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Summary record of the 11th meeting of the EURL ECVAM Preliminary Assessment of Regulatory Relevance (PARERE) Network

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Abstract

The 11th meeting of the Preliminary Assessment of Regulatory Relevance (PARERE) network was held online on 17 February 2022. The purpose of the meeting was to provide updates on 3Rs (Replacement, Reduction and Refinement of animal testing) activities undertaken in EU Member States and by Commission services and relevant EU agencies; to inform and seek input on extended information requirements under REACH and the potential to introduce New Approach Methodologies (NAMs). Regarding the latter topic, the aim was to discuss more efficient ways to introduce NAMs into chemical hazard and risk assessment practices under the REACH regulation as foreseen by the EU Green Deal.

The session on a roadmap for respiratory sensitisation testing included presentations that introduced the topic, a summary of the feedback received from PARERE on various EURL ECVAM consultations on respiratory sensitisation and examples of approaches used for chemical risk assessment of respiratory sensitisers under various EU legislation. Since respiratory sensitisation has been identified as a critical hazard in the EC's chemical strategy for sustainability, the aim was to have an exploratory discussion on the short-, mid and long-term needs for tackling the field of respiratory sensitisation and the use of NAMs to satisfy regulatory information requirements.

1 Introduction

EU Directive 2010/63/EU on the protection of animals used for scientific purposes requires that Member States nominate a single point of contact to provide advice on the regulatory relevance and suitability of alternative approaches proposed for validation. The PARERE network[1] is trans-sectoral and includes regulators of the EU Member States, representatives from EU agencies such as the European Medicines Agency (EMA), the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA), and relevant policy services of the European Commission. Regulators operating within all sectors of relevance to alternative methods are involved as early as possible in providing a preliminary view on the potential regulatory relevance of methods and approaches submitted to EURL ECVAM for validation or peer review or evaluation. PARERE members are consulted on several occasions over the year, either on the regulatory relevance of individual methods or approaches that are submitted to EURL ECVAM or on other topics such as e.g. EURL ECVAM Recommendations, standardisation and validation frameworks for novel technologies developed within research projects funded by the EU Framework Programmes for Research and Innovation.

The 11th meeting of PARERE was held online on 17 February 2022 (the list of participants and the agenda are included in Annex 1 and 2, respectively).

[1] More information on the PARERE network can be found here: https://joint-research-centre.ec.europa.eu/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/alternative-methods-toxicity-testing/advisory-and-consultation-bodies/parere-eurl-ecvam-network-preliminary-assessment-regulatory-relevance_en

2 Welcome and updates

The meeting was chaired by Valérie Zuang, EURL ECVAM. She welcomed all members and invited new members to introduce themselves. New members included Joanna Roszak from Poland, Zuzana Ševčíková from Slovakia, Kaisa Askevik from Sweden, Verena Fetz from Germany, Michael Schaeffer from the European Research Executive Agency (REA), Marco Fabbri from DG Internal Market, Industry, Entrepreneurship and SMEs (DG GROW) and Zinta Podniece from DG Employment, Social Affairs and Inclusion (DG EMPL) of the European Commission. The chair highlighted the different agenda points, which were up for discussions, and approved the draft agenda. She then invited the PARERE members who volunteered to provide updates on activities within the PARERE network in the respective Member States and in the respective Commission DGs and EU Agencies. Additional information on these updates can be found in the respective presentations on CIRCABC.

2.1 Updates on activities within the PARERE network

Updates were provided by the representatives of the European Commission's Scientific Committees, the Scientific Committees on Consumer Safety (SCCS) and on Health, Environmental and Emerging Risks (SCHEER).

Vera Rogiers (SCCS) started her update by mentioning that there were some concerns within the SCCS, and that PARERE would be an appropriate forum to express these concerns. The Committee provides Opinions on health and safety risks of non-food consumer products (e.g. cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products) and services (e.g. tattooing, artificial sun tanning). For the safety evaluation of cosmetic ingredients, two channels are functional within the EU. The safety of the substances in Annexes II to VI of the Cosmetic Products Regulation is evaluated by the SCCS; the safety of cosmetic products with all their ingredients is evaluated by the industry placing them on the EU market. Thus, the Annex substances fall under the responsibility of the SCCS and are directly linked to the Regulation. The mandate to provide an opinion for cosmetics is usually coming from DG GROW. SCCS publishes Notes of Guidance (11th revision, 12th revision under preparation) which are designed to provide guidance to public authorities and to the cosmetic industry to improve harmonised compliance with the Cosmetic Products Regulation.

The Annex substances include colorants, preservatives, UV filters, and all ingredients, which may have an impact on human health. In that case, the Competent Authorities of the MS come together and inform the Commission about any issues. The EC then consults the SCCS (SCCS consultation is compulsory). During the current discussions on EDs, there are two prioritisation lists of cosmetic ingredients which could be potential EDs (28 substances including preservatives, UV filters, hair dyes). Further to the EU Green Deal, there are changes expected to the REACH and CLP legislation but also to the Cosmetic Products Regulation, which are linked to the fate of the SCCS. At ECHA where they deal with industrial chemicals, they are concerned with hazard identification and classification and labelling. For cosmetics, the SCCS looks at human risk assessment and works in accordance with the Cosmetic Products Regulation. The SCCS today is an independent committee but it has become clear that the Committee will soon be under the responsibility of ECHA, and the SCCS is concerned of being taken up in Committees such as e.g. the Committee for Risk Assessment (RAC). The SCCS would like to remain an independent committee following robust scientific principles and avoiding political interferences. The SCCS invest in human relevant and importantly, completely animal-free risk assessment. The Green Deal agrees on "one substance, one "hazard" assessment" however, SCCS is pleading for a first tier where hazard assessment is evaluated, followed by a second tier for risk assessment.

V. Rogiers continued by mentioning that the risk assessment process as carried out by the SCCS is driven not only by toxicity but also by exposure and on the basis of solid scientific evidence. The Committee has developed as a "pre-runner" for innovation in cosmetic legislation, because in the meantime, they have always worked with New Approach Methodologies (NAMs) and became experts in animal-free methodology and innovation in risk assessment (pushing industry to submit dossiers including next generation risk assessment (NGRA)). Political decisions up to now did not play a role in the SCCS opinions. The SCCS' Notes of Guidance (NoG) are also followed outside the EU, e.g. in May 2021, the Chinese authorities have adapted to the NoG for the risk assessment of cosmetics manufactured in China. V. Rogiers finished by informing that the same members of SCCS and SCHEER had been appointed by DG SANTE for the new term of the committees (2022-2026) during the COVID crisis, that there was a new lay-out of DG SANTE's website for the Scientific Committees (europa.eu), that experts could apply anytime to the Database of Experts (europa.eu) and that some of the Committee's opinions were published in Scientific Journals.

Renate Kraetke (SCHEER) presented the activities of the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER). She mentioned that SCHEER would share some of the open words expressed by the representative of the SCCS. SCHEER is also facing a new term from 2022-2026 including some changes such as a potential move to ECHA. The SCCS' mandates are closely related to the Cosmetic Products Regulation, whereas for SCHEER, the mandates come from various fields and various DGs. SCHEER is not bound to animal-free testing, but they are interested and faced with NAM data and are using them as far as possible. SCHEER, on request of Commission services, provides Opinions on questions concerning emerging/newly identified health and environmental risks; risks to consumer safety or public health and issues not covered by other EU risk assessment bodies. The committee is composed of 17 Members, plus external experts in case the permanent members do not cover the expertise. SCHEER is constantly looking for additional experts and the database for experts to apply remains open. The Committee makes use of public information (*in vitro*/*in vivo*/epidemiology), reports from other scientific bodies and sometimes also dossier/confidential information. The current mandates relate to the safety of cobalt and titanium dioxide (prohibited because of CMR properties) in toys with regard to a possible derogation from their prohibition. Another mandate relates to potential health effects of exposure to electromagnetic fields (EMF). In 2021, during the COVID crisis, SCHEER's work was reduced and most of SCHEER's opinions related to environmental risk assessment, in particular on water quality. SCHEER provided scientific advice on Guidance Document n°27: Technical Guidance for Deriving Environmental Quality Standards and on proposed EU minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge. Other opinions in 2021 related to medical devices, i.e., on the safety of breast implants in relation to anaplastic large cell lymphoma for which they analysed epidemiological data; on toxicological reference values for certain organic chemicals emitted from squishy toys with regard to adopting limit values under the Toys Safety Directive 2009/48/EC 'Chemicals in squishy toys'; and on electronic cigarettes for which they used all possible information they could get. For the latter, many public comments had been received during the public commenting period. SCHEER hopes to be able to work as an independent scientific committee also in the future.

Verena Fetz (Germany) asked whether external experts needed to have any specific prerequisites. Renate replied that they should have experience and expertise in the field of the work of the committee. The secretariat is also looking at any potential conflicts of interests. If these conditions are met, the experts are added to the database and will be contacted in case their expertise is needed. The committee is in particular interested in experience with *in vitro* methods and computational modelling, which is less available in the current committee.

Georges Kass (EFSA) mentioned that many of the chemicals showed on the slide related to water quality were also investigated in the large EU-funded project PARC. One of the objective of PARC is to look at the different sources of exposure, including water. EFSA wondered whether there was any link between the work of SCHEER and that of PARC. Renate replied that some SCHEER members were also involved in PARC activities but could investigate further with her ecotoxicity colleagues in SCHEER.

Philippe Castelain (Belgium) mentioned that he was involved in the risk assessment of pesticides for agricultural use. Regarding pesticides residues in water, which is an issue for both surface and ground water, Philippe wondered what the object of SCHEER's research was, considering also the use of *in vitro* methods.

Renate replied that the committee was not carrying out research, but provides advice based on the mandates they receive. The mandate consisted in evaluating the way these pesticides should be regulated regarding water quality. For deriving water quality standards, any available *in vitro* and *in vivo* data were analysed. Renate referred to their webpage and opinions for further information.

Martin Paparella (Austria) presented the Austrian PARERE activities of 2021. An Austrian PARERE workshop on genotoxicity had been organised with the purpose of identifying the current regulatory discussion items for genotoxicity assessment and possible action towards the 3Rs. Genotoxicity could be the next lowest hanging fruit for a full NAM-based Integrated Approach to Testing and Assessment (IATA). For genotoxicity, you look at the mode of action (MoA) and not at an apical adverse effect related to genotoxicity such as cancer, teratogenicity or organ toxicity. Prof. Knasmüller from Vienna, Austria, has identified a new cell line that he has characterized for mutagenicity testing and evaluates current methods with regard to their predictivity and reliability. Austria will also contribute to activities on genotoxicity within PARC and there was a need to discuss if/how important the quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) for NAM-based genotoxicity assessment is. The Austrian PARERE Network and the Austrian Competent Authority were invited and the workshop was supported by two specialized academic scientists (Prof. Knasmüller, Prof. Fürhacker). The outcome of the workshop was that a common understanding with regard to the regulatory relevance to revise the UN GHS text in order to ease category 1 classification without specific germ cell genotoxicity tests was

reached. There was also a preliminary discussion that in case the revision of the GHS test is not successful, another option could be to suggest the development of a new GHS class or category for somatic genotoxicity. This is of similar high concern than the germ cell genotoxicity. Other outcomes included the support to develop a new NAM-based IATA for genotoxicity by supporting further validation of the HuH6 cell-line and the systematic review of Prof. Knasmüller for the performance of current genotoxicity methods which could then also feed into the PARC activities. In case of a successful scientific validation of a full replacement IATA for genotoxicity, the legal text and guidance will need to be adapted. There had also been an Austrian-Belgian PARERE workshop on cardiotoxicity, which is related to the H2020 funded ALTERNATIVE project. An AOP network for cardiotoxicity is being established, which shall support the development of an IATA that will include a new 3D aged cardiac tissue model, as well as other existing methods (developed elsewhere). Both the Austrian and Belgian PARERE representatives are leading several deliverables of the regulatory work package in the ALTERNATIVE project and they are supported by two post-docs. The project aims to interact with regulators to understand their needs in the area of pharmaceuticals, pesticides and industrial chemicals. Other consortium partners will develop three-dimensional tissue engineering *in vitro* models mimicking the human cardiac tissue of young and old people. Multi-omics analyses and contractility will be measured and a machine learning risk assessment tool should be developed.

Raffaella Corvi (EURL ECVAM) reminded that the JRC is leading, on behalf of the EU, the update of the GHS chapter and the revision of the criteria for germ cell mutagenicity at the UN. In that context, Raffaella enquired whether Austria had already a concrete idea how a new UN GHS class or category for somatic germ cell mutagenicity and the IATA on genotoxicity could be moved forward also from a procedural perspective.

Austria replied that in agreement with Prof. Knasmüller, they will prepare a test submission to EURL ECVAM. These activities will also be progressed via the PARC project and Austria would find it useful to also have a wider EU PARERE discussion on options in the field. Austria will also support the UN activity via the OECD. Austria is open to any other suggestions for engagement.

Birgit Mertens (Belgium) reported on the seventh Belgian PARERE network meeting held on 10 May 2021. The network is usually meeting twice a year and focuses on a specific topic. The main outcomes of the EU PARERE network based on the EURL ECVAM status report 2020 were presented at the meeting. They then focused on the topic of the quality control of vaccines based on non-animal methods for both human and veterinary vaccines. There was also an update on the VAC2VAC project and on the PARC initiative as the work undertaken in PARC will be very relevant for the regulators.

There has also been a joint meeting of the Belgian and Austrian PARERE network on 21 December 2021, which focused on cardiotoxicity. The aim was to discuss the lack of data requirements for cardiotoxicity in the different regulatory fields amongst regulators from Belgium and Austria, as well as the partners from the ALTERNATIVE project (see above). Other activities within the Belgian network of relevance to the 3Rs were the development of some replacement methodologies, such as the Genomark biomarker consisting of 84 genes to evaluate genotoxicity. The method has been developed by VUB and Sciensano. HepRG cells are exposed to the compound of interest, gene expression data are then collected for 84 genes of interest mainly by RT-qPCR. Prediction models have been developed with the R programme which allows to classify a compound as genotoxic, non-genotoxic or equivocal. The prevalidation study demonstrated good prediction performance. A test presubmission form has therefore been completed and will be submitted to EURL ECVAM.

Belgium also reported that new features were added to the RE-Place platform to make it more user-friendly to submit NAMs on-line. It focuses on NAMs used in Belgium and currently includes 170 test methods. Belgium hopes that the number of methods included in the database will increase with the optimized tool. Belgium also worked on promotional material (e.g., brochures, leaflets, videos). They use the RE-place website and LinkedIn social media to share a lot of information and knowledge on NAMs and contribute to publications such as e.g. blogs and interviews. Belgium invited the other PARERE members to contact them in case they would like to use these means to share their material. Regarding the IC-3Rs (www.ic-3rs.org), Belgium mentioned that in 2021, a two-day symposium had been organised at which more than 400 people participated from 36 different countries. The recording is available from the IC-3Rs website. A new communication manager has been hired at IC-3Rs in 2022 and two post-doctoral posts are vacant.

Helena Kandarova (Slovakia) appreciated the website RE-place and was wondering if it was exclusively Belgium-oriented. Belgium replied that currently they collect methods mainly from the Flemish and the Brussels regions but will investigate the possibility to extend it to other European countries. Slovakia also enquired on the number of people working on the database to which Belgium replied that the work was done by two colleagues (one post being currently vacant) and coordinated by Birgit Mertens and Vera Rogiers.

The new PARERE representative of Sweden, Kaisa Askevik mentioned that they were in the process of establishing a network and may contact other PARERE members to enquire how it was done in other countries. The Swedish 3Rs centre has a close collaboration with Swedish government agencies, six of which have been compelled to have a 3Rs strategy, namely the Swedish Chemical agency, the Swedish Food agency, the Medical Products agency, the Swedish Environmental Protection agency, the National Veterinary Institute and the Swedish agency for Marine and Water management. They have regular meetings with these agencies. More agencies will follow as part of the PARERE network. The Swedish 3Rs centre is also involved in the Swedish agency-hub for PARC. They also established a network for replacement (called "Replace") in 2019, which is open for stakeholders with an interest in animal-free methods for research, toxicity screening and/or for regulatory purposes. The network should facilitate contact and exchange of information. It is continuously growing and includes now almost 300 registered people. The members of the network are regular guests on webinars organised by the Swedish 3Rs centre. In 2021, a webinar took place where the EURL ECVAM recommendation on non-animal derived antibodies had been presented. Two other webinars on validation had been organised as well, the first webinar covered early steps of method validation – those preceding the hands-on laboratory based study and the second webinar covered further steps – the hands-on laboratory based validation study, as well as the scientific peer review. Finally, the Swedish 3Rs centre is working on infographics on validation which will explain the formal validation process in a broader context and make it available on its webpage in spring 2022.

Vera Rodas (Norway) drew the attention to a project called SWIFT on "A Bayesian network model for predicting fish acute toxicity based on fish embryo testing: a probabilistic weight-of-evidence approach" in which one of the Norway PARERE network representative, Adam Lillicrap, is the leading scientist. The project is aiming at strengthening the weight-of-evidence of fish embryo toxicity data to replace the acute fish toxicity test described in OECD TG 203. The process has been very long. A Bayesian network, which is a mathematical model, has been developed in this SWIFT project. The project has been presented to the Norway Competent Authorities. It uses fish embryos until 96 hours post-fertilisation. Sweden raised a question regarding a note that had been sent to the Competent Authorities. It proposes a potential revision of Annex 3 of Directive 2010/63/EU with a specific zebrafish section, where zebrafish embryos would be protected after 72 hours post-fertilisation. The latter would be in contradiction to the implementing decision and would compromise the fish embryo testing approach as an alternative method as it would mean that the embryos would be protected by the legislation after 72 hours.

Susanna Louhimies (DG ENV) replied that the documents that had been circulated were thought-starters in terms of what scientific evidence has been provided as the basis that DG ENV should consider for revising Annex 3. Once all the feedback will be collected, DG ENV will evaluate it and everything needs to be aligned before a formal Commission proposal to revise the annex can be presented. The annexes however cannot contain definitions, and thus the 72-hour limit will continue to be used as a cut-off for considering zebra fish embryos as independently feeding and under the scope of the Directive, when kept at approximately +28° C.

Laura Rossi (ECHA) presented an overview of the projects in which ECHA is involved. In addition to those, ECHA is also working on the extended REACH information requirements and PARC initiatives. ECHA is heavily involved in the OECD work, such as the harmonisation of reporting templates, for which e.g., it works together with JRC on updates of OHT 66 and OHT 66-1 on *in vitro* skin sensitisation. Several ECHA staff is participating in the OECD TG and GD work via expert groups and in the revision of the OECD QSAR toolbox model. ECHA is also involved in APRCA related activities, in the toxicological relevance of the Metabolic biomarkers (also called the M700+ panel), a collaboration between ECHA and the University of Birmingham, BASF and APCRA partners. ECHA is also involved in the development of a robust *in vitro* ADME/TK model which is a combination of the use of a competent hepatic cell line in which clearance rates and kinetics can be measured *in vitro* and combined with ADME profiling and metabolomics.

The APCRA project that is already ongoing for a couple of years, is a collaboration between ECHA, Heath Canada, US EPA, EFSA, US NTP, A*STAR and many other governmental organisations from Europe and Asia. It consists of retrospective and prospective case studies. Regarding the retrospective study, a comparison of point of departure based on high throughput of bioactivity, exposure predictions and traditional hazard information (*in vivo* data) for 488 substances is being made.

The first prospective study is estimated to be completed for tier 2 in spring 2023 and the follow-up will depend on the results. Tier 2 investigates the performance of the 5-day rodent assay into which multi-omics and benchmark dose (BMD) assessment have been introduced. This is studied for about 20 substances. The expected outcome would be to obtain a quantitative PoD based on the *in vivo* and molecular data to obtain more insight on the hazardous profiles of these substances. The aim is to investigate if a NAM battery can

mimick the toxicological triggers that are usually obtained from a standard subchronic repeated dose toxicity (RDT) study. Depending on the type of outcome from the 5- day rodent study, additional testing might be then triggered in tier 2.

In the APCRA prospective study, in order to demonstrate that an outcome is comparable with the RDT 90d study in the context of hazard characterisation and risk management, NAM testing has to provide an estimate of NOAEL and LOAEL and also information based on observed or predicted effects that indicate if a classification for repeated exposure is needed. This can then also provide indications and triggers for additional testing, which can be helpful in the future for prioritising the testing of selected substances of concern and in the short term will reduce animal testing by avoiding unnecessary testing.

Martin Paparella mentioned that US and Canada would take a slightly different approach in the APCRA project heading to the next generation risk assessment, where they use a huge battery of NAMs to derive points of departure and apply assessment factors, kinetic modelling etc. for the purpose of risk assessment. Martin enquired if and in which way these two projects were aligned which each other.

Philippe Castelain wondered if any investigation for potential respiratory sensitisation was foreseen in the APCRA project as it is not part of the list of triggers that was presented by ECHA.

Vera Rogiers asked whether the people involved in the revision of the OECD QSAR toolbox could be informed that when you investigate the threshold of toxicological concern (TTC) in the Toxtree tool of JRC and in the OECD QSAR toolbox, you obtain different results for Cramer class 1, 2 and 3.

Georges Kass picked up on the TTC question as EFSA published a GD three years ago, where they referred to the two TTC platforms and also highlighted the differences. The OECD Toolbox group is well aware of these (occasional) discrepancies, which have been well analysed in the literature (e.g. Bhatia et al., 2015). The most significant discrepancies result from different chemistry interpretations of vague wording in the original rules (published in 1978 before computational implementations).

Laura Rossi replied that she would contact and feed back to the appropriate people at ECHA.

Georges then provided an overview of the most recent activities of EFSA. EFSA is dealing with food and feed safety and the vast majority of data they receive come from *in vivo* studies. EFSA is working hard to develop a strategy for incorporating non-animal based studies and data into their risk assessment. In the recently published EFSA strategy 2027, it is highlighted that EFSA is committed to develop and integrate new scientific developments focusing on NAM-based methods and the minimisation of animal testing. The development of NAMs and omics approaches for chemical risk assessment is in fact part of EFSA's key actions. In this spirit, some activities have been carried out at EFSA over the last two years. They include the development of a GD on read-across for food safety and its applicability in a regulatory framework, the testing opportunities for using NAMs to underpin the classical read-across for chemicals and bringing in also the biological read-across. EFSA also recently published a GD on risk assessment of nanomaterials to be applied in the food and feed chain. Although the majority of the data are based on *in vivo* studies, EFSA highlighted the importance of *in vitro* toxicity tests as they have the advantage to better understand the fate of the nanoparticles in terms of their cellular internalisation and ability to study what happens in the cell. It also helps to better understand the toxicokinetic and toxicodynamic elements of nanomaterials. Another area in which EFSA invested was the development of an IATA for developmental neurotoxicity at OECD level, which is purely based on an *in vitro* testing battery. EFSA recently published case studies on how it could be applied.

Another area of focus at EFSA is on non-monotonic dose-responses and their implications on human health risk assessments. The EFSA opinion recommends to consider NAM-based studies to facilitate the assessment, and also minimise the need for repeating animal studies. The integration of available animal and human studies with NAMs could provide the mechanistic understanding required for implementing the use of AOP approaches and the implication of non-monotonic dose-responses on human health. A recent published GD of EFSA focuses on human risk assessment of combined exposure to multiple chemicals and how to do the grouping of such chemicals. The GD recommends to support integration of data generated from NAMs as currently investigated world-wide (OECD, US EPA, EFSA) and in Horizon 2020 and Horizon Europe programmes (EuroMix, EU-ToxRisk, HBM4EU, PARC etc.) and to further develop and implement *in silico* approaches that could support grouping of chemicals. This will support the development of NAMs for grouping multiple chemicals based on a) predictions of the interaction between chemicals and their molecular targets and b) predictions of toxicological endpoints. EFSA's increased budget over the recent years also allowed them to outsource a number of collaborative projects on NAMs in fields such as pesticides neurotoxicity and nanomaterials/GIT nanofibres uptake and genotoxicity.

Upcoming collaborative projects will be on artificial intelligence for NAMs (AI for NAMs data search, extraction, appraisal and integration in AOPs); PFAS immunotoxicity; essential oils as feed additives and interspecies metabolic differences.

EFSA is also developing the TKplate 2.0 which is an open-source platform integrating PBTK models and machine learning models for risk assessment of single and multiple chemicals and biological stressors in animal species. Other projects of high priority for EFSA are on 'omics (in particular *in vitro* 'omics) and bioinformatics and how they could be helpful in risk assessments. EFSA is collaborating in a number of EU collaborative projects under H2020 such as ASPIS (in particular RisHunt3R), ALTERNATIVE, PARC (as associate partners together with ECHA). Georges finished by mentioning the EFSA ONE conference to be held in Brussels and online on 21 to 24 June 2022. One session will be on NAMs and their regulatory application.

In her update, Helena Kandarova (Slovakia) mentioned that Slovakia had nominated a new test facility, the Centre of Experimental Medicine (CEM) of the Slovak Academy of Sciences (SAS) to EU-NETVAL since the previous test facility lost its GLP status. The activities of CEM-SAS include a large number of activities and involvement in the work of EURL ECVAM, OECD and ISO, such as e.g. contributing to the chemicals selection for the TH validation study; providing expertise to the PARERE Network on various topics such as topical toxicity testing (skin/eye/phototox), sensitisation, microphysiological systems, tissue engineering of 3D models; providing expertise to OECD in the development of the new OECD TG 498 (published in 2021) on phototoxicity testing, further work on the Performance Standards document related to TG 498 and the phototoxicity AOP; involvement in the medical devices NAM-work, consulting with the US experts on the acceptance of ISO 10993-23 into the MD regulation in USA (FDA); preparing laboratories for *in vitro* inhalation (irritation/sensitisation) studies for 2023.

Slovakia organised an OECD-EFSA supported online symposium on "Dissemination and implementation of the OECD *in vitro* and *in silico* methods applicable to the assessment of chemicals, food and feed" in Bratislava on 24 to 25 May 2021. More information on the scientific programme and participation can be found in the presentation and here. Several young Slovakian scientists and students participated in the JRC Summer School on non-animal approaches in science: the 3R..evolution organised by EURL ECVAM on 17 to 21 May 2021. The event was highly appreciated and one Slovakian early career scientist, Peter Bôbíš, won the PETA Science Consortium International's poster award for his work developing and optimising a three-dimensional human cornea-like model to assess the photoirritancy and eye irritancy of medical devices and drugs without using any animal components.

Another important conference was the TOXCON 2021, organised by the Slovak Toxicology Society SETOX in September 2021 in High Tatras, Slovakia, which brought together *in vivo* and *in vitro* scientists. Helena also highlighted some activities of ESTIV, the European Society of Toxicology in Vitro. ESTIV is collaborating with EURL ECVAM and disseminates the JRC reports related to *in vitro* and *in silico* science on its website. ESTIV is also organising the *in vitro* applied training course for scientists who would like to upgrade their skills in *in vitro* techniques. This is a 6-day course organised at the Luxembourg Institute of Science and Technology (LIST) on 6 to 11 June 2022. The ESTIV congress will be held in Barcelona, 22 to 25 November 2022. Two Congress highlights will be the BEAMS (Bridging across methods in the biosciences: the opportunities of COVID-19) workshop organised by EURL ECVAM/JRC and the ASPIS Cluster meeting where the three H2020-funded projects (Ontox, RisHunt3R and PrecisionTox) will present their scientific outcomes.

S. Louhimies (DG ENV) appreciated the many activities taking place in the PARERE network and updated on five topics of high relevance to the network:

- The EP resolution for a transition to innovation without animals
- The new transparency tools under Directive 2010/63/EU
- The Open access e-training modules
- The Test Method Regulation under REACH

The use of animals in research and testing is a sensitive topic with high public interest. The recent reports of malpractice with undercover footage increased the concern by the public and the European Parliament, and put growing pressure to move towards animal-free research and testing. The EP adopted a resolution including concrete plans and actions to accelerate a transition to innovation without the use of animals in research, regulatory testing and education on 16 September 2021. The European Commission response was transmitted on 10 February 2022 and will be published by the EP at the Documentation gateway. The key message of that resolution is to draw up an EU-wide action plan to drive active phase-out of animal use in research and testing. Susanna presented the European Commission's response (see presentation) and reminded that the ultimate goal of full replacement is enshrined in EU legislation, which is unique in the

world, and that there is a legal obligation to replace the use of animals when non-animal methods become available which provides a step-wise approach as science advances.

A new European Citizen Initiative is underway to collect one million signatures and is open until the 31st August 2022. If the criteria are reached, the EC will need to formally respond.

Regarding the new transparency tools under Directive 2010/63/EU, Susanna mentioned that the collection of statistical data is the basis for analysis and understanding of the status quo and that the non-technical project summaries provide further the context. The EU has taken a real quantum leap in transparency by providing two open access central EU databases permitting anyone who is interested to data-mine these databases for various purposes, including for identification of areas where alternatives are most urgently needed.

Regarding the open access e-training modules, Susanna mentioned that, thanks to the funds provided by the EP, the EC has developed several e-learning modules to support Replacement, Reduction and Refinement through education and training. One specific module is on developing alternatives for regulatory application where GIVIMP is described. These modules are all freely-available. Six modules are already available and ten more are under development. The modules should be understood as additional tools and are not intended to replace face-to-face learning.

The activities of the European Partnership on Alternatives to Animal testing (EPAA) include a project on pyrogenicity testing to try to move away from the rabbit test by revising 59 monographs in the European Pharmacopoeia and recommend *in vitro* alternatives, which are already available since a while. Another EPAA project is about quantitative *in vitro* to *in vivo* extrapolation in which the effectiveness of a computational algorithm for converting *in vitro* concentration-response data to *in vivo* dose-response data.

Regarding the Test Method Regulation, Susanna informed that the update of the TMR was aiming to fasten the uptake of new alternative methods following the adoption at OECD level. The draft proposal is expected to be ready for presentation in the REACH Committee in its next meeting (27-28 April 2022).

Tuula Heikkonen (Finland) informed that in Finland they had broadened and focused 3Rs activities by setting up a 3Rs Centre whose website will be launched in the coming days. Training courses will be organised. Tuula suggested to discuss in future how we could further increase the utility of the PARERE network and the 3Rs Centres (which are sometimes partly overlapping) to fulfil the final call of Directive 2010/63/EU to replace animal tests by alternative methods.

2.2 General update from EURL ECVAM

Raffaella Corvi gave an overview of some selected EURL ECVAM's activities.

The chemicals strategy for sustainability published by the EC on 14 October 2020 is part of the EU's zero pollution ambition and is one of the first deliverable of the European Green Deal. Among its ambitious objectives, one is increasing the amount of information available on potentially hazardous chemicals, while at the same time making the best use of innovative non-animal methods. In this context, EURL ECVAM is involved in the update of the REACH information requirements exploring opportunities to introduce NAMs.

Since the previous PARERE meeting, EURL ECVAM has continued to investigate the field of Organ-on-chip (OOC) and to coordinate the thyroid validation study. In relation to OOC, EURL ECVAM published an analysis on standards for OOC and organised a workshop, which led to the establishment of a CEN CENELEC focus group to advance standardisation of OOC.

Several activities of EURL ECVAM led to the publication of an ESAC peer review Opinion on the scientific validity of the GARDskin and GARDpotency test methods, the adoption by the OECD Guideline No. 497 on defined approaches for skin sensitisation, guidance document No. 331 on the characterisation, validation and reporting of physiologically based kinetic (PBK) models for regulatory purposes.

At the level of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), EURL ECVAM has contributed to the finalisation of the chapters on skin corrosion/irritation and serious eye damage/eye irritation to introduce non-animal testing methods (United Nations, 2021. Globally Harmonized System of classification and labelling of chemicals (GHS) – 9th revised edition). Work is ongoing to revise chapters on skin sensitisation and germ cell mutagenicity.

New reports on advanced non-animal models in biomedical research have been published in neurodegenerative diseases, immune-oncology, immunogenicity testing for advanced therapy medicinal products.

In the field of education and training several resources are now available.

More of EURL ECVAM's work and activities can be found in the 2021 EURL ECVAM Status Report

Discussion with the PARERE members:

Finland asked whether any PARERE consultations were foreseen for 2022. The chair replied that PARERE might be consulted on a few questions which will be presented during the next session on extended REACH information requirements and that it will be discussed during that session.

Additional consultations might be needed during the year if EURL ECVAM receives test presubmissions for which the preliminary regulatory relevance and other aspects need to be discussed with PARERE.

3 Extended REACH information requirements and opportunities to introduce NAMs

Andrew Worth (EURL ECVAM) presented the status of a JRC-led action under the Chemicals Strategy for Sustainability to explore options for extending the information requirements under REACH, including an increased reliance on NAMs. The work is being carried out with a Core Group including representatives from DG ENV, DG GROW and ECHA. The analysis will feed into an impact assessment covering all of the proposed revisions to REACH. He also presented the results of a JRC survey aimed at gaining information on which NAMs, or batteries of NAMs, are already in use within companies.

Andrew also presented an option for a longer-term vision of regulatory information requirements, even though this will not be considered in the impact assessment. The aims of this future option would be to: a) achieve higher safety by having a basic level of knowledge about a higher number of substances while requiring less information for each substance; and b) minimise animal testing with the eventual aim of complete replacement. At the end of the presentation, Andrew described a few questions related to potential future actions and on which EURL ECVAM would be interested to receive the opinion of the PARERE network. The questions raised were the following:

1) In relation to the protection goals of the CSS, are there any gaps or redundancies in the proposed NAM-based Information Requirements?

2) What mechanism could be put into place to ensure the efficient recognition of acceptable NAMs? (e.g., Agency List, Commission List, Test Methods Regulation)

3) In cases where animal testing is conducted as a last resort, what mechanism could be put into place to explore whether the same degree of protection could have been obtained by using NAMs?

4) How can we increase acceptance of NAMs by using standards? What would these standards look like and how would they be adopted?

Discussion with the PARERE members:

Knud Peterson (Denmark) thanked for putting forward these thoughts and highlighted that there were suggestions by some people to get the NAMs put into use through different ways than the OECD Test Guidelines Programme (TGP). To that end, DK was wondering if the acceptance of NAMs through the OECD process was really a serious bottleneck, since DK (as DK's National Coordinator (NC)) was not seeing a large queue of mature NAMs, which would be ready for replacing the animal methods right now. DK felt that at present, and probably in the near future, the OECD TGP was not the bottleneck, but rather the maturity of the science that was holding back the use of NAMs. DK was also wondering what parts of validation people would see as redundant or irrelevant when it comes to accepting NAMs.

Andrew replied that these were very good questions and that he had an open mind on these. It might be a non-problem and issues could be solved through the OECD mechanisms or perhaps we would need to explore a hybrid approach. He mentioned that other PARERE members were involved in the WNT and invited those to express their point of view too. Regarding the OECD TGP, the chair (also EU NC) mentioned that the beauty of the programme was the mutual acceptance of data (MAD), saving a lot of duplicative testing.

Betty Hakkert (The Netherlands) also thanked for the clear and interesting presentation. As the NL's NC and risk assessor at the risk assessment committee at ECHA (RAC), Betty mentioned that she had similar questions as DK. It was not clear for her whether it was just the science that was lagging behind, and information on robustness, reproducibility and transferability of methods was lacking. NL mentioned that it was good to keep in line with OECD and believed that many people working with the OECD had similar views. She then provided the example of the NL's work on kinetic *in vitro* methods where they observed large variability depending on how they conducted the study. This would not mean that these methods should not be used, but rather that there was a need to provide guidance on how to perform them, irrespective if they were used at the level of REACH annexes VIII to X or for waiving. The NL would wish that we would all work together on these important questions. NL also asked how the health benefits are being monitored in the impact assessments. From a human health and environmental point of view, some of the proposed additional endpoints were very important, but it was not clear how their impact on health and costs of testing was assessed. Regarding administrative costs, the NL mentioned that the inclusion of unclear NAMs would lead to many discussions with the registrant and ECHA, and to too high administrative costs. Therefore, it was important to include reproducible (robust) and transferable NAMs.

Andrew thanked for the questions and mentioned that the Core Group had been struggling with the same questions during the internal discussions on REACH information requirements, where e.g. the legal certainty and the tension between legal certainty and the flexibility that you need to introduce NAMs, were highlighted as well. On the other hand, too much flexibility will trigger inefficient discussions, therefore a common understanding of best practices, which would more fall in the rail of guidance documentation rather than legal text, was desirable. Andrew mentioned that the impact assessment (which will be on the whole package of proposals for the REACH revisions) would aim to assess the impacts both qualitatively and where possible quantitatively, but it was acknowledged that the latter is especially challenging.

Regarding the question on robustness and reproducibility of methods raised by the NL and DK, the chair noted that we would all agree that demonstrating the scientific validity of methods would remain an important endeavour and there was no intention to change this. The question was more on how the acceptance process of scientifically valid NAMs could be streamlined. The OECD TGP was also interested to speed up the uptake of emerging technologies into the programme. An OECD workshop on that topic is foreseen in December 2022. The chair noted that there is a need to see how we can deal with a high innovation rate of methods and approaches in the near future from a validation and acceptance procedural point of view.

Martin Paparella (Austria) thanked for the overview and the questions posed and commented that he appreciated the presented proposal of a risk matrix. Regarding the fourth question, Martin concurred that the improvement of the acceptance process of NAMs or the provision of a new framework for their acceptance would be discussed at the OECD WNT workshop in December 2022. Martin noted that an evolution of the concept of the Performance-based TGs would be needed. Future TGs may not need to be so specific to any individual method, but the TG may specify mechanisms that need to be covered, the minimum reproducibility of the method and some reference chemicals in a broader frame. Regarding the gaps and redundancies (question 1), Martin said that we might really need to consider how we will perform risk assessment in future. We cannot replace the animal tests and the specific organ toxicity results that they are providing. The concept of the next generation risk assessment (NGRA) needs to be considered for use in REACH. NGRA does not aim to predict any specific toxicity but rather define a limit value that is likely to be protective. In Martin's understanding, the NGRA builds on and extends the threshold of toxicological concern (TTC[2]) concept, where the assessment is based on no-effect level distributions for different chemical groups, defined by chemical structure (essentially Cramer class). For NGRA, NAMs with a high-throughput performance would be applied and cover a huge mechanistic space and the limit values derived would be less conservative than with the TTC, but sufficiently protective relative to animal testing data. In that sense, Martin was wondering how gaps and redundancies could be defined, as we would not want to replace what we currently have. The risk matrix is a rough approach to regulation. The current approach based on animal tests is quite coarse too, in fact, we do not know the reproducibility of these methods, they are not validated in the same way as the NAMs and we know that there are limitations in their reliability and relevance for the protection of human health. The precision of these set of animal-based TGs is not very high. Regarding the question whether cardiotoxicity would be a relevant additional endpoint in chemical assessment, Martin mentioned that although some evidence had been gathered to demonstrate that it is an issue, the endpoint had not yet been well explored and appreciated. A NAM-based IATA is currently being investigated to potentially address this endpoint.

Denmark commented that often arguments that animal studies in OECD TGs were not validated, had a low precision and were not relevant for human health, were put forward. DK believed that it was used as an argument for being a bit looser on how you validate NAMs. DK thought however that it was a bad argument, as it was not because something was not done properly in the past, that it should not be done properly now. DK repeated that the problem was not a slow OECD process but rather the unavailability of mature NAMs. Regarding the slide on maturity of methods, DK enquired what the time horizon was for NAMs when they were colour-coded in green or red.

Andrew replied that the colour coding was a rough way of comparing the level of maturity of methods for different endpoints. The reason for the questions posed was more process-related, e.g. how do we establish scientific validity and how do we establish acceptability for particular contexts of use. Andrew suggested to tease those two processes apart, now more than we did before. There could be a process in which you quickly demonstrate the scientific validity of NAMs for the critical hazards and it could be discussed what that would look like. However, that might not include extrapolation to existing animal studies or to humans, but would

[2] The Threshold of Toxicological Concern (TTC) is a concept that refers to the establishment of a level of exposure for all chemicals, whether or not there are chemical-specific toxicity data, below which there would be no appreciable risk to human health.

rather focus on mechanistic relevance and reproducibility. This could be the level at which scientific validity is demonstrated, which would be consistent with what is done today. Then would come the question of what combination of scientifically valid NAMs are useful for a particular purpose. It would probably be helpful to decouple the regulatory relevance question from the technical readiness and scientific validity, the idea being to have a large battery of scientifically valid NAMs out of which regulatory solutions (IATA) could be constructed. Then the discussions, wherever they would take place, be it in the Agency, within a cross-agency group, within PARERE etc., would be how do you plug and play, i.e., what combination of scientifically valid NAMs would be fit for a given purpose, and this might be different for different applications (chemical safety assessment, C&L, etc.). At least we would then be shifting the burden of discussion away from the actual scientific validity, which would become a more routine scientific and technical exercise, to the acceptability and utility in different use contexts. Andrew asked whether the business as it was done today would really be fit for purpose or if there were ways to change it to expedite the use of NAMs and ultimately increase the protection levels.

The NL mentioned that it was important to speak the same language. Austria had mentioned that a rough estimate was enough. However if we talk about legal decision-making then we will have a very difficult case in court if we have only rough indications, as this would simply be considered as not enough. Referring to the presentation of the Commission and to the COVID period, the NL mentioned that when there is pressure to get something (such as a vaccine e.g.) developed and approved, there are resources allocated to that task. The lack of prioritisation and allocation of human and financial resources to the development and validation of NAMs might be the issue here. Currently, there is not a whole range of robust NAMs waiting for approval. As risk assessor, Betty sees all kind of *in vitro* methods being used in a wrong way, in particular for investigating modes of action, which demonstrates the importance to have robust data in a regulatory decision-making process. Regarding cardiotoxicity, the NL agreed that it was an important endpoint, which could not adequately be picked up in animal studies and for which no official animal-based TGs exist. This situation would provide a good opportunity to develop and accept NAMs for this endpoint.

The chair concurred that we should all work together to address these questions. She mentioned that although not always considered fully mature, NAMs were already being used by industry. Decoupling scientific validation from regulatory usefulness of the NAMs might expedite the process since, according to the experience made during validation studies, mechanistic relevance and reproducibility of NAMs are usually fine. On the other hand, for regulatory application, additional work and thinking (e.g., how to combine the NAMs in IATA or DAs) is usually required.

Andrew added that Austria picked up on the fact that the generic risk matrix proposed was an approximative way of binning chemicals which was precisely the idea. We should not delude ourselves that we are doing better than that today. Unlike physics, toxicology is a very imprecise science and we have to embrace the uncertainties. We should not be trying to measure potency down to five decimal places, since this is not needed to ensure adequate risk management. Unfortunately, though, the acceptance of promising NAMs is delayed by discussions at OECD about predictivity, reproducibility and requests for testing more chemicals to squeeze in a few more percentage points here and there. What we really want to do is to compare chemicals and just have the standards that give us that means of comparability. We want to discriminate the chemicals of most concern, i.e. the ones that have to be banned, from the ones that have to be restricted and the ones we can live with. Andrew questioned whether it was better to know a huge amount about one chemical or a bit about a thousand of chemicals.

The NL considered that the last point raised was more related to screening issues and less so to final decisions on certain chemicals. Against this, Austria agreed with Andrew and mentioned that he did not like this idea of screening followed up by higher tier animal tests because at the end, it would not increase our capacity to control chemicals. Furthermore, the reliability and relevance of screening methods may need to be quite high to scientifically justify the conduct of follow-up animal testing – with the consequence that the performance of these "screening level NAMs" may easily be already above or in the same range as the performance of the current standard animal tests.

4 Roadmap for respiratory sensitisation testing

The chair welcomed and introduced some risk assessors and experts in the field of respiratory sensitisation who had been nominated by PARERE members and who joined this specific session of the PARERE meeting. Nominations had been received by the Netherlands, Italy, Belgium, EFSA and ECHA. Ireland had mentioned that the PARERE representative was the risk assessor.

Laura Gribaldo (EURL ECVAM) introduced the session by reminding that the first discussion with PARERE on respiratory sensitisation had taken place at the November 2020 PARERE meeting, when discussions on the need to elaborate a validation framework for respiratory sensitisation *in vitro* testing started. At that time, EURL ECVAM had received a test presubmission on the ALIsens test method, an *in vitro* method based on air-liquid interface predicting respiratory sensitisation of inhalable substances. The model was promising and EURL ECVAM consulted PARERE on a series of questions.

The replies from PARERE had been at that time that scientifically valid methods to detect respiratory sensitisers were clearly needed in different regulatory domains (e.g. chemicals, plant protection products, consumer products). Scientifically valid methods that could with high sensitivity and specificity predict respiratory sensitisation would be of great benefit in areas like occupational workers' safety, pesticide exposure in the agriculture, but also for risk assessment of cosmetic products. The submitted test method ALIsens was interesting and might be of potential use in a regulatory context, also for defining classification criteria under GHS/CLP. The method at the time of the presubmission would however need to be further optimised by testing a higher number of both respiratory and skin sensitisers. Additional chemicals to the limited set of chemicals already tested should also be used to evaluate whether the method could distinguish between irritants and sensitisers. Inter-laboratory variability was also crucial to detect the scientific reliability of the test method. Finally, regarding respiratory sensitisation, the scientific evaluations in support of EU OSH occupational exposure limits (OELs) might identify the need for a 'respiratory sensitisation' notation.

The EURL ECVAM assessment report on the ALIsens test presubmission had been prepared in the light of this outcome and sent to the test submitter. The submitter is currently finalising the new testing and EURL ECVAM hopes to receive soon a full submission including the additional data requested.

Laura mentioned that during this PARERE meeting, the intention was not to discuss the ALIsens test method in particular, but rather to discuss more generally the area of respiratory sensitisation testing for REACH. Currently under REACH, there are no requirements for testing chemicals for respiratory sensitisation. However, proposals had been made to include this endpoint in the REACH standard information requirements. Nevertheless, respiratory sensitisers are considered chemicals that pose an equivalent level of concern as substances of very high concern (SVHC). The issue thus exists.

Laura noted that a set of additional questions had been sent to PARERE in view to prepare for this session and facilitate the discussions at the meeting. In the next presentation, Silvia Casati would provide a summary of the replies from PARERE on these questions. The session was meant to discuss the specific needs in this area, in particular in view of PARERE's experience as regulators and risk assessors.

Silvia Casati (EURL ECVAM) provided an overview of the feedback received from PARERE members (Italy, Sweden, Austria, Belgium, ECHA, EFSA, Slovakia, Germany, the Netherlands, Ireland) on the following three questions:

- Which methodologies/information are currently being considered for assessing respiratory sensitisers?
- Besides hazard identification, what are the features/parameters that future NAMs should be able to provide to regulate these chemicals and ensure human health protection?
- Do you think it will be important for future NAMs to be able to discriminate between respiratory sensitisers and respiratory asthmagens?

In relation to the first question, there was consensus on the use of human data as the primary evidence for assessing respiratory sensitisers. Animal and other types of data (e.g. *in vitro*, *in chemico*, *in silico*) are generally used as supporting evidence within a WoE approach. Read-across and grouping is also applied in specific cases.

In response to question two, PARERE indicated that it would be of value if future methods could be applied to both low molecular weight chemicals and proteins. In addition to hazard identification, information on the relative potency that allows to establish a point of departure for derivation of a derived minimal effect

(DMEL[3]) and also potency sub-categorization for classification (GHS/CLP) was deemed necessary. It was also suggested that future methods should help elucidating the mechanisms of action.

The majority of responses to question three indicated that there is value in future NAMs to be able to increase the knowledge on the modes and mechanisms of action, including discrimination between respiratory sensitisers and respiratory asthmagens for prioritizing the regulatory action on those with higher concern and for other purposes e.g. as a basis for grouping and read-across. It was also noted that since the number of respiratory asthmagens is expected to be vast, it would be unreasonable to list them as SVHC. Others felt the distinction should not be an absolute demand for NAMs, since it is not made by the regulation, it may be more important to know at which concentration the mechanisms are triggered.

Silvia highlighted a few additional considerations to keep in mind during the discussions:

The chemicals that can induce respiratory sensitisation are known (i.e., diisocyanates, anhydrides, metals, amines), why is there still so much concern for this endpoint?

Is the incidence and prevalence of asthma known for both respiratory sensitisers and respiratory asthmagens?

Which priority steps should be undertaken to devise a roadmap for progressing the field of NAMs for respiratory sensitisation?

In case a database of *in vivo* (human/animal) data is considered important to support the development of NAMs, would PARERE have access to such data?

Emma Di Consiglio (Italy) presented an overview of the state-of-the-art in the assessment of respiratory sensitisation in the regulatory settings, based on an expert consultation providing a focus for establishing specific information requirements. There are no validated and regulatory accepted models to test for respiratory sensitisation, especially for low molecular weight chemicals. A lack of reference chemicals, together with uncertainty regarding relevant immunological mechanisms, has hampered method development. Currently, within CLP and REACH, chemicals are classified almost exclusively based on observations in workers. The example of protein based allergens (e.g. Amylase, α- plus other enzymes), according to a CoRAP adopted opinion (Community Rolling Action Plan, 2016) was described. Safe exposure levels for workers and consumers were proposed, based on long-term experience and available data. Since the identification of a threshold for such effect is still challenging, within REACH for a quantitative risk characterisation, the derivation of a derived minimum effect level (DMEL) is recommended. According to the Plant Protection Product legislation, if an active substance is a skin sensitiser, it can potentially induce a hyper-sensitivity reaction, and therefore respiratory sensitisation effects (Chapter 5/7, Mammalian toxicology dossier). The example of a microorganism-based insecticide, i.e. Beauveria bassiana strain (EFSA Conclusions, 2020) provided an overview on the assessment of potential sensitiser pesticides and the resulting warning phrase ("Micro-organisms may have the potential to provoke sensitising reactions") was proposed. For biocide authorization purposes, respiratory sensitisation is requested as additional data, considering available existing information (i.e. non-human data: physico-chemical properties, grouping, (Q)SARs, *in vitro*; human and animal data) or immunotoxicity assessment outcome. Supporting information on sensitisation/allergenicity/hypersensitivity in workers and other populations exposed can also be used. The Scientific Committee on Consumer Safety defined a tiered approach for the inhalable cosmetics safety assessment, including the respiratory tract sensitisation assessment. Likelihood of significant inhalation exposure and hazard assessment for systemic/local toxicity should be considered, as well as data from skin sensitisation tests for prioritization. However, some criticisms have been faced, due to the uncertainty in the respiratory irritation/sensitisation evaluation with the available toxicity studies, the lack of non-animal test methods for inhalation toxicity, the applicability of skin sensitisation data (despite similarities, the two mechanisms seem to differ in some crucial points). For pharmaceuticals, the first assumption is that respiratory sensitisation is due to direct contact between chemicals and the respiratory tract (e.g., by inhalation of volatile compounds). No indirect mechanism (e.g., oral ingestion, parenteral administration) is considered relevant. EMA Guidelines state "Local tolerance should be evaluated for those sites that come into immediate contact with the medicinal product as a result of the method of administration." Although the inhalation route is not mentioned, the general principles are applied. In the Medical Device area, in some cases, when there are regulatory needs, the respiratory sensitisation potential of an ingredient is assessed by using *in vivo* testing, in some cases the sponsors shift, and provide skin sensitisation data instead.

[3] The derived minimal effect level (DMEL) is defined as a level of exposure below which the risk levels become tolerable.

Some critical issues remain open, including potential differences between small molecules (chemicals) and macromolecules and between skin and respiratory sensitisation mechanisms; the likelihood of a threshold in induction versus elicitation phases; the lack of predictive toxicological assays and their validation.

Discussion with the PARERE members:

Laura Rossi (ECHA) mentioned that ECHA would be willing to collect a list of all the known respiratory sensitisers as it would be easy to get access to ECHA's dissemination site. There would also be data on a substance in the RAC report, if the substance has a harmonised classification.

Philippe Castelain (Belgium) raised a question on substances being used in plant protection products, such as serine proteases. A threshold limit value of 60 ng/m3 had been established for that class of proteases. However, in literature there are controversies about the robustness for the establishment of this reference concentration for this class of enzymes, as sensitisation already occurred at much lower doses, such as e.g. 0.9 ng/m3, for some other enzymes of this class. There is agreement that this type of substances are respiratory sensitisers and their mode of action is known. The problem is to quantify their respiratory sensitisation potential. Philippe enquired if it would be possible to assess potency in *in vitro* test systems and extrapolate to the *in vivo* situation for establishing an appropriate threshold limit. Without being able to define such a threshold dose, substances could not be approved and put on the market.

Italy added that it was important to also distinguish between the threshold dose for induction and that of elicitation. The elicitation could occur at lower doses than those required for induction. Some exposure levels would thus not always protect already sensitised individuals.

Silvia thanked for raising this specific case, and explained that EURL ECVAM would like to understand from PARERE if they are confronted with other chemicals which are respiratory sensitisers than those that have already been identified.

Belgium replied that in their portfolio of plant protection products, it is evident that at the moment, in the absence of any robust data, there are no new substances that have been approved on the European market, as the substances of low molecular weight are extremely reactive substances, which can cause also other effects such as genotoxicity. Methodologies being able to assess the respiratory sensitisation potential of new types of substances, such as e.g., enzymes and microorganisms, are thus urgently needed.

Vera Rogiers (SCCS) suggested to include the area of perfumes under cosmetics because these are inhalable substances which can cause allergies. A quantitative risk assessment framework (QRA) has been developed under the EU-funded project IDEA. Vera provided some contacts of people who are involved in the IDEA project. Vera also suggested to involve some experts in exposure modelling from the SCCS.

Besides the area of plant protection products, cosmetics and perfumes for which respiratory sensitisation was clearly an issue, Laura enquired about a potential concern for respiratory sensitisation in the chemicals arena.

ECHA explained that since there were no available tests for that endpoint, ECHA would usually receive human data when the concern for an existing substance was already present and in that case it was already too late from a personal protective point of view. ECHA is currently trying to group substances that are similar in nature to see whether they have similar hazards. This might result in new classifications. ECHA hopes to obtain information also on new chemicals if there is a link with older existing substances for which the hazard already exists.

Regarding the question on incidence and prevalence of asthma due to respiratory sensitisers or respiratory asthmagens, Laura Gribaldo enquired if PARERE had an idea on the number of cases of asthma that are due to respiratory sensitisers or due to respiratory asthmagens. She asked whether evidence or data existed, that could discriminate between these two cases to understand the degree of seriousness in terms of the dimension of the risk.

Austria mentioned that in case this is not known, it would be worthwhile to investigate it by a systemic literature review. For the occupational safety levels, it is often the sensory irritation threshold that is driving the occupational limit value derivation. Sensory irritation is a nervous system reaction with the biological function to alert the organism on very low levels of concentration of chemicals that could become dangerous in terms of real local effects at a slightly higher concentration. Sensory irritation is a different mode of action that cannot be readily caught in these types of assays, but could eventually be extrapolated from such assays using assessment factors. It would be helpful to develop an AOP network to describe/understand the MoA and investigate the common key events between respiratory sensitisers and respiratory asthmagens.

ECHA mentioned that they had no list indicating the number of respiratory asthmagens or sensitisers, probably mainly due to the fact that CLP does not make a distinction between them. For substances that are clear respiratory sensitisers and for which human data were available, ECHA noticed that it was not always possible to measure the IgE levels. Without having methods which can reliably assess these levels, it is extremely difficult to say whether there is an immunological or non-immunological reaction, even in the presence of human data.

Silvia mentioned that in case no tools are available that could distinguish between those chemicals inducing hypersensitivity through an immunological mechanism and those that are irritant, it would be interesting to know how many irritant chemicals would be classified as SVHC. This information would also be useful in the framework of the impact assessment on REACH updates.

Emanuela Corsini (Italy) reminded about the ECVAM workshop of 2007 (ECVAM Workshop 60), where numbers in terms of chemicals, effect and prevalence in occupational settings had been provided. The prevalence of work-related asthma derived from cross-sectional studies of working populations exposed to chemicals and ranged from four to 54%. However, in most cases, the estimates were below 10% (median 8.5%). Considering only the low molecular weight chemicals, the list of recognised respiratory sensitisers is quite limited compared to that of the skin sensitisers. In the report, the combination of the immunological and non-immunological respiratory reactions accounted in total for 350 chemicals, which is far below the number of chemicals causing skin sensitisation. Nevertheless, chemicals inducing respiratory sensitisation are high risk chemicals inducing an IgE-mediated event eventually leading to an anaphylactic choc. Emanuela agreed that it was important to have methods that were able to discriminate between the two. The ALIsens method is very complex with regard to the type of cells included. In fact, there are not only the surrogates of alveolar macrophages but also the surrogate of mast cells, which could bind and degranulate in an IgE independent manner and trigger an asthmatic reaction on top of the chemicals that could go directly to the alveoli. It would be interesting to see how the ALIsens method will perform for irritants that could lead to an asthmagenic non-immunological mediated event compared to chemicals that classify as immunological-mediated respiratory allergens.

The chair mentioned that the test submitter of the ALIsens test method communicated that they are currently testing additional 25 to 30 chemicals representing respiratory sensitisers and irritants, innocent chemicals, skin sensitisers and a few proteins and would re-submit to EURL ECVAM around May/June.

Andrew mentioned that it was fascinating to listen to this discussion and a bit worrying because the Commission is effectively committed to include respiratory sensitisation as an information requirement at the low tonnage end. However, it seemed that the biology was not fully understood yet and that the technology to assess this endpoint at this stage was missing. Andrew wondered if, in the case that we could not introduce methodologies today to detect respiratory sensitisers, respiratory irritation could be a stepping stone as part of this roadmap. Prevalence is important too considering public health impacts, benefits and costs. If the prevalence of respiratory sensitisers is very low, maybe it is not worth the costs at the low tonnage end of REACH annexes. It would be good to get a handle of what these figures really are. Andrew also asked if a respiratory sensitiser was a kind of subset of respiratory irritants or if they were partially overlapping. If they were a subset, this would give us an idea of how to build a testing strategy and maybe we would start first by identifying respiratory irritants and they might not be considered as SVHC, but substances of concern. Then at some point in the future, we would be able to narrow down those respiratory irritants to identify which ones of those were respiratory sensitisers and would become SVHC.

Emanuela replied that there was a possibility for a strong sensitiser to be also an irritant but then they do not trigger the same activation in dendritic cells. The idea to partially explain the strong versus weak sensitiser is the irritant potential that the strong sensitisers could have, but not the weak sensitisers.

Helena Kandarova (Slovakia) mentioned that the irritants could be relatively easily identified with the 3D lung models because, if the dosimetry and the exposure were done correctly, respiratory irritation was a matter of cytotoxicity most of the time. For respiratory sensitisation, the mechanism was different and more complex, as it is the case also for the skin. Strong sensitisers will also be irritants but many sensitisers will not be acutely irritating the lungs. An additional complexity for the *in vitro* assays for the lung is the need for a correct dosimetry and exposure, which are important aspects that need to be considered during the development of *in vitro* assays. It will not be possible to apply certain materials in the same way as for other assays such as pipetting the material on the surface of the cells, since this would not be a representative exposure in the lung and would lead to too many false positives. Advanced systems such as e.g. VitroCell mimicking inhalation would be preferred. The whole set up would then become part of the validation study.

Emanuela explained that another issue common to many respiratory allergens was their instability in water. Positive responses could be obtained in the same assays as those used for skin sensitisation in dendritic cells, as respiratory sensitisers upregulate the same molecules. However, a higher concentration of the sensitiser would be required due to the instability, which means that a real estimation cannot be made when extrapolating to the *in vivo* exposure. It would depend if a yes/no answer was desired or hazard characterisation. In the latter case, there is a need to consider the technical limitations of some of the current assays.

Rob Vandebriel (NL) mentioned that both skin and respiratory sensitisers are positive in the LLNA, but when measuring cytokine expression, respiratory sensitisers induce IL-4, while skin sensitisers do not. You could say that respiratory sensitisers are a subset of skin sensitisers in a way. Regarding the question on stability in water, isocyanates are prone to hydrolysis and in the case of an air exposure, the moisture in the air needs to be controlled. Too much moisture induces hydrolysis and less reactivity of isocyanates.

Regarding the distinction between respiratory sensitisers, respiratory irritants and those that are strong sensitisers and may also have some irritation potential, Silvia enquired whether it was possible to roughly quantify them in terms of percentage.

Emanuela replied that there would be a need to make a literature search to obtain such numbers. Referring to the ECVAM workshop report 60, most of the chemicals are respiratory irritants. It has been shown that the elicitation phase depends on the exposure and dose during the induction phase. The problem is that the induction phase is clinically asymptomatic so that the dose that is sensitising a person is not known. In allergic individuals, the threshold at which a person does not develop a clinically relevant allergic reaction is only investigated for limited agents such as proteins and food allergens.

Helena mentioned that the irritation of a lung may not always be reversible because the structure of the lung is different from that of skin where irritation is reversible. Strong irritation of the lung can provoke scarring of the tissue.

Silvia enquired whether respiratory irritation would cause effects in the upper airways. Helena replied that it would, but that the effects would later on also affect the alveoli. Clinicians would need to be consulted on the clinical effects.

Silvia mentioned that we do not exactly know how the respiratory sensitisers behave in the *in vitro* methods because there had not been a consistent testing with all the available methods, but we would know that all respiratory sensitisers turn out to be positive in the LLNA. If we have a positive result in a skin sensitisation assay, we do not know if it is a skin or a respiratory sensitiser. However, if we add a respiratory irritation test, we could start excluding things, which could be a strategy to start from. Silvia enquired if this would be appropriate.

Vera Rogiers replied that it would depend if the method was good enough for making such distinctions. Vera wondered about the size of the problem in the absence of robust data and methodologies. She suggested to enquire this first before adding respiratory sensitisation as additional endpoint in the REACH standard information requirements. She added that other factors might play a role such as metabolisation and that there was a difference between acute and chronic exposure. In the case of a chronic irritation, the tissue becomes more susceptible to other compounds that could induce respiratory sensitisation. There could be a combined problem of respiratory sensitisation and irritation at the same time.

Laura mentioned that the problem of inhalation toxicity exists and had been discussed for many years and that there was a need to take action. However, before investing human and financial resources, the different steps within such a roadmap would need to be discussed in order to be efficient. In the area of occupational health, a lot of data is available. The issue would be to link the epidemiological data on chemical hazard identification and assessment with the data collected in other areas of legislation.

The first step in such a roadmap could e.g. be the collection of all relevant data and the establishment of a database with human data. One could also start with the selection of relevant chemicals, which were classified for that endpoint. Then, in a second step only, a discussion on the methods and models could take place.

Rob explained that 25 years ago when they started to perform the LLNA to detect both skin sensitisers and respiratory sensitisers, for skin sensitisation, the LLNA was put forward and included in an OECD TG. For respiratory sensitisation on the other hand, the proposal of several research groups to combine the test with measurement of IL-4, was never pursued and taken up, most probably because there was no regulatory

information requirement. Rob was thus of the opinion that in the absence of a regulatory information requirement, the interest to further develop and adopt a method in an OECD TG would be lower.

Vera R. mentioned that at the SCCS they received more and more safety dossiers related to inhalation toxicity of aerosols, sprays and nanoparticles, which were very complex and that there was a need to consult with industry manufacturing and assessing these types of products.

Laura enquired whether the PARERE members could contribute or facilitate the access to data on respiratory sensitisation in case EURL ECVAM would pursue the idea of data collection.

Vera replied that she could put EURL ECVAM in contact with the relevant people that she knows. EURL ECVAM could then get access to data under a confidentiality agreement as most of the data in the cosmetics industry were confidential.

Helena asked if EURL ECVAM was interested to get access to the epidemiological data from the facilities where people manufacture e.g. nanoparticles of titanium. Large biomonitoring studies were performed in these facilities.

Silvia thanked and replied that ECVAM would first need to identify the type of data that are needed and then reach out in a strategic way with specific requests to the relevant people.

In relation to data collection and input from industry, the chair mentioned that the EURL ECVAM stakeholder forum (ESTAF), including various industrial associations, could also be consulted.

Austria noted that it would very much support the shift from chemical pesticides to biopesticides such as microorganisms, and that such a shift could eventually reduce the difficulties of toxicological assessments, since for such types of pesticides you would not expect carcinogenicity or developmental neurotoxicity. Respiratory sensitisation might become the main issue where we would need data and tools to assess potency.

Silvia thanked for the very useful input received by PARERE which will serve as a good starting point to better understand the issues in the area and start to define a roadmap.

The chair thanked all the participants and wrapped up the meeting before closing.

5 Actions

- PARERE members will be consulted by written procedure on the questions related to the extended REACH information requirements.
- PARERE members will be informed about EURL ECVAM's contribution to any follow-up activities in the field of respiratory sensitisation.

Annex 1 – Participants

Austria

Belgium

Czech Republic

Denmark

Estonia

Finland

Germany

Hungary

Italy

Latvia

Luxembourg

Norway

Poland

Slovak Republic

Spain

Sweden

The Netherlands

European Commission:

DG ENV

DG GROW

DG RTD

DG EMPL

DG SANTE

JRC

EU Agencies:

ECHA

EFSA

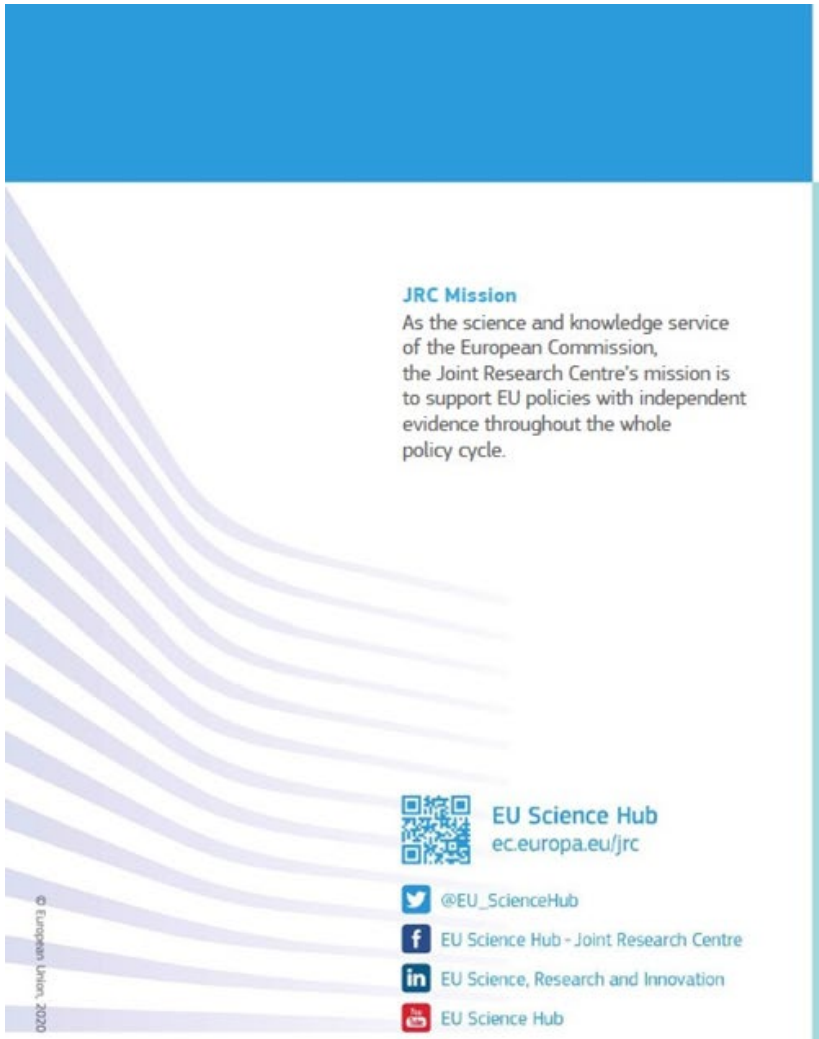
REA

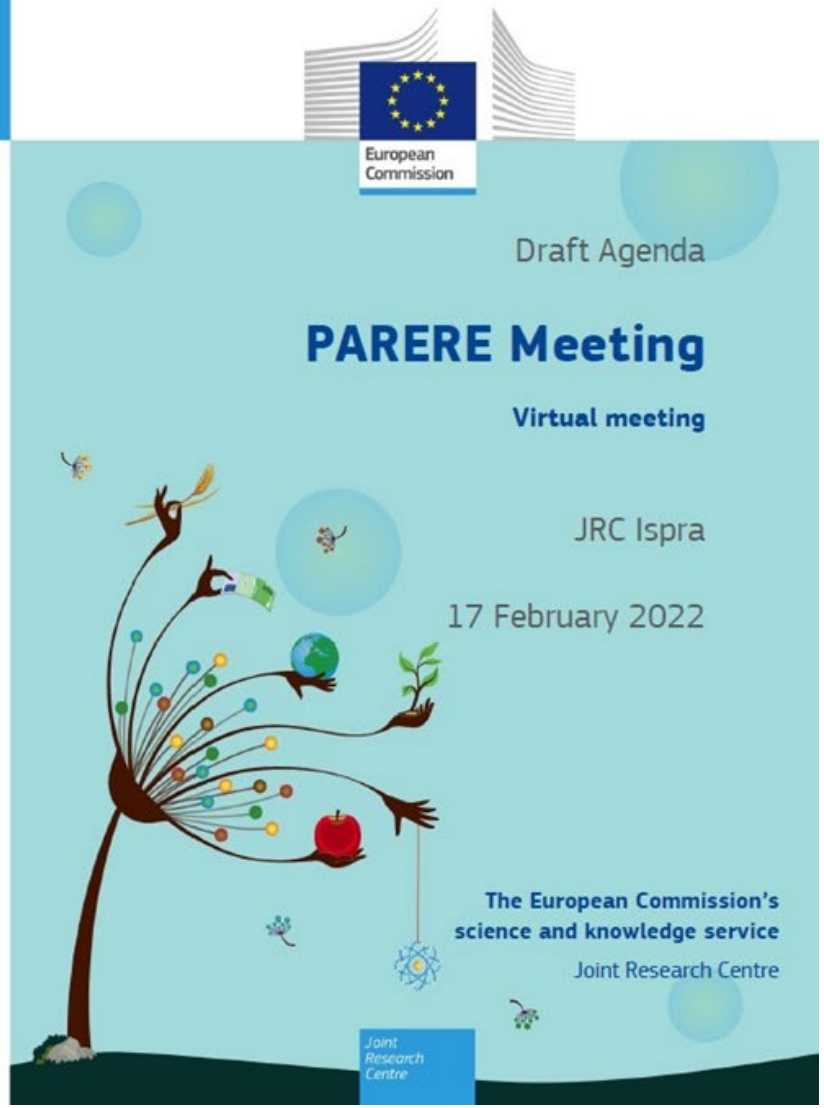
Scientific Committees:

SCHEER

SCCS

Annex 2 – Agenda





PARERE meeting
JRC Ispra, 17 February 2022

10:00-10:05	**Welcome** (*Chair: Valérie Zuang*)
10:05-10:15	**Adoption of the agenda**
10:15-11:15	**Round-table of brief updates from PARERE members** (on a voluntary basis)
11: 15-11:30	**General update from EURL ECVAM** (Maurice Whelan)
11:30-11:45	Discussion
11:45-12:00	*Break*
12:00-13:00	**Extended REACH information requirements and opportunities to introduce NAMs** (Andrew Worth) The aim is to discuss more efficient ways to introduce NAMs into chemical hazard and risk assessment practices under REACH.
13:00-14:00	*Lunch Break*
14:00-16:50	**Roadmap for respiratory sensitisation testing** (Laura Gribaldo, Silvia Casati) Presentations will include an introduction, summary of the feedback received from the PARERE consultation, and an example of a particular approach used for chemical risk assessment of respiratory sensitisers. The aim is to have an exploratory discussion on the short-, mid and long-term needs for tackling the field of respiratory sensitisation and the use of NAMs to satisfy information requirements.
16:50-17:00	**AoB and closure of meeting**

Annex 3 – List of references

This annex has been added for information purposes only. It includes references to documents which were mentioned during the PARERE meeting.

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Kimber I, Agius R, Basketter DA, et al. Chemical Respiratory Allergy: Opportunities for Hazard Identification and Characterisation: The Report and Recommendations of ECVAM Workshop 60. *Alternatives to Laboratory Animals*. 2007;35(2):243-265. doi: 10.1177/026119290703500021

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Regulatory Toxicology and Pharmacology, Volume 71, Issue 1, 2015, Pages 52-62, ISSN 0273-2300. https://doi.org/10.1016/j.yrtph.2014.11.005.

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