from publicly funded research.

- A great number of open calls looking for inputs on specific Three Rs or regulatory needs would be highly beneficial.

Bibliography

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Annex I – Agenda

Joint meeting of the Preliminary Assessment of Regulatory Relevance (PARERE) and the EURL ECVAM Stakeholder Forum (ESTAF) networks

1st day: Wednesday 28 November 2018

Building 58 Auditorium

14:00-14:15	**Welcome and introduction** *Maurice Whelan*
14:15-15:00	**Flash presentations on EURL ECVAM highlights** *EURL ECVAM*
15:00-15:30	**Question and answer session**
15:30	Group photo
15:30-15:45	Coffee break
15:45-18:15	**Plenary presentations on validation (20 min presentations+10min discussion)**
15:45-16:15	**The principles of validation** *Andrew Worth, EURL ECVAM*
16:15-16:45	**Outcome of BfR-RIVM workshop held in June 2018** *Michael Oelgeschläger, BfR*
16:45-17:15	**Outcome of ICATM workshop held in October 2018** *João Barroso, EURL ECVAM*
17:15-17:45	**Validation strategies within the EU-ToxRisk project** *Costanza Rovida, EU-ToxRisk*
17:45-18:00	**Working instructions for the breakout groups**
18:15	Departure from JRC to Hilton Hotel
19:40	Departure from Hilton Hotel to Restaurant Corte Visconti
20:00-22:30	Dinner

JRC Ispra, 28-29 November 2018

2nd day: Thursday 29 November 2018

Break out rooms: building 100, room 1102; building 101, rooms 2002 and 2302

09:00-11:00	**Break out groups for discussion on the following question:** *Relevant methods for relevant problems:* *There is currently a multitude of non-animal methods available and under development which do not have a defined use for safety assessment.* *What multi-stakeholder initiatives can you propose that would lead to more purpose-driven development of new methods and the successful application of existing methods?*

Building 58 Auditorium

11:00-11:30	Coffee break
11:30-12:30	**Reporting back from break out groups and discussions**
12:30-13:00	**Wrap up**
13:00	Light buffet lunch and departures