

ANNUAL REPORT 2022

*European Partnership for Alternative
Approaches to Animal testing (EPAA)*



The European Partnership
for Alternative Approaches to Animal Testing





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1. Foreword

On 15th Sept 2021 the EU Parliament adopted a resolution to ‘Accelerate a Transition to Innovation without the use of Animals in Research, Regulatory Testing and Education’ calling for an action plan with ambitious objectives, reduction targets and replacement timelines. This resolution has not only set the tone for EPAA activities throughout 2022 but has also reset expectations within Europe as implementation of the European Green Deal (EGD), EU Chemical Strategy for Sustainability (CSS) and the Pharmaceutical Strategy for Europe continue at pace.

This ‘reset’ was clear during the [Helsinki Chemicals Forum 2022](#) (8th-9th June 2022) when several EPAA partners participated in a panel on ‘How to accelerate the replacement of animal toxicity testing?’ hosted by Dr Patience Brown (OECD). It was also palpable in the session on ‘[New Approach Methodologies: Moving Beyond Animal Testing](#)’ at the [ONE - Health, Environment, Society Conference 2022](#) (21st-24th June 2022), chaired by Dr Maurice Whelan (JRC), where Dr Sylvia Escher (Fraunhofer ITEM) presented the [EFSA roadmap](#). This renewed focus on replacement has re-energised the unique collaborative partnership at the heart of the EPAA as we seek to accelerate the transition to animal-free, sustainable innovation together.

Our EPAA Partners Forum this year (6th May & 14th November 2022) has focussed on the broad topic of ‘Exposure Consideration for Human Safety Assessment’ aiming to identify opportunities for refinement, reduction or replacement of regulatory animal testing through increased cross-sector collaboration, focus areas for industry-regulator exchange and remaining challenges for further research investment as we review the role of exposure science in the human safety assessment of chemicals, foods and medicines.

In parallel, our EPAA ‘Use of NAMs in Regulatory Decisions for Chemical Safety’ project has begun addressing the conclusions of the 2021 Deep-dive workshop ([Westmoreland et al. 2022](#)) through establishing three workgroups starting with ‘Frameworks for the use of NAMs for regulatory decisions on chemical safety’ who have been discussing the recent ECETOC publication ‘A

framework for chemical safety assessment incorporating new approach methodologies within REACH’ ([Ball et al. 2022](#)). The second EPAA NAMs project workgroup will scope and establish an EPAA ‘NAM User Forum,’ building on the success of the ongoing EPAA Skin Sensitisation User Forum, and a new workgroup will identify opportunities for early application of ongoing scientific research that seek to address regulatory testing requirements in 2023.

On 13th Sept 2022, representatives from EPAA visited the EU Parliament in Strasbourg to participate in an EPAA Lunchtime Debate on ‘Accelerating the Transition to Animal-Free, Sustainable Innovation’ hosted by Tilly Metz, MEP. The MEPs who attended welcomed the progress that EPAA has made in the last year whilst reiterating their expectation to receive a roadmap providing details on the implementation of the actions outlined in the EP Resolution on ‘Accelerate a Transition to Innovation without the use of Animals in Research, Regulatory Testing and Education’.

Finally, as EPAA co-Chairs we would like to thank all EPAA partners and mirror group members for their contributions, help and support that have collectively made 2022 a landmark year for the partnership.



Giacomo MATTINÒ

EPAA European Commission
Co-Chair



Gavin MAXWELL

EPAA Industry Co-Chair

2. Overview of the Project Platform in 2022

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 pooling knowledge and resources to accelerate the development, validation and acceptance of alternative approaches. Replacement methods embrace increasing knowledge of toxicity mechanisms together with data from New Approach Methodologies (NAMs) that are utilised in Defined Approaches (DAs) and Integrated Approaches to Testing and Assessment (IATA), to allow less and less dependence on animal tests for assessment of human and environmental safety. The EPAA projects overseen by the Project Platform (PP) aim to develop NAMs that fill critical information gaps, demonstrate applicability of NAMs to regulatory decision-making (often supported by case studies), and engage and communicate with stakeholders in EU and globally. For some of the most complex systemic toxicity endpoints complete replacement of animals in safety studies using NAMs approaches is not yet possible however, PP projects such as Monoclonal Antibody Safety and Acute Toxicity are providing objective evidence to enable very welcome reductions and refinements of animal use in regulatory studies.

The PP is composed of EPAA partners and associates that either lead the individual projects agreed upon by the EPAA Steering Committee or are there to supervise them ensuring scientific quality and effectiveness. In 2022, the PP has supported nine project teams which synergistically combine the expertise and collaboration available across industry sectors, academia, NGOs and regulatory agencies. Of the nine projects, three are in the dissemination phase and approaching completion, namely Clostridial Vaccines (for veterinary use), PBK¹ Modelling in Safety Assessments and Monoclonal Antibody Safety. Throughout the 2020-2022 period, each team has worked effectively to maintain

excellent progress despite the often major constraints of the COVID-19 pandemic and it has been pleasing in 2022 to

a. Projects in 2022

- 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. Skin Sensitisation Dissemination (User Forum on use of NAMs)
- h. PBK Modelling in Safety assessments
- i. Non-animal science (NAMs) in regulatory decisions for chemical safety

gradually return to in-person discussions for some of the more important meetings, workshops and conferences.

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 2022) on each individual project which are provided in orange, italicised text.***

a. Clostridial Vaccines for veterinary use

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

All laboratory work for the project has been completed and the project has resulted in appropriate and important revision of European Pharmacopoeia (Ph. Eur.) monographs.



¹ Physiologically Based Kinetic

The project is now focused on dissemination of results.

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 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 was 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.

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 reproducibility and showed excellent concordance with the animal-based tests currently used by vaccine manufacturers. Furthermore, based on the results presented and discussed at a dedicated workshop² it was postulated that optimisation of the *in vitro* assays evaluated in the first study³ 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 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 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 assays⁴ for in-process toxicity and antigenicity testing of *C. septicum* vaccines outperform the animal-based methods. This will allow full advantage of the superior sensitivity and accuracy of the *in vitro* MLD and TCP tests to be taken when manufacturers implement these alternatives as in-process controls.



2 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

3 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

4 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>

5 https://proceedings.altex.org/data/2017-01/WC10_entire_issue1.pdf, page 234

6 https://proceedings.altex.org/data/2021-01/altex_WC11.pdf, page 140

7 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>

8 <https://freepub.edqm.eu/publications/PUBSD-168/detail>

In light of the results of the project, the European Pharmacopoeia (Ph. Eur.) Group of Experts 15V revised the monographs for veterinary vaccines against cytotoxic Clostridia to introduce in vitro methods. Subsequent to the Ph. Eur. public inquiry the revised monographs were adopted by the Ph. Eur. Commission in June 2021, published in Ph. Eur. 10.8 and implemented on 1st July 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 of final product.

The results of the validation of the Vero cell line-based methods were discussed at an EDQM - EPAA workshop² and were presented at the 10th World Congress on Alternatives and Animal Use in the Life Sciences (Seattle, 2017)⁵. The regulatory consequences of the study were presented at 11th World Congress on Alternatives and Animal Use in the Life Sciences (Maastricht, 2021)⁶. The outcomes of the first and second collaborative studies have now been published^{2,3}. 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 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 online⁷ together with the webinar proceedings⁸. An additional paper on the optimisation of the in vitro TCP and MLD methods is planned for publication in a peer reviewed journal.

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)⁹.

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.

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.

Phases 2 and 3 of the study are being conducted in 2022; an inter-laboratory comparison of different vaccines, statistical analyses and a report are expected to be completed before the end of the year. 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. Preliminary results are very encouraging including across different vaccine manufacturers. Statistical analysis showed very good transferability of the ELISA method between laboratories (variability of ELISA results well within expected limits). A final report of Phase 2 is anticipated at the end of 2022 and an international workshop on Phase 2 results is planned for the beginning of 2023. Phase 3 which includes testing in production will continue through 2023.



9 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>

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). *Further publication and dissemination of the results is planned for 2023.*

c. Acute Toxicity *Identification of clinical signs predictive of mortality*

Mammalian acute toxicity testing remains a requirement for chemicals, agrochemicals and biocide 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.)¹⁰ were revised in 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 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.

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” based on clinical signs (in contrast to mortality) appears to be preventing wider uptake of the TG 420, and it is not currently the test of choice.

Data (including mortality, clinical signs and body weight) from previous acute oral toxicity studies have now been 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 90 studies (from an initial 250) suitable for statistical analysis and which provide wide coverage of different chemical classes and industry sectors (agrochemical, cosmetics, chemicals, food, pharmaceuticals and others). The results are very encouraging, indicating that certain individual clinical signs or combinations of 2-3 clinical signs can be predictive of mortality at the higher dose. If these signs are observed in more than one animal during an acute oral toxicity study, there is no need to use a higher dose, since the lower dose demonstrates that evident toxicity has been reached. Testing at a higher dose will provide no additional information and will likely result in animal death or severe suffering. The project has provided objective data demonstrating that death is not a necessary endpoint, allowing substantial avoidance of morbidity and mortality in acute toxicity studies. This enables the development of guidance to aid the recognition of “evident toxicity” to support wider use of the FDP over other currently accepted methods and has the



¹⁰ 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

potential to reduce the suffering and numbers of animals used when in vivo acute oral toxicity studies are required.

The project dissemination plan includes a peer-reviewed publication, and presentation at international conferences which has already included poster presentations at Society of Toxicology (San Diego 2022) and ICT / Eurotox (XVth International Congress of Toxicology, joint meeting of the International Union of Toxicology (IUTOX) and the European Society of Toxicology, Maastricht 2022). The project advocacy plan includes further liaison with OECD regarding guidance in TG 420, publication of the project findings together with additional information on the NC3Rs website and reviewing with regulatory authorities.

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 recommendations of an EPAA hosted international workshop¹¹ for vaccine **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. Considerable progress has been made in the area of potency testing, although achieving fully aligned global regulatory approaches to testing requirements based on alternative methods

remains a challenge. The project initially focused on, and has successfully contributed to the deletion of regulatory requirements for *in vivo* tests of innocuity in Ph. Eur. Monographs and WHO recommendations.

The EPAA workshop had 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. 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



11 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>

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, WOA (World Organisation for Animal Health, founded on 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/WOA, two chapters of the WOA Terrestrial Manual¹² 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 disregarded¹³. WHO have communicated to testing laboratories in several countries to emphasise that the general toxicity tests are no longer required¹⁴. 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)¹⁵ 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).

Two new areas of project activity were identified through earlier consultation with users in Member states and agencies.

Progress in these areas has continued in 2022:

*Pyrogenicity testing is relevant to a wide range of products including vaccines, chemicals and blood products. The Ph. Eur. monographs encourage replacement of **pyrogen testing in rabbits** by suitable alternative methods, however, more than 50 Ph. Eur. product-specific monographs mention the rabbit pyrogen test (RPT) and not the alternatives. As such, the rabbit test continues to be used widely. Moreover, *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 was the need for more training of users in non-animal methods, in particular the monocyte activation test (MAT).*

Stimulated by the EPAA project team, EDQM defined a strategy in June 2021 to amend Ph. Eur. monographs

12 <https://www.woah.org/en/what-we-do/standards/codes-and-manuals/>

13 (<https://apps.who.int/iris/bitstream/handle/10665/325184/9789241210256-eng.pdf?ua=1>)

14 https://www.who.int/immunization_standards/vaccine_quality/who_nnb/en

15 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>

(removal of RPT) in the years to 2026¹⁶. New chapters on MAT for vaccines and on pyrogenicity were published for comments in July 2022. Revision of various chapters will then begin in 2023.

In addition, a new chapter of the 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 blood extract.

*The EDQM and EPAA are evaluating the potential application of next generation sequencing and other technologies for detection of endotoxin. A publication in *Pharmeuropa Bio* is planned for January 2023 to be followed in February by a joint EDQM - EPAA dedicated workshop to discuss deletion of RPT together with a training in MAT. The event will be public, open to all stakeholders impacted by RPT deletion (e.g., health authorities, industry users, service providers) and would seek international perspectives to support global alignment by promoting alternative assays such as MAT and BET. 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.*

In 2022, the project is also exploring further 3Rs opportunities in the area of human vaccines (for example, rabies, tick borne encephalitis, pertussis) including those arising from the recently completed EU Vac2Vac project.

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 and is nearing completion in 2022 (see below).

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 (mAbs) 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, Determine the value of 6-month repeat-dose toxicity studies and the potential to replace or refine these. (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 the UK National Centre for the 3Rs (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 86% of cases, long-term toxicity studies did not identify novel toxicities of human concern. New toxicities of potential concern for human safety or that changed trial design were identified in 13.5% of cases, with 7% being considered critical and 2% leading to program termination.

A technical workshop which included industry and regulators was held virtually over two days in 2021 to discuss interpretation of the data and to develop an evidential approach to support the conduct of fewer studies. Opportunities to further optimize study designs to reduce animal usage were identified. An iterative, weight-of-evidence (WoE) model which considers

factors that influence the overall risk for a mAb to cause toxicity was developed. This model enables an evidence-based justification, suggesting when 3-month toxicity studies, rather than longer term toxicity studies, are likely sufficient to support late-stage clinical development and registration for some mAbs.

An assessment of reversibility from adverse findings is required during pharmaceutical development, but there is flexibility around how and when this is performed and if recovery animals are necessary. Additional analysis of the projects' data led by NC3Rs, found that recovery animals are included in a high number of toxicity studies with mAbs and often in multiple studies across the mAb development programme. However, the results supported regulatory guidance outlining the acceptance of alternative scientific assessment and/or the use of recovery animal groups in only one study, when warranted.

The project is nearing completion and is now focused on dissemination. A paper based on the workshop and the WoE approach to chronic toxicity studies for human therapeutic monoclonal antibodies has been submitted for publication in Regulatory Toxicology and Pharmacology¹⁷. A second manuscript on recovery data is being prepared by NC3Rs and will be submitted to a peer reviewed journal in 2022. The project was presented to WC11 (Maastricht, 2021), Biosafe (virtual, November 2021), Society of Toxicology (SOT, San Diego, March 2022), Dutch Toxicology Society (NVT, Ede, May 2022), the Preclinical Assessors meeting (Zagreb, June 2022), ICT-Eurotox (Maastricht, September 2022) and will be presented to the American College of Toxicology (ACT, Denver, November 2022). The European Medicines Agency (EMA) Safety Working Party has been kept updated on the project's progress.

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).



17 Hsiao-Tzu Chien et al. (2022) Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach. Regulatory Pharmacology and Toxicology (submitted)

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 safety¹⁸. 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. 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 project¹⁹ and another on the workshop²⁰.

A second agrochemical project was begun in March



18 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>

19 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>

20 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>

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, for example, in some instances tumour findings may be related to high dose and excessive toxicity and thus are not relevant. Based on a database of 115 tumors in various organs, involving 72 substances, consensus on criteria for filtering of high dose findings has been reached; these were applied to the set of 115 tumor cases for which the MOA involved was unknown. This has resulted in the definition of “known unknowns” together with a consolidated list of MOAs. The project team is now focusing on predicting carcinogenic potential based on defining a WoE approach together with disseminating the results for “known unknowns”.

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

Sensitisation of human skin to chemicals is a potential danger to human health and therefore reliable hazard and risk assessments need to be performed to ensure these ingredients can be used safely. 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 work of many stakeholders has been the assessment without the use of animals, and as a

result a number of validated non-animal tests or 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 “Building Confidence in Skin Sensitisation Potency Assessment Using New Approach Methodologies” held in Brussels in October 2019²¹.

Recommendations from a previous Workshop²² and the Partners Forum have been followed-up in 2020-22 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 Alvertox 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 was evaluated by the Skin Sensitisation group in 2020-21. Six successful Skin Sensitisation User Forum sessions took place each focused on a case study presentation followed by Q&A with 10+ organizations (EPAA members) participating each time.

A case study from Cosmetics Europe has been accepted as an OECD IATA²³. To maximise the impact of the User Forum, the team is inviting other interested parties to share knowledge and to involve a wider audience. A second round of User Forum sessions is being discussed to gain confidence in NAMs with some complex case studies and involvement of a wider audience. Identification of sector-specific needs and gaps is ongoing. User Forum sessions on medical devices and on agrochemicals assessments are planned next, and the potential to share case studies from the pharmaceutical sector is being explored.

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21 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>

22 Basketter D. et al (2019) Applying non-animal strategies for assessing skin sensitisation report from an EPAA/cefic-LRI/IFRA Europe cross sector workshop, ECHA Helsinki, February 7th and 8th 2019. Regulatory Toxicology and Pharmacology <https://doi.org/10.1016/j.yrtph.2019.104477>

23 <https://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm>

h. 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 Forum²⁴, 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 products) 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, which is now complete, 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 previous QIVIVE project has been 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 chemicals²⁵. A protocol for the formal systematic review had been previously published²⁶. 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 tool²⁷ which facilitates hierarchical searching of existing models; this tool has been added to the JRC / ECVAM catalogue²⁸.

The influence of physico-chemical property estimation and analogue selection on model quality has been investigated. The KNIME platform²⁹ has been used to develop a workflow that assists selection of analogues (with PBK models) that

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24 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>

25 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 49:197–208 <https://doi.org/10.1177/02611929211060264>

26 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

27 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>

28 <https://data.jrc.ec.europa.eu/dataset/f98e9abf-8435-4578-acd6-3c35b5d1e50c>

29 Konstanz Information Miner, a free and open-source data analytics, reporting and integration platform

may be used to derive a PBK model for a similar chemical lacking a model. The workflow uses chemical fingerprints and physico-chemical properties to identify 'similar' chemicals, although users have the option to adapt the workflow to use other metrics. The workflow has been tested using case studies (i.e., atenolol (pharmaceutical) and flumioxazin (herbicide)) and similarity metrics have been used to identify PBK modelling-relevant analogues. The tools allow development of an initial PBK model for a target chemical based on a model for an analogue, following optimization of the analogue selection process.

Results from the project have been disseminated in oral and poster presentations at the following conferences: ASCCT (virtual, 2020), QSAR 2021³⁰, the WC11 (Maastricht, 2021), EURL ECVAM JRC Summer School on Non-Animal Approaches in Science (2021), SOT (San Diego, 2022) and ICT / Eurotox (Maastricht, September 2022). The KNIME workflow and user guide will be made available via Github (linked to a publication) in 2022.

i. Non-animal science in regulatory decisions for chemical safety

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. In light 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). In addition, The European Food Safety Authority (EFSA) has recently published a roadmap on the use of NAMs in risk assessment³¹ with a goal to routinely use NAMs to address data gaps by 2027.

Over a period of initially, one year from 4Q 2021, this project aimed to provide a cross Industry/EC environment for creative appraisal of current use of NAMs / non-animal science 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 has opened 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.

The focus of this project is on actual experience of EPAA partners in the use of NAMs for decision-making and

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³⁰ <https://www.ascctox.org/qsar2021/qsar-2021-program>

³¹ Escher SE. et al. (2022) Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment <https://doi:10.2903/sp.efsa.2022.EN-7341>

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 CSS.



The project began with a “deep-dive” workshop (virtual, 23-24 November 2021) to share information from groups evaluating NAMs for different regulatory purposes. A workshop flash report is available³², and a poster summarising the workshop conclusions was presented at ‘One – health, environment, society’ (EU Conference, Brussels, June 2022). The full report of the workshop³³ has been published “Open access” in the Regulatory Toxicology and Pharmacology journal.

The workshop shared information including case studies from groups that are using NAMs for various purposes associated with safety decision-making. It explored circumstances where NAMs could be used, whether NAMs could provide alternative DNELs (derived no-effect level of exposure) with consideration of appropriate uncertainty factors, and the potential of NAMs to contribute to EU CSS. Scientific exchange focused on programmes particularly relevant to EPAA partners and the discussions aimed to identify major challenges faced by policy makers and NAM users. The following key areas for further development of NAMs were identified:

- 1. Building trust through defining criteria for robust, reliable and reproducible use of NAMs, and level of acceptable variability. (Scientific)*
- 2. Existing regulation could be revised to further explore tiered schemes that include exposure and NAMs without seeing animal studies as the gold standard. (Regulatory)*
- 3. Increasing opportunities to use NAMs that are fit for regulatory needs (e.g., Annexes of REACH). (Regulatory)*
- 4. Industry and regulators to find ways to explore more NAM-based assessments in regulatory submissions to increase confidence in the use of NAMs in regulatory decisions. (Education, training and exchange of knowledge).*

Since the workshop, two working groups (WG) have been established to progress the NAMs related follow-up activities:



32 EPAA Workshop, virtual 2021. Deep-Dive Workshop on «Use of New Approach Methodologies (NAMs) in Regulatory Decisions for Chemical Safety» <https://ec.europa.eu/docsroom/documents/48034>

33 Westmoreland C. et al. (2022) Use of New Approach Methodologies (NAMs) in regulatory decisions for chemical safety: Report from an EPAA Deep Dive Workshop <https://doi.org/10.1016/j.yrtph.2022.105261>

WG1 is exploring frameworks that could be used for regulatory purposes including building on the ECETOC Framework for chemical safety assessment incorporating NAMs within REACH (based on Ball et al., 2022)³⁴ and linking to the long-term objectives suggested by the EC JRC. Three areas are proposed for follow up with EPAA - ECETOC: (i) to examine how exposure-based approaches could fit into REACH revision discussions, building on the concept of “classification of exposures”; (ii) to survey existing weight of evidence (WoE) approaches and evaluate their potential utilization to characterise chemical hazards (case studies), and (iii) to investigate a tiered approach as an alternative classification system for risk management / Classification and Labelling (C&L) without using animal data.

WG2 is progressing the implementation of the NAMs Users Forum using case studies for dialogue on the application of NAMs to regulatory decisions on chemical safety.

In addition to the project activities, a recent EPAA Partners Forum³⁵ (Brussels, 6 May 2022) on “Exposure Considerations for Human Safety assessments” included discussion on opportunities to harmonise and standardise approaches to accelerate the use of NAMs in regulatory testing. The importance of exposure-based approaches in facilitating the use and acceptance of NAMs approaches was highlighted. The conclusions from the Partners Forum will be developed further in a second Partners Forum held in November 2022.



34 Ball N. et al. (2022) <https://doi.org/10.1007/s00204-021-03215-9>

35 EPAA Partners Forum. (2022) Exposure considerations for human safety assessments. Flash report: <https://ec.europa.eu/docsroom/documents/50194>

3 Dissemination and Communication

a) Science Prize 2022



The EPAA 3Rs Science Prize of €10000 is granted every other year to a scientist with an outstanding contribution to 3Rs. The aim is to promote positive contributions from industry or academia and encourage more scientists to focus their research on the 3Rs goals. Assessment is conducted over 6 selection criteria defined by the EPAA Steering Committee:

1. Impact on the 3Rs (reduction, refinement, replacement of animal uses) *weighted x2*
2. Innovation/contribution to meeting an urgent, yet unmet scientific need
3. Possible applicability of the method/approach for regulatory testing (including for safety or potency) *weighted x2*
4. Impact on predictive safety science (better data/science is obtained thanks to the work of the applicant compared to the current animal method)
5. Work potentially applicable widely e.g. to other methods and endpoints and across sectors
6. International recognition (already published work, number of publications, rankings in peer-reviewed journals etc.)

In 2022, a total of 6 high-caliber applications were submitted to the EPAA secretariat and evaluated by the selection committee. The highest score was attributed to Dr Amer Jamalpoor from Toxys. His case study focuses on „ReproTracker: an animal-free platform for developmental toxicity testing“.



Quotes from the Selection Committee

“Highly innovative and impactful work”

“Well thought platform and concept to achieve animal-free DART”

“Much needed area for replacement, cross sectorial, in use”

b) 3Rs Student Grants 2022

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 2022, six grants were given in total.

ICT (International Congress of Toxicology) 2022

A full grant: **Samuel Madureira Silva**, *RETHINKING HUMAN TESTICULAR ORGANOID FORMATION: THE MISSING TOXICOLOGICAL MODEL FOR THE HUMAN TESTIS?*

A half grant: **Tianyi Li**, *IDENTIFICATION OF BIOMARKERS AND OUTCOMES OF ENDOCRINE DISRUPTION IN ADULT HUMAN OVARIAN CORTEX*

EUSAAT 2022

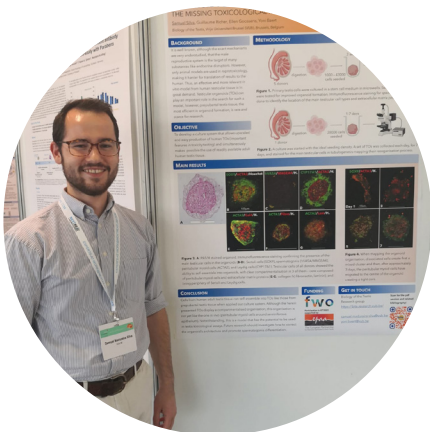
A full grant: **Ashesh Chakraborty**, *A HUMAN IN VITRO MODEL FOR AIRWAY EPITHELIAL INJURY AND REGENERATION*

A half grant: **Barbara Jozef**, *A SERUM-FREE MEDIUM THAT SUPPORTS CULTIVATION OF FISH CELL LINES: CASE STUDY ABOUT RTGILL-W1 GOING SERUM-FREE*

ESTIV 2022

A full grant: **Kaat Leroy**, *CONNEXIN-BASED CHANNEL ACTIVITY IS NOT SPECIFICALLY ALTERED BY HEPATOCARCINOGENIC CHEMICALS*

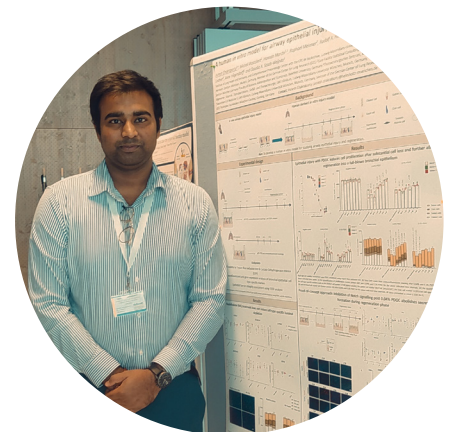
A half grant: **Axelle Cooreman**, *EFFECTS OF DRUGS FORMERLY REPURPOSED FOR COVID-19 TREATMENT ON CONNEXIN43 HEMICHANNELS AND PANNEXIN1 CHANNELS*



Samuel Madureira at ICT 2022



Barbara Jozef at EUSAAT 2022



Ashesh Chakraborty at EUSAAT 2022

c) EPAA events

- EPAA/NC3Rs working session on Acute oral toxicity (21 March)
- Partners Forum on “[Exposure Considerations in Human Safety Assessment, Part I](#)” (6 May 2022)



- EPAA lunch-debate in the European Parliament “Accelerating the transition to animal-free, sustainable innovation” (13 September)



- Partners Forum on “Exposure Considerations in Human Safety Assessment, Part II” (14 November)
- Annual Conference 2022 “Accelerating the Transition to Animal-Free, Sustainable Innovation”



- EPAA NAMs working session in Ispra, Italy (5-6 December) “Use of NAMs in Regulatory Frameworks: Scientific Working Session for potential future EPAA WG1 activities

d) External events

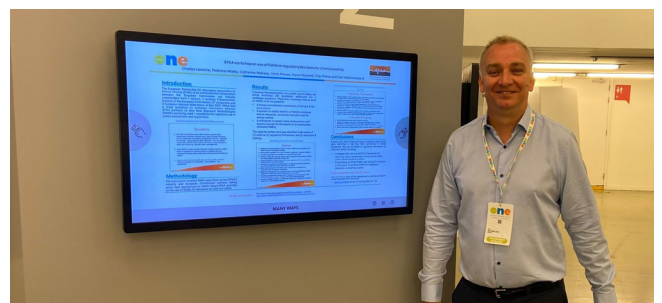
Posters:

SOT 2022, (27-31 March, San Diego, California, US)

- “Guidance to support the use of evident toxicity in acute oral toxicity studies (OECD TG 420)”
Fiona Sewell, David Andrew, Marco Corvaro, Thomas Holmes, Irene Manou, Boris Mueller, Tim Rowan, Graham Horgan.
- “Development of a tool to assist selection of chemical analogues to facilitate development of new physiologically-based kinetic models using a read-across approach”,
Courtney V Thompson, Steven D Webb, Joseph Leedale, Peter E Penson, Judith C Madden, Alicia Paini.
- “Evaluating optimal study designs for toxicity studies with monoclonal antibodies: results from a MEB/ Industry/NC3Rs survey”
Hsiaotzu Chien, Helen Prior, Fiona Sewell, Katrin Schutte, Lucinda Weir, Peter van Meer
- “The use of recovery animals across monoclonal antibody development packages: opportunity for further optimization remains”
Helen Prior and Fiona Sewell

ONE - Health, Environment, Society Conference 2022, **(21-24 June, Brussels, BE)**

- “[EPAA Workshop on use of NAMs in regulatory decisions for chemical safety](#)”,
Charles Laroche, Federica Madia, Catherine Mahony,



Irene Manou, Gavin Maxwell, Pilar Prieto and Carl Westmoreland.

ICT / Eurotox 2022 (18-21 September, Maastricht, NL)

- “New data supporting recognition of evident toxicity in acute oral toxicity studies (OECD TG 420)”
Thomas Holmes, Fiona Sewell, David Andrew, Marco Corvaro, Irene Manou, Boris Mueller, Tim Rowan, Graham Horgan.
- “Predicting non-genotoxic carcinogenic potential of agrochemicals: a mechanistic approach (Step 1: Analysis of Tumor Occurrence to Identify Targets for Future Investigation of Currently Unknown MoAs)”
Joantine van Esterik, Harm Heusinkveld, Marco Corvaro, Jan Willem van der Laan, Dick Lewis, Federica Madia, Irene Manou, Philip Marx-Stoelting, Stephanie Melching-Kollmuss, Elodie Pasquier, Frédéric Schorsch, Guy Steiblen, Christian Strupp, Gerrit Wolterink, Ruud Woutersen, Raffaella Corvi, Jyotigna Mehta, Mirjam Luijten
- “Criteria for selecting physiologically based kinetic models for use in developing new models for data poor analogues via a read across approach”
Judith C Madden, Courtney V Thompson and Alicia Paini
- “An automated tool for selection of chemical analogues to facilitate development of new physiologically-based kinetic models using a read-across approach”
Courtney V Thompson, Steven D Webb, Joseph A Leedale, Peter E Penson, Alicia Paini, David Ebbrell, Judith C Madden
- “Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight-of-evidence approach”
Hsiaotzu Chien, Helen Prior, Fiona Sewell, Katrin Schutte, Lucinda Weir, Peter van Meer

Netherlands Society of Toxicology (24 May 2022, Ede, NL)

- “Evaluating optimal toxicity study designs with monoclonal antibodies: results from a MEB/Industry/NC3Rs survey”
Hsiaotzu Chien, Helen Prior, Fiona Sewell, Katrin Schutte, Lucinda Weir, Peter van Meer

Oral Presentations:

16th Preclinical Assessors Meeting (PAM) (2-3 June 2022, Zagreb, Croatia)

“New insights into NHP use in Toxicology Studies with Biopharmaceuticals: An MEB/Industry/NC3Rs Project Sponsored by EPAA” by Peter van Meer.

Biosafe virtual conference (4-6, 11, 13 October 2022)

“Further Opportunities for NHP use in Toxicology Studies with mAbs: Update from an MEB/Industry/NC3Rs project sponsored by EPAA” by David Clarke

ACT (American College of Toxicology) (13-16 November 2022, Denver, Colorado)

- “New insights into NHP use in Toxicology Studies with Biopharmaceuticals: An MEB/Industry/NC3Rs Project Sponsored by EPAA” by Peter van Meer.
- “Inclusion of Recovery Animals: Opportunity for Further Optimization Remains” by Helen Prior

Webinars:

[13th Helsinki Chemicals Forum](#), panel discussion on “How to accelerate the replacement of animal toxicity testing”, Gavin Maxwell (8-9 June)



“Leveraging Information from Existing Physiologically-Based Kinetic (PBK) Models to Assist Development of New Models”, Judith Madden. Webinar hosted by Liverpool John Moores University (29 September)

“Does no animal testing mean less protection from dangerous chemicals?”, Gavin Maxwell. Cruelty Free Europe online event “Target Zero: Routes to a toxic-free Europe without animal testing” (27 October)

e) Publications

Chien Hsiao-Tzu, et al. (2022) Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach. Submitted to Regulatory Toxicology and Pharmacology, August 2022, awaiting review.

EDQM Council of Europe (2022) Novel *in vitro* model as alternative to *in vivo* toxoid vaccines testing: Clostridium septicum vaccine as proof of concept, Joint EDQM-JRC-EPAA virtual workshop Webinar sessions, 9 and 10 March 2021, Proceedings <https://freepub.edqm.eu/publications/PUBSD-168/detail>

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(570).

<https://doi.org/10.3389/fphar.2021.630457>

Loizou G, McNally K, Paini A, Hogg A. (2022) Derivation of a Human *In Vivo* Benchmark Dose for Chlorpyrifos from ToxCast *In Vitro* Concentration Response Data using RVis, an Open-Source PBK Modelling Platform and a Computational Workflow for Probabilistic Quantitative *In Vitro* to *In Vivo* Extrapolation. Front. Pharmacol. - Predictive Toxicology

<https://doi.org/10.3389/fphar.2021.630457>

Loizou G., McNally K., Paini A., Hogg A (2022). 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

<https://doi.org/10.3389/fphar.2021.754408>

Prior H. et al. (2022) The use of recovery animals in nonclinical safety assessment studies with monoclonal antibodies: further 3Rs opportunities remain. Planned submission to Regulatory Toxicology and Pharmacology, October 2022 (*in progress*)

Thompson C.V. et al. (2021) A Systematic Review of Published Physiologically based Kinetic Models and an Assessment of their Chemical Space Coverage. Alternatives to Laboratory Animals 49:197–208 (First published online November 26, 2021)

<https://doi.org/10.1177/02611929211060264>

Westmoreland C. et al. (2022) Use of New Approach Methodologies (NAMs) in Regulatory Decisions for Chemical Safety: Report from an EPAA Deep Dive Workshop

<https://doi.org/10.1016/j.yrtph.2022.105261>



f) Social media

The image shows a screenshot of the LinkedIn profile for EPAA (@EPAA3Rs). On the left is the LinkedIn navigation menu with the following items and counts: Connections (1,311), Contacts (2,155), People I Follow (4), Groups (18), Events, Pages (67), Newsletters, and Hashtags (5). The main profile area shows the EPAA banner for the 2022 Annual Conference, the profile picture, the name EPAA (@EPAA3Rs), a bio describing the organization's mission, the join date (March 2013), and follower counts (436 Following, 1,586 Followers).

Manage my network

- Connections 1,311
- Contacts 2,155
- People I Follow 4
- Groups 18
- Events
- Pages 67
- Newsletters
- Hashtags 5

EPAA
870 Tweets

SAVE THE DATE
EPAA ANNUAL CONFERENCE 2022

“Accelerating the Transition to Animal-Free, Sustainable Innovation”

EPAA
@EPAA3Rs

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

Joined March 2013

436 Following 1,586 Followers

04 Future Prospects

EPAA's 2023 activities will continue to address the six challenges outlined in our [EPAA Action Programme 2021-2025](#) (detailed below) to increase use of AAT/NAMs for regulatory safety testing in the context of the [EU Chemical Strategy for Sustainability](#) (CSS), [Pharmaceutical Strategy for Europe](#) and in consideration of the EU Parliament resolution to 'Accelerate a Transition to Innovation without the use of Animals in Research, Regulatory Testing and Education' (P9_TA(2021)0387).

In addition to the ongoing EPAA project activities, a number of new activities are planned for 2023:

I. Address science and technology gaps

- EPAA's 'Use of NAMs in Regulatory Decisions for Chemical Safety' project will seek to identify priority research challenges in collaboration with the [APCRA](#), [ASPIS](#) and [PARC](#) research consortia.

II. Improve intra and inter sectorial collaboration and coordination

- Building on the 2022 EPAA Partners Fora, EPAA will work with [SETAC](#) to organise a 2023 Partners Forum exploring 'Exposure considerations and use of NAMs in Environmental Safety Assessment' (title tbc).

III. Optimise translation from research to regulatory practice

- EPAA 'Use of NAMs in Regulatory Decisions for Chemical Safety' WG2 NAM User-Forum team will organise a workshop to review initial case studies, discuss learnings from the Skin Sensitisation NAM User Forum and agree priority topics for future sessions.

IV. Facilitate acceptance of additional sources of evidence in the current regulatory framework

- EPAA will partner with EDQM in February 2023 to run a three-day workshop in Brussels on 'The Future of Pyrogenicity Testing: Phasing out the Rabbits Pyrogens Test' with presentations from industry and regulatory authority experts and an interactive training session.
- Coordination of the European Commission services responsible for the relevant regulatory framework will be carefully followed, including via the Steering Group regular meetings

V. Communicate scientific opportunities and challenges

- EPAA partners will collaborate to develop a roadmap with an initial focus on replacing the use of Animals for Regulatory Testing of Chemicals. The roadmap should identify critical needs for an animal-free system to steer NAM methodological developments.
- EPAA has submitted a proposal to the organisers of World Congress on Alternatives and Animal Use in the Life Sciences (WC12) for holding a NAMs session during the Congress in Canada in August 2023.

VI. Promote education and knowledge-sharing

- EPAA will organise an EU Parliament workshop and poster exhibition on 'Accelerating a Transition to Animal-Free, Sustainable Chemical Innovation' (title tbc) to discuss EPAA partner and collaborator progress and challenges.

05 Membership update

In 2022, EPAA welcomed 1 new member: DOW. The Partnership includes now 5 Directorates-General of the European Commission, 38 companies, and 8 European industry federations, representing 7 industrial sectors. Further information is available at the EPAA website:

https://ec.europa.eu/growth/sectors/chemicals/epaa/partners_en

38 Companies (including 1 SME)



Partner Agencies



8 Sectoral Associations



Mirror Group (Advisory body)

- M. Pereira (Chair)
- A. Barros
- T. Heinonen
- C. Hohensee
- H. Kandarova
- E. McIvor
- S. Pietikäinen
- B. Pintado
- V. Rogiers
- H. Spielmann

5 DG's of the EC

- DG GROW
- DG ENV
- DG SANTE
- DG JRC
- 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

BCOP: Bovine Corneal Opacity & Permeability Assay

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

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

WHO: World Health Organisation





Visit to know us

GROW-EPAA@ec.europa.eu