EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F – Health and Food
Systems Toxicology
European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM)

Summary Record

PARERE Meeting 29 May 2024, Ispra, Italy

The 13th meeting of the Preliminary Assessment of Regulatory Relevance network (PARERE) was held online (Webex) on 29 May 2024 from 9.00 to 13.30 h. It was organised by the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of the European Commission's Joint Research Centre, Ispra, Italy (the list of participants and the agenda are included in Annex 1 and 2, respectively).

Welcome and introduction

The meeting was chaired by Valérie Zuang, EC/JRC/EURL ECVAM. She welcomed all members and called for attendance. Participants from Austria, Germany, Italy, Finland, Belgium, Spain, Poland, the Netherlands, Sweden, Denmark, Slovakia, Luxembourg, Ireland, Czech Republic and Latvia followed the meeting. Representatives from Commission services, EU agencies and Scientific Committees participated as well (see Annex 1).

After the introduction, the chair highlighted the different agenda points, which were up for discussion, and approved the draft agenda. She then invited the PARERE members from the Member States who provided a power point presentation to provide 5-minute updates on activities within their national PARERE network. Additional information on these updates can be found in the respective presentations on CIRCABC. Updates were delivered by Austria, Germany, Italy, Finland, Belgium, Luxembourg and the Netherlands. Due to time constraints, other Member States (who had not provided a power point presentation) were invited to present updates at the next PARERE meeting or to provide slides to be shared on CIRCABC. Presentations from EURL ECVAM included updates on the European Partnership for the Assessment of Risks from Chemicals (PARC; Horizon Europe project), the Animal-free Safety assessment of chemicals: Project cluster for implementation of novel Strategies (ASPIS; Horizon 2020 project), the EC roadmap towards phasing out animal testing for chemical safety assessments, the European Partnership on Alternative Approaches to

Animal testing (EPAA) designathon, emerging technologies, qualification framework for organ-on-chip and updates on the revision of OECD GD 34 on the validation and international acceptance of new or updated test methods for hazard assessment (see Annex 2). Most of the presentations from EURL ECVAM included some questions to be addressed by the PARERE network through written consultation after the meeting (see Annex 3).

Updates from the Member States

Martin Paparella (Austria) discussed three projects focusing on innovative approaches in the field of toxicology and risk assessment.

1. Alternative Project: The project aims to build an innovative platform for assessing the cardiotoxicity of chemicals, using AOP network development, computational methods, and machine learning. It includes a 3D microphysiological model for the young and aged heart, and an integrated approach to testing and assessment in line with the next generation risk assessment. The project is led by Gianluca Giardelli (Politecnico di Torino, Italy) and Federico Vozzi (Consiglio Nazionale delle Ricerche, Italy) and will last until September 2024.

The Alternative project is also part of the Green Deal Health Cluster, and the team recently discussed what they share within this cluster, especially considering the need for non-animal methods to support the Green Deal initiatives. The current reliance on animal testing in regulatory frameworks is a significant challenge, but there are numerous alternative methods and assessment procedures available, including risk matrixes. The team plans to release a policy brief summarizing the shared perspective on the need for non-animal methods in the context of the Green Deal.

2. CHIASMA Project: This Horizon Europe project, led by Tommaso Serchi from the Luxembourg Institute of Science and Technology, will progress the technical readiness level of several in vitro methods and work towards their validation. It involves case studies and integrates experimental with computational data. The project aims to extend the next generation risk assessment approach to the safe and sustainable by design concept, incorporating life cycle assessment and socio-economic aspects.

3. INSIGHT Project: this project, led by Dario Greco from Tampere University of Finland, aims to evolve the AOP concept towards safe and sustainable by design. It focuses on impact outcome pathways and provides regulatory assessment workflows for human health, ecotoxicology, and the economic and societal impact of materials. The project will generate fair data and computational models, which should be operable by regulators. Martin encouraged interested parties to reach out and stay updated by subscribing to newsletters and following the projects.

Katrin Schutte (DG ENV) enquired whether the work under the CHIASMA project could be categorized as prevalidation work to which Martin replied positively.

The chair asked for some clarifications around the policy brief. Martin explained that it is aimed at European policy officers to raise awareness about the importance of non-animal methods and the potential actions that can be taken at the policy level to support their development and implementation. The policy brief is intended to reach beyond regulatory bodies and influence policymakers at the European level. Milena Mennecozzi (EURL ECVAM) mentioned working on a similar policy brief for biotech projects focusing on non-animal methods. Martin informed that the Green Deal Health Cluster's policy brief would most probably be ready by June 2024 and might be available for sharing soon.

Verena Fetz (Germany) provided an update on the German Centre for the Protection of Laboratory Animals (Bf3R) and its various areas of competence, including the Centre for Documentation and Evaluation of Alternative Methods (ZEBET); research groups focusing on reducing severity and improving animal welfare; alternative methods in toxicology; and coordinating and promoting research on alternative methods. The Bf3R hosts the database AnimalTestInfo, which contains non-technical summaries of animal tests which also feed into the EU Allures database. The database allows for the classification and prediction of experimental animal numbers based on disease types and research areas. The centre also maintains the online tool SMAFIRA, which helps users retrieve alternative methods from the literature and the Animal Study Registry, a pre-registration platform for animal studies to prevent selective reporting. Verena explained their involvement in EU research projects such as PARC, RiskHUNT3R and Panoramix at their Department of Experimental Toxicology and their support to the PEPPER validation platform. The German Federal Ministry for Education and Research provides funding for development and validation with a strong focus on the Three Rs and also for the transfer of developed methods into OECD test guidelines or into Pharmacopeia. The Ministry also created the Federal Network 3R, which connects Three Rs centres, provides seminars, and exchanges information and knowledge throughout Germany. Additionally, there is a trend of universities creating their own 3R centres, with a strong accumulation in the Berlin region, where the Bf3R is located.

The chair asked if the Bf3R was the overall coordinator of all these Three Rs Centres in Germany to which Verena replied that there was no overall coordination.

Helena Kandarova (Slovakia) informed that all the Three Rs Centres were united in a network coordinated under a COST action and that EUSAAT, the European Society for Alternatives to Animal Testing, was also running a network of Three Rs Centres of which many of these Three Rs Centres in Germany were part.

In response to a question from Lucia Selfa-Aspiroz (EURL ECVAM) about the Animal Study Registry, Verena clarified that its use is currently voluntary and not mandatory for researchers. The number of users is not available at the moment, but Verena offered to provide the information upon request. She expressed hope that the registry's usage will increase over time. Lucia requested further details on implementation ideas and offered to provide her email address for follow-up discussions with the responsible colleague.

Emma Di Consiglio (Italy) provided a detailed update on the ongoing activities in Italy related to the development of alternative testing strategies for chemical risk assessment and the involvement of Italian entities in various work packages within the European Partnership for the Assessment of Risks from Chemicals (PARC).

Other activities involve collaboration with the Organisation for Economic Co-operation and Development (OECD), European Food Safety Authority (EFSA), European Chemicals Agency (ECHA), the European Medicines Agency (EMA) non clinical working party (through AIFA) and the European Commission's Joint Research Centre (JRC).

In summary, some of the key projects Italian entities are working on include: PARC work packages 2, 5, 6 and 8; activities within the OECD (membership in various groups supporting NAMs development and application, such as the omics expert group and the development of the OECD QSAR Assessment Framework as well as supporting the revision of guidance document 34 for validation); activities with EFSA (ISS co-coordinates along with BfR, the EFSA-funded action NAMs4NANO); the EPAA/JRC NAM designathon project for systemic toxicity; the EMA non-clinical working party (this collaboration involves the Italian Agency for Medicine (AIFA), concept papers, reflections, and papers on new approach methodologies within EMA, and a focus on reducing the use of live animals and reusing

animal study results); the Italian National Reference Centre for Alternative Methods (this centre is involved in research activities, including the development of a 3D system for neurotoxicity, storage of cells without animal-derived reagents, and training activities for operators in the field of animal experimentation); and the Conference on One Health (this conference was organised by the Italian 3Rs Center, with the current director, Professor Arti Devi Ahluwalia, as co-chair for the course section and ongoing activities in education, dissemination, and training at various universities).

Emma finished by thanking the Italian PARERE network and the respondents to their survey for their contributions and mentioned that Professor Anna Maria Bassi, a member of the national PARERE network, received a prize within the "researchers and innovators" category for her work in bringing non-animal-based research into universities.

Tuula Heinonen (Finland) discussed the PARERE network in Finland, which consists of members from the Finnish Safety and Chemical Agency, ministries, including the Ministry of Agriculture and Forestry, the Ministry of the Environment, the Finnish Medicines Agency and the Finnish Environment Institute. Key organisations in Finland include:

-The Finnish 3R Center (FIN3R), established in 2022, it focuses on organising educational events like webinars, workshops, and courses related to the 3Rs. These events cover topics like refinement, non-animal methods, and advanced courses for students.

-Fincopa, established in 2003, promotes education, training, and communication on 3Rs and related topics.

-The Finnish Centre for Alternative Methods (FICAM): Founded in 2003, FICAM acts as an umbrella organisation for promoting the 3Rs in Finland. Its activities include: collecting, sharing, and exchanging scientific information on the 3Rs; organising training programs on 3Rs and ethics; arranging meetings, symposiums, and workshops on various 3Rs topics; publishing information on the 3Rs for both scientific communities and the public; participating in national committees under EU Directive 2010/63 on the protection of animals used for scientific purposes.

Other important players are:

The National Committee for the Protection of Animals Used for Scientific or Educational Purposes (TOKES). This committee supervises the Finnish 3R Center and is responsible for implementing EU Directive 2010/63.

3R Observatory Group: established in 2023, this group develops indicators to monitor Finland's progress on the 3Rs, benchmarks progress against other EU member states, and sets goals for further advancement.

Finnish Network of Animal Welfare Bodies: formed in 2023, this network focuses on animal welfare in research.

New Legislation on Animal Identification Methods: implemented in 2024, this legislation mandates the use of analgesia for marking rodents and rabbits, and provides a list of permitted identification methods for wild animals used in research. Tuula is still the Finnish PARERE delegate and continues to contribute to the cause during her retirement.

Maude Everaert (Sciensano, Belgium) presented the activities of the Belgian PARERE network. Two virtual network meetings were held in 2024. The first meeting covered updates on the FELASA (Federation of European Laboratory Animal Science Associations) meeting, ongoing 3Rs projects, and the EMA 3Rs Working Party. The second meeting focused on the development of organoids from

transgender women testes and on the use of *in vivo* assays for independent batch release of vaccines.

Three other 3Rs events were organised in Belgium: 1) a study day on laboratory animals and alternatives by the Brussels Region, attracting over 200 participants; 2) a joint 3R symposium with Twinalt (H2020 project) and the European project REPLACE on emerging topics in the 3Rs; 3) a federal agency event focused on advancing the 3Rs in regulatory testing of medicines.

The Belgian presentation highlighted the Flemish government's action plan to reduce animal testing, published in April 2023. The plan involved 20 organisations in Flanders and included 33 diverse actions, such as e.g., developing an online platform within universities; educational initiatives; knowledge sharing on alternative approaches and technologies. The plan was presented to the scientific community in November 2023 and is being monitored for progress. Maude also provided updates on the REPLACE Project. The project has collected over 280 methods from 182 experts across 33 Belgian organisations. The project actively promotes the use and development of NAMs through social media, newsletters, website content, and educational webinars.

Martin (Austria) enquired about the scope of the action plan, specifically whether it covered areas beyond the regulatory use of animals, such as e.g. biomedical research. Maude confirmed that the plan included actions in various fields, including biomedical research, and she offered to share the link to the plan and to the final report with more details. She also clarified that the plan focused on reducing animal testing, not necessarily replacing it entirely.

Milena (EURL ECVAM) asked about the quality control process for methods included in the REPLACE database. Maude explained that the primary goal of the project was to create an inventory of methods with associated experts and organisations. Due to limited resources, the project does not conduct in-depth quality checks on all submitted methods. The focus is on providing a contact point for further information and assessment. The database has recently added a feature allowing for internal quality control within research groups.

Axelle Cooreman (IC-3Rs, Belgium) presented the activities of the Belgium 3Rs Innovation Centre. The centre focuses on promoting the 3Rs in animal research through various initiatives such as:

- Research Projects: the centre coordinates research projects focused on developing non-animal alternative methods. Three PhD students have been hired for 2023/2024 with one project completed and two others nearing completion.

- Action Plan: the centre is leading the IC-3Rs action plan for the VUB (Vrije Universiteit Brussels) in the context of the Flemish Government.

- Collaboration with REPLACE: the centre works closely with the REPLACE project.

- Symposium: an annual symposium is organised, with a focus on reduction and refinement challenges in 3Rs research. The upcoming symposium will be held on September 19th, 2024.

- Hands-on workshops: these workshops cover various topics related to the 3Rs and are limited to 50 participants for optimal learning.

- Webinars: the centre offers webinars to promote the implementation and understanding of the 3Rs. A webinar on retrospective analysis as important tool is scheduled for June 12th, 2024.

- Educational Activities:

- Researchers: a new course on the environmental safety of cosmetics is being introduced in September 2024.

- Secondary School Students: the centre is developing teaching materials to promote critical reflection on animal testing among students aged 17-18.

The centre's efforts have resulted in a 7% reduction in animal use at the VUB and a shift towards milder animal procedures.

Martin (Austria) asked who the target audience for the new environmental safety of cosmetics course was. Vera Rogiers (SCCS) replied that the course is designed for professionals already working in safety assessment or regulatory fields within the cosmetics industry. The course will cover environmental safety aspects of cosmetics, including water treatment and regulatory challenges like REACH and CLP. This course aims to provide participants with knowledge and tools to reduce animal testing in the environmental safety assessment of cosmetics.

Arno Gutleb (Luxembourg) presented Luxembourg's contribution to 3Rs research. The Luxembourg Institute of Health (LIH) and the Luxembourg Institute for Science and Technology (LIST) are involved in WP5 of the PARC project, focusing on immune toxicity and respiratory sensitisation. They also contribute to the development of norms and standards related to NAMs.

LIST participates in over 10 EU-funded projects related to NAMs development and application in various fields like pharmaceuticals, materials science, and cosmetics. It is involved in the EFSA "NAMS4Nano" project, contributing expertise in advanced microscopy technology. It is a partner in an EFSA tender project on multi-omics workflow from quantitative in vitro data. It participates in various OECD working parties such WNT, WPMN, WPRM.

LIST together with the University of Luxembourg (UniLu) focuses on animal-product-free cell culture for liver and lung models. They received a PETA early career scientist award for a coworker's work in this area.

Betty Hakkert (NL) presented an overview of the Netherland's involvement in various 3Rs-related activities. The Dutch National Institute for Public Health and the Environment (RIVM) is co-leading the update of OECD guidance document 34. It participates in various OECD WP and expert groups, contributes to the PARC project and is involved in the PEPPER project. Betty in particular mentioned the Dutch National Growth Fund, the project VHP4Safety and the RIVM publications on Landscapes NAMs chemical substances and pharmaceutical products.

Dutch National Growth Fund: Centre for animal-free biomedical translation (CPBT)

› The Dutch National Growth Fund will invest 124.5 million euros in the Centre for Animal-Free Biomedical Translation. Its aim is to generate safer, more effective treatments, while reducing animal suffering. RIVM, in collaboration with Utrecht University, UMC Utrecht and Utrecht University of Applied Sciences, is one of the initiators of the centre and involved in strengthening the acceptance and use of animal-free biomedical innovations. The project will start in 2025 for a duration of 10 years.

National project VHP4Safety collaboration with the European H2020 ONTOX project

› The Dutch VHP4Safety project recently signed a memorandum of understanding with the European H2020 ONTOX project. Together, both projects kicked-off their collaboration during the first VHP4Safety-ONTOX day, held on April 24th, 2024. The aim of VHP4Safety is to build a platform for safety assessment of chemicals and pharmaceuticals based on human

data. VHP4Safety is funded by the Netherlands Organisation for Scientific Research (NWO) with a budget of 10 million Euros, for a duration of 5 years. The project started in 2021 and will end in 2026.

RIVM publications on Landscapes NAMs chemical substances and pharmaceutical products to facilitate the process from development towards acceptance and regulatory uptake of NAMs

- In 2023 and 2024 RIVM published two factsheets that provide an overview of the steps NAMs need to take from development towards acceptance and implementation into regulatory frameworks for chemical substances (2023) and pharmaceutical products (2024). Currently, the Dutch funding organisation ZonMw uses these factsheets as a basis to develop an online tool, the NAM Navigator, with the aim to guide researchers in development of fit-for-purpose NAMs. Furthermore, RIVM currently collaborates within the COST action IMPROVE network to develop a landscape NAMs for the biomedical field (Landscape New Approach Methodologies | RIVM)

Netherlands/RIVM is also involved in the implementation of 3R Methods in CLP/GHS.

Betty expressed interest in future opportunities to share information and discuss activities within the PARERE network.

Update from EURL ECVAM on selected topics

Sharon Munn (EURL ECVAM) provided an update on the PARC project, a large EU Horizon project focused on assessing risks from chemicals. The goal is to advance scientific knowledge and inform policymaking. PARC is funded by the European Commission and Member States, involves 24 Member States, 4 associated countries, 1 non-associated third country, and 200 partners. The project focuses on advancing scientific knowledge of risk assessment from chemicals. It includes 8 work packages, with a particular focus on hazard assessment (WP5) and innovation, regulatory risk assessment (WP6).

JRC has a cooperation agreement with PARC. Its involvement includes providing advice, feedback, review of deliverables, review of proposed projects and participating in workshops. Its focus is on work packages related to non-animal methods.

- Work Package 5 (Hazard Assessment):
    - Focuses on substances with high concern, innovative methods for human health and environment, and computational approaches.
    - Emphasises regulatory readiness of non-animal methods (NAMs).
    - JRC is supporting the development of test readiness criteria for NAMs.
- Work Package 6 (Innovation, Regulatory Risk Assessment):
    - Focuses on Integrated Approaches to Testing and Assessment (IATAs).
    - Involves developing IATAs for specific health effects, such as endocrine disruption, genotoxicity, and liver fibrosis.

- Collaborates with other EU-funded projects, like the EURION project, developing methods to improve the identification of endocrine disruptors. EURION will come to an end and the final event will be held in Brussels on June 13th, 2024. A policy brief is in preparation. A lot of methods from EURION are being fed into and taken up by PARC and a lot of the same people are involved. Nevertheless, there may well be methods coming already out of EURION.

Sharon mentioned that as highlighted in previous presentations from the PARERE representatives and since 24 MS were involved in PARC, it was clear that most of the EU MS were heavily involved in the project. She asked what the level of connection of PARERE representatives with PARC was.

Then, Sharon asked the more specific question on whether MS would have an interest in taking the lead in bringing promising methods identified in the thyroid AOP network onto the OECD work plan for test guideline development. These methods would eventually be used in IATA.

The chair invited PARERE representatives to consult their internal PARERE network and reply in written after the meeting. She also invited PARERE representatives to share any information/opinion already at the meeting if they wished so.

Martin (Austria) informed that Austria is actively participating in the PARC project. Contributions include:

In the framework of a genotoxicity IATA, Austria is leading a subtask to assess the variability of genotoxicity data. It is aiming to collect databases to understand variability within and between laboratories. This research is expected to contribute to the acceptance of new approaches in the field.

In the framework of replacing the fish test, Austria is collaborating with EAWAG, the Swiss Federal Institute of Aquatic Science and Technology, to find alternatives to the acute fish test. They combine cell tests and fish embryo tests to assess their protective capacity for neurotoxins. This work is in progress and results were presented at the SETAC meeting, with a publication forthcoming.

In the context of respiratory sensitisation, Austria is contributing to the mechanistic knowledge and method development for respiratory sensitisation. This work is linked to a detailed review paper on respiratory sensitisation, a project within the OECD Test Guidelines Programme.

Austria is also involved in biomonitoring-related activities.

Martin mentioned that Austria would provide a written summary of their contributions to the PARC project.

Helena Kandarova (Slovakia) mentioned that Slovakia was involved in the PARC project through the work of the Slovak Institute of Public Health but also directly involved through collaborations with the ONTOX and RiskHUNT3R projects. Helena informed about PARCOPEDIA (https://www.parcopedia.eu/) and mentioned that Slovakia had participated in the PARC synergies questionnaire (https://www.eu-parc.eu/synnet) and found it valuable for identifying potential collaborations. She encouraged other participants to complete the questionnaire to foster further cooperation within the PARC project and with other organisations.

Vera Rogiers (SCCS) informed that Belgium is actively participating in the PARC project through Sciensano (Birgit Mertens is the coordinator for Belgium) and Tamara Vannaka from VUB's research group is involved in thyroid function research. A doctoral student has been hired for this research

project. The team is involved in WP6 and is currently in week five of this work package. A future goal is to expand the project to include genome markers.

Emma Svensk (Sweden) acknowledged Sweden's involvement in the PARC project, but the Swedish 3R Centre is not formally a part of it. However, they are engaged through the national hub for governmental agencies, which allows them to maintain a general overview of PARC activities in Sweden without being directly involved. In addition, they participated in organising a meeting and workshop for Swedish researchers and governmental agencies related to PARC, where they listened to discussions and gained insights into the project.

Verena Fetz (Germany) mentioned her personal involvement in PARC Work Package 5, on a non-genotoxic project. She also knows that from BfR, there is involvement in multiple work packages. She offered to provide a list of BfR's involvements and added that some German universities are involved in different work packages as well, suggesting it would be easier to share this information via email.

Regarding the second question, PARERE members preferred to consult back home prior to provide an answer.

Elisabet Berggren (EURL ECVAM) provided an update on the EU-funded project ASPIS on Animal-Free Safety Assessment of Chemicals. It involves three projects with about 70 partners contributing and a budget of €60 million. The project is very active at the moment, getting results and finalising their work. Elisabet referred to the PARERE-ASPIS workshop of 2023 where ASPIS and ASPA, the ASPIS Safety Profiling Algorithm, had been presented. The safety assessment framework has been applied to several case studies and a version 2.0 of the framework is under development. There had been a follow-up PARERE-ASPIS Webex meeting in June 2023 to continue the discussion with PARERE. Elisabet mentioned the fourth annual ASPIS Open Symposium taking place in Copenhagen, Denmark on 11-12 September 2024 for updates on the progress. ASPA is also being used outside of the ASPIS project and applied also in PARC. It is an activity that will also feed into the Commission's roadmap towards phasing out animal testing for chemical safety assessments.

Elisabet asked whether PARERE was interested in a PARERE-ASPIS webinar to receive an update on ASPIS and continue the discussions initiated with PARERE in 2023.

Helena mentioned another event, where the progress of ASPIS will be presented, namely the 2024 European Society for Alternatives to Animal Testing Congress taking place in Linz, Austria on 18-20 September.

In general, PARERE members expressed interest in a follow-up PARERE-ASPIS webinar.

The chair mentioned that they would look for potential dates with the ASPIS coordinators.

Katrin Schutte (DG ENV) provided an update on the roadmap towards phasing out animal testing for chemical safety assessments. She reminded that the Commission had committed to develop such a roadmap in its communication, replying to the European Citizens' Initiative 'Save cruelty-free cosmetics' in July 2023. The more general underlying obligations are in Article 13 of the EU Treaty and the obligation to protect animals as sentient beings and in EU Directive 2010/63, which already has the ultimate aim of phasing out of all animal use for research and regulatory purposes. The roadmap furthermore responds to a resolution that the European Parliament sent to the Commission in September 2021, in which they also asked to accelerate the transition to non-animal testing.

The roadmap will be a policy document that aims to analyse and describe the steps to replace animal testing; outline a path to expand and accelerate the development, validation, and implementation of non-animal methods, and make the roadmap applicable to all relevant EU chemical legislation. It will not specify how to replace each individual animal method, but will instead define interim actions and milestones.

The roadmap is expected to be finalised by the end of 2025 and will be published under the new Commission. It will include a set of short-, mid-, and long-term actions to replace animal testing.

The Commission is forming specific working groups to develop the roadmap, including groups focused on human health, environmental safety assessment, and change management. The first meetings of these groups are scheduled for June 2024.

The Commission is also conducting outreach activities to involve stakeholders and gather their input. These activities include workshops and a public consultation.

Helena Kandarova (Slovakia) enquired whether the EU wanted to develop their own guidance on how to do validation to which Katrin replied that the aim was still to work according to the principles that the OECD is applying, but to not necessarily wait until there is sufficient regulatory interest from all the OECD member countries before starting validation/acceptance work. That could lead to acceptance of a method under the EU regulatory framework.

Helena expressed some worries regarding eventual loss of the OECD “mutual acceptance of data” principle. She was also wondering if from a regulatory perspective, it would not mean double work.

Katrin replied that the idea would not be to conduct double work, but to find a more efficient way of preparing the work that then later could be approved or taken over at OECD level.

The chair clarified that the principles of validation as defined in OECD GD34 and its updated version, would still be maintained and informed about the upcoming presentation on that topic later in the meeting.

Joao Barroso (EURL ECVAM) confirmed that the point was not to devise a parallel validation process, but rather to discuss ways towards accelerating acceptance and that there was a need to differentiate between the two. The aim was to try to find mechanisms to go ahead to get at least faster approval within the EU, which could also facilitate later on international acceptance. This process is already in place and has already happened in certain situations, but the point was to discuss whether we could formalise a process that could be applied not just exceptionally, but more routinely.

Betty Hakkert confirmed that there had been cases where test methods had been introduced in the EU Test Method Regulation before becoming OECD test guidelines and that it was good to work towards this aim. She wondered who would be in charge of this process. PARERE and in particular the National Coordinators of test methods have experience with this issue, so that it could be relevant to inform and involve them in the development of this process at an early stage.

Katrin replied that it had been the very first meeting of the inter-service group on the roadmap discussing about validation and acceptance and that she would be personally very interested to have PARERE or the National Coordinators more closely involved. She would take note of the NL’s interest to involve more these two groups.

Knud Petersen (DK) highlighted the challenge of determining when a chemical poses a significant enough risk to warrant regulation. This decision often involves political considerations and the level

of scientific evidence required. While it is theoretically possible for the EU to regulate chemicals based solely on non-animal methods (using e.g. risk or safety factors), the reality is more complex. The main issue lies in the political aspect of regulation. We can only regulate chemicals based on their potential harm, and the question is how much evidence of harm is necessary to justify regulation. This is a frequent point of discussion in courtrooms, even with current in vivo methods. The difficulty lies in determining the level of proof needed to convince a chemical company that regulations are necessary. This question is often overlooked in discussions but is fundamentally important and should also be tackled in the roadmap.

Martin Paparella (Austria) concurred that it was a policy question. There is a need to determine the acceptable level of uncertainty and how to address it. The scientific process should also be based on full transparency of current uncertainties and limitations of animal-based methods.

This is also one of the milestones in Austria's European Green Deal cluster policy. Austria recommended working on this to achieve clarity and common understanding, since once the current uncertainties and the level of pragmatism used for the current animal-based methods are understood, it would be possible to frame and accept a new system.

Betty Hakkert concurred and emphasised the importance of policy changes such as modifying information requirements and classification criteria at the same time, to facilitate the adoption of non-animal testing methods and being able to implement them in decision-making. She mentioned that at the GHS level, it was even more difficult to achieve any changes in criteria than at the European level.

Andrew Worth (EURL ECVAM) provided the second part of the presentation on the roadmap towards phasing out animal testing for chemical safety assessments. He focused on the aspect of "change management" in the context of transitioning to animal-free assessments for chemical safety. Three working groups have been established, including a change management working group, which will be co-led by JRC and DG GROW. The aim of the change management working group is to identify principles and practices to facilitate the structuring of a roadmap and the subsequent transition to animal-free assessments. Andrew highlighted that there were already ideas about what a future assessment framework could look like, but the challenge was how to make the transition towards that, starting from where we are today. Andrew proposed using a model that illustrates the idea that different activities form synergies and lead to the final impact. He also proposed periodic checkpoints to monitor and report progress along the way. He suggested conducting targeted stakeholder consultations, developing a set of indicators to monitor progress, and identifying data gaps that may impede monitoring of progress. He also introduced the idea of a "recognised initiative" which is a unit of change or a pathway to impact and a means of giving directionality to various activities (in subsequent discussions of the change management working group, this was reformulated as a "transitional initiative"). Andrew concluded by asking two questions: 1. How do PARERE members foresee the transition to animal-free safety assessments? 2. What steps are being undertaken to facilitate change management in their own organisations and Member State? Andrew was seeking feedback and ideas from the audience to inform the work of the change management working group.

Helena informed that she works in an organisation where the majority of research and assessment is done in vivo, and she is trying to implement the switch to in vitro. This process was going very slowly and more targeted trainings were needed to different fields where in vitro assessments can be done, such as food, medical devices, and chemicals. She also informed that she was organising training for the technical normalisation group the following month on the biocompatibility assessment of medical devices using NAMs. Helena believes that more of these trainings and support for the broader community are needed.

The chair asked Helena if she had priority groups that she would train to which Helena responded that they are undergoing multiple trainings for different groups. She mentioned that they are conducting a one-week training in a specific field at their institute and also conducting trainings for medical devices testing and chemical assessment using OECD-adopted methods. The chair asked Helena if she thinks regulators should be trained, and Helena responded that regulators need to be trained as well as CROs that will offer these types of methods.

Vera Rogiers (SCCS) recommended to look at what was done for the assessment of cosmetics, which needs to be animal-free.

Emma di Consiglio (Italy) commented that it was useful to have collaboration between research and regulatory fields to reach the goals described by Andrew.

Betty Hakkert (NL) commented that regulators should also train developers on what are the needs and what are the hurdles, and that it was essential to work together to tackle these issues. She also mentioned that it was crucial to think about how to assess the hazard so that the information can be used for various uses of the chemical.

Marco Fabbri (DG GROW) commented that interaction between scientists and regulators was essential, and they have started interacting with PARC and ASPIS. He believed it was essential to think about how new methods will be applied by regulators from the early stage of development.

Georg Streck (DG GROW) commented that firm evidence like applications that show non-animal methods and approaches are working better than animal methods would be very helpful. He also mentioned that regulatory acceptance is crucial, and education is important, but it was also essential to make these tests reliable and trustworthy through validation.

Martin commented that he posted the reference to their publication in the chat (https://doi.org/10.1002/etc.5578; see in particular figure 2) where several ideas for what is needed for the change that could be interesting to consider for the roadmap in terms of change management.

The chair concluded the discussion by thanking everyone for their comments and mentioned that they would look at the information provided in the chat box.

Pilar Prieto (EURL ECVAM) presented an activity called “Designathon” that is being done in close collaboration with the European Partnership on Alternatives to Animal Approaches (EPAA), and which is part of a large EPAA project on systemic toxicity that started a few years ago. The focus of the activity is on exploring the use of new approaches to hazard classification, specifically for systemic toxicity. Pilar listed the names of people in the unit who are contributing to this activity and provided a link to the EPAA website where key documents and relevant information are being uploaded. The pilot phase of the challenge was launched in May 2023, inviting submissions of non-animal-based solutions that would inform the development of a future classification system for systemic toxicity. The aim was to create a classification system that would reflect levels of concern related to the current classification system, with three levels: high, medium, and low. The team provided a list of 150 reference chemicals to the providers of the solutions, which were previously divided into three groups of high, medium, and low concern. The challenge was open for a few months, and 23 solutions were submitted, which were diverse and rich in methodologies and approaches.

Pilar explained that the team is now working on designing a new classification scheme that will be based on new approaches and will ensure equivalent levels of protection as the current system. The

hope is to use the different non-animal-based solutions that were submitted as a starting point for designing this new classification scheme. Pilar mentioned that a workshop had been organised in March 2024 to discuss the solutions with the submitters. The workshop was organised into breakout sessions, where participants discussed toxicokinetic (TK) and toxicodynamic (TD) properties, identified the top five properties that should be included in the new classifier, and discussed different ways of integrating bioactivity and bioavailability. Pilar concluded that the team is now identifying three key areas for moving the project forward, which are chemical space, biological space, and classification strategies. These areas will be the focus of working groups under the umbrella of the EPAA, which will include members of the EPAA and representatives of different sectors. She mentioned that the next steps will be discussed with the EPAA Steering Committee in June 2024 to have a better idea of how to go ahead with these working groups. She also mentioned that the activity is still in its beginning stages, but would hope to have something more elaborated in the near future that can be put forward for consultation with stakeholders.

The chair mentioned that no questions on the Designathon were asked to PARERE at this stage and introduced the next presentations on emerging technologies.

Julia Malinowska (EURL ECVAM) started by explaining that the next presentations on emerging technologies were focussed on omics, imaging, stem cells, and organ on chip. Julia explained that omics is a promising tool for application in regulatory toxicology, as it may be used for chemical grouping and read-across, identification and characterisation of hazards, and elucidation of modes of action of chemicals. However, there are challenges to using omics in regulatory toxicology, including lack of formal standardisation. Julia noted that there are omics-relevant standards available in the form of best practices from scientific communities, but there is an urgent need for formal standardisation. One example of such formal standard is the OECD Omics Reporting Framework, which is currently being expanded to cover the reporting of omics data when used to form a group of chemicals, called Chemical Grouping - Application Reporting Module (CG-ARM).

There is also an ongoing OECD project on sample collection for omics, which is a welcome contribution to the landscape of standardisation. Julia emphasised the importance of communication with research communities and noted that standardisation is not just about rules, but also about valuable knowledge products. With respect to EURL ECVAM's internal activities, these have been focused on investigating the existing standards for transcriptomics and metabolomics to address standardisation in a systematic, efficient, and collaborative manner. Julia further explained that interpretation of omics data requires further standardisation activities as it is often challenging, especially in the context of future OECD test guidelines, which could incorporate omics measurements. Therefore, this aspect is an important area of investigation for EURL ECVAM. Julia welcomed any input by PARERE members on these questions and handed over to Milena.

Milena Mennecozzi (EURL ECVAM) presented another emerging technology, namely imaging, and more specifically, cell-based imaging. Milena mentioned that imaging is extremely powerful and enables us to test multiple endpoints at the same time on the same samples. Imaging can be performed on fixed samples as well as in kinetic mode, and it can be performed in 2D and 3D. Imaging is extremely useful for evaluating the health of the cells and should be implemented more in regulatory toxicology. She informed that the DNT (Developmental Neurotoxicity) in vitro battery was based mainly on imaging. The battery includes a series of tests using different cell types, including neurogenetic cells, astrocytes, radial glia, neurons, and oligodendrocytes. The endpoint by imaging includes proliferation, migration, or differentiation.

The DNT in vitro battery has been developed and used by the EFSA for risk assessment of pesticides. The methods had been reviewed by OECD, and there were some initial recommendations on the evaluation of the methods. However, the real transferability of the methods from a non-naive laboratory to a naive laboratory had not yet happened. Milena explained that imaging is a complex, multi-step process that starts with the collection of the sample, the staining of the sample, the acquisition, the analysis of the images, and the reporting of the data. To facilitate the uptake of image-based in vitro methods into regulatory toxicology, a workshop on "Facilitating the uptake of image-based in vitro methods in regulatory toxicology" had been organised at the JRC in March 2024. Experts from academia, industry, and software or instrument providers had been invited. Different topics were covered during the two-day workshop, including setting the scene on DNT, learning from CROs and industry, and best practices for standardisation of image acquisition and analysis. One key recommendation that came out of the workshop was that most of the variability in image-based in vitro methods comes from the biological model. Milena mentioned that it was therefore essential to minimise the biological model variability by improving model characterisation and making sure that the right expression markers are evaluated. There was also a need to improve the image acquisition by ensuring that instrument calibration is performed, and if people work with fluorescence, a calibration of the fluorescence should be performed using a calibration plate. Milena recommended that all this information should be properly reported to facilitate the transferability of the methods.

She informed that for image analysis, there was a need to increase transparency by sharing information on the software used, the version of the software, and all the parameters used. In addition, metadata should also be reported to ensure that the data can be comparable and reused by other laboratories. The end goal was to facilitate the uptake of image-based in vitro methods in regulatory toxicology by having a very detailed SOP that should be shared, using well-characterized reference chemicals throughout the process, and sharing all the information regarding the instrument. It was clear during the workshop that there were certain areas of imaging, in particular imaging pathology, that were more advanced than others. Milena advised to learn from these more advanced fields of imaging. Milena concluded her presentation by inviting PARERE to stay tuned to learn more on the recommendations that came out of the workshop. She informed that they were writing a manuscript which would most probably be published by the end of the year 2024.

Lucia Selfa-Aspiroz (EURL ECVAM) then introduced a project on standardising the biological part of NAMs, and more specifically, induced pluripotent stem cells (iPSC). Lucia explained that iPSC can have intrinsic biological variability that may not match the requirements of reproducibility of test methods. The project undertaken at EURL ECVAM aims to find a balance between the intrinsic biological variability and the reproducibility required for regulatory testing. Lucia noted that there are already published guidelines and criteria for standardising different aspects of iPSC, but there was a need for more implementation of these standards. The project has interacted with different stakeholders, including developers of iPSC, academic researchers, biotech companies, regulators, and standard-setting organisations. Lucia mentioned that a panel discussion is being organised in the context of the conference of the International Society on Stem Cell Research to discuss the use and implementation of standards for stem cells in non-clinical research. The discussion will cover topics such as the advantages and challenges of implementing standards and possible solutions or pathways to forward the implementation of standards. Lucia also mentioned that a detailed review paper was being prepared within the OECD test guidelines programme on the use of liver iPSC and liver organoids for liver toxicity testing. The detailed review paper will cover many aspects, including the characterisation of the cells, their availability and instability.

Monica Piergiovanni (EURL ECVAM) presented the organ on chip activities carried out in the unit together with collaborators. She explained that the term "organ on chip" refers to microscale cell culture platforms that have been engineered to enable complex interactions between organs or to enable specific microenvironment features. She discussed the standardisation activities that have been initiated in 2021 with an online workshop and an expert group of about 120 people. The goal was to identify priorities, synergies, and timing for standardisation activities.

Monica also mentioned that EURL ECVAM was working with the European Medicines Agency's 3Rs working party to steer the use of organ on chip in regulatory assessment. They are working on revising the guideline on the principles of regulatory acceptance of 3Rs, that will include two annexes to provide regulatory acceptance criteria for liver on a chip for drug induced liver injury, and heart on a chip for safety pharmacology testing.

Monica explained that EMA was also trying to simplify the concept of context of use for developers so that they can submit their proposals in a structured way. She mentioned that they had a workshop in Berlin last year to understand how to practically implement the qualification framework.

Monica also discussed the collaboration with the European organ on chip society (EUROoCS) and the creation of a database of relevant documents to support developers in understanding what it means to qualify or validate an organ on chip device. Finally, she mentioned that EURL ECVAM is involved in a liver on a chip ring trial, an industry-led activity where six pharmaceutical companies are working together to qualify a liver on a chip device for a specific context of use.

Monica asked the following questions to PARERE which were related to the emerging technologies which had been presented:

- In relation to omics, what would be the one key aspect of the omics workflow PARERE would like to see standardised most immediately to increase likelihood of using omics data in chemical safety assessments?
- Should omics-based methods in future test guidelines produce only binary-type results (e.g., classifying a chemical as a skin sensitiser based on a decision criteria; see e.g. TG on GARD^{TM}skin) or could there be expert judgement allowed (such as e.g. in animal-based test guidelines)?
- Have PARERE members, or regulators in their national network, assessed data from complex in vitro systems (ex. iPSC-based models, organ-on-chip models) in dossiers? If yes, how is the process of assessment? Who is involved?
- Do PARERE representatives know assessors with expertise on complex in vitro systems?
- Have PARERE representatives assessed dossiers with omics or high content imaging data?

The chair asked PARERE members to reply to these questions in written.

Joao Barroso (EURL ECVAM) presented the status of the OECD project on the revision of OECD Guidance Document (GD) 34 on the validation and international acceptance of new or updated test methods for hazard assessment of chemicals. The Joint Research Centre (JRC) proposed to revise GD34 in November 2022, and the project was approved in April 2023. The US and the Netherlands expressed interest in co-leading the project.

Work began with a virtual meeting in December 2022 using a live Slido survey to identify key discussion areas for subsequent meetings, including a face-to-face meeting in Paris in 2024. The project group identified the main priorities for GD34's revision, focusing on validation of defined approaches, practical guidance on validation, the concept of technical validation, relevance

assessment of New Approach Methodologies (NAMs), validation of new technologies like organ-on-chip and AI, and revising reproducibility and transferability assessment processes. The revised GD34 will primarily cover non-animal or new approach methodologies for inclusion in test guidelines with mutual acceptance of data. It will cover in vitro, in vivo, and in silico methods, but may require additional expertise for in vivo methods. A significant change in practice was noted: formal pre-validation studies have largely been replaced by more streamlined approaches due to resource constraints and the evolving nature of validation processes.

The revision process also aims to make GD34 more practical, flexible, and accessible for method developers, who are now often the primary users and validators of their own methods. The group is considering various scenarios for validation and acceptance processes, especially for methods that are part of Defined Approaches (DAs) or Integrated Approaches to Testing and Assessment (IATAs).

Discussions include how to streamline validation and international acceptance and whether to maintain both aspects in GD34.

Readiness criteria are being developed to assess the readiness of methods at different stages of the validation process, with a focus on creating a template for inclusion in the guidance document. Subgroups have been formed to tackle validation of defined approaches and their information sources, readiness criteria, and transferability and reproducibility assessment. The aim is to ensure GD34 provides clear, practical guidance and remains adaptable for future developments in validation practices.

The chair thanked the speakers and proposed a timeline of one month for replying to the questions asked in the different presentations to which the network agreed. PARERE members were invited to reply to the consultation by 1 July 2024 using the template in Annex 3.

Annex 1 - Participants

Austria
Belgium
Czech Republic
Denmark
Finland
France
Germany
Ireland
Italy
Latvia
Luxembourg
Poland
Slovak Republic
Spain
Sweden
The Netherlands

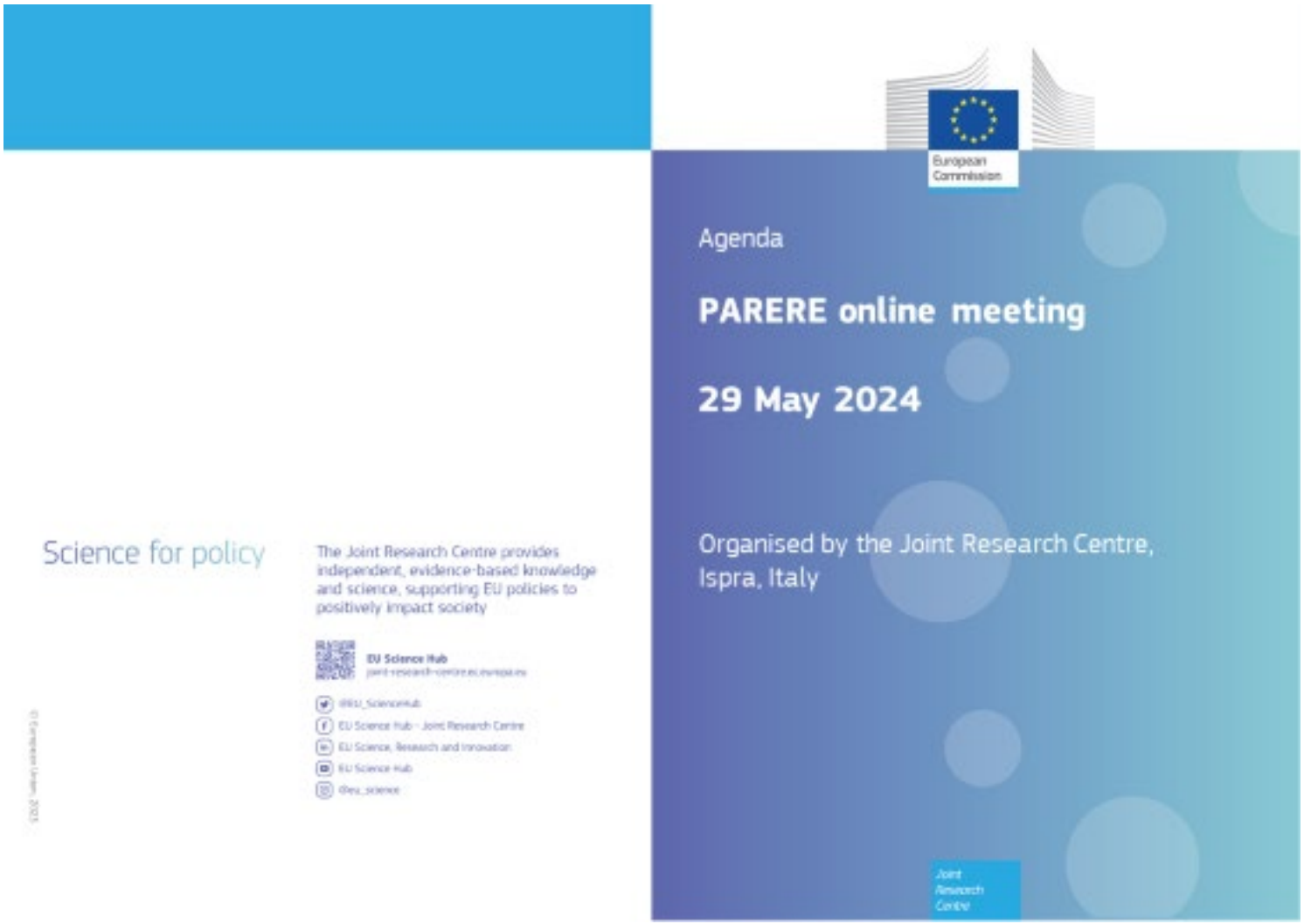
European Commission:
DG ENV
DG GROW
DG EMPL
JRC

EU Agencies:

ECHA
EFSA

Scientific Committees:
SCCS
SCHEER

Annex 2 – Agenda



Participants:

PARERE Network

EURL ECVAM

Agenda – PARERE online meeting (WebEx)

Wednesday 29 May

09:30-09:35 **Welcome and introduction**

09:35-10:45 **Updates from the Members States (5 minutes per MS)**

10:45-11:00 *Break*

11:00-12:45 **EURL ECVAM updates on:**

- **PARC**
- **ASPIS**
- **Roadmap for phasing out animal testing**
- **Designathon**
- **Emerging technologies (organ-on-chip, 'omics, imaging-based assays)**
- **Qualification frameworks**
- **Update of OECD GD 34**

12:45-13:00 **AoB and conclusion**

Annex 3 - Consultation template

PARERE Consultation - Follow-up to PARERE Meeting (29/5/2024)

(Please check the presentations on CIRCABC for additional information)

Member State/Commission Service/EU Agency/Scientific Committee: [to be filled in]

Topic	Question	Response
1. PARC	1a. What is your level of involvement in PARC?	
	1b. Considering methods identified in the thyroid AOP network and eventual use in IATA would there be an interest of MS to take a lead in bringing such methods onto the OECD work plan for test guideline development?	
2. ASPIS	2a. Are you interested in a PARERE-ASPIS +1 webinar for update and discussion?	
3. Roadmap for phasing out animal testing	3a. How do PARERE members foresee the transition to animal-free safety assessment?	
	3b. In the context of the Commission's Roadmap, what steps are being undertaken to facilitate change management in your own organisations and MS?	
4. Designathon	No question	N/A
5. Emerging technologies	*omics-specific questions*: 5a. What would be the one key aspect of the omics workflow you would like to see standardised most immediately to increase likelihood of using omics data in chemical safety assessment? 5b. Should omics-based methods in future test guidelines produce only binary-type results (e.g., classifying a chemical as a skin sensitiser based on a decision value, see GARD™skin) or could there be expert judgement allowed (e.g., animal-based test guidelines)?	

	Questions related to all emerging technologies presented: 5c. Have you assessed data from complex in vitro systems (ex. iPSC-based models, Organ-on-Chip models) in dossiers? If yes, how is the process of assessment? Who is involved? 5d. Do you know assessors with expertise in complex in vitro systems? 5e. Have you assessed dossiers with omics or high content imaging data?	
6. Qualification framework	No question	N/A
7. Update of OECD GD34	No question	N/A

Abbreviations/Acronyms:

AOP	Adverse Outcome Pathway
ASPIS	Animal-free Safety assessment of chemicals: Project cluster for implementation of novel Strategies (EU Horizon 2020 project)
GD	Guidance Document
GARD™skin	Genomic Allergen Rapid Detection (GARD™) test method for assessment of skin sensitisers
IATA	Integrated Approaches to Testing and Assessment
iPSC	induced Pluripotent Stem Cells
MS	Member State(s)
OECD	Organisation for Economic Cooperation and Development
PARC	European Partnership for the Assessment of Risks from Chemicals (EU Horizon Europe project)
PARERE	Preliminary Assessment of Regulatory Relevance network