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

PARERE Meeting 27-28th November 2018, Ispra, Italy

The meeting of PARERE was held on 27-28th November 2018 (the agenda is included in Annex I).

Welcome and Updates

The JRC's EURL ECVAM welcomed all members and briefly highlighted the different agenda points which were up for discussions. The draft agenda was approved. EURL ECVAM then invited PARERE to give updates on activities within the PARERE network in the respective Member States.

Round-table on activities within the PARERE network

Italy informed that different dissemination and education activities on 3Rs are ongoing in universities and schools. A Working Group consisting of a pool of national experts has been established to inform the Animal Welfare Bodies. The PARERE network collaborates with the new 3Rs Centre established by the University of Pisa and the University of Genova.

Finland explained that a 3Rs consortium to promote the development of alternative methods has been established. There will be activities involving universities on education and training, promotion of alternative methods and validation.

France mentioned that sharing of knowledge on alternative methods between disciplines was an important activity in France.

In Ireland a national expert group on the protection of animals has been established.

Sweden allocates one million euro per year to proposals for replacement applications and refinement projects. A 3Rs Centre was established in Sweden in November 2017. It is the acting body of the National Committee established in 2013. The PARERE contact person is also a member of the National Committee. There is some outreach towards Baltic States to create a network with different expertise. A Swedish EU-NETVAL laboratory is funded for participating in the ECVAM validation study on the AR-CALUX test method to detect endocrine disrupting chemicals.

In Slovak Republic more engagement with universities is planned. A 3Rs platform was established in June 2018 to promote implementation and development of alternative methods and operating with the support of the Slovak Society of Toxicology (SETOX). The platform includes experts in 3Rs and relevant Ministries. The PARERE contact person acts as chair.

A PARERE representative has also now been nominated by the Luxembourgish Ministry of Agriculture, viticulture and rural development. The Luxembourg Institute of Science and Technology (LIST) developed an *in vitro* method on respiratory sensitisation that they will soon submit to EURL ECVAM for evaluation.

Representatives from two scientific committees advising the European Commission, namely the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environment and Emerging Risks (SCHEER) have also been appointed to the PARERE network. The topic of alternative methods is highly relevant for both scientific committees.

In Belgium, a database, called Re-place, that collects the knowledge on alternative methods available in the Flemish and Brussels region, has been developed.

The Netherlands informed that they were leading together with the UK, the working group "Use of non-animal testing methods for classification of health hazards" established under the UN sub-committee on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) and that they were active in the OECD Test Guidelines Programme.

EFSA informed that risk assessment uses data generated from *in vivo* studies because legislation stipulates that these are required. They keep an eye on progress with alternatives and are involved in the OECD TG adoption.

Austria aims to bring science and regulation together and started to map what competence is available in order to target funding. The PARERE work will be discussed with the Austrian Ministry of Science.

EURL ECVAM informed about the survey on the PARERE network that it had carried out in 2018 with the National Contact Points for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. Sixteen Member States replied so far to the survey (Belgium, Finland, Ireland, Slovenia, Spain, Czech Republic, Germany, Denmark, Greece, Hungary, Italy, Latvia, Norway, Poland, Sweden, Slovak Republic). All respondents know their PARERE contact person. PARERE networks seem to have been established in most of the MS. Some MS reported sometimes an insufficient involvement of representatives from all regulatory areas (the chemical sector is predominant) and sometimes a lack of expertise in some areas of consultations. Some MS reported that more face to face meetings within their MS were needed. A few MS said that it would need more financial support. All the respondents found the network useful, in particular for knowledge sharing on the Three Rs and being informed about the latest developments in the field. The survey as well as the complete replies received from the MS will be shared with the PARERE network.

Collaboration between the different bodies at national level and outreaching to National Committees and Animal Welfare Bodies was deemed to be crucial by the PARERE meeting participants.

Many MS reported that communication was good between bodies, with some cross-over of representatives. Some lack of involvement from University side was raised.

Further discussion is now needed on how to proceed in an environment where the definition of an alternative approach is more complex, and regulatory information requirements are evolving. What constitutes regulatory relevance becomes more challenging.

Test method submissions, assessments and related issues

The assessment of the γH2AX/pH3 pre-submission was completed in February 2018 and the test submitter was informed that EURL ECVAM does not intend to progress the submission further at the present time. The assessment of the full submission of the SENS-IS assay was finalised with some shortcomings being identified. The method was not considered for peer review at this stage and the test submitter was invited to address the identified issues and submit a revised full submission.

Following a preliminary EURL ECVAM assessment and PARERE consultation on the pre-submissions, the test submitters of the Toxtracker® (genotoxicity) and of the EDITOX (psychiatric adverse effects) assays were invited in 2017 to provide a full submission but none was received in 2018.

A new pre-submission on TR MARCoNI (thyroid hormone disruption) and three new full submissions on the GARD (skin sensitisation), on the Bioelution testing of metals and metal-containing materials in synthetic gastric fluid, and on RTgill-W1 (acute fish toxicity) were received by EURL ECVAM in 2018. The assessments of the pre-submission on TR-MARCoNI was completed in March 2018 and the test submitter was invited to complete a full submission. The assessment of the GARD full submission was put on hold pending outcomes of discussions that are on-going at the OECD and the method was not considered for peer review at the present time. The assessment of the two other new full submissions will progress during 2019.

Several scientific issues associated with the assessment/validation of *in vitro* methods were identified and discussed. EURL EVAM is receiving an increasing number of single, mechanistic "mini-methods" that cannot be used stand-alone for regulatory decision making. The assessment of their regulatory relevance is therefore becoming increasingly difficult, because often it is not known how these single mechanistic methods will be used in an integration context at the time they are submitted to EURL ECVAM. Assessing relevance purely on the basis of comparisons with reference animal data is also difficult due to its scarcity for some endpoints (e.g. developmental neurotoxicity) and its poor/unknown relevance and quality. There is currently a too high focus on "gold standards" and predictive capacity in the assessment of the relevance of an *in vitro* method. There needs to be a shift from purely empirical assessments to a more epistemic or knowledge-driven process where understanding of biological processes/systems underpins the assessment of the relevance of a method. Chemical selection in external validation studies managed by method developers is generally poor and may introduce bias in the assessment. More guidance is needed to facilitate chemical selection for validation purposes. SOPs developed by method developers are usually too specific to their laboratory conditions/equipment, which hinders transfer of the method to other laboratories, and their automation if applicable. Higher level protocols that only specify what is strictly required should be developed and made available by method developers.

Finally, non-scientific issues associated with the regulatory acceptance of *in vitro* methods were also identified and discussed. Currently the process of acceptance of new methods in a regulatory context is very much restrained by existing regulatory requirements, which were developed several

decades ago on the basis of animal data. However, new approach methodologies are substantially different than those animal methods and will not provide the same type of data. It is difficult, if not impossible, to fit new approaches into an old framework. Regulatory requirements should therefore evolve in parallel with the development and availability of new methodologies. Tackling intellectual property and confidential business information to ensure (i) commercial availability under fair, reasonable and non-discriminatory conditions, (ii) transparency and (iii) GLP implementation are also important issues currently being discussed at OECD level.

Updates on relevant activities within different OECD groups

Working Party on Hazard Assessment

EURL ECVAM explained how various OECD expert groups are organised under the OECD Council. Reference was made to four OECD projects in which JRC has (played) a leading role within the Working Party on Hazard Assessment (WPHA). Within the IATA Case Studies project, JRC had developed a case study on the grouping and read-across of Nano-TiO_2 genotoxicity[1], which illustrates the use of chemoinformatics in grouping, and also evaluates the applicability of the ECHA Read-Across Assessment Framework (RAAF). In a joint project between the WPHA and the Working Party on Exposure Assessment (WPEA), the JRC co-led the drafting of a chapter on hazard characterisation, emphasising the use of New Approach Methodologies[2]. Finally, two ongoing projects were presented, one of which is developing an overview of concepts and guidance related to Integrated Approaches to Testing and Assessment (IATA), while the other is developing guidance on the characterisation, validation and reporting of physiologically based kinetic (PBK) models.

Working Group of the National Coordinators of the OECD Test Guidelines Programme

EURL ECVAM described the different sections and organisation of the Workplan of the OECD Test Guidelines Programme (TGP) that is managed by the Working Group of the National Coordinators of the OECD Test Guidelines Programme (WNT). The different projects which are led or co-led by the EC-JRC through EURL ECVAM were described. Recently, JRC has co-led with the US and ICAPO the update of OECD Guidance Document 23 on Aqueous-phase Aquatic Toxicity Testing of Difficult Test Chemicals[3]. The aim of this project was to update GD 23 with new techniques available for testing poorly water-soluble test chemicals while avoiding the use of solvents, thereby eliminating the need for a solvent control group and thus reducing the number of animals used. The Guidance Document was approved at the WNT30 meeting in April 2018. The second part of the project, co-led with ICAPO, is aiming at reducing control fish in OECD TGs and is still on-going.

Two Test Guidelines (TGs 319 A and B[4]) on the determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes/liver S9 sub-cellular fraction were also approved at

[1] http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2018)28&docLanguage=En

[2] http://www.oecd.org/chemicalsafety/risk-assessment/considerations-for-assessing-the-risks-of-combined-exposure-to-multiple-chemicals.pdf

[3] http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2000)6/REV1&doclanguage=en

[4] https://www.oecd-ilibrary.org/docserver/9789264303218-en.pdf?expires=1553079920&id=id&accname=oid031827&checksum=045A1F1B9A5827B049EF7BCD126D7FC5 and https://www.oecd-ilibrary.org/docserver/9789264303232-en.pdf?expires=1553080018&id=id&accname=oid031827&checksum=148786A16A3FB6D3CF91E7D75A3F3815

WNT30. The associated GD 280[5] provides information on how to best perform the two *in vitro* methods and describes how the *in vitro* intrinsic clearance can be extrapolated to *in vivo* intrinsic clearance (i.e. to a whole-body metabolism rate constant). Regarding human health effects, the status of the project aiming at developing a guideline on defined approaches for skin sensitisation (JRC co-lead with US and Canada) was described, as was the scope of the project on a new guidance document on developmental neurotoxicity (DNT) *in vitro* assays that EFSA/JRC are co-leading with US and DK. These methods permit evaluation of a chemical's impact on critical neurodevelopmental processes, mimicking different windows of human brain development. The *in vitro* assays will be incorporated in IATA(s) with other information sources for different regulatory purposes. Finally, JRC has contributed to the development of Guiding Principles on Good Practices for the availability/distribution of protected elements in OECD Test Guidelines. The Guiding Principles are up for approval at WNT 31 in April 2019.

Extended Advisory Group on Molecular Screening and Toxicogenomics

EURL ECVAM described the AOP programme at the OECD which was created in 2012 and is managed by the OECD Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST), which the JRC / EURL ECVAM co-chairs on behalf of the EU/EC together with the USA (represented by the EPA). The AOP framework provides a comprehensive means of gathering, synthesising and validating collective knowledge about key toxicological processes and therefore provides a very strong basis for the design and development of integrated approaches to testing and assessment using alternative methods. Several AOPs have been endorsed by the OECD and many more are in the stages of development and review. The AOP programme has achieved international visibility and recognition, however future impact will rely on greater outreach, particularly to regulatory and research communities. Opportunities for PARERE to engage in this international endeavour included: assessing the (preliminary) regulatory relevance of AOPs; providing input to discussions on prioritisation; linking up with EAGMST country representatives and ensuring crosstalk between OECD expert groups; supporting the scientific review of AOPs; general promotion of the AOP programme with Member States; organisation of information days and training courses (with support from JRC/EURL ECVAM); and identifying potential contributors to AOP development projects.

General discussion at the end of day 1

Slovakia raised the question about *in vitro* testing in the area of medical devices and suggested to have a discussion on that topic in another PARERE meeting. EURL ECVAM pointed to the ISO standard 10993-10:2010 for the biological evaluation of medical devices. It describes the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and skin sensitisation. It was felt that safety testing of medical devices was a topic of general interest for PARERE.

EURL ECVAM mentioned the role of 3Rs centres which are many and with a variety of expertise, and overall the 3Rs centres' landscape is quite heterogeneous. The question whether PARERE was interacting with 3Rs centres was raised.

Italy mentioned that they just started the process of sharing opinions with them, but a proper exchange of information from them has not yet happened.

[5] http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2018)12&doclanguage=en

Germany mentioned that it would be important to have complementary interests. Several 3Rs centres are popping up and there is the likely need of creating a network.

DG ENV mentioned that during the last meeting of the European Society for Alternatives to Animal Testing (EUSAAT) in September 2018, an entire session had been dedicated to 3Rs centres. There had been a discussion whether the centres should do all a bit of everything or need to work more strategically. A lack of funding and of coordination of 3Rs centres had been raised.

The 3Rs centres also represent a mix of different entities (e.g. universities or governmental institutes) with different orientations and focus areas that are to be considered a richness to exploit. A congress of the Federation of European Laboratory Animal Science Association (FELASA) will take place in June 2019 and could be an occasion to bring forward a more strategic approach.

EURL ECVAM also asked about education and training of new generations of regulatory toxicologists. The SCCS representative mentioned the example of the course organised for regulators at the Free University of Brussels (VUB).

Introduction to "Adverse Outcome Pathways (AOPs)"

In this agenda item, EURL ECVAM presented three aspects associated with AOPs: (i) why the AOP concept is needed, (ii) what AOPs actually are, and (iii) how AOPs are captured, managed and disseminated.

Why Adverse Outcome Pathways?

In order to achieve the goal of using data generated at molecular and cellular level to predict adverse outcomes at the whole organism level, a unifying concept or framework to convert the data into knowledge that can capture, visualise and connect mechanistic information from all sources is desirable. To create such a knowledge base would require a common platform connecting basic researchers, technology developers, regulatory risk assessors and decision makers to create an interdisciplinary community of practice. Such a knowledge framework should have certain attributes, such as 1) being accessible to all to facilitate the sharing and synthesis of data and ideas, 2) providing assurances of quality through a transparent process of peer review, and 3) being able to represent the state of the science at a given point in time and yet allowing constant evolution. The Adverse Outcome Pathway concept can fulfil many of these requirements and can contribute to an increased understanding on how biological processes can be disrupted by an external stressor. The concept was adopted by the OECD in 2012 to help member countries to make better use of increased knowledge of how chemicals induce adverse effects in humans and wildlife**.**

What are Adverse Outcome Pathways?

An AOP describes a sequential chain of causally linked events starting on the molecular level, spanning multiple levels of biological organisation, to an adverse health or eco-toxicological outcome of regulatory relevance. This concept facilitates the paradigm change in toxicology from the observation of adverse effects to an understanding of the underlying mechanisms and supports the safety assessment of chemicals in many ways, including supporting the evaluation of combined effects of chemical mixtures. AOPs provide insight into the relationships between *in vitro* data and the probability of occurrence of adverse outcomes *in vivo*, as well as for species-to-species extrapolation and life-stage specificity. Specifically, AOPs can support read across and categorisation of chemicals, hazard identification, prioritisation and targeted testing. AOP development allows for identification of relevant data gaps and molecular/cellular targets for the development of *in vitro*

screening assays and, ultimately, integrated testing systems. AOP networks, defined as AOPs that share at least one common element, represent systems biology more realistically, provide information on interactions between AOPs and can reveal links between biological pathways.

How are Adverse Outcome Pathways captured, managed and disseminated?

In real life, AOPs are text-based documents that can be retrieved from the OECD's AOP Wiki website (https://aopwiki.org), where each AOP consists of a series of hyperlinked documents describing the AOP main information, and its elements, i.e. Molecular Initiating Events (MIE), Key Events (KE), Key Event Relationships (KER) and Adverse Outcomes (AO). Depending on the access rights AOP Wiki users have, they can read AOPs (anonymous access), comment on AOPs (user account created via self-registration), or enter and edit an AOP (user account upgraded via the SAAOP, the Society for the Advancement of AOPs, http://www.saaop.org/). Special user privileges are needed for officially reviewing an AOP or gardening the AOP Wiki (i.e. keeping it clean from inconsistencies and "orphan" entries). More info is available at http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm. The AOP lifecycle consists of several steps: After an AOP is entered and has reached a certain maturity, it undergoes an OECD internal review (formal correctness), after which an external review (scientific correctness) can follow, which is a prerequisite for official adoption of the AOP so that it can be used in regulatory affairs. A current bottleneck in this life cycle is identifying parties willing to execute the external review, and PARERE is invited to collaborate with JRC on finding ways to improve this situation.

How can we move the AOP framework forward with PARERE?

Relevant aspects of the OECD programme to consider

Several examples of how PARERE could contribute to progress the AOP framework were highlighted during the different presentations. One obvious role would be to assess the regulatory relevance of AOPs, in particular during the early stages of AOP development, and to provide input to discussions on prioritisation.

Another useful role could be to pull the country representatives of the different OECD groups (EAGMST-WNT-WPHA) closer together to ensure better communication and more coordinated interactions and actions.

The scientific review process of AOPs also needs support from experts. In November 2018, the OECD secretariat sent a letter to WNT and WPHA calling for expression of interest regarding the organisation of the scientific review of six AOPs following their development on the Wiki platform. However to-date, no member country has volunteered.

In addition, information days and training courses could be organised (with support from JRC/EURL ECVAM). In that context the formal training provided to EFSA was cited as a successful example. Finally PARERE could contribute to the identification of potential contributors to AOP development projects.

Discussions

The AOP describing the linkage between inhibition of complex I (CI) of the mitochondrial respiratory chain and motor deficit as in parkinsonian disorders[6] was cited as a good example of a regulatory

[6] Bal-Price, A. *et al.* (2018), "Adverse Outcome Pathway on Inhibition of the mitochondrial complex I of nigro-striatal neurons leading to parkinsonian motor deficits", *OECD Series on Adverse Outcome Pathways*, No. 7, OECD Publishing, Paris. http://dx.doi.org/10.1787/b46c3c00-en

relevant AOP. Its development was triggered by the EFSA External Scientific report (Ntanzi *et al.*, 2013[7]) where an exposure to pesticides was associated with Parkinson's disease. In support of this epidemiological study, the AOP concept has been applied to verify whether on the basis of the available data, there is biological plausibility and mechanistic understanding in support of such a link. EFSA plans to use this AOP as a basis for building *in vitro* testing strategies.

The use of this AOP in other sectors depends on the question posed and the re-purposing of the "knowledge package". Regulators can reflect on how useful this knowledge is for the question they are confronted with and how this knowledge can be adapted to become useful for their purpose.

Another example was the application of AOPs by the British-American Tobacco Association to e-cigarette additives.

Some MS use AOPs in their risk assessments and refer to the AOP rather than to the very many scientific papers which were used to develop the AOP.

EURL ECVAM informed that it had launched a study to conduct an in-depth analysis of the AOP environment. It will be a stocktaking exercise of the current adoption rate of the framework and include recommendations for the way forward.

With regard to PARERE's involvement in the AOP framework by assessing the regulatory usefulness, several factors that need to be considered prior to the launch of such a consultation were raised such as e.g., what is the best phase during AOP development for consulting, what criteria should be used, should several AOPs related to an endpoint be grouped? In general, it was not yet clear what such a consultation should look like and therefore further engagement with PARERE on this topic is needed. It was decided that EURL ECVAM will design a draft consultation and share it with PARERE for further refinement.

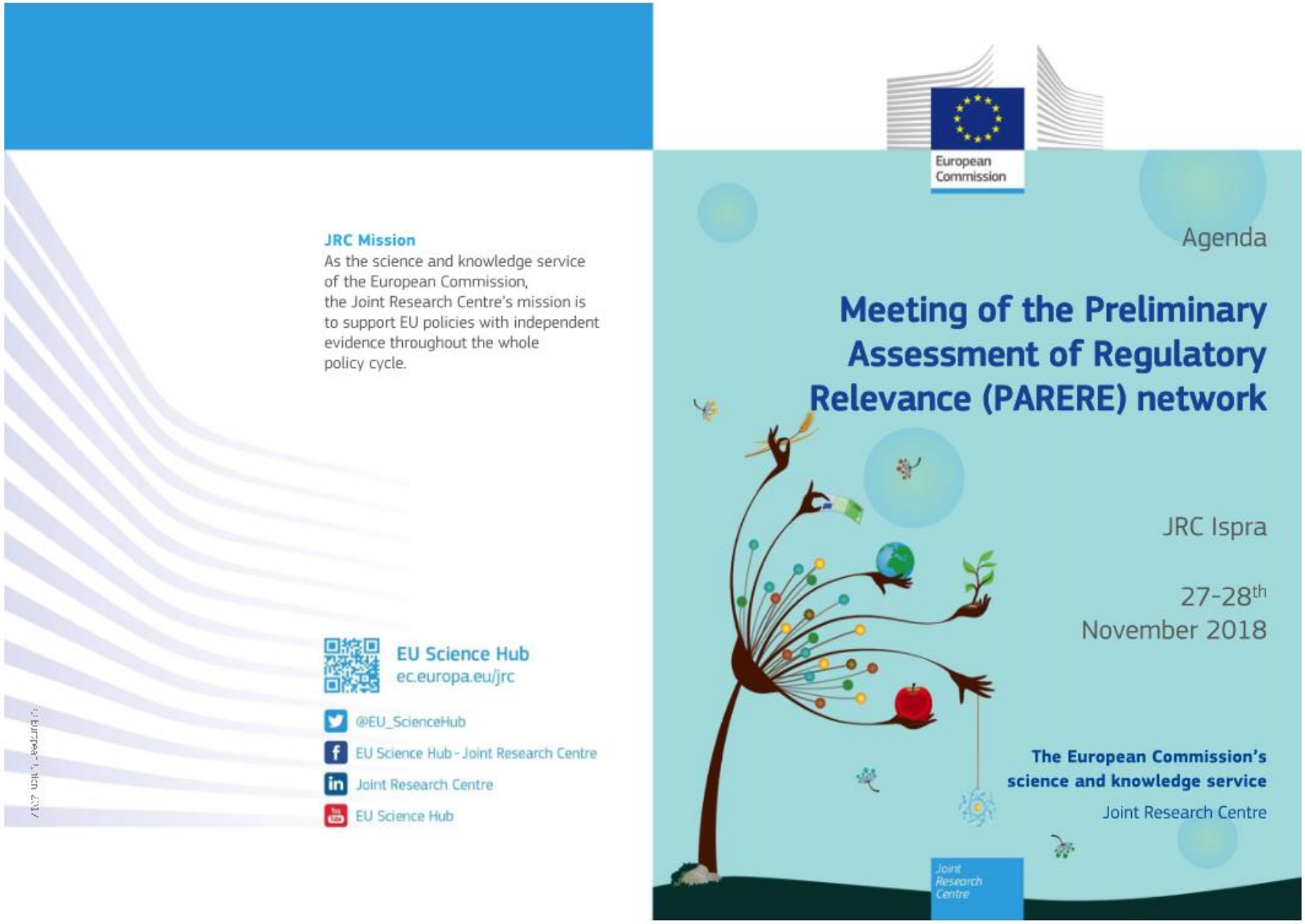
Actions

EURL ECVAM will share the outcome of the NCP consultation on interactions with the PARERE network with PARERE.

EURL ECVAM will devise a consultation with PARERE on AOPs but will consult with PARERE on the approach and questions to be used to ensure the consultation is as efficient and effective as possible.

[7] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4691

Annex I – Agenda



Meeting of the Preliminary Assessment of Regulatory Relevance (PARERE) network

1st day: Tuesday 27 November 2018

Building 36 Room 2

13:30-15:00 **Welcome and updates**

- **Approval of draft agenda**
- **Round-table on activities within the PARERE network**
  PARERE representatives and EURL ECVAM
- **Test method submissions, assessments and related issues**
  Joao Barroso, EURL ECVAM

15:00-16:00 **Updates on relevant activities within different OECD groups:**

- **Working Party on Hazard Assessment**
  Andrew Worth, EURL ECVAM
- **Working Group of the National Coordinators of the Test Guidelines Programme**
  Valérie Zuang, EURL ECVAM
- **Extended Advisory Group on Molecular Screening and Toxicogenomics**
  Maurice Whelan, EURL ECVAM

16.00-16:30 *Coffee break*

16.30-17.30 **Discussion**

18.00 *Transport to hotel*

JRC Ispra, 27-28th November 2018

2nd day: Wednesday 28 November 2018

Building 36 Room 2

09:00-10:30 **Introduction to "Adverse Outcome Pathways (AOPs)"**

- **Why Adverse Outcome Pathways?**
  Sharon Munn, EURL ECVAM
- **Developing Adverse Outcome Pathways**
  Brigitte Landesmann, EURL ECVAM
- **The AOP Knowledge Base**
  Clemens Wittwehr, EURL ECVAM

10:30-11:00 *Coffee break*

11:00-12:30 **How can we move the AOP framework forward with PARERE?**

- **Relevant aspects of the OECD programme to consider**
  Maurice Whelan, EURL ECVAM
- **Discussion**
  Moderators: Maurice Whelan and Valérie Zuang, EURL ECVAM

12:30-13:00 **Wrap-up and close**

Building 58 Auditorium

13.00-14.00 *Buffet lunch*

14:00 *Start of joint PARERE-ESTAF Meeting*