EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Systems Toxicology Unit
EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

Summary Record

PARERE Meeting

20th October 2015, Ispra, Italy

The PARERE meeting was held on the morning of 20th October 2015 (agenda included in Annex I) and was followed by a joint PARERE-ESTAF meeting (summary record available).

The meeting commenced with a brief tour-de-table for members to introduce themselves.

1. Status of the establishment of PARERE in MS

A feedback session on the results from the survey on the establishment of the PARERE network within Member States (MS) was presented with an overview of the responses to each question.

- For the majority of MS, the PARERE Contact Point (CP), EU National Contact Point (NCP) and OECD National Coordinator (NC) are not the same person. However, for Austria (AT), the Netherlands (NL) and Belgium (BE), the PARERE CP is the same as the OECD NC whilst Lithuania (LT), Latvia (LV) and Portugal (PT) currently have no OECD NC.
- Regarding the current format of the network, members discussed the difficulties relating to the communication exchanges. Some MS experience specific difficulties (see the slides for the meeting). Spain mentioned that the main problem is that there is an issue with uniformity in the responses to consultations from their experts. However, these heterogeneous responses can be very informative and, when viewed in context, can inform on the type of responses coming from different sectors.
- Progress has been made with the network: there are now 24 MS represented. The main purpose of the network is to gather feedback on the regulatory relevance and potential utility of alternative methods/approaches, tapping into all the valuable knowledge and vast experience distributed across numerous MS regulatory bodies. Communication is the key: PARERE contact points need to strive for ever stronger communication links within the network and to learn from each other. MS could do more at the OECD level to promote alternative approaches. The PARERE network can assist with this aspect. More face-to-face meetings within MS organised by PARERE contact points, as opposed to just circulating documents, would be ideal to further engage representatives of the regulatory community to consider the use of alternative approaches in decision-making processes that they typically oversee.
- How to strike the balance between scientific evaluation and regulatory relevance assessment in order to provide a holistic opinion on a new method/approach is essentially up to individual MS to decide. Interesting perspectives from a variety of sectors can be channelled through

PARERE. However, the participation of different regulatory sectors is often not equal and so there is a need for more engagement, cross-talk and knowledge-exchange between these sectors.

- For questions about regulatory relevance, PARERE is the network for consultation. For an industrial/user viewpoint, ESTAF is typically consulted by EURL ECVAM.
- EURL ECVAM concluded this session by commending PARERE on the quality and relevance of their responses which demonstrates that the network is working.

2. Feedback from PARERE members on their experience in carrying out their roles

This discussion session highlighted the following points in relation to consultations on regulatory relevance of proposed alternative methods:

- The PARERE network could do more in terms of communication and engagement activities to increase political awareness of the financial challenges involved in 3Rs.
- There is a sense that whilst many regulators are keen to be involved, there are some regulators who are not interested in alternative approaches and these pose barriers to progress in this area. Sometimes it is not clear to regulators how best to combine *in vitro* methods to replace *in vivo* methods to address specific key events (KEs) in adverse outcome pathways (AOPs).

3. Consultation of PARERE on the devTOX^{qP} method

EURL ECVAM presented the results of the consultation with PARERE on the regulatory relevance of devTOX^{qP}. The summary report of this consultation will be shared with PARERE following the meeting.

A few major concerns about the method were highlighted, one of which regards the mechanistic relevance of the two metabolites, ornithine and cysteine, which are measured and the rate of false negative results. Some minor concerns were also indicated by some PARERE members, namely toxicokinetics; patent issues (although this can be solved where there is a motivation to proceed); and some practical issues concerning the cell line used and the epigenetic programming of the donor cells.

In summary, the feedback from PARERE was very positive in relation to the potential of the method as a screening tool for detecting teratogens and there is some biological and mechanistic relevance for the method. However, there are still some concerns relating to the classification of the reference chemicals and the question of how to use it in a regulatory context remains unclear. There may be a need for a guidance document to summarise the regulatory context of the method. EURL ECVAM concluded that the summary of the PARERE consultation will be finalised and transferred to the test submitter. The summary will be circulated following the meeting and PARERE will have two weeks to make any final comments before sending to the test submitter.

Other feedback during the discussion included:

- The test method uses an expensive cell line to measure ROS/a stress response pathway.
- It is necessary to know where this assay fits into the landscape of mechanistic and biological responses.

- There is not enough information to conclude on the regulatory relevance of the assay. There is a lack of convincing evidence to progress: mechanistic concerns, chemical space concerns.
- It could be a very good screening tool, or under a WoE approach as part of an IATA. Therefore it could be necessary to have an IATA established before the method is approved.
- The consensus was that although there are many merits to the test method, there is not enough evidence to propose it as a test guideline.
- It was noted that the name of the assay is misleading since the scope of the assay appears to be teratogenicity, not developmental toxicity. One suggestion was to name the assay for the specific mechanistic information it provides ("stress response/polyamine synthesis assay"), evaluate the performance of the assay comparatively to other in vitro methods addressing this mechanistic information (as far as available) and mechanistically validate its use for a systemic toxicity assessment beyond developmental toxicity. This may prove especially helpful considering that testing for a series of key events may lead to multiple adverse outcomes in terms of classification and that this may even induce considerations if and how adaptations of GHS to key event based testing approaches may be necessary in future. Finally, four PARERE members expressed the need to address the patent that covers the assay as well as the cell line.
- However, it is important to encourage SMEs as they are the main developers of AMs. It is necessary and proper therefore to communicate the deliberations of PARERE on this method to the test submitter in a positive way and encourage them to continue with its development and further evaluation.

4. Priority Areas for 3Rs

The priority needs identified by the OECD include:

- improve *in vitro* metabolism for EAT assays (OECD 97, 2008[1])
- *in vitro* thyroid pathway assays (OECD 207, 2014[2])
- *in vitro* non-genotoxic carcinogenicity IATA (OECD 2015, SPSF[3])
- *in vitro* methods addressing epigenetic modes of action (MoAs, Annex 1 to OECD 178 2012[4]). This is an emerging research area which could provide more opportunities to look across the different sectors.
- uncertainty characterisation of standard *in vivo* methods and reference data, adversity *in vitro* (OECD 2015, SPSF background document[3])

Austria raised the question on a letter to DG Research asking for funding to address urgent requirements.

[1] Detailed Review Paper On The Use Of Metabolising Systems For In Vitro Testing of Endocrine Disruptors
[2] New Scoping Document on in vitro and ex vivo Assays for the Identification of Modulators of Thyroid Hormone Signalling
[3] OECD Project 4.94; see OECD Work plan for the Test Guidelines Programme (TGP): http://www.oecd.org/env/ehs/testing/TGP%20work%20plan_declassification_July%202015.pdf
[4] Detailed Review Paper on the State of the Science on Novel In Vitro and In Vivo Screening and Testing Methods and Endpoints for Evaluating Endocrine Disruptors

Discussion

EUToxRisk, which starts in Jan 2016 and will run for six years, addresses mechanism-based toxicity testing and risk assessment for repeated dose and reproductive toxicity testing with a focus on integrating AOPs into a read-across setting.

It is important to encourage regulatory scientists to contribute and to identify activities to enable a regulatory scientific community to perform a fair comparison of methods (*in vitro* versus *in vivo*). The area of hazard classification is a particularly challenging area for advancing alternative approaches since the regulatory approach usually taken is rather traditional (i.e. very strong reliance on animal data to classify hazard).

EURL ECVAM identified:

Bioelution Test Method[5]

- Flagged by some MS in the context of CLP and REACH, there is an apparent need for a standardised and internationally recognised (non-animal) method to determine the bioelution of metal ions from alloys. The Commission service in charge of CLP Regulation has launched activities on the bioelution methods for metal compounds and their applicability in the framework of CLP - Art. 12(b). DG ENV/DG GROW have organised two workshops aimed at discussing the industry proposal on the use of bioelution data for classifying alloys for human health endpoints.
- CARACAL[6] is the policy arena to discuss REACH and CLP. They will discuss this industry proposal and, if agreed, the bioelution method will be submitted to EURL ECVAM.

Discussion

- There are different bioelution protocols and these are not standardised.
- There is confusion regarding bioavailability and bioaccessibility. This lack of clarity is in the context of art. 12 (b) of the Regulation N° 1272/2008 on Classification Labelling and Packaging of substances and mixtures (CLP). This article states that the classification conclusion should take into account "conclusive scientific experimental data show that the substance or mixture is not biologically available and those data have been ascertained to be adequate and

[5] Bioavailability is defined as the extent to which a substance is taken up by an organism and is available for metabolism and interaction.
Bioaccessibility is defined as the fraction of a substance that dissolves under surrogate physiological conditions and therefore is “potentially available” for absorption into systemic circulation.
Bioelution tests are carried out to calculate the bioaccessible concentration in the alloy by comparing metal ion releases from the alloy versus metal ion releases from the reference material (pure metal in case of an alloy).

[6] This expert group advises the Commission and ECHA on the implementation of REACH and CLP. The group is composed of representatives of national Competent Authorities for REACH and CLP, representatives of Competent Authorities of the European Economic Area and European Free Trade Association (EEA-EFTA countries), as well as a number of observers from non-EU countries, stakeholders from industry and trade associations, NGOs, trade unions, and international organisations.

reliable". The possible use of data from a bioelution method in the context of art 12 (CLP) is under discussion. The regulatory position needs to be clarified.

- PARERE asked if this is really of 3R relevance. However, there is some overlap with the toxicokinetic strategy, (exposure based waiving).

Reproductive and developmental toxicity

- EURL ECVAM will produce a draft strategy document by the end of 2016. It is hoped that this will provide some guidance with respect to the development and validation of in vitro methods such as devTOX^{qP}.
- Endocrine Disruption is obviously related inter alia to reproductive toxicity. The current AOPs for ED and reproductive toxicity (www.aopkb.org) could be expanded to include the whole of the reproductive toxicity area. EURL ECVAM asked PARERE if they knew anyone in their networks who could contribute to ideas for this area. There could be a scoping or a regulatory science workshop before writing the strategy document.
- The proposed deletion of OECD TG 478 (proposed by the EC) on the Dominant Lethal Assay was mentioned. Whilst this TG has undergone significant improvements, it has scientific limitations and uses a large number of animals. The conduct of a Rodent Dominant Lethal Test is not a regulatory requirement in any EU legislation, and across sectors. European agencies, including the European Chemical Agency (ECHA), the European Food Safety Agency (EFSA) and the European Medicines Agency (EMA) have been consulted to seek their view on the impact of a possible TG deletion. All agencies replied that old data from Rodent Dominant Lethal test are only rarely found in the submitted dossiers and it has not been requested, nor it has been indicated as data gap in the past years. Moreover, the assay is not explicitly mentioned in the EFSA Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Journal 2011;9(9):2379) and in the EMA Guidelines. It is mentioned in the ECHA Guidance Document (Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance, Version 3.0, August 2014), but ECHA has never requested such test. Furthermore, there are very few facilities in each region that currently conduct the assay. Thus, practical experience is very limited. The majority of the laboratories will not have historical data and will have to rely on published control data. Thus, proficiency will have to be demonstrated by the ability to reproduce dominant lethal frequencies from published data.

Follow up

- *Strategy for 3Rs impact in developmental and reproductive toxicology – PARERE members can ask their networks to contribute ideas for this area*
- *PARERE members were asked by Austria if they could support an action to request funding for research priorities (feedback to be given directly to the Austrian contact point)*

5. AOB

- The European Citizens' Initiative will provide an opportunity to establish a mapping of the knowledge sources with potential 3R relevance and their networks currently operating successfully.

- Although there is significant investment in the 3Rs, this is not necessarily being translated into tangible validated methods. There is a gap between research and the validation pipeline. EU-NETVAL was mentioned as it is being expanded - this is a strong instrument for the deployment of these new methods.
- It is important to provide incentives to companies to take up new methods.

Actions:

i. *In coordination with EURL ECVAM's continued commitment to engage all key actors in the 3Rs area, PARERE members were asked to discuss the matters reported here within their networks.*

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Annex 1

PARERE Meeting

20th October 2015

Agenda

Item	Time	Description	Format
	9:00-9:15	**Opening of the meeting and welcome**	
1	9:15-9:45	**Status of the establishment of PARERE in MS** Results of the mapping exercise and practical issues encountered regarding the establishment and operation of the network in Member States.	Presentation (EURL ECVAM) Discussion
2	9:45-10:15	**Feedback from PARERE members on their experience in carrying out their roles** In particular in relation to consultations on regulatory relevance of proposed alternative approaches, providing feedback on EURL ECVAM "strategy" reports and commenting on draft EURL ECVAM Recommendations following ESAC Peer Review of Validation Studies.	Discussion
	10:15-10:45	*Coffee Break*	
3	10:45-11:30	**Consultation of PARERE on the devTOX^{qP} method** Brief overview of the devTOX method and the submission from the developer; Results of the written PARERE consultation; Conclusions regarding the relevance and utility of the devTOX^{qP} method in a regulatory context.	Presentation (EURL ECVAM) Discussion Agreement
4	11:30-12:30	**Identifying priority areas for the 3Rs** Open discussion on priority areas and topics where PARERE could contribute, including for example, the development of a EURL ECVAM strategy for reproductive toxicity.	Proposals (PARERE) Discussion Agreement
	12:30-13:00	**AOB**	