ANNUAL REPORT 2021

EUROPEAN PARTNERSHIP FOR ALTERNATIVE APPROACHES TO ANIMAL TESTING (EPAA)

The European Partnership for Alternative Approaches to Animal Testing

European Commission

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1. FOREWORD

In this Annual Report we are looking back at an exciting year with many EPAA activities in the 3Rs area of Reduction, Refinement and Replacement. We are pleased to say that the agreed EPAA Action Program for the years 2021-2025 constitutes our roadmap for progress on the development, validation and mutual recognition of alternative approaches. This is available for reference on our website. The uniqueness of the EPAA is the direct partnership between industry and the regulators. It is our firm belief that this partnership fuels trust in the use of alternative approaches addressing the safety of ingredients. This dialogue is exemplified in the Skin Sensitisation User Forum, where actual case studies on ingredient safety for this endpoint are presented and discussed among industry and regulators. The past year we have also introduced a new project, on New Approach Methodologies [NAMs], building on the successful Blue-Sky Workshop on repeated dose toxicity from a few years ago. This project is developed in very close partnership with the European Commission's Joint Research Center and with full support by other Commission services. The goal here is to work through case studies on how we could ensure protection for consumers and workers for ingredients using approaches that use best-in-class science and all available data, without necessarily generating new animal toxicity data for all endpoints.

We will need to do this in a balanced way, and there will be situations where new animal testing may still be required, but we are sure that the time is right for another step forward that Europe can take in this area.

As EPAA we have had good discussions with ANSES/France [L'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail] and with BfR/Germany [Bundesinstitut fur Risikobewertung] who are also very interested in NAMs through the so-called PARC initiative – the Partnership on the Assessment of Risk from Chemicals. During the virtual 11th edition of the World Congress on Alternatives and Animal Use in the Life Sciences (WC11) we are proud to say that EPAA had a strong presence, through a training session on Skin Sensitisation and a session where we talked about some of the ongoing EPAA projects. Both sessions were well attended and sparked a lively debate on future direction for safe and sustainable use of the 3Rs. We as the EPAA co-chairs continue to welcome and encourage all these efforts, and the partnership will continue to contribute to implementing the 3Rs principle in all relevant areas. The EPAA, as a unique platform for voluntary cooperation and pooling of expertise and knowledge amongst regulators and industry, will continue to strive for replacing animals and reducing their numbers and suffering in regulatory testing.

Giacomo Mattinó

DG GROW, EC Co-Chair

Rob Roggeband

P&G, Industry Co-Chair

2. OVERVIEW OF THE PROJECT PLATFORM IN 2021

The EPAA aims to replace animal testing by innovative, non-animal methods, to reduce the number of animals used and to refine procedures where no alternatives exist or are not sufficient to ensure the safety of substances (the '3R principle'). The partners are sharing knowledge to accelerate development, validation and acceptance of alternative approaches. Replacement methods embrace the increasing knowledge of toxicity mechanisms of action together with data from in silico and *in vitro* tools that are utilised in integrated testing strategies and model systems, to allow less and less dependence on animal tests for assessment of human and environmental safety. The EPAA Project Platform (PP) aims to develop new approach methodologies (NAM) that fill critical information gaps, demonstrate application of NAMs to regulatory decision making (often supported by case studies), and engage and communicate with stakeholders in the EU and globally.

The PP is composed of EPAA partners and associates that supervise and assess the individual projects agreed upon by the EPAA Steering Committee to ensure scientific quality and effectiveness. In 2021, the PP has supported 10 project teams which synergistically combine the expertise and collaboration available across industry sectors, academia, NGOs and regulatory agencies. Throughout 2020 and 2021, each team has worked hard to maintain excellent progress despite the continued constraints of the COVID-19 pandemic. Results from the work of the project teams led to several peer-reviewed publications and/or presentations at the 11th World Conference on Alternatives and Animal Use in the Life Sciences held in Maastricht (WC11, 2021). In autumn 2021 we initiated a new project related to NAMs and entitled “Non-animal science in regulatory decisions for chemical safety”.

Projects in 2021

a. Clostridial Vaccines for veterinary use

b. Human Rabies Vaccines

c. Acute Toxicity

d. Harmonisation of 3Rs in Biologicals

e. Monoclonal Antibody Safety

f. Carcinogenicity of Agrochemicals

g. Applying Non-Animal Strategies for assessing Skin Sensitisation (User Forum)

h. *In vitro* to *in vivo* Extrapolation

i. PBK Modelling in Safety assessments

j. Non-animal science in regulatory decisions for chemical safety (new NAMs project)

Typically, each project has a duration of more than one calendar year in which methods and data are developed and analysed, and results are discussed, disseminated and published. For each project summarised here, a brief background and overview is given together with the most recent developments (for 2021) on each individual project which are provided in blue, italicised text.

a. CLOSTRIDIAL VACCINES FOR VETERINARY USE

Novel in vitro methods to replace animal-based in-process control tests

Vaccines for protection against diseases caused by Clostridial species in animals are used widely. Their pharmaceutical quality is controlled by vaccine manufacturers in accordance with the specifications of the European Pharmacopoeia (Ph. Eur.) monographs for clostridial veterinary vaccines and with their market authorisation dossiers. For many of these vaccines both the toxin and toxoid bulk (obtained by detoxification of toxin and used to produce the final vaccine batches) are currently controlled by animal-based tests. This is the case for toxicity and antigenicity in-process controls which are performed in mice by using the minimum lethal dose (MLD) and the total combining power (TCP) tests, respectively. The tests account for the use of large numbers of animals and therefore *in vitro* methods to replace them are very desirable. In addition, because of their potentially higher sensitivity and precision, *in-vitro* assays may offer better tracking of production consistency and allow more accurate vaccine blending.

Therefore, a project was undertaken on Clostridium (C.) septicum vaccine for veterinary use, aiming at validating *in vitro* assays for toxicity and antigenicity and at proposing their inclusion in the Ph. Eur. This species was chosen to perform a proof-of-concept study since C. septicum is a common component of veterinary combination clostridial vaccines, and since a manufacturer had already developed candidate alternative methods for the control of this component. As other components of combined veterinary clostridial vaccines are also based on detoxified cytotoxic antigens (cytotoxins), it is expected that the alternative assays developed for C. septicum could be adapted to all cytotoxin-based clostridial antigens with the potential to greatly reduce the total animal usage for in-process control testing of veterinary vaccines.

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The project on C. septicum vaccine for veterinary use was launched in 2014 and is now nearing completion. It benefited from the joint support of the EPAA and of the European Directorate for the Quality of Medicines & Healthcare (EDQM, Council of Europe). For this project, two successive collaborative studies were run by the EDQM in the framework of the Biological Standardisation Programme (BSP, an applied research programme which is co-sponsored by the EU and the Council of Europe). The first study, involved 11 laboratories, including 6 vaccine manufacturers and 5 public sector control laboratories from 7 countries. Results obtained demonstrated that the proposed *in vitro* assays were suitable in terms of repeatability and showed excellent concordance with the animal-based tests currently used by vaccines manufacturers. Furthermore, based on the results presented and discussed at a dedicated workshop[1] it was postulated that optimisation of the *in vitro* assays evaluated in the first study[2] would allow the establishment of improved assay procedures.

Therefore, the project was extended to develop optimised cell-based assays that would fully exploit the precision and greater sensitivity of the cell-based methods and to evaluate them in a second collaborative study. This project extension consisted of *in vitro* testing only; it was again supported jointly by EPAA and the EDQM, and coordinated by the EDQM with the help of a large project management team together with 14 participants including vaccine manufacturers and official control laboratories in Europe, USA, Morocco and Mexico. The experimental work was successfully completed in 2018 and the final results demonstrated that the optimised *in vitro* tests are very consistent, with intra- and inter-laboratory variations far lower than those for the analogous *in vivo* tests.

This indicates that the non-animal, cell line-based assays[3] for in-process toxicity and antigenicity testing of C. septicum vaccines outperform the animal-based methods. This will allow full advantage to be taken of the superior sensitivity and accuracy of the *in vitro* MLD and TCP tests when manufacturers implement these alternatives into the current in-process controls.

1 Validation of alternative/3Rs methods for the in-process quality control of Clostridium septicum vaccines BSP130: Participants workshop report. Egmond aan Zee, Netherlands. 15-16 September 2015. https://circabc.europa.eu/sd/a/48c4e459-44e5-4231-bd4f-c8d4e05d4952/Clostridials_report_2015_BIO_15_9_DEF.pdf

2 Daas A, Behr-Gross ME, Bruckner L, Redhead K. (2020) Collaborative study for the validation of cell line assays for in-process toxicity and antigenicity testing of Clostridium septicum vaccine antigens - Part 1. Pharmeur Bio Sci Notes (2020) 53-124
https://pharmeuropa.edqm.eu/app/BioSN/content/BioSN-0/2020-4_Clostridium_septicum_vaccine_antigens-Part_1.pdf

3 Behr-Gross ME et al. (2021) Collaborative study for the validation of cell line assays for in-process toxicity and antigenicity testing of Clostridium septicum vaccine antigens - Part 2: Optimisation of cell line assays. Pharmeur Bio Sci Notes (2021) 101-156
https://pharmeuropa.edqm.eu/app/BioSN/content/BioSN-0/2021-5-Clostridium-septicum-vaccine-antigens-Part-2.pdf

In light of the results of the project, the European Pharmacopoeia (Ph. Eur.) Group of Experts 15V revised the monographs for vaccines based on cytotoxic Clostridial antigens to introduce Vero cell line-based methods and this was followed by completion of the Ph. Eur. public inquiry. Subsequently, the revised monographs were adopted by Ph. Eur. in June 2021 (implementation date 1st April 2022). Importantly, the revisions allow not only replacement of in vivo by in vitro tests but will also require only residual toxicity testing of antigens rather than final products.

The results of the validation of the Vero cell line-based methods were discussed at an EDQM - EPAA workshop1 and were presented at the 10th World Congress on Alternatives and Animal Use in the Life Sciences (Seattle, 2017). *The regulatory consequences of the study were presented at 11th World Congress on Alternatives and Animal Use in the Life Sciences (Maastricht, 2021). The outcomes of the first and second collaborative studies have now been published2,3. The project has stimulated considerable interest including the potential for application of the optimised protocol developed in this project to other, in vitro replacement, assay validation research work. To disseminate the study results and to promote the implementation of new methods, a joint EPAA - EDQM – JRC EURL ECVAM workshop on study outcomes and new in vitro methods implementation was held in Strasbourg in March 2021 in webinar format and with over 200 registered participants from more than 40 countries. Presentations and session summaries of the webinar have been published online[4] and the proceedings are being drafted. It is anticipated that the two studies and workshops will be published in 2021-22 in a special issue of Pharmeuropa Bio & Scientific Notes.*

b. HUMAN RABIES VACCINES

Replacement of animal-based potency tests

Before vaccines are released for use, their quality must be assured. The current *in vivo* potency test for the release of human rabies vaccines (the National Institutes of Health mice intracranial challenge test) is problematic and involves the use of large numbers of animals, of which half develop distressful rabies symptoms. Clearly, replacement of the NIH test will have a high impact on animal use and it is therefore a priority for the implementation of the 3R principles. The aim of this project is the replacement of the NIH *in vivo* test with an *in vitro* antigen (G glycoprotein) quantification assay using an ELISA technology. A specific ELISA was selected as a suitable replacement method in a pre-collaborative study. The method recognizes most vaccine strains used worldwide for human rabies vaccines (including from Chinese manufacturers)[5].

An international collaborative study to validate the transferability and robustness of the selected ELISA began in 2017 with the support of EPAA. The study is being coordinated by EDQM as part of the Biological Standardisation Programme (BSP) of the Council of Europe and the EU Commission. It is expected that the study will generate data supporting the revision of the Ph. Eur. monograph on Human Rabies vaccines as well as global acceptance of the replacement method.

4 Novel in-vitro model as alternative to in vivo toxoid vaccines testing: Clostridium septicum vaccine as proof of concept https://www.edqm.eu/en/proceedings-international-conferences#3R

5 Morgeaux S. et al. (2017) Replacement of in vivo human rabies vaccine potency testing by in vitro glycoprotein quantification using ELISA – Results of an international collaborative study. Vaccine 35 https://doi.org/10.1016/j.vaccine.2016.12.039

Phase 1 of the study, the transfer of the assay and protocol to study participants and relevant regulatory agencies has been completed successfully. Negotiations for the production and world-wide distribution of the two standardised monoclonal antibodies to be used as reagents in the ELISA has been concluded. The two antibodies are adequate for almost all human rabies vaccine strains and in 2020 became commercially available from two manufacturers, world-wide. Qualification of the monoclonal batches and sample predilution choice has been achieved.

Phase 2 of the study is ongoing; it includes an inter-laboratory comparison of different vaccines, statistical analyses and a report which is expected to be completed in 2022. The study has 31 participants including 8 vaccine manufacturers and laboratories from Europe and other regions (South, Latin & North America, India, Indonesia, Philippines, Japan, China, Vietnam, North Africa). The large number of international participants is a strength of the study but has also brought additional challenges not least because of the pandemic and the need to adapt the method to different laboratories and equipment.

The project has prompted considerable interest from international regulators and NGOs. It has been presented to many national and international meetings including a joint meeting of National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods (NICEATM) and the International Alliance for Biological Standardization (IABS), Rabies workshop (USA, October 2018), French Days of Virology meeting (March 2019), and at the IABS Global Congress on Animal Testing for Vaccines, in Bangkok, Thailand (December 2019). Publication and dissemination of the results is planned for 2022, after completion of Phase 2.

c. ACUTE TOXICITY

Identification of clinical signs predictive of mortality

Identification of clinical signs predictive of mortality
Acute toxicity testing remains a requirement for chemicals and agrochemicals in order to establish their overall hazard profile and to meet classification, labelling and packaging (CLP) requirements that are relevant to human safety, for example, in emergency situations. Acute toxicity testing is no longer needed in the pharmaceutical sector and is banned in the cosmetics sector.

The REACH standard information requirements for the endpoint of acute toxicity (REACH Annex VIII, point 8.5.3.) were revised in May 2016[6] allowing a waiving of acute toxicity testing via the dermal route under certain circumstances. Acute toxicity by the oral route is still the most common testing requirement and therefore this route has been prioritised by EPAA. This project has identified opportunities to waive the acute oral toxicity animal testing requirements completely or, where this is not possible, to refine the decision-making steps or assessment strategies so as to minimise suffering of animals. Recommendations on a 3Rs-based classification & labelling decision framework to include replacement of death as an endpoint will be developed at the end.

[6] Commission Regulation (EU) 2016/863 of 31 May 2016 amending Annexes VII and VIII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards skin corrosion/irritation, serious eye damage/eye irritation and acute toxicity OJ L 144, 1.6.2016, p. 27–31

The objective of this project is to determine whether or not observed clinical signs (evident toxicity) are predictive of mortality at higher dose levels in acute oral toxicity studies and are an appropriate alternative to death as an endpoint. The findings are being analysed and applied to develop guidance on use of evident toxicity as an endpoint and to support use of the Fixed Dose Procedure (FDP) for acute oral toxicity studies (OECD Test Guideline (TG) 420). This test uses fewer animals than other accepted methods (TG 423 and TG 425) and does not use death as an endpoint, giving clear animal welfare benefits. Unfortunately, the subjective nature of evident toxicity (in contrast to mortality) appears to be preventing wider uptake of the TG 420, and it is not currently the test of choice.

Data from previous acute oral toxicity studies is being mined and statistically analysed in collaboration with the UK National Centre for the 3Rs (NC3Rs), the UK Chemicals Regulation Directorate and EPAA member companies. This has delivered data on approximately 200 substances which provide wide coverage of different sectors (agrochemical, cosmetics, chemicals, food, pharmaceuticals and others). Earlier in the project there were set backs in accessing studies and many of the studies initially identified were found to be unsuitable for a variety of technical reasons including incomplete information for the purposes of the project. However, these difficulties have now been overcome and good progress is being made with a sufficiently large, anonymised dataset from studies shared by EPAA member companies covering different chemical classes. The studies have been screened for suitability (approximately 90 usable studies). Robust statistical analysis of the data will be completed in 2021.

The project dissemination plan includes a workshop, publication, and presentation at international conferences such as Eurotox in 2022.

d. HARMONISATION OF 3Rs IN BIOLOGICALS

Deleting international regulatory requirements for in vivo general safety tests

International divergence of testing requirements is common in the field of biological products. As a consequence, companies developing, manufacturing and distributing products globally may be required to conduct both animal and non-animal tests to have access to all markets. This is ethically unsound, increases development costs, and may delay patient access to essential vaccines and medicines. The EPAA Biologicals project aims to facilitate harmonisation of 3Rs in biologicals regulatory testing requirements between countries / regions. Specific actions continue to be progressed for harmonisation and international convergence of 3Rs in regulatory testing requirements for biological products. The project has initially focused on deletion of regulatory requirements for tests of innocuity in Ph. Eur. Monographs and WHO recommendations. In addition, the project now also includes pyrogenicity testing which is relevant to a wide range of products including vaccines, chemicals and blood products.

Previously, the EPAA hosted an international workshop[7] that defined the most effective pathways for international convergence of testing requirements and provided recommendations including for prioritised actions to delete the regulatory requirements for specific animal-based tests.

[7] Schutte K. et al. (2017) Modern science for better quality control of medicinal products "Towards global harmonisation of 3Rs in biologicals": The report of an EPAA workshop. Biologicals 48 http://dx.doi.org/10.1016/j.biologicals.2017.05.006

The workshop recommendations for safety tests included active engagement with regulators and international bodies to encourage deletion of *in vivo* tests of innocuity (including abnormal toxicity test (ATT) / general safety test (GST)) from international and national regulatory requirements as well as from guidelines for human vaccines and other biologicals (e.g. specific biological substances derived from animal sources). Similar recommendations were made to encourage deletion of the ATT / GST, target animal batch safety test (TABST) and laboratory animal batch safety test (LABST) for veterinary vaccines and other biologicals.

The Biologicals project team has submitted formal requests to WHO, OIE and Ph. Eur. to encourage deletion of specific tests (GST / ATT, LABST and TABST) from their recommendations or requirements. Notable successes have already been achieved. Most importantly, the Ph. Eur. Commission endorsed the complete suppression of the test for abnormal toxicity (ATT) from 49 monographs in the Ph. Eur. and this has been implemented.

Following requests from EPAA to OIE, two chapters of the OIE Terrestrial Manual[8] now refer to VICH Guidelines for live and inactivated vaccines that include waiving of target animal batch safety tests (TABST) when consistency of manufacturing process has been demonstrated. The WHO Expert Committee on Biological Standardization (ECBS) has recommended the discontinuation of the inclusion of the innocuity test in all future WHO Recommendations, Guidelines and manuals for biological products published in the Technical Report Series. It is further stressed that the requirement for the innocuity test in the published WHO Technical Report Series documents should be disregarded[9].

WHO have communicated to testing laboratories in several countries to emphasise that the general toxicity tests are no longer required[10] . The substantial progress already made in deletion of tests for innocuity by EPAA working in collaboration with Humane Society International (HSI), EDQM and EC JRC was presented at a HSI Symposium held in Rome (Spring 2019)[11] and at the IABS Symposium in Bangkok (December 2019). Roadmaps including country-specific activities were published for the elimination of the tests. In addition, WHO is partnering with NC3Rs to review animal testing requirements in WHO Guidelines and Recommendations for biologicals with proposals to identify evidence-based opportunities to extend implementation of 3Rs strategies and application of non-animal testing approaches.

The Biologicals project continues to (a) encourage deletion of *in vivo* ATT/GST/TABST/LABST from national / jurisdictional and legal requirements as well as international guidance (WHO) and (b) implement outreach activities in other prioritised non-EU countries (Japan, South Korea, China and Russia) by the most efficient channels, including joint activity with EDQM, EC JRC and HSI, and (c) coordinate dissemination activities on deletion of ATT/TABST/LABST by EPAA, industry and HSI. An overview of progress achieved in the Harmonisation of 3Rs in Biologicals project was presented at WC11 (Maastricht, 2021).

[8] https://www.oie.int/standard-setting/terrestrial-manual/access-online/

[9] https://apps.who.int/iris/bitstream/handle/10665/325184/9789241210256-eng.pdf?ua=1

[10] https://www.who.int/immunization_standards/vaccine_quality/who_nnb/en

[11] Viviani L. et al. (2020) Global harmonization of vaccine testing requirements: Making elimination of the ATT and TABST a concrete global achievement. Biologicals 63 https://doi.org/10.1016/j.biologicals.2019.10.007

The 2015 workshop[7] recommendations for potency tests had the overall aim to achieve international convergence on the scientific principles for the use of appropriately validated *in vitro* assays to replace *in vivo* methods. The area of potency testing and achieving aligned global regulatory approaches to testing requirements based on alternative methods remains a challenge. In 2018, two new activity areas were identified through consultation with users in Member states and agencies. In the following areas progress has continued in 2021:

Pyrogen testing in rabbits, for which the Ph. Eur. monographs encourage replacement by suitable alternative methods but the rabbit test continues to be used widely. *In vitro* tests require product-specific validation and are often not accepted outside EU. A survey of users' experiences with *in vivo* and *in vitro* tests for pyrogens has been completed and analysed. A key finding is the need for more training of users in non-animal methods, in particular the monocyte activation test (MAT).

In vitro tests require product-specific validation and are often not yet accepted in several non-EU countries. In general, Ph. Eur. encourages replacement by suitable alternative, non-animal methods however, >50 Ph. Eur product-specific monographs mention the rabbit pyrogen test (RPT) and not the alternatives. Stimulated by the EPAA project team, EDQM defined a strategy in June 2021 to amend several Ph. Eur monographs (removal of RPT) in the next 5 years[12] . In addition, a new chapter for Ph. Eur was introduced in July 2020 for the bacterial endotoxin test (BET) using a recombinant factor C assay as a potential replacement of the existing test based on horseshoe crab extract. There are also developments in non-EU countries including China and South Korea to prepare for future use of the MAT alternative; the in vitro MAT assay entered the Chinese pharmacopoeia in 2020.

Monoclonal antibody safety testing. Animal studies are currently required in the non-clinical development of monoclonal antibodies. However, therapeutic monoclonal antibodies exhibit a safety profile that is almost exclusively based on their pharmacological properties and immune responses. The latter are known to be poorly translated to humans from animals. This offers the possibility to reduce animal studies for safety assessment including the potential to improve and reduce 6-month repeat-dose toxicity studies. Following detailed discussion with representatives of industry, Dutch MEB (Medicines Evaluation Board) and EC, an EPAA project was begun in 2019 for monoclonal antibody safety *(see below)*.

[12] https://www.edqm.eu/en/news/european-pharmacopoeia-put-end-rabbit-pyrogen-test

e. Monoclonal Antibody Safety

Optimal duration of non-clinical studies to assess safety of monoclonal antibodies

The translational and predictive value of animal studies is increasingly being debated and questioned in the public, scientific and regulatory community. However, evaluation of safety and efficacy of new drugs or indications often require conduct of animal studies that have evolved over time and are embedded in (inter)national guidance and legislation. It is highly desirable to restrict the use of animals in safety and efficacy studies to those which provide essential, meaningful information that is relevant to humans. Research suggests that in specific cases, such as monoclonal antibody products for humans, opportunities exist for optimized non-clinical programmes with reduced animal use.

One of the central observations made is that therapeutic monoclonal antibodies exhibit a safety profile that is almost exclusively based on their pharmacological properties and immune responses. The former are generally predictable based on pharmacology data obtained in short-term studies, while the latter are known to be poorly translatable from animals to humans. While research is still ongoing, this would suggest that long-term animal studies are not always needed. In particular, products with a highly defined pharmacological space (e.g. bio-betters or follow-on products) would be amenable to abbreviated approaches.

This project aims to improve and reduce the use of animal studies by re-evaluating regulatory practices from a non-clinical perspective, focussing on monoclonal antibodies (for human use), and to build on the previous research experience at the Dutch MEB. Importantly, that research was based only on approved marketed products which are considered safe. Products that did not progress beyond animal or clinical studies and were never submitted to regulatory agencies for review had remained out of scope. To make firm conclusions on the criteria for reduced non-clinical testing, data from studies on products that were never submitted for marketing authorization are also needed to provide a more complete body of evidence. The specific objectives of this project are to (a) establish criteria for decision making on the need and duration of non-clinical safety studies for monoclonal antibodies based on drug development programmes for both marketed and non-marketed molecules, (b) establish regulatory consensus based on scientific facts that these criteria are acceptable as a justification to deviate from the current guidelines in future marketing authorization applications, and (c) initiate discussions to document these new criteria in EMA guidance.

This EPAA project is led by the Dutch MEB with strong support from EC DG ENV, 14 pharmaceutical companies, industry experts and UK NC3Rs. *This has ensured that the project can rely on a database of adequate size and includes a substantial quantity of proprietary data for non-marketed molecules that have been made available by industry working in collaboration with UK NC3Rs which is participating in the project as a neutral intermediate organization. Data submission to NC3Rs by companies has been completed, and NC3Rs have anonymised and coded the data before passing it to the Dutch MEB for analysis and interpretation. Data has been received for 142 unique mAbs (>103 non-marketed products) which combined with data for marketed products was sufficient for completion of the final analysis. In 85% of cases, long-term toxicity studies did not identify novel toxicities of human concern. Of the 15% that did, approximately 50% were considered to be clinically important findings.*

A technical workshop which included regulators was held virtually over two days in April 2021 to discuss interpretation of the data and to develop an evidential approach to support the conduct of fewer studies. The team is currently evaluating weight of evidence models to determine the requirement for longer term toxicity studies.

The project was presented to WC11 (Maastricht, 2021). The output of the project will be published in a peer reviewed journal in 2021/22.

f. Carcinogenicity of Agrochemicals

Waiving of two-year carcinogenicity studies

Two-year carcinogenicity studies are part of the regulatory requirements for pharmaceuticals, additives and chemicals (mainly agrochemicals and biocidal products). These studies entail the use of large numbers of animals. Currently, to assess the potential for a non-genotoxic compound (i.e. not inducing DNA damage) to increase the risk of cancer in humans, 2-year carcinogenicity studies in rats and/or mice are performed. Although the relevance to human safety of data from rodent carcinogenicity studies has often been questioned, thus far this type of study remains the default requirement. Regulatory requirements also include repeated dose toxicity studies of 3 to 6 months duration for compounds intended for long-term administration.

This is a follow-up to a previous, successful EPAA project on the prediction of carcinogenicity of pharmaceuticals which provided evidence that in many cases a 2-year carcinogenicity study in rats could be waived without compromising human safety[13]. The waiver could be granted based upon prior knowledge of the pharmacological properties of these compounds integrated with histopathological findings from 3 to 6-month repeated dose toxicity studies and together with evidence for lack of genotoxic potential and lack of hormonal perturbation. The conclusions were based on data analysis of 289 pharmaceutical compounds and demonstrated a prediction rate of 92% and 98% for non-carcinogens and for carcinogen compounds, respectively.

This follow-up consists of two sequential projects that aim to identify opportunities for improving the science supporting the regulatory testing of agrochemicals, and to achieve reduction in the use of animals when assessing the potential for carcinogenicity. The projects anticipate (i) the enhanced prediction of carcinogenic potential of agrochemicals in humans using mechanistic information together with 3-month repeated dose toxicity data to reduce or replace the need for 2-year carcinogenicity studies, and (ii) establish a virtual waiver for 2-year agrochemical carcinogenicity animal studies.

The two agrochemical carcinogenicity projects are supported by EPAA and are being conducted by RIVM (National Institute for Public Health and the Environment, The Netherlands). The project team includes some of the same researchers as in the previous pharmaceutical-focused project. In the first project on agrochemicals, data was collected for >400 agrochemicals.

[13] van der Laan JW. et al. (2016) Prediction of the Carcinogenic Potential of Human Pharmaceuticals Using Repeated Dose Toxicity Data and Their Pharmacological Properties. Frontiers in Medicine 3 https://doi.org/10.3389/fmed.2016.00045

Of these, 170 are considered to be non-genotoxic carcinogens and thus relevant to the projects' objective of providing an overview of modes of action (MOA) and key events in carcinogenicity. Analysis of data has been completed to identify the most relevant MOAs and target organs involved in agrochemical carcinogenesis, and to determine potential parameters and assays for detecting MOA, non-genotoxic compounds, and target organs.

From the MOAs identified in this first agrochemical project a subset was discussed in an EPAA expert workshop (June 2019, Brussels) with participants including toxicologists, regulators, industry and NGOs. The main outcome of the workshop was that the MOA-driven approach was strongly supported and was considered the way forward, complementing other relevant international activities such as those by the OECD and US-EPA. Although the project identified a selection of 10 MOAs or MOA networks underlying non-genotoxic carcinogenic potential of agrochemical compounds, some crucial data gaps were also identified. These include the observation of treatment-related tumours for which no MOA information could be identified ('known unknowns') as well as assessment of the human relevance of each of the MOAs. For the majority of the MOAs, an alternative approach (i.e. without the need for a 2-year carcinogenicity assay) remains to be developed.

This first project has been completed and two papers have been published in peer reviewed journals: One manuscript on all the work completed in the project[14] and a second on the workshop[15].

A second, subsequent agrochemical project was begun in March 2020 with the objectives of (a) identification of 'known unknowns' and consolidation of MOAs, and (b) development of a weight of evidence approach to predict carcinogenic potential of agrochemicals without the need for two-year rodent studies. *An approach for the identification of 'known unknowns' has been established. This approach primarily includes filtering of irrelevant findings. In some instances, tumour findings may be related to high dose and excessive toxicity and thus not relevant. For this, criteria and relevant considerations have been drafted; these are currently under discussion with the project team. Meanwhile, the approach is being applied to the 'known unknowns' (116 tumors in various organs, involving 74 substances). The aim is to complete the first objective in this project by the end of 2021, which will result in a consolidated list of MOAs.*

g. Applying Non-Animal Strategies for assessing Skin Sensitisation (User Forum)

Sharing knowledge and experience on the use of NAMs for skin sensitisation decision-making through a User Forum

Skin Sensitisation to chemicals can result in Allergic Contact Dermatitis and therefore reliable hazard and risk assessments need to be performed to ensure that sensitising ingredients can be identified and used safely.

[14] Heusinkveld H. et al. (2020) Towards a mechanism-based approach for the prediction of nongenotoxic carcinogenic potential of agrochemicals. Critical Reviews in Toxicology 50 https://doi.org/10.1080/10408444.2020.1841732

[15] Luijten M. et al (2020) A comprehensive view on mechanistic approaches for cancer risk assessment of non-genotoxic agrochemicals. Regulatory Toxicology and Pharmacology 118 https://doi.org/10.1016/j.yrtph.2020.104789

The current legislation in Europe for the safety evaluation of chemicals (REACH: 1907/2006) and cosmetics (EU Cosmetics Regulation: 1223/2009) includes the requirement to assess the skin sensitisation potential of a substance or formulation. The focus of intensive previous research of many scientists has been the assessment without the use of animals, and as a result several validated NAMs are accepted as OECD Test Guidelines (TGs). These and other approaches are being increasingly used as part of defined approaches to inform Integrated Approaches to Testing and Assessment (IATA) for skin sensitisation.

This project has focused on training and peer-to-peer knowledge-sharing since the EPAA Partners Forum (PF) on "Skin Sensitisation new approach methodologies (NAMs)" held in Brussels in October 2019[16].

Recommendations from the 2019 Workshop and Partners Forum have been followed-up in 2020-21 through (a) an exchange of ideas in a "User Forum" including practical experience for regulatory decision-making and (b) EPAA-sponsored training sessions including an online training successfully completed at WC11 (Maastricht, 2021) in collaboration with Altertox academy. Presentations were given by NICEATM and Industry members of EPAA.

The User Forum as a mechanism to build confidence in the use of NAMs is being evaluated by the Skin Sensitisation group. Three successful, trial Skin Sensitisation User Forum sessions took place in 2020 and three more in 2021; each focused on a case study presentation followed by Q&A with 10+ organizations (EPAA members) participating each time. For example, in 2021 the three User Forum sessions focused on Next Generation Risk Assessment case studies for Skin Sensitisation provided by Cosmetics Europe.

h. *In Vitro* to *In Vivo* Extrapolation

Testing an algorithm for quantitative in vitro to in vivo extrapolation (QIVIVE)

The overall context of this work is the development of reliable non-animal, *in vitro* bioassay-based testing strategies for human safety testing of chemicals. Predictions of human exposure and safety assessments of chemicals are increasingly based on *in vitro* data using software applications and have the potential to improve human safety assessments by, for example, application of ADME (Absorption, Distribution, Metabolism and Excretion) prediction capacity with important reductions in the number of animals used for metabolism and toxicity studies. However, the existing PBTK (physiologically based toxicokinetic) models have limited utility particularly in accounting for human inter-individual variability. Therefore, for more widespread adoption of these models, it is important to address the limitations of the existing algorithms.

The aim of this project is to test the effectiveness of a computational algorithm developed to convert *in vitro* concentration-response data to *in vivo* dose-response data (known as quantitative *in vitro* to *in vivo* extrapolation or QIVIVE) and to evaluate its applicability to Bisphenol A, Chlorpyriphos and Perfluorooctanoic acid.

[16] Basketter D. et al. (2020) Building Confidence in Skin Sensitisation Potency Assessment Using New Approach Methodologies: Report of the 3rd EPAA Partners Forum, Brussels, 28th October 2019. Regulatory Toxicology and Pharmacology 117 https://doi.org/10.1016/j.yrtph.2020.104767

This is intended to show applicability of the algorithm to a diverse range of chemicals from several industrial sectors. Regulatory acceptance will most likely depend on the biological relevance of the alternative-to-animals assay systems and not on the computational algorithm used for QIVIVE. The early involvement of regulators from EC or EU Agencies in the project will facilitate the future steps needed to achieve regulatory acceptance of the data generated with this approach.

This EPAA project builds on an earlier EPAA and Cefic LRI supported project to develop software applications for user-friendly *in vitro* / *in vivo* exposure prediction. It is carried out by the same team in the UK Health and Safety Executive Science and Research Centre with advice of experts from EC, DG JRC, EFSA and industry. The project complements the project on PBK Modelling in Safety assessments (see below).

The project has translated in vitro concentration-response relationships to in vivo dose-responses for toxicologically important chemicals selected as case studies, determined in vivo benchmark dose (BMD) values from the translated data, and compared the predicted in vivo BMD to existing experimental BMD values used in chemical safety assessments by regulatory agencies such as EFSA and US EPA. PBPK models for Perfluorooctanoic acid, Chlorpyriphos and Bisphenol A have been built. Algorithms have been tested including in vitro concentration – response data and data with known adverse outcome pathways (AOP) associations; results include population estimates and global sensitivity analysis in using in vitro dose data to characterise in vivo dose effects. The outputs from this work include characterisation of specific uncertainties associated with the computational tools and in vitro data used which have been presented according to the OECD PBK template currently under development. The suitability, utility and concordance of in vitro assay systems have been examined.

In the project, a major computational challenge was the use of R (open-access statistical modelling language) which had significant limitations (long execution times) when used for large and complex physiologically based pharmacokinetic (PBPK) models. This has been resolved by using MCSim syntax[17] to provide short execution times. To maximise significance, the project has used RVis (originally funded by EPAA and Cefic LRI) which has been updated to run, visualise and optimise PBPK models in both MCSim and R syntaxes[18].

A paper for derivation of human BMD using QIVIVE for Perfluorooctanoic acid has been published[19] , a paper for Bisphenol A has been accepted for publication[20] and the manuscript for Chlorpyrifos is in preparation. The potential for development of QIVIVE training courses is being evaluated.

[17] https://www.gnu.org/software/mcsim

[18] http://cefic-lri.org/projects/aimt7-rvis-open-access-pbpk-modelling-platform/

[19] Loizou G, McNally K, Dorne J-LCM, Hogg A (2021) Derivation of a Human *In Vivo* Benchmark Dose for Perfluorooctanoic Acid From ToxCast *In Vitro* Concentration–Response Data Using a Computational Workflow for Probabilistic Quantitative *In Vitro* to *In Vivo* Extrapolation. Frontiers in Pharmacology 12. https://doi.org/10.3389/fphar.2021.630457

[20] Loizou G, McNally K, Paini A, Hogg A (2021) Derivation of a Human *In Vivo* Benchmark Dose for Bisphenol A from ToxCast *In Vitro* Concentration Response Data Using a Computational Workflow for Probabilistic Quantitative *In Vitro* to *In Vivo* Extrapolation. Frontiers in Pharmacology: Predictive Toxicology in press

i. PBK Modelling in Safety assessments

Tools to support application of physiologically based kinetic (PBK) modelling in safety assessment

Read-across is increasingly being used by regulators and others as a non-animal alternative, whereby data from one or more source chemicals is used to predict the effect of a target chemical of interest. However, as identified in the first EPAA Partners Forum[21], the main barrier to greater uptake is the limited kinetic data available for source and target chemicals. Increased utilisation of read-across through improved PBK modelling is expected to lead to less reliance on animal testing and greater confidence in safety predictions.

For any chemical (food additive, drug, cosmetic, pesticide etc.) to have an effect, the chemical (or its transformation product) must not only possess intrinsic activity but must also reach the relevant site of action at sufficient concentration. Hence, for more reliable risk assessment, consideration must be given to both intrinsic activity and internal exposure. Physiologically based kinetic (PBK) models are used to predict the overall time-concentration curves for chemicals in blood / organs, and are increasingly used by industry, academia and regulators. The models can be used in conjunction with pharmacological or toxicological information to determine the true potential of a chemical to elicit an effect, desirable or undesirable. One of the advantages of using information from PBK models, is that organ-level concentrations and effects on sensitive individuals can be identified and taken into consideration.

This project began in 2019 and is led by Liverpool John Moores University working in conjunction with EC, EURL ECVAM, CEFIC LRI, Cosmetics Europe and industry partners together with US EPA, and US and EU advisors. Coordination between this project and the QIVIVE project is being facilitated.

The project has four aims to support PBK modelling applications in safety assessment, as follows: (a) conduct and publish a complete systematic review and collation of existing, published PBK models in rats and humans (and other mammals) to provide a readily updatable resource for PBK model developers and users, (b) assess the chemical space coverage of existing PBK models in relation food additives, drugs, cosmetics, pesticides and industrial chemicals, (c) investigate similarity assessment metrics (e.g. chemical fingerprints) to determine the most appropriate for selecting analogues for PBK development and (d) develop a freely available software tool to assist the identification of appropriate analogues via an automated workflow.

The project has completed and published a systematic review of PBK models and a comparison of chemical space with datasets for pharmaceuticals, botanicals, pesticides, cosmetics, food additives and REACH chemicals[22]. A protocol for the formal systematic review had been previously accepted and published[23].

[21] Laroche C. et al. (2018) Finding synergies for 3Rs – Toxicokinetics and read-across: Report from an EPAA Partners' Forum. Regulatory Toxicology and Pharmacology 99 (2018) 5–21. https://doi.org/10.1016/j.yrtph.2018.08.006

[22] Thompson CV. et al. (2021) A systematic review of published physiologically-based kinetic models and an assessment of their chemical space coverage. Alternatives to Laboratory Animals in press

[23] Thompson C, Madden J, Penson P. (2020) Systematic review to determine the chemical space of existing physiologically-based kinetic (PBK) models. PROSPERO 2020 CRD42020171130 https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020171130

The data extraction was made from 1638 papers, and resulted in 7533 individual models, for 1888 chemical names and 1186 InChIKeys (representing unique chemicals), in ≥21 species; it is compiled in a large spreadsheet tool[24] which facilitates hierarchical searching of existing models. It is anticipated that additional models will be added to this spreadsheet and a further update released in future.

The influence of physico-chemical property estimation and analogue selection on model quality is being investigated and case studies are being developed for Atenolol and selected pesticides. Further work is planned to include development of an initial PBK model for a target chemical based on a model for an analogue and optimization of the analogue selection process. Initial results from the project have also been disseminated in oral and poster presentations at the conferences QSAR 2021 (https://www.ascctox.org/qsar2021/qsar-2021-program), the WC11 (Maastricht, 2021) and at the EURL ECVAM JRC Summer School on Non-Animal Approaches in Science (2021).

j. Non-animal science in regulatory decisions for chemical safety (new project)

Opportunities to use non-animal science in regulatory decisions for chemical safety in the EU

The European Union has long been committed to promoting the development and validation of approaches to assuring safety that do not rely on animal testing. Considering of the EU Directive on the protection of animals used for scientific purposes (Directive 2010 /63/EU), the use of guideline and non-guideline test methods not requiring experimental animals is encouraged in all sectors of EU Chemicals Policy.

A large number of animals is currently used in the EU to comply with the demands of REACH. It is anticipated that this number could increase with the current ambitions of EU Chemical Strategy for Sustainability (CSS). If new approach methodologies (NAMs) can be used to fulfil the information requirements of this legislation in areas where animal tests are currently demanded this would cause a decrease in the number of animals used in the EU for chemicals registration. There is a commitment to non-animal approaches in REACH, which can provide the same level of information as current animal tests. However, it is quite possible that similar (or better) protection of human health could be provided using the modern science and understanding of human biology from NAMs without necessarily predicting the effects seen in the current, high-dose rodent studies.

NAMs are increasingly used within industry to make decisions about the human safety of chemical exposures prior to manufacturing new products. NAMs, as well as next generation risk assessment (NGRA) methodologies, are already used in the cosmetics sector for regulatory purposes (where the ban on animal testing for cosmetics purposes has driven innovation in risk assessment). Recently, there has also been uptake of the NGRA approach into the 11th Revision of the Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation from the Scientific Committee of Consumer Safety (SCCS/1628 /21).

[24] Sayre R. et al. (2019) Existing physiologically-based kinetic (PBK) models, identified via PubMed searches (with associated EndNote library). figshare. Dataset. https://doi.org/10.6084/m9.figshare.10075574.v1

In addition, The European Food Safety Authority (EFSA) has recently published a scientific opinion on Development of Integrated Approaches to Testing and Assessment (IATA) case studies on developmental neurotoxicity risk assessment as well as initiating an activity to define a roadmap on the use of NAMs in risk assessment with a goal to routinely use NAMs to address data gaps by 2027.

Over a period of one year from 4Q 2021, this project aims to provide a cross Industry/EC environment for creative appraisal of current use of NAMs for decision making and to define what is needed to increase the confidence to use NAMs more routinely for Chemicals Registration. In particular, the project will open a discussion around safety decision-making using information from NAMs that may not be direct surrogates for the output from traditional animal data since this is perceived as a hurdle to progress with regulatory uptake (e.g., REACH Annex XI).

The focus of this project is on actual experience of EPAA partners in the use of NAMs for decision-making and exchange of this between the Industry sectors and Commission partners. EPAA is well placed to do this work as the partners represent both industry sectors currently working with NAMs for decision-making and the EC scientists involved with discussions on use of NAMs, e.g. in the APCRA programme (Accelerating the Pace of Chemical Risk Assessment). The topic is very relevant to the reduction of animal usage in REACH and the implementation of the Chemical Strategy for Sustainability.

The project will start with a 'deep-dive' workshop (virtual) in the last quarter of 2021. This will facilitate information exchange between the partners of how NAM's are being used / considered for regulatory use in safety assessment and registration (excluding non-animal approaches currently used routinely in regulatory submissions e.g. in vitro OECD TG studies). The discussion will involve NAM users talking about their experiences and should allow an exploration of:

a. Are there circumstances where NAMs could be used for safety assessments in different sectors or to provide information for the classification and labelling of ingredients in the EU – regardless of tonnage – across different safety endpoints? (e.g., low tonnage compounds in REACH where industry still needs to make decisions on safety prior to use e.g., systemic safety, carcinogenicity);

b. How could NAMs be used to provide alternate DNELs (derived no-effect levels of exposure with consideration of appropriate uncertainty factors) for decision-making in a different way from traditional toxicology testing whilst still providing robust information on safety?
c. Could a and b contribute significantly and more quickly to the EU Chemicals Strategy for Sustainability: 'One substance – one assessment'?

This scientific exchange will focus on programmes particularly relevant to EPAA partners including:

- *Cosmetics Europe LRSS Case Studies/Framework using NAMs for Decisions on Consumer Safety*
- *ECETOC Case Studies/Framework from the Transformational Programme on NAMs for use in a tiered approach to REACH gap-filling*
- *The Commission's APCRA Case Studies including the work on Margin of Safety with NAMs*
- *EFSA activities on NAMs including the work on an IATA for DNT*
- *OECD IATA for non-genotoxic carcinogens*
- *Other relevant EPAA partner activities (e.g. from the pharmaceutical sector)*

Discussions will aim to identify what the biggest challenges are that face policy makers and users of NAMs. Challenges that may be particularly relevant include (but are not limited to) knowing when to embrace NAMs, the competency of risk assessors to use NAMs in decision-making, the market availability of NAMs and building trust in the use of NAMs to deliver risk assessments which will maintain the EU's reputation for protection of human health. A workshop report would identify areas for follow-up.

Following the initial 'deep-dive' workshop in 2021, it is anticipated that subsequent working sessions will be held in 2022 to develop recommendations regarding what is needed (scientific, political, legal, operational) to gain confidence in using NAMs within Chemicals Registration. The possibility of shaping follow-up practical projects under the EPAA would also be discussed if appropriate opportunities emerge.

3. Dissemination and Communication

a) Refinement Prize 2021

The EPAA Refinement Prize of €6000 is granted every other year to a laboratory technician, animal care- taker or technologist who has demonstrated outstanding achievements in new, novel approaches to advance implementation and/or awareness raising of refinement of animal testing.

Refinement PRIZE 2021 | epaa The European Partnership for Alternative Approaches to Animal Testing

Assessment is conducted over 5 selection criteria defined by the EPAA Steering Committee:

a) Impact on animal welfare – evidence based

b) Creativity and innovation

c) Practicability, applicability and implementation potential to regulatory testing and/or quality control

d) Potential for wider impact beyond immediate area

e) Publication of data or potential for publication

In 2021, a total of 9 high-caliber applications were submitted to the EPAA secretariat and evaluated by the selection committee. The highest score was attributed to Inês Mendes Preguiça from the Faculty of Medicine of the University of Coimbra. Her case study HaPILLness focuses on « Precise voluntary oral drug dosing in rodents – an innovative 3Rs approach ».

Quotes from the Selection Committee

> "Improvement in terms of animal welfare & study quality, thus potentially avoiding repetition of study"

> "Proven suitability for different drugs"

> "Thorough protocol, well designed and applicable to a wide range of substances"

b) 3Rs Student Grants 2021

Every year, several high-profile international meetings bring together world-class scientists working on the development and acceptance of 3R alternatives to animal testing (Replacement, Reduction or Refinement). Costs linked to participation may prevent students with promising work or young scientists at the beginning of their career from attending these events. The EPAA partners are therefore happy to sponsor the 3Rs student grants to facilitate the participation of students and young scientists in such events.

In 2021, four grants were given in total.
Two grants were offered to students presenting their work at the 11th World Congress on Alternatives and Animal use in Life Sciences (WC11).

2021 3Rs in transition From development to application VIRTUAL CONGRESS - AUGUST 2021

1. Carlo Alberto Paggi "The paradox of chondrocytes and chondrosarcoma cells: do they behave similarly when exposed to mechanical cues?"
2. Melanie Pahl "In vitro screening for developmental neurotoxicity by using a human cell-based testing battery: A case study of flame retardants"

Two more grants were given to students presenting at the EUROTOX 2021.

EUROTOX 2021 VIRTUAL CONGRESS 27 SEPTEMBER — 1 OCTOBER 2021

1. Julia Hartmann "Human induced pluripotent stem cell (hiPSC)-derived neural progenitor cells as brain region-specific models for neurotoxicity testing"
2. Saskia Galanjuk "Characterization of the Human Induced Pluripotent Stem Cell (HIPS)-Test to Predict Embryotoxicity"

c) EPAA at the 11th World Congress on Alternatives and Animal Use in Life Sciences(WC11)

The 11th World Congress was postponed due to COVID-19 pandemic and was held from 23 August – 2 September 2021 with the theme: "3Rs in transition: from development to application". This emphasizes the increasing focus on the use of better, more human-relevant models for personalized and preventive health care, safer products and innovative research.

The scientific program of the congress was drawn around four main themes:

- Safety
- Disease
- Innovative technologies, and
- Ethics and Welfare

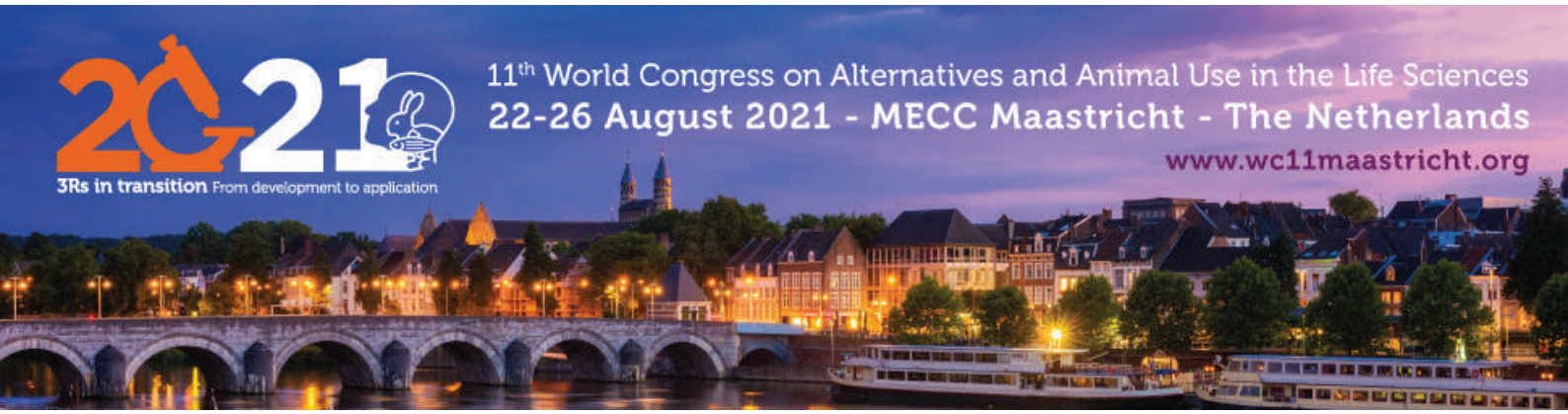
EPAA's participation at the WC11 was three-fold:

1. EPAA training session on Skin Sensitisation in collaboration with Altertox Academy.

2. A sponsored session called “Promoting the use of 3Rs through partnership: EPAA”
3. An EPAA virtual booth with information about the Partnership, its membership and activities

Promoting the use of 3Rs through partnership: EPAA

Timing CET	Title of presentation	Speaker
18:30-18:45	A public private partnership facilitating the development and uptake of 3R approaches	Giacomo Mattino’, EPAA Co-Chair (EC, DG GROW, BE)
18:45 -19:00	New ideas for assessing systemic toxicity	George Daston (P&G, US)
19:00-19:15	Harmonisation of 3Rs in Biologicals	Katrin Schutte (EC, DG ENV, BE)
19:15-19:30	Optimal duration of non-clinical studies to assess the safety of monoclonal antibodies	Peter van Meer (Medicines Evaluation Board, NL)
19:30-19:45	Tools to Support Application of Physiologically-Based Kinetic Modelling in Safety Assessment	Judith Madden (Liverpool John Moores University, UK)
19:45-20:00	Promoting and building confidence on the use of 3Rs	Rob Roggeband, EPAA Co-Chair (P&G, BE)
20:00-20:30	Q&A	All speakers



d) EPAA events

- Annual Conference, “How can EPAA help the successful implementation of the EU Chemical Strategy for Sustainability” (27 October 2021)

- EDQM-EPAA-DG JRC Workshop on “Phasing out animal testing for in-process control of veterinary vaccines: Clostridium septicum as a proof of concept” (9-10 March 2021)
- EPAA-NC3Rs-MEB “Workshop on optimal duration of non-clinical safety studies for human mAbs” (26 & 28 April 2021)
- 3 User Forum sessions on skin sensitisation on 24 April, 2 July and 15 October 2021
- EPAA-Altertox Academy Skin sensitisation training session at WC11 (27 October 2021)
- EPAA “NAMs Deep-Dive Workshop” (planned for 23-24 November 2021)

e) External events

EPAA projects and achievements were presented to a number of scientific events:

- EPAA booth at the 11th World Congress on Alternatives and Animal Use in life Sciences (23 August – 2 September 2021)

- Participation of Rob Roggeband at the BfR Symposium: Challenges in Public Health Protection in the 21st Century: New Methods, Omics and Novel Concepts in Toxicology; Workshop on Future concepts and strategies in Berlin (15-17 November 2021)

- Presentation by Marie-Emmanuelle Behr-Gross of "Update on the EPAA projects on vaccine" at the Flanders Vaccines Conference (14 October 2021)

f) Publication

In 2021, four (4) peer-reviewed publications have been produced reporting results from EPAA-supported projects:

Behr-Gross M-E, Siklodi B, Le Tallec D, Halder M, Manou I, Sinitskaya N, Bruckner L, Dalmadi B, Kiss L, Redhead K. (2021) Collaborative study for the validation of cell line assays for in-process toxicity and antigenicity testing of Clostridium septicum vaccine antigens - Part 2: Optimisation of cell line assays. Pharmeuropa Bio Sci Notes (2021) 101-156 https://pharmeuropa.edqm.eu/app/BioSN/content/BioSN-0/2021-5-Clostridium-septicum-vaccine-antigens-Part-2.pdf

Loizou G, McNally K, Dorne J-LCM, Hogg A (2021) Derivation of a Human *In Vivo* Benchmark Dose for Perfluorooctanoic Acid From ToxCast *In Vitro* Concentration–Response Data Using a Computational Workflow for Probabilistic Quantitative *In Vitro* to *In Vivo* Extrapolation. Frontiers in Pharmacology 12. 630457. https://doi.org/10.3389/fphar.2021.630457

Loizou G, McNally K, Paini A, Hogg A (2021) Derivation of a Human *In Vivo* Benchmark Dose for Bisphenol A from ToxCast *In Vitro* Concentration Response Data Using a Computational Workflow for Probabilistic Quantitative *In Vitro* to *In Vivo* Extrapolation. Frontiers in Pharmacology: Predictive Toxicology (in press)

Thompson CV, Firman JW, Goldsmith MR, Grulke CM, Tan YM, Paini A, Penson PE, Sayre R, Webb S and Madden JC (2021) A systematic review of published physiologically-based kinetic models and an assessment of their chemical space coverage. Alternatives to Laboratory Animals (in press)

g) Social media

Twitter

Edit profile

EPAA
@EPAA3Rs

European Partnership for Alternative approaches to Animal testing. One of the leading PPPs to promote 3Rs.

Joined March 2013

410 Following **1,479** Followers

LinkedIn

Manage my network

Connections	1,182
Contacts	2,155
People I Follow	2
Groups	18
Events	
Pages	52
Newsletters	
Hashtags	4

4. Future Prospects

The EPAA activities planned for 2022 will follow the priorities identified in the Action Programme (2021-2025). Special attention will be paid through the new EPAA project on NAMs to help the implementation of the Chemical Strategy for Sustainability (CSS). The EPAA also plans to collaborate with important EU-funded research consortia, like PARC and ONTOX, with which it shares common goals for the promotion and regulatory acceptance of 3Rs approaches.
As soon as the public health situation related to the COVID crisis allows, the EPAA will hold a face-to-face Partners Forum on “Exposure considerations in Human Safety Assessment”.

The EPAA Mirror Group remains a core element of the partnership’s structure. Based on its composition, consisting of experts from a wide range of external stakeholders having an interest in the 3Rs and alternatives to animal testing, the group brings clear added value to the work of EPAA.
The EPAA SC highly appreciates the great input provided by the Mirror Group in 2021, with better organized and coordinated contributions thanks to the appointment of a MG Chair. The partnership hopes to continue receiving the support and active participation of all the MG members, including from the MEP Sirpa Pietikäinen (Honorary Chair of the EP Intergroup on animal welfare).

5. Membership Update

In 2021, EPAA welcomed 2 new members: SASOL and GOWAN Crop Protection. The Partnership includes now 5 Directorates-General of the European Commission, 38 companies, and 8 European industry federations, each representing a separate industrial sector. Further information is available at the EPAA website: https://ec.europa.eu/growth/sectors/chemicals/epaa/partners_en

38 companies (incl. 1 SME)

8 Sectoral associations

5 DG's of the EC

1. DG GROW
2. DG ENV
3. DG SANTE
4. DG JRC
5. DG RTD

6. Acronyms and Abbreviations

3Rs: Replacement, Reduction and Refinement of Animal Testing
3T3 NRU PT: Neutral Red Uptake Photo-toxicity assay using the 3T3 mouse fibroblast cell line
AAT: Alternatives to Animal Testing
ANSES: Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, FR
BCOP: Bovine Corneal Opacity & Permeability Assay
BfR: Bundesinstitut fur Risikobewertung, DE
BSP: Biologicals Standardisation Programme
CEFIC: European Chemical Industry Council
CLP: Classification and Labelling of Products
CMR: substances that are carcinogenic, mutagenic or toxic to reproduction
CSS: Chemical Strategy for Sustainability
DG: Directorate General (of the European Commission)
DG ENV: European Commission Directorate-General for Environment
DG GROW: European Commission Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs
DG JRC: European Commission Directorate-General Joint Research Centre
DG RTD: European Commission Directorate-General for Research and Innovation
DG SANTE: European Commission Directorate-General for Health and Food Safety
EC: European Commission
ECHA: European Chemicals Agency
EDQM: European Directorate for the Quality of Medicines & HealthCare (Council of Europe)
EFPIA: European Federation of Pharmaceutical Industries and Associations
ELISA: Enzyme Linked Immunosorbent Assay
EMA: European Medicines Agency
EP: European Parliament
EPAA: European Partnership for Alternative Approaches to Animal Testing
EURL ECVAM: The European Union Reference Laboratory for Alternatives to Animal Testing
EUROTOX: Association of European Toxicologists and European Societies of Toxicology
EUSAAT: European Society For Alternatives To Animal Testing
EUToxRisk: An Integrated European 'Flagship' Programme Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st century
IATA: Integrated Approaches to Testing and Assessment
IMI: Innovative Medicines Initiative
ITS: Integrated testing strategies
JEG 3Rs: Joint Expert Group on 3Rs
MGEN: Model Equation Generator software
MEB: Medicines Evaluation Board
NAMs: New Approach Methodologies
NC3Rs: National Centre for 3Rs (UK)
OECD: Organisation for Economic Co-operation and Development
PBTK: Physiologically-Based Toxicokinetic
REACh: Registration, Evaluation, Authorisation and Restriction of Chemicals
RVis: R Visual; a prototype for the analysis of structure and performance of PBPK, and other models, written in the free, open source syntax R or C++
SEURAT-1: Safety Evaluation Ultimately Replacing Animal Testing
WC11: 11th World Congress on Alternatives and Animal Use in Life Sciences
WHO: World Health Organisation

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https://ec.europa.eu/growth/sectors/chemicals/epaa_en