EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Health, Consumers and Reference Materials
Chemical Safety and Alternative Methods Unit
EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

Summary Record

PARERE Meeting 21st October 2019, Ispra, Italy

The meeting of PARERE was held on 21st October 2019 (the agenda is included in Annex I).

Welcome and Updates

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

Round-table on activities within the PARERE network

Germany (BfR) informed about a new registry for animal experiments launched by the German Centre for the Protection of Laboratory Animals (Bf3R). The Animal Study Registry is an online registry for scientific studies involving animals conducted around the world. The registry has been launched as a reaction to the reproducibility crisis and provides scientists with a platform to register a study plan prior to the start of experiments in order to prevent selective reporting. This allows reviewers or other scientists to compare the initially registered contents with the final publication. Thereby, Animal Study Registry encourages transparency, reproducibility, and animal welfare.

Slovakia (Slovak Academy of Sciences) mentioned that the newly formed Platform of 3Rs is currently recruiting experts and organising its activities. The platform is operating with the support of the Slovak Society of Toxicology (SETOX). The platform includes experts in 3Rs and relevant Ministries. The PARERE contact person acts as chair.

Spain informed about the activities of REMA, the Spanish network for the development of non-animal alternative methods, that falls under the Ministry of Agriculture. REMA co-organised with the Ministry of Health a meeting on the activity of the European Committees related to Chemical Risks (http://www.remanet.net/noticias/archivos/PROGRAMA.pdf). The event was organised to disseminate the activities of the different European Committees that carry out tasks related to the evaluation of chemical risks, the protection of laboratory animals and the promotion of alternatives. Around 150 participants were involved, including researchers, representatives from the Spanish

chemical industry, Institutions and NGOs, members of the Board of REMA and the Spanish representatives of the Biocidal Products Committee, the Member State Committee, the Committee for Risk Assessment, the EFSA Scientific Committee/Panels and EURL ECVAM.

The SCCS pointed out that the 10th revision of the SCCS Notes of Guidance was published. It includes novel approaches for the testing of cosmetics ingredients, as well as weight of evidence approaches for genotoxicity by allowing the use of new test methods which have not yet been formally validated (e.g. Toxtracker, gH2A). In reference to the EU forum on EDs organised by EC on 8th November 2019, in the SCCS view, ED identification will be a challenge with *in vitro* methods only, in particular for preservatives. No single new preservative has been put on the market in recent years.

The Danish Environmental Protection Agency deals with all the aspects of health and environmental issues. With a strong tradition in the implementation of non-animal methods, the Danish EPA has recently been involved in activities related to allergens, which have become a major health concern. Costs for therapies are second only to carcinogens. They are also involved in the development of QSARs for skin sensitisation. Denmark has a 3Rs Centre. DK EPA is currently supervising the development of 3D models for skin sensitisation carried out at the Danish Technology University.

Belgium (Sciensano) informed that during the year, there had been face-to-face meetings of the Belgian PARERE network and discussions on regulatory aspects. An important project of Sciensano in partnership with the VUB, is an online tool to collect New Approach Methodologies (NAMs) in one central database (called RE-Place). The RE-Place project aims to collect the knowledge on alternative methods available in the Flemish and Brussels regions and centralise this information into a database to make it more accessible to the public. By mapping and centralising the existing expertise, the project will enhance the application of existing methods and stimulate the development of new alternative methods. The RE-Place database can also evolve into a broader platform where researchers can connect with peers and potential partners in order to initiate new collaborations. Eighty methods were collected so far (www.RE-Place.be). Sciensano also collaborates closely with the Innovation Centre-3Rs (IC-3Rs) at the Vrije Universiteit Brussel (VUB), which is involved in education and training in the field of 3Rs. In November 2019, the IC-3Rs organised together with BELTOX and the International Society for *In Vitro* Methods (INVITROM) a joint Symposium on alternative methods followed by a joint Workshop on how to make an animal-free research project.

SCHEER is involved in drafting Opinions on different health and environmental issues. During the formulation of their opinions they often use toxicological data/assessments. Of interest to the PARERE Network could be the recent update (2017) of the 2009 opinion on the *"Need for non-human primates in biomedical research, production and testing of products and devices"*. This Opinion responds to six main issues in the mandate and highlights the many scientific approaches that could significantly contribute to the replacement, reduction and refinement (3Rs) of Non-Human Primates (NHP) studies and tests.

The Netherlands (RIVM) highlighted that the Dutch Ministry of Agriculture will stop animal testing by 2025, which offers challenges and opportunities. NL is involved in several OECD projects and the regulators are closely working with method developers. NL is also involved in the implementation of 3Rs methods in the Globally Harmonised System on Classification and Labelling of Chemicals (GHS).

Latvia represented by the Institute of Food Safety, Animal Health and Environment (BIOR) informed that a new Platform for the National Committee and PARERE Network was created. This platform includes 3Rs experts and National Committee members. New Guidelines for the judicious and humane use of experimental animals and the designing of the experiments have been developed. Furthermore training on experiment designing and selection of appropriate statistical methods was organised with experts from Porto University.

The activities of Estonia (Ministry of Rural Affairs) focus on dissemination and promotion of the 3Rs. The establishment of the PARERE network at national level is still work in progress.

In Finland (Tampere University, FICAM), the newly founded 3Rs Consortium is coordinated by FICAM, the Finnish Centre for Alternative Methods. FICAM works on the development of new methods, promotion of good practices and regulatory uptake. The interest of FICAM lies primarily in alternative methods for biomedical research purposes, in the design of disease models (e.g. angiogenesis), and in education and training courses. They now also finally received funds for validation with a budget of 300,000 euro per year for four years. FICAM will present their activities at a one-day conference on research innovation and 3Rs, organised by Eurogroup for Animals at the European Parliament.

Sweden (Swedish Centre for Animal Welfare) mentioned that the Swedish Chemical Agency has been very active in the area of the 3Rs whereas other regulatory authorities have been more difficult to engage. Other sectors however have to report now what they did in the context of the 3Rs (e.g. the Swedish Food authority is working in this area but it is not well known). The Swedish Research Council had allocated 1.5 million euro per year to the 3Rs and they will now evaluate how the money was spent and what the outcome was. The Research Institute of Sweden RISE is a known established EU-NETVAL laboratory.

France (INERIS) is involved in several activities and projects on human health effects at OECD level. France mentioned that the French platform FRANCOPA, dedicated to the development, validation and dissemination of alternative methods to animal testing was auditioned by the Parliament and National Assembly, with the outcome of two reports, one on animal experimentation and the second on substance evaluation. INERIS is also involved in the APCRA (Accelerating the Pace of Chemical Risk Assessment) initiative, running a case study on endocrine disruptors.

Ireland has nominated a new member, Dr Alan Breen from the Department of Agriculture, Food and the Marine of the Irish government, who is replacing Professor Alan Baird. The Department of Agriculture, Food and the Marine is working towards a policy that does no longer accept *in vivo* studies for biocides authorisation.

Luxembourg (LIST) informed about the first 3Rs symposium that will be organised in Luxembourg in November 2019 with the involvement of the Ministries of Health, Economy and Research and Universities. LIST is supporting the development of disease models and one alternative method on respiratory sensitisation has recently been submitted to ECVAM for evaluation.

UK (NC3Rs) is involved in the application of NAMs. They are supporting the advancement of AOP development. More than 30 projects have been funded with a budget of 7 million euro over the recent years. More recently, they have been involved in the assessment of nanomaterials and of endocrine disruptors in ecospecies (planned).

Czech Republic (National Institute of Public Health) mentioned that the 3Rs centre has been established recently and that the cooperation with the government was good. Dissemination of the 3Rs goes through the webpage of the ministry. They are working with FELASA, the Federation of European Laboratory Animal Science Associations and cooperate with Norecopa. They published the PREPARE guidelines on how to plan, design and conduct animal experiments of Norecopa.

Italy (Istituto Superiore di Sanità) collaborates with the National Committee established in the framework of Directive 2010/63/EU. It is involved in different activities acting to share available databases, collecting alternative methods and in education activities in secondary schools. It also contributed to the organisation of the workshop “The 3Rs principle for a common vision”, on the implementation of 3Rs, together with the Italian 3R Centre and the National Reference laboratory

ISZLER. At this event, it presented the PARERE activities over the last nine years. It participated in the ECOPA Symposium with the Italian Platform on alternative methods (IPAM) and also participated in discussions related to 3Rs aspects in the EU Regulation on Medical Devices.

Austria (Medical University of Innsbruck) stated that there were no new developments regarding the 3Rs and the PARERE network in Austria compared to last year.

EC Directorate-General Environment informed that they were working on the Commission report on the implementation of Directive 2010/63/EU by the Member States and on the Statistical report on the use of animals for the scientific purposes in the EU. The new reports will also include aspects of animal use, which have not previously been available, for example, on the genetic status of animals and the actual severity experienced by the animals during their use in procedures. Both reports will be published soon.

The Directive requires that non-technical summaries of authorised projects are published to inform the public on live animal use. From 2021 onwards, the publication of non-technical project summaries will be required through a central EU database and within six months of the authorisation of the project in line with the amendments made to Directive 2010/63/EU by Regulation (EU) 2019/1010.

EC Directorate-General GROW pointed out that the European Partnership on Alternatives to Animal Approaches (EPAA) is currently funding ten projects related to the 3Rs. A 3Rs Refinement Prize will be awarded at the annual EPAA Conference that will take place in November 2019.

EC Directorate-General RTD mentioned that in H2020, which is the current 7-year (2014-2020) Framework Programme for research, there are around 70 projects in the area of Three Rs with a total funding of more than 200 M€ from the Commission. Within the new EU research framework programme Horizon Europe that succeeds Horizon 2020, research and innovation missions are incorporated. These missions are meant to increase the effectiveness of funding by pursuing clearly defined targets. One mission will be on cancer.

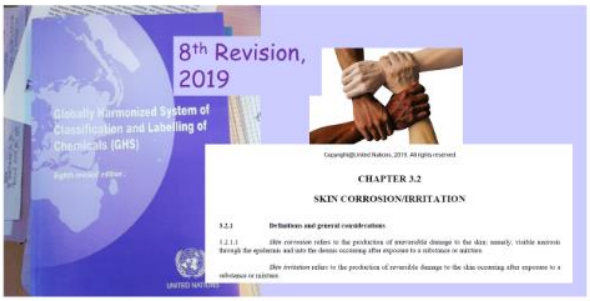
Updates on the AOP framework

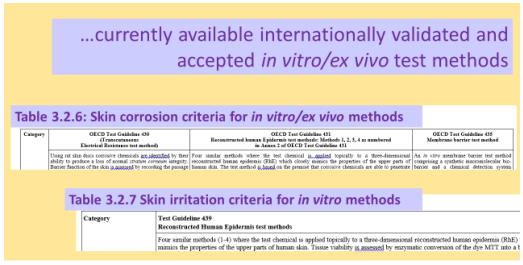
Clemens Wittwehr presented an ongoing study analysing how the AOP Framework can improve regulatory decisions. Although the AOP Knowledge Base now features more than 200 AOPs (30 of which are at an endorsed or pre-endorsed stage), real life translation of the AOP knowledge into tangible regulatory measures is lagging behind expectations. Preliminary results of the study show that regulators seem less impressed by the scientific findings accumulated in an AOP, but rather by its translation into novel testing strategies, e.g. in IATAs or Defined Approaches. Increased focus on AOP-based IATAs and DAs can therefore boost the AOP framework's impact on regulatory decisions. Early findings also indicate that the dichotomy between the "in vivo" and the "in vitro" communities, and the resulting mistrust and lack of scientific agreement between these two groups, can be overcome by using the AOP framework as the driver for integrating the best aspects from both communities. The final report from the study will become available in the first half of 2020 and will be published to a wider audience.

Non-animal approaches under the EU CLP Regulation[1] and the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)[2]

Elisabet Berggren provided a short overview of the activities of the Non-animal working group established at the UN GHS in 2017, co-chaired by the NL and UK. The task of the working group is to revisit the current GHS text and update it with non-animal approaches. The working group is open-ended, and is composed of about 50 members representing governments, European Union, OECD, UN specialised agencies, UN related organisations and NGOs.

During the first biennium of activity, 2017-2018, the working group discussed Chapter 3.2 on skin corrosion and skin irritation. The drafting was coordinated on the initiative of the NL. This activity was successfully concluded when the UN subcommittee agreed with consensus to include the revised text in the 8th Revision of the GHS (2019).



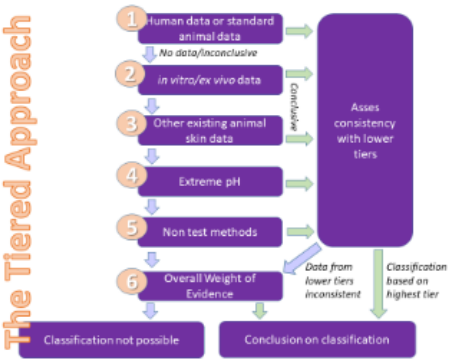


In the guidance part of Chapter 3.2, a table of current accepted OECD test guidelines for skin corrossion and skin irritation applying *in vitro/in vivo* methods are included. In addition, a large part of the text of the chapter was revised to include both *in vitro/ex vivo* and computational models accurately to enable classification also without any animal data.

[1] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1272-20190726&from=en)

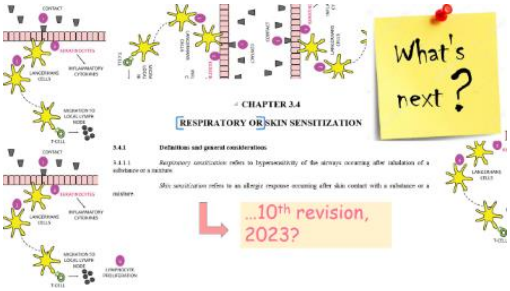
[2] Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Eighth revised edition, United Nations, 2019 (https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev08/ST-SG-AC10-30-Rev8e.pdf)

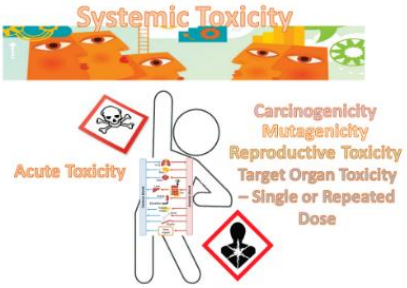
Elisabet stressed that it is important to remember that GHS classification is based on existing data, which is the reason why a tiered approach is applied which allows for classification in a rather straightforward manner except when lower tiers might provide contradictive data, and a weight of evidence approach must be applied.

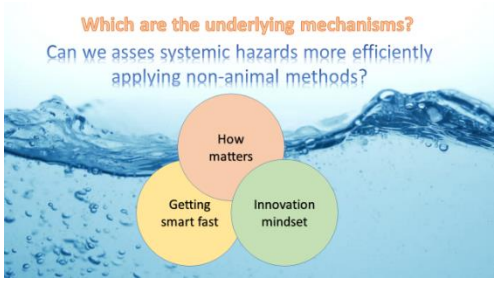


Currently the working group is working on the revision of Chapter 3.3, serious eye damage and eye irritation. JRC is leading the discussion with support from the NL. The draft Chapter 3.3 is following the same structure as the already revised Chapter 3.2, and discussion in the group is currently focusing on the classification for eye irritation, for which currently no stand-alone *in vitro/ex vivo* methods exist. The working group is aiming to conclude the drafting of Chapter 3.3, for inclusion in the 9th revision of GHS, to be published in 2021.

The next endpoint to tackle will logically be skin sensitisation, where the OECD is currently finalising the guidance on defined approaches for skin sensitisation, typically based on the combination of three methods including both *in vitro* and computational methods. The revision of Chapter 3.4 is planned to be included in the 10th revision of GHS in 2023.



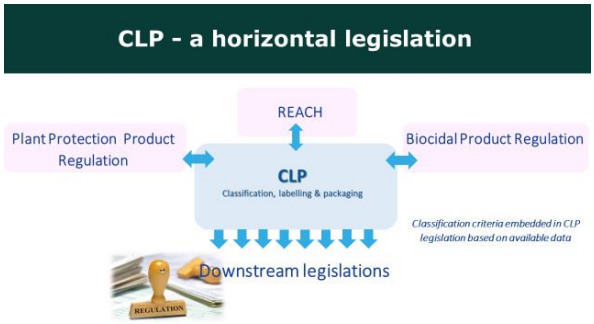




The next challenging endpoint to address in the GHS will be systemic toxicity in 2023. None of the systemic toxicity endpoints, apart from perhaps genotoxicity could be directly substituted by non-animal methods. It might be timely to start considering the possibility of new more upstream endpoints, reflecting the mechanism of action of a chemical, such as genotoxicity or endocrine disruption.

It could also be worthwhile to reflect on the mechanisms involved in the current endpoints, to try to understand how many of those are overlapping between different classifications, and if a new, more rational and more complete classification system could be developed for systemic toxicity applying more relevant and knowledge driven approaches.

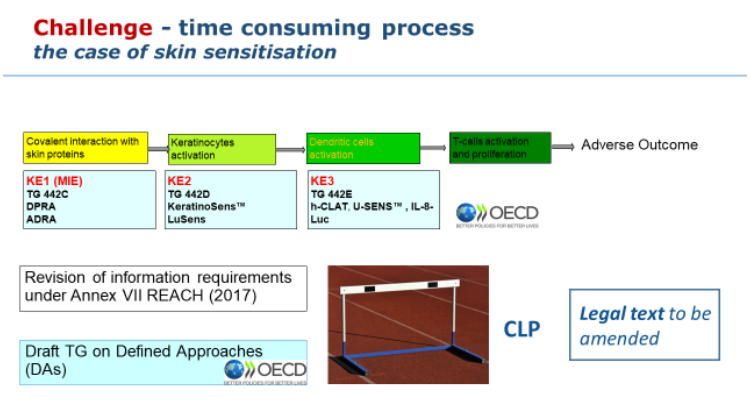
Raffaella Corvi continued to discuss the challenges to reach compliance with GHS and CLP with less or no animal testing. Within the EU the GHS is implemented through the CLP Regulation. CLP is an essential part of our current chemicals legislation, as the classification triggers risk management measures related to chemicals through several other legal acts in the EU, like restrictions and bans.



The CLP Regulation is directly applicable to all industrial sectors and any chemical or mixture regardless of the volume that is put on the market or present at a workplace, must be classified and labelled accordingly. It is a horizontal legislation, and it is directly interconnected with the risk assessment under other pieces of legislation such as REACH, the Plant Protection Product Regulation (PPPR) and the Biocidal Product Regulation (BPR). There is a long range of downstream legislation, such as that for workers and consumers protection, that use the CLP classification for risk management measures.

The classification criteria, based on the GHS and implemented through the CLP, is further explained in the ECHA guidance. In general, classification is based on human evidence and animal studies, but non-animal methods are encouraged to be used in a weight of evidence approach. However, besides topical toxicity and in cases when read-across can be applied, the non-animal methods are difficult to use as a stand-alone solution.

At the joint workshop of the Member State Committee and the Committee for Risk Assessment at ECHA in October 2018, the topic of discussion was related to the endpoints skin sensitisation and genotoxicity and the challenges to comply with CLP.

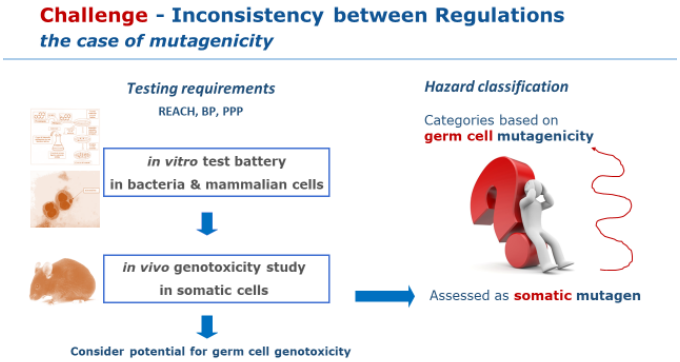


Skin sensitisation was the first example of a more complex endpoint for which the standard animal test could be completely replaced with a combination of *in vitro* and/or *in silico* methods. Although alternative approaches for skin sensitisation have been adopted as OECD TGs and REACH

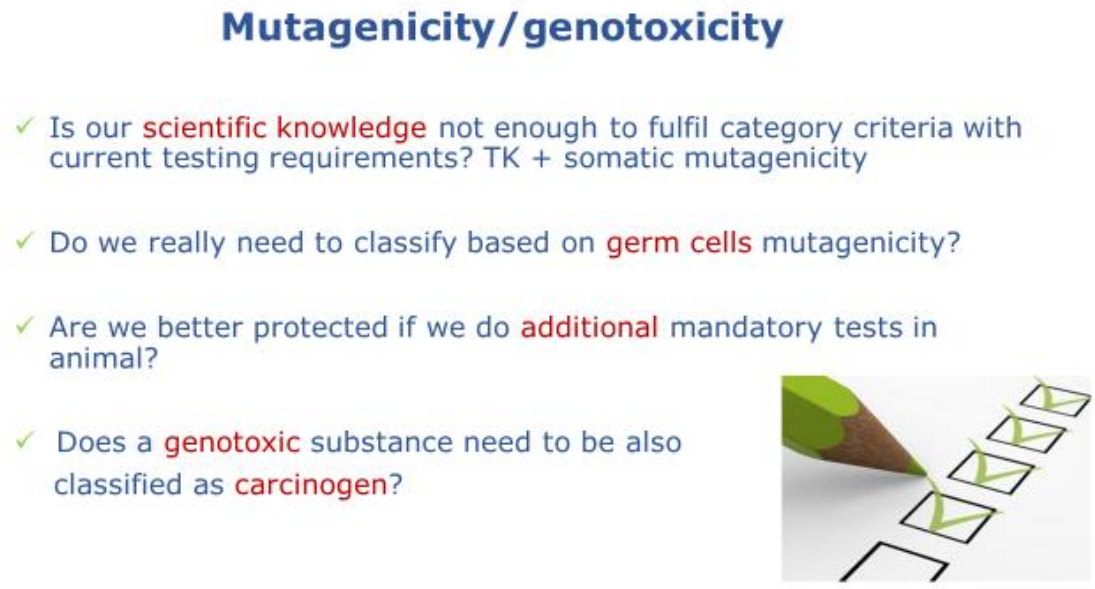
Annexes have already been updated to include them, their use in classification will be challenged until GHS and CLP have been updated accordingly.

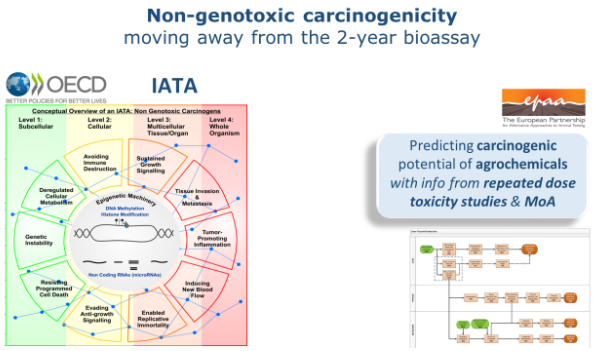
There is still a challenge to fulfil the CLP criteria and whether this must be done with more *in vivo* tests or if it is possible to introduce further replacement methods for more endpoints, also for the systemic ones.

For genotoxicity, it is even difficult to fulfil the criteria with current available methods, including data from *in vivo* studies in somatic cells. While testing requirements rely on mutagenicity in somatic cells, classification is based on mutagenicity in germ cells.



The *in vitro* data based on bacteria and mammalian models provide a good protection level, as these methods are very sensitive, however they might lead to false positive results. Therefore, when there are positive results from *in vitro* studies, REACH, PPPR and BPR, all require an additional *in vivo* genotoxicity study, to confirm genotoxicity to somatic cells, or in case of negative results from the *in vivo* study determining no concern for genotoxicity. However, this is triggering the less severe Category 2 classification, unless it can be proven that the substance is also toxic to germ cells, where traditionally no reliable methods have been available, and classification in Category 1B, has rather been based on the consideration of the potential of the substance to reach germ cells. Raffaella proposed several questions for reflection:

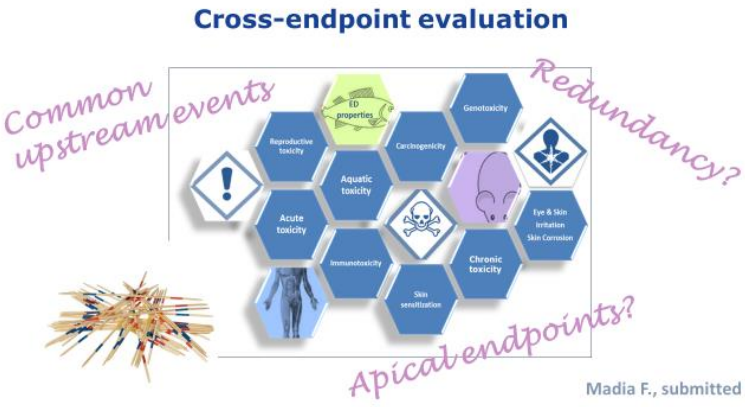




For more complex endpoints such as e.g., non-genotoxic carcinogenicity, several questions are currently being investigated. Which are the mechanisms leading to non-genotoxic carcinogenicity and how much do they overlap with other systemic long-term toxicities?

How can we fit the mechanistic data to the current classification system? The mechanistic data is more knowledge driven and could assist in understanding different diseases, and their relationship.

At EURL ECVAM, we are currently investing more time and resources in activities aimed to avoid redundancy of *in vivo* testing and to facilitate the integration of novel non-animal methods in regulatory settings. We therefore like to share these ideas with the PARERE network, and start a discussion on where to go when we need to tackle the reduction and replacement of animal methods used for the assessment of systemic toxicity.



Discussion with the PARERE members:

Subject: Current scientific progress & non-animal methods are not consistent with current GHS/CLP criteria for systemic toxicity

Questions put forward by ECVAM for the discussion:

- *Is it possible to adapt the current classification system to introduce non-animal methods for systemic toxicity?*
- *Are different classes needed?*
- *Focus on risk management & protection measures?*

The experts round the table confirmed that it was an impossible task to substitute, for example a 90-day study in rodent with a battery of *in vitro* methods, in contrast to the possibility of using in *vitro/in silico* methods for skin sensitisation. Some experts considered the new approach methods as supplementary methods to be applied for strengthening a read-across assessment or provide useful information in a weight of evidence analysis. The experts agreed that CLP is currently the best instrument to guarantee a sufficient risk management within the EU. In order to reach the same level of protection as that provided by the hazard assessment of chemicals and mixtures under the CLP, the risk for each use of each chemical or chemical mixture on the EU market would need to be assessed, which is an impossible endeavour.

The experts recognised that genotoxicity would probably be the next GHS chapter to be tackled after skin sensitisation, when introducing non-animal methods, as it is already a mechanistic endpoint rather than an adverse outcome and classification is not based on potency. Moreover, genotoxicity is well understood from the mechanistic point of view and *in vitro* methods have been applied since decades. Some experts considered it as an opportunity to start to identify other mechanistic endpoints and reflect on a different classification system.

Participants also discussed whether it would be useful to further investigate the current protection levels, to ensure that the same protection is kept in a future system and also to highlight possible gaps in the current system. Such an analysis could also be helpful to understand to which level we need in depth evaluations in our hazard assessment, and when classifications are overlapping and do not provide any additional value.

It was agreed that the best point for starting to discuss systemic toxicity, and also to look at protection levels, would be the CMR substances, since the classification for these endpoints is not potency based (though available information on C- and R- potency should be used for defining specific concentration limits (SCL) for substances. SCLs are part of the harmonized classification of substances and used for mixture classification).

The discussion was open ended and all experts around the table were invited to send further ideas and thoughts to ECVAM that could be shared within the PARERE network and feed into a discussion agreed of common interest to continue.

Actions

PARERE to share any additional thoughts on the questions raised related to CLP/GHS with EURL ECVAM.

Annex I – Agenda

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

JRC Ispra, Monday 21 October 2019

BUILDING 101, ROOM 1302

12.30-13.30 *Buffet lunch (building 101, 2nd floor)*

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

- **Approval of draft agenda**
- **Round-table on activities within the PARERE network**
  PARERE representatives and EURL ECVAM
- **Updates on the AOP framework**
  Clemens Wittwehr, EURL ECVAM

15.30-16:00 *Coffee break*

16:00-16:30 **Non-animal approaches under the EU CLP Regulation* and the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)**

- **Introduction of GHS criteria based on non-animal methods**
  Elisabet Berggren, EURL ECVAM
- **Challenges of GHS/CLP-compliance with less or no animals**
  Raffaella Corvi, EURL ECVAM

16.30-17.30 **Discussion** (*co-animated by E. Berggren and R. Corvi*)

18.00 ***Transport to Restaurant at Hotel Lido, Angera***

18.30 ***Social get-together at Hotel Lido, Angera***

19.30 ***Social dinner at Hotel Lido, Angera***

*EU Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures.