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 25th November 2020, Ispra, Italy

The meeting of PARERE was held on 25 November 2020 by Webex due to the COVID-19 pandemic (the list of participants and the agenda are included in Annex 1 and 2, respectively).

Welcome and Updates

The meeting was chaired by Valérie Zuang, EURL ECVAM. She welcomed all members and introduced new members or additional participants from Hungary, Sweden, Directorate-General Employment, Social Affairs and Inclusion (DG EMPL), DG Health and Food Safety (DG SANTE) and DG Internal Market, Industry, Entrepreneurship and SMEs (DG GROW) of the European Commission and the European Medicines Agency (EMA) and briefly explained the procedures for the on-line meeting. The chair highlighted the different agenda points, which were up for discussions, and the draft agenda was approved. She then invited PARERE to give updates on activities within the PARERE network in the respective Member States and in the respective Commission DGs and EU Agencies, on a voluntary basis.

Updates on activities within the PARERE network

Italy provided an overview on the state of the art of alternative methods/approaches and their regulatory acceptance in Italy. The pandemic has forced them to explore different ways to go forward. Italy launched a mini-survey within their national network in order to investigate what initiatives have been undertaken in the field of alternative approaches and what has been accepted at regulatory level. Italy is very satisfied of the outcome of this survey. A lot of input has been received from the vaccines area but also from other fields, such as e.g. the validation of specific thyroid hormone methods within EU-NETVAL; the development of AOPs related to altered vascularization of the placenta and a project on an integrated *in vitro* approach for intestinal fate of oral ingested nanomaterials at OECD level; modelling human variability in toxicokinetic and

toxicodynamic processes using Bayesian meta-analysis, physiologically-based modelling and *in vitro* systems in the framework of EFSA's activities; and finally, work on Microphysiological Systems (MPS) – organ on a chip (OOC) with EMA. A summary of the feedback on the activities is provided in the below presentation.

[presentation Italy]

Belgium informed that within the national PARERE network that was set up two years ago in Belgium, they usually organise two meetings a year with members from the different regulatory fields. One meeting takes place prior to the EU PARERE meeting and one meeting afterwards, so that the different outputs of the EU PARERE meeting can be transferred to the appropriate regulators efficiently. The first meeting in 2020 had been foreseen in March, but was then postponed to summer due to the COVID-19 pandemic. During that meeting, each regulator made a presentation on the regulatory requirements in his/her field of competence and the place for alternative methods. This had been very appreciated by the different participants who were now able to be in direct contact with other regulators from different fields. The second meeting of the Belgium PARERE network took place in November where an update on the ALISENS consultation was provided and discussed, as well as new approach methodologies (NAMs) for genotoxicity testing. Regarding the RE-Place project, already described at the last PARERE meeting (it started three years ago on the initiative of the Flemish region and was later joined by the Brussels region), the funding by the Flemish region ended in April 2020. It was however agreed to fund a follow-up four-year project that started in April. The Brussels region will also continue to support the funding until the end of 2021. The third region of Belgium, i.e. the Walloon region, now also expressed interest in funding the project so that the RE-Place project can be considered as a real Belgium initiative. A special issue on new approach methodologies in toxicology included in the RE-Place database was published in the scientific journal MethodsX. Another initiative of Belgium is the IC3Rs Centre that has been established at the Free University of Brussels (VUB) and that is chaired by Prof. Vera Rogiers. The IC3Rs received considerable funding and will be expanded with Ph.D projects in collaboration with other universities and institutes, dissemination activities on the 3Rs and training events.

France mentioned that the private-public platform for the prevalidation of testing methods on endocrine disruptors (PEPPER), which was officially established in December 2019, became operational. The goal of the platform is to organise and fund prevalidation studies and as such, fill the gap between the development of new test methods and their international validation, in particular for EDs. It will also allow to prepare better submissions for validation. There is currently a lack of validated methods at European level and a lack of funding of prevalidation and validation activities. The creation of PEPPER, which is a non-profit organisation, is based on the French National Strategy on EDs and is supported by a programme on "investments for the future". The funding from industry and ministries of the government was allocated to PEPPER in June 2019. In 2020, time was spent to identify, review and document candidate methods that could be selected for prevalidation. During this rigorous process, seventeen methods were selected and ranked according to different criteria. The first three methods, the *in vitro* hPlacentox-PE, *in vitro* LCMs/MS steroidogenesis and *in vivo* Zebrafish obesogenic test, were ranked as high priority. More information on these three test methods and the prioritisation procedure can be found in the below presentation.

[presentation France]

The Swedish PARERE contact person is located at the Swedish Centre for Animal Welfare (SCAW). An additional representative, Viveka Hillegaart, joined this PARERE meeting and she will take over from the current PARERE representative. In 2020, several meetings between Swedish Competent authorities (Chemical Protection Agency, Food Protection Agency, Medical Protection Agency) and

the Swedish Centre for Animal Welfare had been organised. Discussions revolved around how the PARERE contact person and the 3Rs Centre can support the agencies with information on the use of alternative methods. Another way to support the 3Rs was through the Swedish Research Council, which has a special funding opportunity (of about 60 000 euro/year for three years) for the 3Rs and alternative methods. In 2020, two refinement projects for translational research have been funded as well as other reduction and replacement projects (the projects are listed in the below presentation). Whilst previously the funding was specifically allocated to the development of alternative methods for chemicals toxicity testing, it is now spread over a broader range of 3Rs activities.

[presentation Sweden]

Spain mentioned that the National Contact Point for PARERE had circulated all the EURL ECVAM documents to its network. The network had faced difficulties to comment on the latest consultation on an *in vitro* test method for respiratory sensitisation due to the pandemic. They received a series of consultations from different members of their network. They have been consulted on the current renewal of the guide for reviewing the competence of the people involved in laboratory animal procedures, on a draft decree including the changes in the non-technical summaries of animal procedures, and on a manual on the preparation, collection and people management related to the use of animals in research. With regard to the activities of REMA, the Spanish network for alternatives, it will meet with the director of the Spanish Agency on Research to promote research activities on alternatives. REMA has conducted a survey on the different teams involved in the development of alternatives where they identified many groups, not only in the area of toxicology but also in biology, molecular biology, biochemistry etc. They also identified the main cell lines being used and that several of the scientists were working on computational models. In late 2019, they had two workshops, one on chemical lists and the evaluation by European Committees and the second one was to promote a national strategy for alternatives. They have several collaborations, which are ongoing, particularly for a project on computational tools for the prediction of the environmental fate of biocides under degradation and with the Spanish Society for Laboratory Animals for the publication of a book on animal experimentation and for contribution on articles, which are published in their journals. They are also promoting dissemination activities on alternatives on their website, forum and on social media.

[presentation Spain]

Finland highlighted two important activities, the activities of FICAM, the Finnish Centre for Alternative Methods, at the Tampere University, that develop cellular models and methods, act as a validation laboratory within EU-NETVAL, disseminate information on the 3Rs and provide education and training. Another significant activity financed by the Ministry of Agriculture and Forestry with one hundred thousand euros per year is the setting up of a 3Rs consortium in Finland, which will be coordinated by FICAM. Aspects of refinement and reduction will be added to FICAM's activities. This consortium is collecting the best practices from the animal laboratory sites so that scientists perform the laboratory animal tests as adequate as possible and include the 3Rs in their research. They are also collecting the *in vitro* cellular models and methods and the respective expertise in Finland in order to share best practices here as well. They are organising advanced 3Rs training courses on *in vitro* testing and on refinement disease models. The 3Rs consortium focuses on toxicity testing, basic research and disease models. FICAM is also a member of the EPAA.

DG ENV informed that two new pieces of legislation had been adopted since the last PARERE meeting. Under Directive 2010/63/EU the Commission adopted in February 2020 the report on the implementation of the Directive by Member States and the EU Statistical report on the use of animals for scientific purposes. Since the legal document is a directive, the Member States (MS) are

free to decide on the best way to implement it and deliver on its objectives. Therefore, an implementation report, published every 5 years, is needed. It looks at the MS structures and procedures to deliver on the objectives of the directive. A novel aspect in the latest report is looking at all the other animals that are not reported annually in the statistics in the EU, i.e. the animals that are bred and not used for scientific purposes. In the EU in 2017, there were 12,597,815 animals, which were bred, not used and consequently killed.

Regarding the EU statistical report, the data are divided into the number of animals that are directly used in research, testing, routine production, and education and training, and animals that have been used in order to provide genetically altered animals (GAA) for the research and testing communities. Furthermore, since some animals are reused (even three or four times) the Commission is also looking at all animals used in support of EU research and testing. For the GAA, both the creation of new lines and maintenance of existing lines are considered. In this category, again the number of animals, as well as the number of uses are detailed. Looking at numbers of animals in detail over the last three years of the reporting period, there has been a continuous reduction in time. Besides numbers of animals used, the statistical report provides a wealth of other information and opportunities to analyse the data in many different ways. For example, for the non-human primates, the report describes their origin (e.g. from self-sustaining colonies or from first or second generation purposed breeding) and for regulatory testing, the legislative drivers are described. An important novelty is to be able to look at the severities linked to the uses. Comparing, where feasible, the data from Directive 86/609/EC with the data collected under the new directive, indicative trends show that there is about 20% decrease in the number of animals used in research, testing, routine production and E&T between 2008 and 2017. With the annual statistics report together with information on data on animals that are bred, not used and killed, the EU is a world leader in transparency. However, the Commission went even further and amended the directive in 2019 through Regulation (EU) 2019/1010 to improve the speed of publication, the accuracy and access to MS statistical data and to speed up the publication, and improve the quality and access to the Non-technical Project Summaries (NTS). This will be achieved by providing open access, searchable database for MS statistical data on animal use and for all NTS. The aim is to move from data to information and from information to knowledge to ultimately arrive at insight and wisdom.

Insight can e.g. be obtained through the NTS which are summaries of projects in a layman language that have been authorised by the authorities. Within six months after authorisation, NTS have to be published. This should allow to get a better understanding of the different animal use areas and more insights on why e.g. in certain areas the severities are so high. It should be possible to assess the 3Rs efforts and to data mine this information. When a project ends and there is a requirement for retrospective assessment, certain MS are obliged to publish this retrospective assessment through the same database. Part of the questions that are being asked in the retrospective assessment is whether any new techniques were identified to refine or replace a specific procedure, which will permit to get information on innovation at everybody's reach.

Regarding the timelines for the whole process, for the statistics of 2020, users are currently (end of 2020) finalising the collection of data on the animal uses. They have to report these data at the end of the year so that the data can be transferred to the authorities when the new year starts, i.e., in Q1 latest Q2. In Q3, the authorities look at all the data making sure that the data quality is accurate after which they pass the information to the Commission. The Commission will then do the same quality checks and perhaps have some bilateral discussions on some data, and during the following year, the Commission will publish the data. The data collected in 2020 will be published in 2022. The Commission has adopted the new data requirements in 2020. The MS will start to collect the new data from beginning of 2021, submit to the Commission in 2022 and latest by 2023, MS level statistical data will be available to anybody who has access to internet.

For the NTS, the adoption of the new timelines are at the same time as for the statistics (April 2020). The Commission has developed the electronic submission tools, which should be ready for the MS early 2021. The public will however probably not see the NTS before July 2021 because the legislation says that you have to start publishing them latest 6 months from the authorisation. In conclusion, the transparency is high, but the information will only be as good as the input of the information by the users will be, so progress is only possible with engagement and commitment by all.

DG ENV continued to report on a different subject, namely the regulatory uptake of new test methods. The Test Method Regulation (TMR), Commission Regulation No 440/2008, is a daughter legislation of REACH and it lays down the applicable test methods for the purpose of REACH. Most of the listed methods are internationally agreed test methods, mostly OECD test guidelines, as far as tests for toxicology and ecotoxicology are concerned. It is a bit different for physico-chemical methods where ISO methods are referred. Keeping the TMR up to date has proven difficult in the past, as the full text of the test method was reproduced in the annex of the TMR. This led to long preparation and translation periods for amendments, with the consequence that the EU test methods were often not aligned with the latest version of corresponding OECD test guidelines (and other international methods). The newly developed test methods appeared in the TMR only after a prolonged period. This was a source of frequent complaints, including an ombudsman case. In order to improve the situation, the Commission analysed the legal possibility that it could have and it is envisaging changing to a simpler system. The latter consists in listing, in the annex of the Regulation, the references (i.e. the title, the number) to applicable internationally agreed test methods for which the full test protocols are available elsewhere (e.g., in OECD test guidelines). Only test methods for which no such reference exists in an external source will be taken up in full in the annex. The concept was presented to MS and stakeholders in the CARACAL (expert group on REACH and CLP) in June 2020. The overall approach was welcomed and supported by the CARACAL members during the meeting and in subsequent written comments. The comments also pointed to some open questions and issues that still need some considerations, which were very helpful to identify the issues that still need to be considered and addressed. They are taken into account in the currently ongoing development of a proposal to change the TMR, which will be presented for discussion and eventual adoption at the REACH Committee in the next months.

[presentation DG ENV]

DG GROW informed that Mr Salvatore D'Acunto (Head of Unit of DG Grow.E.2) has temporarily taken over the Commission co-chairmanship of the European Partnership for Alternative Approaches to Animal Testing (EPAA) from Mr Franz Lamplmair after his departure. Mr Roman Mokry of the same unit will provide technical and administrative support for the activities under the EPAA. Regarding the assessment and registration of cosmetic ingredients under REACH, for the first time in August 2020, the Board of Appeal (BoA) of ECHA took two compliance check decisions on registration files for two UV-filters, homosalate and 2-ethylhexyl salicylate, used exclusively in cosmetics. The BoA confirmed that ECHA might conclude that to comply with REACH, even if the substances are used exclusively in cosmetic products, the applicant must provide studies on animals. The Cosmetics Regulation does not deal with the protection of the health and safety of workers handling the substances used in the production of cosmetics. Workers may handle these substances in greater quantities, with higher concentrations, more frequently, and consequently also with higher exposure than the end-users of cosmetic products. Therefore, to protect workers working in the industry, animal testing may be required under REACH as a last resort. The marketing ban of cosmetic products containing a substance exclusively used in cosmetics which has been tested on animals is triggered only if the results of a study on animals required under the REACH regulation are relied on in the cosmetic product safety report (under the Cosmetics Regulation), in order to demonstrate the safety for the end-user of the cosmetic product. Therefore, the Commission is of

the opinion that the BoA's decisions are based on both REACH and the Cosmetics Regulation and are fully in line with the Commission communication of March 2013 and the joint ECHA-Commission statement of October 2014. The concerned company Symrise, that is also a member of EPAA, launched a legal challenge against the two decisions of ECHA's BoA on 27 October 2020 at the EU General Court that could have widespread impact on the sector. If the court rules in the company's favour, the BoA's decisions could be voided and ECHA will need to pay a compensation to Symrise. This procedure could however take up to two years with more information becoming available after the summary of pleads is published in the Court's official journal.

ECHA provided an update on the use of alternatives under REACH. The REACH regulation stipulates that every three years, ECHA needs to publish a report on how alternatives have been used in the REACH registration dossiers. In 2020, ECHA published its fourth report on the topic. The number of substances that ECHA has to analyse is increasing with every reporting period due to differing registration deadlines for different tonnage bands. It started with less than 2000 substances to reach more than 12000 substances. In the two first reports, only the higher tonnage substances were analysed whilst in the two last reports, all tonnage bands have been investigated. The data mining used by ECHA is based on algorithms, no manual data mining is performed due to the large amount of substances which need to be assessed. This can cause errors, as it relies on the accuracy of the data that the registrant includes in the registration dossier. Errors could e.g. be on the type of test material that the registrant has used with misread-across cases, or whether data have been generated or data of existing publications were used, and when the study has been performed. The algorithm is usually improved to overcome these recurrent errors. ECHA does not see if the data has been generated for REACH purposes or for other purposes, and if the registrant used the alternative approach appropriately. In the latest report and in the previous reports, experimental data, read-across and categories were the main sources of information used, with read-across being the most used. ECHA showed how alternatives have been used for those endpoints where they exist. For skin and eye irritation e.g., a clear increase in their use could be seen when the respective OECD TGs were adopted and included in the EU Test Method Regulation. Moreover, their use increased again from the years 2015 to 2017 when the REACH annexes were amended to specify that for these endpoints *in vitro* methods should be used primarily and *in vivo* methods only in very exceptional cases when *in vitro* methods are not suitable. All the ECHA reports are published on the ECHA website.

[presentation ECHA]

EFSA shared some of their recent activities and their future plans related to NAMs. Read-across has developed as a very common alternative to animal testing. In read-across the assumption is that source and target chemicals are considered to behave in a similar way on the basis of structural similarity. At EFSA, it has been used occasionally in several sectors such as flavourings, food contact materials (FCM), and for some metabolites of pesticide in the context of residue definition. EFSA started to develop an EFSA guidance on the use of read-across in chemical risk assessment. It will also explore what kind of complementary information, such as e.g. metabolism data, should be obtained to reduce the uncertainty linked to a pure chemical read-across. In addition, it will further explore what kind of *in vitro* data can be provided to identify the toxicological signature of a chemical including some mechanistic input to decrease the uncertainty linked to read-across. Another activity of EFSA is the developmental neurotoxicity (DNT) project in which the JRC is also intensively involved. At OECD level, an *in vitro* DNT testing battery is being developed and it is expected that a first draft of an OECD guidance document becomes available by mid-2021. This project on developmental neurotoxicity and the use of NAMs to identify this adverse effect is very important for EFSA.

EFSA is also heavily engaged in the development of AOPs for endocrine disrupting chemicals, one activity focusing on uterine adenocarcinoma and linking ED mechanisms. They will launch a negotiated procedure before the end of the year 2020, the scope being to identify endocrine-active criteria for uterine adenocarcinoma without the need of carcinogenicity study. They are also planning to develop some more AOPs for EDs to maximise the link between endocrine activity (and assays thereof) and adverse event from *in vivo* studies (EATS mediated).

A new project on NAMs has been initiated at EFSA under the "SPEEDO" umbrella. The primary focus of that project is on the use of *in vitro* and *in silico* alternatives, but connected to modern technologies and data models to facilitate harmonisation and reuse of data. The project focuses on human relevant models as the project is dealing with human health, and develop AOPs to get mechanistic understanding for a paradigm evolution from a purely *in vivo* based risk assessment towards a more informative risk assessment that takes advantage of NAMs information. Another EFSA project is on "proof of concept" cases. A platform of researchers and risk assessors in EFSA, EU agencies, and MS is established to define the AOPs or the health concerns, to develop IATAs and test designs. EFSA is working with Article 36[1] organisations to incorporate the data that have been generated into the risk assessment. The two major case studies that are being launched is on pesticides/neurotoxicity drawing back on the AOP developed for Parkinsonian effects where the molecular initiating event is at the level of the mitochondrial complex 1 (i.e., binding and inhibiting complex 1) and on nanofibres/gastro-intestinal tract (GIT) uptake and local effects (inflammation, genotoxicity). More case studies will come and will be reported at the next PARERE meeting.

Another EFSA activity is under the "TK plate" umbrella and is linked to the issue of metabolism and getting PBK models for risk assessment. EFSA was looking for open source PBK models of other species than fish (open source PBK models for fish species have already been incorporated) such as farm animals. Under the same TK plate umbrella, EFSA is working on getting more information from human *in vitro* metabolism data and QIVIVE models for extrapolating from *in vivo* data to an external exposure model that can be used in risk assessment. Activities on predicting human kinetics for EFSA relevant compounds (e.g. pesticides, contaminants) particularly focusing on human *in vitro* metabolism and the issue of inter-human variability in TK, all for use in human risk assessment, are also taking place. EFSA is developing the various guidance documents over the coming years to incorporate this type of data.

Finally, EFSA is currently developing a strategy for the toxicological assessment of proteins based on NAMs. Protein toxicity is often left behind, but it should be reminded that protein toxicity (i.e. prion linked to mad cow disease) was the basis for the establishment of EFSA and the related legislation in the past. Activities include an *in silico* risk assessment strategy to predict protein toxicity; consolidation of the information on protein structural/functional elements relevant in the molecular initiating events leading to toxicity; grouping toxins in a structured, specific manner and; development of a read-across strategy for proteins to predict their toxicity.

[presentation EFSA]

General update from EURL ECVAM

Valérie Zuang presented some of EURL ECVAM's activities and their link to the European Green Deal.

[1] Article 36 organisations are a network of Member State organisations comprised of universities, institutes, governmental, public and other scientific bodies and active in fields within EFSA's mission.
The term originated in Article 36 of EFSA's founding regulation (Regulation 178/2002), which is why these organisations are also known as "Article 36 organisations".

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. It provides an opportunity to introduce and use non-animal methods and approaches. In future, within the Green Deal, the unit will also contribute to the new "Farm to Fork Strategy" on sustainable food along the whole value chain. As indicated before by DG ENV, in February 2020, the Commission published its report on the statistics on the use of animals for scientific purposes. It covers the statistics of the EU MS from the years 2015 to 2017. The report indicates that there have been 9.58 million uses of animals in research and testing for the year 2017. Research (thus basic, applied and translational research) represents 68% of all animal uses while regulatory testing represents 23%. Since most of the animals are used in basic, applied and translational research, EURL ECVAM also invested in activities in these areas beside the area of regulatory testing. More particularly, it undertook a series of studies to review non-animal models in several disease areas. The first studies focused on respiratory tract diseases and breast cancer and were published in 2020. More studies in other disease areas such as e.g. neurodegenerative diseases and cardiovascular diseases were to come. The published studies consist of an executive summary, a technical report and are complemented by a dataset of collected models that is published in the JRC data catalogue. This inventory covers *in vitro* models, such as human-specific cell and tissue-based models including two or three-dimensional cultures, organotypic models; stem-cell technologies, such as human-induced pluripotent stem cells (iPSC), organ-on-a-chip devices with microfluidic systems, (*ex*) *vivo* approaches and computational models. The diseases of the reviews were selected according to their incidence or prevalence. Respiratory tract diseases for example are one of the leading causes of morbidity and mortality globally. They include diseases such as asthma, chronic obstructive pulmonary disease (COPD) and lung cancer. Other selection criteria were the number of animals used for the investigation of the mechanisms and drug discovery, as well as potential causal links between exposure to chemicals and disease development. The study outcomes are useful for several actors involved in the implementation of Directive 2010/63/EU.

As highlighted in the same statistics report**,** every year in the EU, close to 1 million animals are used for antibody generation and production despite the availability of technologies that do not use animals. This number is high and the procedures employed often cause severe suffering. Therefore, EURL ECVAM published a Recommendation that proposes concrete actions for key actors including end-users, commercial providers, authorities, research funding bodies and journal editors. The Recommendation is based on the opinion of our Scientific Advisory Committee (ESAC) and states that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic applications. It also challenges misconceptions existing in the scientific community about non-animal-derived antibodies and highlights the scientific and economic benefits of their use.

At regulatory toxicity and international level, EURL ECVAM is either leading or contributing to several projects at OECD level, which aim at the development of Integrated Approaches to Testing and Assessment (IATA). These include an overview document on concepts and available guidance for IATAs, already published in the OECD series on testing and assessment, a guidance document on the characterisation, validation and reporting of physiologically based (PBK) models for regulatory applications, which will be published late 2020, a guidance document on the application and interpretation of *in vitro* Developmental Neurotoxicity (DNT) assays and, a guidance document on an IATA for non-genotoxic carcinogenicity. The aim is to move away from a check-list approach based on *in vivo* test guidelines that does not efficiently meet legislative mandates which require increased numbers of chemical assessments without a parallel increase in the use of animals and resources. These new approaches are necessary to close the gap between the number of chemicals in use and the number assessed to date and a proper implementation of the new chemicals strategy for sustainability as described before.

Other projects that EURL ECVAM is leading or co-leading at the OECD are the guideline on defined approaches for skin sensitisation. An extensive amount of work was conducted in 2020 to finalise the curation of the LLNA and human *in vivo* reference data classifications; analyse the performance of the DAs against these curated reference data; characterise the applicability domain of the DAs and define the level of confidence in DA predictions. An updated draft Guideline and Supporting Document addressing the work conducted in 2020 will be provided to the expert group and WNT members for review and commenting within the year, with the aim of endorsement by the WNT in 2021. EURL ECVAM has also taken the lead in developing an updated OECD test guideline for Androgen Receptor Transactivation Assays (ARTAs). This TG includes now three ARTAs, one of which using the AR-CALUX cell line that was validated by EURL ECVAM with three laboratories of EU-NETVAL and the test method developer. The updated TG 458 was adopted in April 2020 at the WNT meeting and published.

A draft test guideline on the Hydrochloric acid (HCL) 0.032 Molar method for the determination of relative metal release using a simple simulated gastric fluid, also known as the bioelution method, has been prepared in 2020 and is currently under discussion at an OECD ad-hoc expert group on metal release. The regulatory application of the method is discussed in parallel at a CARACAL subgroup on bioelution.

Another project is the development of an OECD Harmonised Template 201 (OHT 201) for intermediate effects. This template allows the reporting of mechanistic information mainly from *in vitro/in chemico* testing, but also *in silico*, or *ex vivo* testing, obtained with either OECD test guideline methods or non-guideline methods. It also allows the reporting of other classes of methods which provide mechanistic information, including *in vivo* testing or read across. Mechanistic information means effects on molecular, subcellular, cell, tissue or organ level that can be relevant to the hazard assessment. It is important that mechanistic data are captured and reported in an internationally agreed and useful template so that data exchange between data producers and regulatory authorities is as smooth and transparent as possible.

Finally, the project on the use and analysis of control fish in toxicity studies aims to reduce the number of control fish in fish toxicity studies. This is the second part of a project that ECVAM has been co-leading with ICAPO, the International Council on Animal Protection in OECD programs, and with the US. The first part related to the update of GD 23 on Acqueous-Phase Aquatic Toxicity Testing of difficult test chemicals. At present, OECD test guidelines require the use of a water and a solvent control group, when a solvent is used. Data generated according to OECD TGs in the presence of a solvent have been collected and are being analysed to determine if the use of only one control would have had an impact on the outcome of the study. A Detailed Review Paper will be drafted summarising the results, conclusions and recommendations. ECVAM has transferred the co-lead in November 2020 for this second part to the US.

Remaining at international level, the UN Subcommitte on the Globally Harmonised System of Classification and Labelling of Chemicals establishes a biannual working programme, and the GHS is updated every second year. GHS is implemented in the EU through the CLP Regulation as had been reported at the previous PARERE meeting.

In 2017, the Informal Working Group on Non-Animal Testing Methods was established on the initiative and under the co-lead of the NL and UK.

Its first task was to update the Chapter on Skin Corrosion/irritation, which was revised with ECVAM's support, to enable classification based on non-animal methods in the 8th Revisions of GHS published in 2019. After that, and under our lead, the Working Group has revised the chapter on serious eye damage/eye irritation, which became more complex partly due to the introduction of other

validated *in vitro*/*ex vivo* non-guideline methods some of which may be useful to classify in Category 2, since the currently available *in vitro*/*ex vivo* OECD Test Guidelines for serious eye damage/eye irritation often result in an outcome that is inconclusive according to the Test Guidelines' criteria. In addition, the concept of Defined Approaches (DAs) was introduced into the Chapter because it was recognised that single *in vitro* methods would not be able to fully replace the *in vivo* method. This is the first time the concept of DAs is introduced in GHS and it will have a major impact on follow-up chapters covering other endpoints. The Working group has almost finalized its task. The revised chapter will however not be included in the 9th Revision of GHS, as initially foreseen, but in the 10th Revision to be published in 2023. The next endpoint that will be tackled will be skin sensitisation, again under the lead of JRC through EURL ECVAM. In addition, as mentioned at the previous PARERE meeting, ECVAM has discussed the need to clarify the classification criteria for germ cell mutagenicity. Moreover, it became clear that this chapter would also benefit from an update with respect to the current state of the art including newly available test guidelines. Therefore, on behalf of the European Union, JRC has submitted a proposal to the GHS to revise chapter 3.5 on germ cell mutagenicity, which will be discussed at the UN GHS subcommittee meeting in December 2020. To this end, the EC has suggested to establish an informal GHS working group to discuss the proposed revisions in the next biennium with the aim of achieving a coherent and clear text. ECVAM has volunteered to take the lead of that informal working group. The proposal was supported by several Member States.

More of EURL ECVAM's work and activities can be found in the 2019 EURL ECVAM Status report and soon in the 2020 edition to be published in February 2021.

[presentation EURL ECVAM]

Discussion with the PARERE members:

A discussion revolved around the EURL ECVAM Recommendation on non-animal derived antibodies. Some misinterpretation on the recommendation and how it could be implemented in the framework of Directive 2010/63/EU had been observed. Furthermore, a poor scientific understanding about non-animal-derived antibodies and some polarization of the discussion had also been noticed. EURL ECVAM informed that FAQ to tackle the most common misunderstandings/arguments will be issued on its website. In addition, the recommendation had been presented and was discussed at the 19th Meeting of the National Contact Points (NCP) for the implementation of Directive 2010/63/EU, where stakeholders such as the European Animal Research Association and EFPIA also participated.
Germany pointed out that more guidance and communication were needed on this topic and that the individuals and scientific organisations that raised concerns, including the German Society for Immunology, did not lack scientific understanding but were particularly experienced with the generation and use of antibodies and that their concerns should be taken into account and discussed further in some kind of dedicated platform.

The Commission explained that it is the Competent Authorities' responsibility and the project evaluation process that they oversee, to ultimately decide on a case-by-case basis, if a project involving the use of live animals should be approved or not. The applicant need to demonstrate on the basis of scientific evidence why the use of non-animal derived antibodies is not possible for his/her project. The immediate follow-up to the publication of the recommendation had been to engage with NCPs and stakeholders who expressed their concerns and opinions to ECVAM. Several CAs informed ECVAM that they do not have access to the right expertise to include in project evaluation committees e.g. to be able to make the kind of judgements that are required. However, these issues are common to any other non-animal procedure or method being proposed in a project context and which needs to be addressed by MSCAs. DG ENV concurred that there had been very good discussions and pragmatic proposals with the MSCAs on the practical measures that MS are

considering doing and how they can contact those who currently carry out production of antibodies using animals and looking at the justifications that they are providing. Another element was the availability of good expertise to CAs. In this respect, it is important to publish new scientific information on that topic in peer-reviewed journals for a broad dissemination. The National Committees set up in the framework of the Directive, and whose task is exactly to exchange information and assist in the project evaluation process, is also important. The role of some of the 3Rs Centres, who are straight ford coming in specific fields, had also been discussed, but the set-up of these centres are voluntary initiatives by some MS. Some PARERE members felt that the exchanges ought to be continued and that the CAs needed support and have access to the knowledge, as it was difficult for them to decide on a case-by-case basis. DG ENV informed that some MS had already been very proactive, e.g., by organizing a meeting with the National Committee on the topic, others organized a meeting with their 3Rs Centres and between the authorities and the specific industry sector using antibodies for certain purposes. The Commission will continue to provide scientific advice and information on the state-of-play through ESAC opinions, recommendations etc. but ultimately, the responsibility lies with the MS according to Directive 2010/63/EU. The good practices and experience used to handle the topic by one MS can be transferred to another MS.

Validation framework for *in vitro* test methods for respiratory sensitisation

The session on respiratory sensitisation was chaired by Maurice Whelan, EURL ECVAM. This session had been inspired by the rich and informative feedback received during the PARERE consultation on the ALISENS test presubmission. The session was meant to continue the discussion on what elements would be necessary and important for the validation of methods for respiratory sensitisation.

Laura Gribaldo, EURL ECVAM, introduced a few concepts to inform the discussion on a validation framework for this type of test methods. Respiratory diseases are a major concern worldwide. The WHO report on air pollution concluded that every year around 7 million deaths are due to exposure from both outdoor pollution and household air pollution. Apart from air pollution, many chemicals and particles need to be assessed for pulmonary toxicity in the context of REACH and sector-specific regulations. Furthermore, due to COVID-19 and other infectious diseases, there is a need to better understand and account for adverse effects caused by the interplay between exposure to biological and chemical stressors, particularly in vulnerable populations. This is also the reason why the respiratory tract disease area had been included as one of ECVAM's seven systematic reviews on non-animal models used in basic and applied research. About 21000 publications dedicated to respiratory tract disease models over the last five years had been identified in the review. Among these papers, ECVAM was able to select 284 models dedicated to the different diseases of the respiratory tract. In the package of information on the published systematic reviews, there had also been a technical report including some meta-analysis of the data collected, which described the models and methods along different categories. An analysis of the methods broken down by application revealed that most of the methods were used for disease mechanism investigation and drug development, but that a certain amount of methods were also used for toxicity testing. The air-liquid interface model (called ALI model), such as the ALISENS, are considered to be the most relevant ones amongst the methods used for inhalation toxicology. In fact, already in 2016, a workshop convened more than 60 experts to discuss potential validation studies for ALI models. As stated in Article 57 of the REACH regulation, respiratory sensitisers are considered substances of

very high concern (SVHC) and thus it is extremely important to have good methods that pick those up. These substances are now receiving increasing attention in the chemical risk assessment, however an early identification of these substances is problematic nowadays. The LLNA is used, but the extrapolation of the data from rodents to humans is difficult. Furthermore, the LLNA is also using a different route of exposure. Thus, it has revealed important to optimise and validate other methods for this purpose. The ALISENS is an *in vitro* method based on this ALI model for predicting respiratory sensitisation following nebulisation of the substances. The 3D culture model of ALISENS is a relevant model because it stands between the simpler 2D cultures and the *in vivo* situation and consists of alveolar-type II epithelial cells, endothelial cells, dendritic-like cells and macrophage-like cells, and thus able to mimic well the real situation at the level of the alveoli in human beings. When exposed to nebulised chemicals, sensitisers and irritants, the exposure to sensitisers induce the dendritic cell activation and the specific cytokine release pattern, whereas the exposure to irritants does not. ECVAM did an internal assessment of the submission and consulted PARERE, and both assessments were in agreement. The final assessment of the submission asking for further optimisation of the method has been sent to the submitter. Questions to the submitter revolved around the type and number of chemicals tested, the purpose (e.g., sector, information requirements), the context of use (e.g., IATA, WoE), the exposure scenario, the reference chemicals, the reference data, the scientific basis of the method/mode and the technical complexity. However, it had been definitely recognised by both PARERE and ECVAM, that there was a need for this type of methods for the purpose of different regulations. ECVAM wants to start a discussion with PARERE on how to elaborate on a validation framework for respiratory sensitisation methods.

Discussion with the PARERE members:

ECVAM would be interested to involve PARERE in providing further guidance on validation aspects to test method developers of respiratory sensitisation methods. Frequently, we have a situation where validation studies are conceived and designed by test method developers, who then undertake these validation studies and submit the results to ECVAM and to regulators for evaluation. Sometimes it works, but sometimes there are deficiencies in the validation approach that only come out much later in the process. In the interest of trying to make this process as efficient and effective as possible, ECVAM's suggestion is to delve into these validation criteria/principles together with PARERE in view to provide better guidance to test method developers. However, depending on the regulated sector or regulatory community and experts who are consulted, different people have different views on what is important to see in a validation study, thus the importance for an open dialogue. ECVAM was able to share all of PARERE's detailed feedback with the test method developer (as annex to the summary outcome of the consultation). Many of PARERE's comments related to advice to the test submitter to further validate his method. Starting with the regulatory needs, many PARERE members commented that different sectors would potentially require information on respiratory sensitisation, e.g. the biocidal products regulation, REACH, the plant protection products regulation, the cosmetics regulation etc. It was also mentioned that classification criteria could potentially be developed for that endpoint under GHS/CLP at an appropriate moment. ECVAM asked the PARERE members in which regulatory sector they would think would be the most need for providing toxicological data for respiratory sensitisation in a regulatory decision making context.

Slovakia replied that it was important to investigate this area because there were significant needs for these methodologies for instance within the occupational health sector. In the Czech Republic and Slovakia e.g., where titanium is produced, workers are considerably exposed to that substance. From the standpoint of the test method developer, support to retrieve reference data (human and animal data) would be very helpful. The test method developer should also consult a statistician to select an appropriate number of chemicals to be used in the validation study and clearly separate the training set from the chemicals testing set. These points are described in OECD GD 34 on the validation and international acceptance of new or updated test methods for hazard assessment. According to the Slovakia, the major support to the test method developer would be the provision of the reference chemicals for the studies and the supporting *in vivo* data so that no animal tests are conducted in parallel.

The chair replied that this was an interesting scenario because there are requirements to assess chemicals for this human health effect in different legislation but there are no existing guidelines on methods for specifically generating data for this endpoint. If you want to benchmark the results of these data with reference data, what would these reference data be? In some of their comments, Italy and Austria referred to human data that sometimes is used in making a decision about a chemicals' potential for respiratory sensitisation.

Austria mentioned that we would need to agree first on the mode and mechanisms of action that the ALISENS method could capture. There had been long discussions on what respiratory sensitisation really was and the CLP regulation currently covers a very broad section of mode of actions. For this method, the test method developer should clarify which mechanisms the method is covering and describe them. The method could be used straightforward once the reproducibility and the mechanistic applicability domain are known, as we do not have a reference model that we need to replace. We should thus start to use such a method and enrich it later with further methods if needed. For Austria, it was not clear why the applicant asked in the first place for a validation of the method for respiratory sensitisation, whereas in his publication he was writing that the method is equally applicable to respiratory irritation. Austria wondered if the respiratory irritation was a lower hanging fruit as there were other established alternative methods on irritation for skin and eye and all that was needed to add was the kinetic modelling for the respiratory tract, i.e., what is the concentration gradient from the inhaled air down to the alveoli.

The chair mentioned that it would indeed be important to clarify the purpose of the validation first before being able to design the validation strategy. He asked whether there was a need to do both, respiratory irritation and sensitisation, in the same testing strategy or separate?

Defining the purpose of the validation study in terms of what types of toxicological pathways and modes of action are covered by this test and trying to anchor those back to some AOPs being currently developed at the OECD, was also commented during the PARERE consultation. Other comments related to the need for potency evaluation, either by dose-response evaluation or potency–based classification by applying a data interpretation procedure (DIP).

Austria mentioned that potency differentiation between chemicals was important in future, as it has become important for the skin sensitisation assessment, too. What would be useful from a regulatory perspective was to have an *in vitro* method that could be applied to many thousands of chemicals. However, in that case, there will be a high amount of positives and it would be beneficial

to be able to differentiate and prioritise between them according to their potency and thus the concern that one should have. If the outcome of the method will be used to protect the workers, there will also be a need to derive some safe values.

The chair referred to the PARERE comments on the relevance of the LLNA data. If we were looking at the method from a purely scientific and technical perspective, we would be able to draft a list of essential requirements that such a method would need.

Regarding the question on the scientific basis of the method by Austria, Laura Gribaldo mentioned that a decision on the degree of information that is required on the mechanistic understanding of a method should be made at a certain stage, to be able to further validate the method.

For respiratory skin sensitisation, we know that some key events are already described in an AOP, proposed by Kimber et al., which is based on four steps: the protein binding and reactivity, the epithelial inflammatory response, the dendritic cell line activation and the T cell line activation and proliferation. The developer of the ALISENS method claims that the method is able to detect two out of the four steps, i.e., the induction of the cytokines at the epithelial barrier and the activation of the dendritic cells. Laura raised the question if it was enough for a method on respiratory sensitisation to cover two out of four steps described in an AOP. Should we include such a method in an integrated approach covering all four steps? Alternatively, should we go further in the investigation of the mechanisms at the basis of the method?

It is important to take such a decision, as at a certain point we need to be able to optimise and validate the method with reference chemicals.

The chair highlighted that with respect to reference chemicals, almost all PARERE respondents commented that only a handful of chemicals had been tested. However the question on the number of true respiratory sensitisers was also raised by a number of respondents. Are there enough reference chemicals that one could use to carry out a kind of data-driven validation of such methods? In addition, if there were no sufficient respiratory sensitisers, how would we be able to establish the scientific validity based purely on the predictive capacity of such methods? At the moment, respiratory sensitisers are evaluated on the basis of available human and LLNA data and considerations of physico-chemical properties, which is not a perfect approach neither.

Slovakia replied that the situation of lacking good animal reference data because no standardised animal test exist, had also been true several years ago when the *in vitro* phototoxicity test was validated. This was a unique case where people investigated the human data and the exposures from the clinical praxis, and there had also been a number of pharmaceuticals that had been evaluated in a first step. Later on, people from different industries joined with their materials, such as e.g. cosmetic UV filters. Gradually, we may be able to find sufficient chemicals but people need to start somewhere. For instance, if we know three to four human respiratory sensitisers with sufficient potency, the method that is complex enough to cover both, irritation as well as sensitisation, could be investigated. In fact, the model includes five to six different cell types that should be able to address both irritation and sensitisation and distinguish between them. If the model is also able to generate potency data is still an open question. The human potency data are assumed to be quite variable.

The chair asked PARERE if they knew how many respiratory sensitisers existed. In the Biocidal Product Regulation and in REACH under article 57, such chemicals could be flagged as substances of very high concern (SVHC).

Laura Rossi from ECHA mentioned that many valid points had been raised for respiratory sensitisation. For SVHC identification, the potency information would be important. For REACH, respiratory sensitisation is currently not a standard information requirement, but if the data is available, it should be used.

Under substance evaluation, if there is a concern, ECHA could ask for respiratory sensitisation data, but it is then always a specific case.

The chair asked if it was a chicken or the egg question, i.e., had there not been any regulatory requirements because no test existed? Laura concurred and mentioned that since respiratory sensitisation is a very severe human health endpoint, ECHA would appreciate if there would be such a method that could be used to investigate the respiratory sensitisation potential and not to wait until human data would become available.

The chair mentioned that Sweden had commented extensively on the scientific basis of the method, observing that there were several cancer cell lines being used, which we would not consider normal physiology. Italy and Belgium commented about the potential lack of metabolism or that it should at least be characterised. The NL commented on the technical exposure.

Laura Gribaldo commented that the exposure scenario was extremely important and that sometimes it is not taken into account as it should be. For this particular type of adverse effect, we need to make sure that the exposure route is the right one. Nebulisation or an aerosol system for exposing the cells to substances requires a particularly complex and costly equipment. In its comments, Austria had mentioned that it was an acute exposure scenario that was modelled, whereas the method developer claimed that chronic effects were mimicked. Exposure scenarios would thus need to be clarified in a validation framework.

The chair informed that the test method developer would like to follow-up on the feedback and comments received and we will hopefully see a revised version of the test pre-submission in the future. We are however also conscious that several other methods exist, based on different technologies and approaches, but ultimately trying to solve the same problem. In that sense, the soft validation guidance that we could give would help many developers. We should try to harmonise the validation approach as far as possible.

Sweden mentioned that there was a lot of development in that field driven by the occupational health people but there are also several academic groups who work with different cell systems which are more or less sophisticated which can also address different issues/questions.

The chair concluded that the fact that several developments took place in that field should be embraced and that scientists working in that field should come together as they did back in 2016.

ECVAM would like to explore the possibility of developing further the idea of a validation framework or guidance with PARERE, which would be useful for developers and also ultimately, if ECVAM receives more methods to ask PARERE's opinion again.

Laura highlighted that the paper on an expert workshop on respiratory sensitisation mentioned in her presentation was still today one of the most read ones, and that there was a lot of interest in this topic. Since ECVAM is at the interface between developers and regulators, it has the duty to stimulate the discussion and go further in that direction, and that since 2016, not many steps had been taken with regard to the validation of these methods for toxicology. The chair mentioned that validation was a major undertaking for developers and apart from being highly resource-intensive, it is also scientifically challenging to design good validation strategies. ECVAM was hoping to work with PARERE to provide more practical guidance to the development community to help them in the design of their validation strategies. A proper assessment of the reproducibility of the method had been considered important during the PARERE consultation, which is a challenging technical task where ECVAM's EU-NETVAL could also be involved at a certain time point once the relevance of the method had been established.

The chair asked whether anyone from the PARERE members thought that PARERE had no role to play in supporting the development of a validation framework. All PARERE members agreed to support such a framework. Austria mentioned that they would be very happy to contribute to this as it was essential for the future, not only for this regulatory field but also for developmental neurotoxicity, non-genotoxic carcinogenicity and other fields where few reference data were available and where the classical correlation for predictive capacity was not possible. Even for the skin sensitisation area where an AOP was available and where we had thought to base the validation of the DAs on that AOP, in the end, it had also been a massive data correlation exercise to define the boundaries. The chair reminded that, in fact, in the area of skin sensitisation a huge amount of validation had already been conducted by different parties and that the OECD project on the DAs had been extremely challenging to try to get acceptance of these DAs incorporated into a guideline. There had been huge emphasis on the data and in that situation, we were in a luxury position of having more than a hundred reference chemicals and there was still a heavy debate about the relevance of the animal and human data. In areas such as DNT, carcinogenicity etc. we will never have hundreds of reference chemicals and we need to find alternative ways of building confidence.

Organ-on-a-chip and possibilities for regulatory application

The third session of the meeting was on organ-on-a-chip (OoC) and possibilities for their regulatory application and was chaired by Raffaella Corvi, EURL ECVAM. She introduced the session by mentioning that this emerging technology raises a lot of interest in the scientific community and that ECVAM has also started to explore the technology in-house. In the first session, there had been mentioned that the Safety Working Party at EMA was taking action in this field. Because of this evolution, ECVAM considered it timely to discuss the potential translation of organ-on-a-chip to the regulatory domain with PARERE.

Sofia Batista-Leite, EURL ECVAM, set the scene in a presentation and provided some food for thought in the area for follow-up exploratory discussions. Sofia mentioned that in our daily life we are constantly exposed to chemicals and products, for which we need to know if they are safe or effective at certain doses. It is thus important to test them. However, there is still a gap between the test methods that are available and the human effects that they can cover. The work of ECVAM is focused on complementing or replacing the existing animal models with more human-relevant models for testing the effects on humans. In terms of *in vitro* methods, the models have become

more and more complex for better representing how the cells behave in the human body. They have transitioned from 2D into 3D structures and more recently they have become more dynamic, which means that they include the fluid flow that can mimic the blood flow and increase the performance of the cells *in vitro*. Different types of bioreactors and OoC or microphysiological systems (MPS) exist. One may wonder why they are part of the same family if they are so different between them. The definition of OoC by ORCHID, the EU Horizon 2020-funded consortia, includes a "fit for purpose microfludic device containing living engineering substructures in a controlled microenvironment, that recapitulates one or more aspects of the organ dynamics, functionality and (patho)physiological response *in vivo* under real-time monitoring". How do OoC devices provide a benefit when compared to the previous systems? The dynamic fluid flow of OoC devices allows mimicking the blood flow and the device allows for a specific cell composition, like co-cultures, tissue-tissue interface, as well as physiological mechanical cues, which affect the performance of the cells like sheer stress. They also allow having a physiological-relevant ratio between the cells and between the media and the cells. This particular setting, together with the possibility to include sensors and probes for a real-time monitoring of the cells, both by the samples that are taken, or by the measurements made by these probes, allows for a better *in vitro* to *in vivo* extrapolation and improved cell differentiation for stem cells and tissue-specific functions. All these characteristics increase the relevance to the human physiology *in vitro*. It is now possible to investigate some typical endpoints performed in animal tests, which could normally not be done in static or 2D cultures. However, do we really want to continue to focus on what is investigated in animals and keep comparing our *in vitro* results with the animal data? A recent paper published by Donald Ingber, one of the pioneers of OoC, suggests that reviewers of papers start to ask authors to validate their *in vitro* experiments using human OoC data instead of animal data. The paper also mentions that this does not mean that we can fully replace the animal model but rather that we should focus more on the human relevance.

The question is now whether there is a place for OoC devices in the regulatory sciences. In 2018, ECVAM conducted a survey on the validity of complex *in vitro* models including OoC. We received 645 replies, 61 of them were from regulators who did not have much experience with OoC (only 1.6% had experience). Fifty-three % of these regulators mentioned that they were only slightly satisfied with this new technology, whereas 13% and 27% said that they were very satisfied and satisfied, respectively. The majority of regulators mentioned that there were very few publications, which were relevant for regulatory purposes. In their opinion, the factors that hamper mostly the broader acceptance of this technology are the costs, the questionable relevance and appropriateness of these models.

During the presentation, some scenarios where OoC could fit were explored. Some complex endpoints for which no complete regulatory solution yet exists in terms of alternative methods, were selected, namely non-genotoxic carcinogenicity, developmental neurotoxicity and metabolism.

In the area of non-genotoxic carcinogenicity, several experts convened at OECD level and identified some key aspects of cancers, not based on genotoxicity, that would need to be recreated in non-animal models. They then tried to map these key aspects to available non-animal models and identified the gaps. We have taken the same key events and investigated how OoC could fit in terms of what is currently available as models. The majority of cancer-on-a-chip devices have been developed for drug efficacy testing and not for testing if a chemical can cause cancer or not.

However, with the cancer-on-a-chip devices, it is possible to grow cells in an environment that is suitable for the development of tumorigenic cells. They also offer the possibility of connection with other organs (e.g. with liver and heart), inclusion of metabolism, control of temperature, oxygen and pH, as well as sampling on-line and live monitoring. It is possible to detect early events of inflammation with these types of sensors and you can combine different key events. You can observe the recruitment from other cell types located in a different chip, invasiveness of the cells, angiogenesis and migration. Other key events like proliferation can easily be modelled in other more simple *in vitro* systems.

A similar exercise has been carried out for DNT, where the full mapping of all the assays to the different neurodevelopmental processes described in AOPs has already been performed at OECD level. The aim was to check if OoC could be applied in this case or not. We focused on the brain-on-a-chip device, which is also currently more deployed for drug efficacy. The functionality of glial cells, which are very important in the DNT process, could be maintained and specific responses of these cells, not seen in neuronal cells, were obtained. The functionality of the cells can be combined with electrical activity and you can see the calcium oscillation. All four key events, namely proliferation, migration, differentiation and maturation can be observed in one plate. Some collaborators have shown that human iPSC-derived endothelial cells and microengineered organ-chip enhance neuronal development.

For ADME, most of the work that has been carried out is on metabolism and kinetics. ECVAM had taken several initiative in the field such as the evaluation of the human hepatic clearance methods and the validation of the Cyp-induction methods. However, there is still a gap for modelling the proper metabolism that is needed. Liver-on-a-chip devices, are often combined with other organs. This is important since metabolisation can change the toxicity of drugs and chemicals. Some OoC which combine four different types of organs, were able to differentiate between compounds that were toxic to the kidney and those that were not and if the compound was able to pass the blood-brain barrier. The latter event cannot be detected in animal studies. A good inter-organ communication could be shown, as well as the possibility to assess the kinetics of the compound and to measure the metabolites and their potential effects. It is however still important to improve the liver performance. Few studies have focused on chips where they combine four types of hepatic cells and check their specific functions, which is usually very difficult to do. As such, it has been possible to investigate different types of drug-liver injuries, which is normally not possible in static cultures. Comparing chips for different species, like dogs, mice and humans, different cell responses have been obtained based on the species-specific drugs.

Sofia finished her presentation by providing some thoughts and questions on whether OoC could be deployed for regulatory purposes. From the analysis presented, it could be observed that most of the studies focused on drug efficacy. There are many different devices, which is important as there is not one device that fits all. The main advantage of OoC is that they can integrate several key events and key events relationships. They can combine multiple organs and integrate metabolism. The devices are usually used for measuring downstream events. For early events in an AOP, simpler models can be used as they provide satisfactory responses without the need for such complexity.

ECVAM continues to collaborate with EUROoCS, the OoC society, created in the framework of ORCHID, where it is chairing the regulatory advisory board.

The questions raised to PARERE were the following:

- Is this new technology still too futuristic or does it have a place in regulatory toxicology?
- Have you come across any OoC? Do people talk about it in your domain?
- Are there particular areas where this would have a real added value?

Discussion with the PARERE members:

Germany mentioned that they also had many discussions on OoC in-house and some projects running in that area. It is a complex field because it is a mix of materials science, cell biology and engineering. Furthermore, OoC development also seems to never stop, i.e., the developers continue to improve their chip, which does not seem to always come into use. Therefore, Germany found it a good idea to start to investigate those chips that are steadier and are mainly used in pharma, to get an idea of their robustness and reliability. For carcinogenicity testing, beside the fact that every cancer is different, Germany questioned the usefulness of the current OoC models, since they are used to test the efficacy of a drug on a cancer, whereas we are more interested to see whether normal cells transform into carcinomas when exposed to xenobiotics. Germany also wondered if the current cancer models could be used to detect non-genotoxic carcinogens.

Sofia replied that she tried to do a reverse analysis. In fact, the models are already used for efficacy testing. The chip needs to create the conditions for a cancer to be able to develop in 3D, including the process of angiogenesis and migration and recruitment of cells from a different device to develop into a cancer.

Germany mentioned that you need a trigger to induce the cancer and therefore you bias the whole system for this trigger. If one thinks of OoC in the regulatory field, the cancer area is probably the most challenging one. For the kinetic, we probably face again the problem of reference data that is available in the drug development area but not for industrial chemicals, because it is not a standard information requirement. The chair mentioned that these were in fact challenging points and that the aim of the presentation was to explore what type of OoC were currently available. For carcinogenicity, the devices were in fact used in the pharma industry for a different purpose. In the OECD expert group on non-genotoxic carcinogenicity, no OoC has been considered to be integrated in the overall framework so far.

The chair concurred about the multi-disciplinarity in the OoC area which needs to be taken into account by involving experts from the different domains.

EMA commented that in order to be able to use OoC at regulatory level, in particular in the ADME area, the experimental system needs to be standardised. E.g. if you use primary human cells for forming the organoids, they can display significant batch-to-batch or donor-to-donor variability. It is thus a key step to qualify the cell source in terms of quality in the growth and differentiation potential. On the other hand, the donor variability is much more relevant in terms of mimicking the responses of the normal human population. In addition, one should be critical when evaluating the kinetics in these extremely complex experimental systems, considering e.g. the distribution of the

chemical within the experimental system, like e.g. lipophilic compounds that cannot dissolve or contaminants or active substances used in plant protection products. According to EMA, these were the two critical points that needed to be considered before applying OoC in the regulatory domain.

Sofia mentioned that regarding the liver systems, the possibility to better control the culture conditions has lead in some cases to reduce the variability between different studies, also for primary cells. For the kinetic part, it has been explored whether the device can be combined with computational models to understand the kinetics of the compound within the system. However, a lot still needs to be done and the community is well aware about the current limitations.

Regarding standardisation of OoC, Monica Piergiovanni, EURL ECVAM, informed about the analysis on the standardisation needs of OoC that ECVAM is undertaking. ECVAM is collaborating with CEN and CENELEC, the standardisation authorities in Europe, in the organisation of a workshop on standardisation of OoC to be held in April 2021. Updates on this workshop will be provided in early 2021.

Belgium enquired if ECVAM was following up on this topic and working on it closely. Belgium is sometimes requested to participate in projects on OoC and asked to provide regularly input on whether these models are relevant or not. Could input e.g. be requested to EURL ECVAM?

Raffaella replied that the activities so far had been exploratory and that it was still early days. However, EURL ECVAM plans to further develop these activities and also consult PARERE on specific topics in future. Maurice Whelan added that on the regulatory advisory board of EUROoCS, there will a representation from various sectors with a dominant representation from the pharmaceutical sector, including a number of members who are currently on the EMA Safety Working Party. The regulatory advisory board will try to tackle the main issues and requests for support in a way that all the community can access that knowledge and make it efficient for the regulatory community. Slovakia mentioned that this was an excellent initiative because these advanced systems could help us a lot in the future for correctly predicting, in particular in areas where it is not yet possible to address everything with static models. Including dynamics in simple models can already improve the model a lot. They had seen this in recent studies with the intestinal tissues, where the tissue had a completely different absorption profile under dynamic conditions. This had of course an impact on the uptake of the drugs into the system. Slovakia was very much looking forward to hear more about these activities and agreed that standardisation would be an important issue.

At the end of the third session, Valérie wrapped up the meeting. PARERE members will receive all the presentations through CIRCABC. Regarding the second session on respiratory sensitisation, an internal discussion on how to progress the validation framework for methods will take place. One possibility would be to draft a validation guidance intended for the developers of methods on respiratory sensitisation and consult PARERE on that guidance. Another possibility would be to organise a workshop with the PARERE members. In any case, PARERE members will be informed and consulted, as usually.

With regard to the third session on OoC, the main points raised were that the technology was under continuous development and that standardisation was very important. ECVAM will continue to explore the area and investigate how we can increase confidence, in particular by the regulators, in

this new technology. PARERE will be involved in further discussions or activities. A draft summary record on the whole meeting will be sent to PARERE.

The chair thanked all the participants and closed the meeting.

Annex 1 - Participants

Austria
Belgium
Czech Republic
Denmark
Estonia
Finland
France
Germany
Greece
Hungary
Italy
Latvia
Luxembourg
Norway
Poland
Portugal
Slovak Republic
Spain
Sweden
The Netherlands

European Commission:
DG ENV
DG RTD
DG GROW
DG SANTE
DG EMPL
JRC

EU Agencies:

ECHA
EFSA
EMA

Scientific Committees:

SCHEER

Annex 2 – Agenda

PARERE meeting
JRC Ispra, 25 November 2020

13:00-13:05	**Welcome** (*Chair: Valérie Zuang*)
13:05-13:15	**Adoption of the agenda**
13:15-14:15	**Updates from PARERE members**
14: 15-15:00	**General update from EURL ECVAM** (20 min presentation by Valérie Zuang followed by discussion)
15:00-15:10	*Break*
15:10-16:00	**Validation framework for *in vitro* test methods for respiratory sensitisation** (scene-setting presentation (15 minutes) by Laura Gribaldo; *Chair: Maurice Whelan*)

The aim is to start to conceive a validation framework together with PARERE for defining some guidance and concepts that could be communicated to the test method development community.

16:00-16:10	*Break*
16:10-16:50	**Organ-on-a-chip and possibilities for regulatory application** (scene-setting presentation (15 minutes) by Sofia Batista-Leite; *Chair: Raffaella Corvi*)

The aim is to have an exploratory discussion on organ-on-a-chip technologies and their potential for translation into the regulatory arena.

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 but only published afterwards. In addition, it includes references which served as a basis to prepare the presentation on organ-on-chip devices.

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