European Commission

Summary Record: Meeting of the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL)

Bowe, G., Corvi, R., Langezaal, I., Malinowska, J., Milcamps, A., Munn, S., Piergiovanni, M., Whelan, M.

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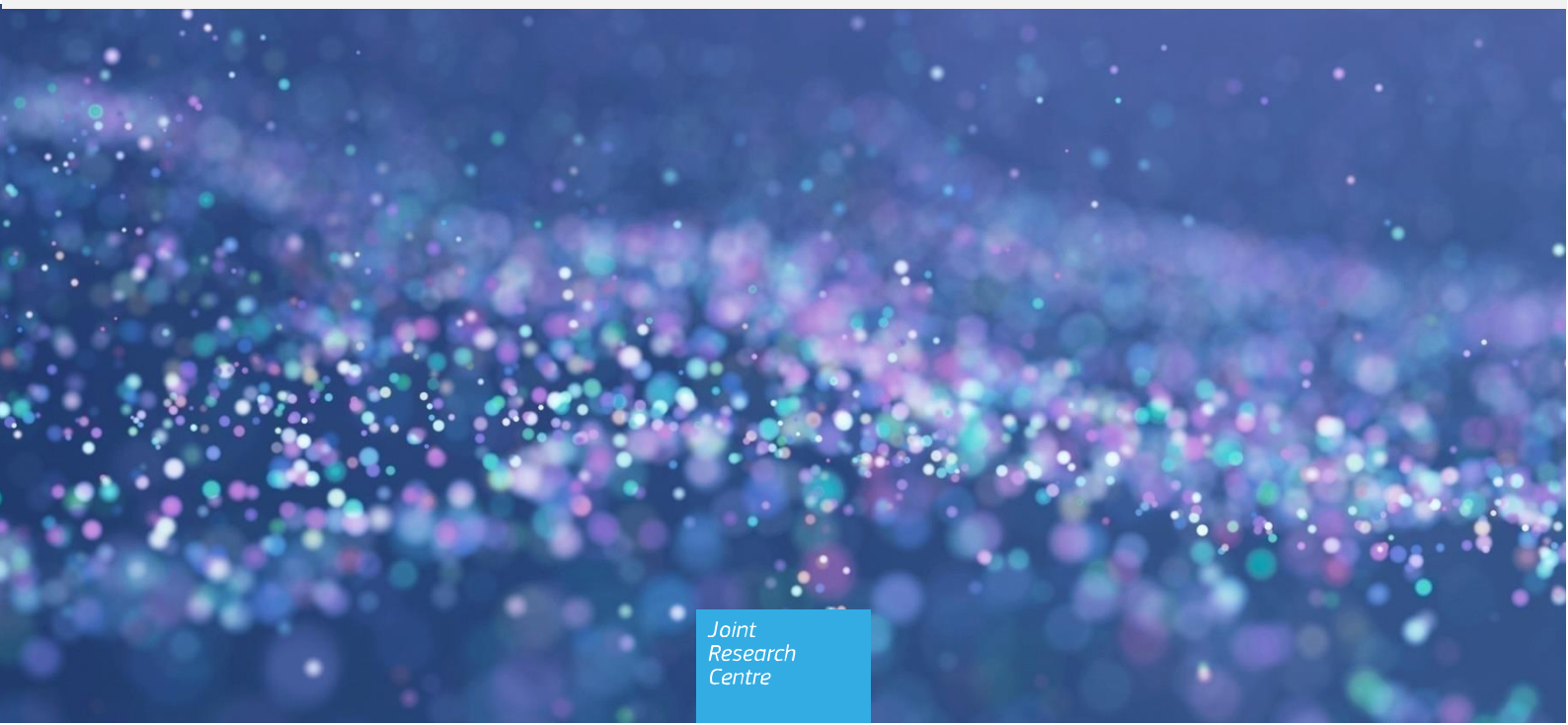

Joint Research Centre

Contact information
European Commission, Joint Research Centre (JRC), Systems Toxicology Unit (F3)
Via Enrico Fermi 2749, 21027 Ispra (VA), Italy
Email: JRC-F3-ENQUIRIES@ec.europa.eu

EU Science Hub
https://joint-research-centre.ec.europa.eu

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Contents

Abstract

The first day of the meeting focused on method validation topics, including the regulatory need for validated methods, organisational and funding challenges in validation studies, the transferability of methods including methods with complex test systems and endpoints, and perspectives from CROs and end-users. The second day concentrated on the recently completed validation study of Thyroid Hormone System Disruption (THSD) methods.

1 Introduction

EURL ECVAM and DG RTD presented updates of their activities. It was emphasised that Member States (MS) are required under Directive 2010/63 to support *in vitro* method validation. However, only some EU-NETVAL members had contact with their National Contact Points for the Directive (NCPs), while other facilities had little or no contact with their NCP.

In relation to the development and validation of New Approach Methodologies (NAMs), including ones towards the development of OECD Test Guidelines (TGs), it was acknowledged that there is a shortage of NAMs specifically tailored for ecotoxicology testing.

2 Validation principles and practice

The OECD presented efforts to address the challenges related to organisational and funding aspects of validation studies. This included sharing preliminary results of a survey on this topic and announcing plans for a follow-up workshop in December 2023.

EURL ECVAM is co-leading a revision of OECD Guidance Document no. 34 to reflect scientific and technical progress and evolving approaches to validation. An analysis of validation studies coordinated by EURL ECVAM showed comparable results between intra- and inter-laboratory reproducibility, suggesting that large-scale inter-laboratory reproducibility assessments may not always be necessary for a well-designed and well-executed validation study, which would make things more cost-effective and efficient. Emphasis was placed on the importance of data integrity and transparency in its generation, processing and reporting.

PEPPER, a French public-private platform for the pre-validation of endocrine disruptor characterisation methods, discussed the time-consuming nature of organisational aspects of validation and how developers unfamiliar with validation requirements often underestimate the necessary time and support needed for validation activities.

Discussions focused also on challenges related to validation funding. Different perspectives were shared regarding the impact of insufficient dedicated funding on laboratories' ability to prioritise validation studies over their other tasks and achieve timely results.

Concerns were raised about the unavailability of certain test (cell) systems due to EU legislation on animal by-products preventing their import. This highlighted the need to ensure long-term availability of test systems.

The recently adopted OECD initial guidance on the *in vitro* battery of methods for developmental neurotoxicity (DNT) was presented. This has been a resource-intensive activity that achieved successful outcomes through early and continuous involvement from the regulatory community, particularly from EFSA and OECD member countries.

3 Transferability of methods

Test readiness criteria, which assess for example the completeness of an *in vitro* method before transfer to another laboratory, can assist both method developers and end-users in identifying potential gaps that need to be addressed. These criteria can be used for tracking method development in EU-funded projects, as was done in the EURION cluster. Participants recognised that these criteria are very useful. Their further uses beyond the development phase of a method is under discussion. The criteria could be amended to incorporate additional criteria for different purposes if considered valuable.

The importance of well-written Standard Operating Procedures (SOPs) was highlighted. Standardisation of common procedures was also discussed, e.g. a standardised procedure for measuring solubility of test items was proposed.

The increasing availability of new methods, measurement techniques, and data analysis streams and the challenges they pose for standardisation and regulatory application was considered.

An EU-NETVAL survey on the use and transferability of *in vitro* methods based on complex test systems or technologies was presented.

The need for standardisation and ongoing related activities for complex biological test systems, organ-on-a-chip, high-content imaging, and omics technologies were also discussed.

4 Perspectives from CRO and industrial end-users

A panel discussion on emerging technologies took place with representatives from CROs and industrial end-users, which included Abich, BASF, Charles River Laboratories, Eurofins, and Labfit. The panellists generally implement test methods that have a good business case and a high customer demand. These methods usually have a clear regulatory use such as those included in OECD Test Guidelines (TG), but sometimes there are specific methods requested by the client. Their clients are mainly companies from the pharmaceutical, chemical, and medical device industries. Emerging technologies are scarcely used within the panellists' CROs. However, two panellists reported that they utilise emerging technologies like genomics and metabolomics analysis in their research services.

The discussion also covered the potential regulatory utility of investigating the impact of chemical exposure on the microbiome, as well as the increasing availability of organ-on-a-chip devices, and the need for their harmonisation and scientific evaluation. The panellists acknowledged that clear regulatory requirements drive the demand for their investment in methods. They expressed concern over potential scenarios where a battery of methods (e.g. DNT or THSD) becomes a regulatory data requirement due to the challenges it presents for CROs in terms of investment, equipment, training and resources. Concern was also voiced about the challenges of transferring these methods and interpreting the data generated. The participants recognised that CROs play a crucial role in the ecosystem of emerging technologies and new non-animal methods. However, it was highlighted that there is a communication gap between method developers and implementers, emphasising the need to address this issue.

5 Validation of methods relevant for Thyroid Hormone System Disruption (THSD)

EURL ECVAM coordinated a major EU-NETVAL validation study to simultaneously assess 18 *in vitro* THSD methods. This effort was made possible with the support of 15 EU-NETVAL facilities and 14 test method developers. The study primarily evaluated test definition, within-laboratory reproducibility and aspects of mechanistic relevance. Some methods will require further optimisation and evaluation.

Representatives from the participating EU-NETVAL facilities presented their results, the challenges encountered, and lessons learned. Several laboratories had to perform (extensive) method optimisation. Some participants found the process of finalising legal agreements lengthy, while others struggled with the notification process for the genetically modified cell lines. Some also indicated a lack of funding for validation and the corresponding difficulties in allocating sufficient staff effort. It was suggested that increased exchanges among participants during the project could have benefited progress by sharing experiences.

EURL ECVAM compared the results of two methods measuring the binding of thyroid hormone to serum proteins, which yielded similar results, and two assays measuring thyroperoxidase (TPO) inhibition, which gave very different results.

The results of this validation study are being further evaluated by the OECD's Thyroid Disruption Methods Expert Group and next steps will be proposed. Additionally, the EU Partnership for the Assessment of Risks from Chemicals (PARC) is developing an IATA for Thyroid Hormone System Disruption, based on an AOP network to which the methods validated by EU-NETVAL are mapped.

Questions were raised about the endogenous expression of the receptor TRα and/or TRß in the GH3 cell line used in one method, and about the comparison of results with two TR transactivation assays. EFSA

noted the absence of assays for capturing liver-mediated thyroid toxicity (e.g. induction of enzymes increasing TH clearance), the most common mechanism in pesticide ED assessment.

PEPPER (France) and BfR (Germany) plan to submit a project proposal (SPSF) to the OECD suggesting the development of a TG for the DIO1 method.

6 Conclusions and reflections on future activities

Certain thyroid hormone disruption methods require further work to demonstrate their transferability to one or two laboratories. Member States (MS) are encouraged to take a leading role in this by proposing SPSFs to the OECD and coordinating the work. Germany and France's initiative, taking up the work of PEPPER, to submit a SPSF proposal to the OECD could set a precedent for future submissions by MS. EURL ECVAM will offer practical support if needed, without necessarily taking a coordinating role.

Future activities may involve methods supporting new IATAs on endocrine disruption, ADME, developmental neurotoxicity, immunotoxicity, and non-genotoxic carcinogenicity.

EU-NETVAL members should consider proposing methods for validation by the network, preferably supported by an explanation of the regulatory and/or business demands, and an outline of funding requirements for the validation study.

Other activities proposed included further enhancement and application of readiness assessment for methods, drafting general guidance for preparation of SOPs, and development of SOPs for solubility measurement or cytotoxicity testing.

It was acknowledged that there is a lack of mutual awareness of the existence and specific roles of the EU-NETVAL facilities, MS NCPs and the National Coordinators of the OECD's TG Programme. This should be addressed since securing funding poses a challenge for future validation studies. MS are therefore encouraged to look for opportunities to improve internal networking and to identify priorities and sources of funding for validation activities.

The participants highlighted the value of exchanges between EU-NETVAL partners including EURL ECVAM, and it was agreed that such exchanges should continue on a more regular basis, possibly through an online channel.

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