ANNUAL REPORT 2023

European Partnership for Alternative Approaches to Animal Testing (EPAA)

European Commission

The European Partnership for Alternative Approaches to Animal Testing

Contents

1. Foreword

The last year has represented a period of rapid regulatory change with important policy initiatives related to the implementation of major EU actions linked to the European Green Deal (EGD), the EU Chemical Strategy for Sustainability (CSS) and the Pharmaceutical Strategy for Europe that have all been the subject of careful scrutiny.

The 2021 EU Parliament resolution to 'Accelerate a Transition to Innovation without the use of Animals in Research, Regulatory Testing and Education' remains an important document of reference for EPAA, coupled with the European Citizens' Initiative 'Save Cruelty-Free Cosmetics – Commit to a Europe without Animal Testing' submitted in January 2023 and the following European Commission Communication in July 2023. There, the Commission launched a series of actions to accelerate the replacement of animal testing including activities that will increase cooperation with Member States; to provide funding to support research on alternative methods to animal testing and to prepare a roadmap defining milestones to reduce and replace animal testing, aiming for a transition to complete animal-free testing for chemicals legislation.

This evolving regulatory landscape combined with an ever-engaged EU Parliament has created the perfect environment for EPAA partners to work together to accelerate the transition to animal-free, sustainable innovation through promoting the refinement, reduction and replacement of regulatory animal testing. The 2023 EPAA annual report captures the collective achievements of the partnership with the following notable highlights:

- Launch of the EPAA NAM Designathon Challenge 2023 & NAM User Forum
- EPAA Lunch Debate in EU Parliament, hosted by MEP Tilly Metz (12th Sept 2023)
- EDQM: EPAA Future of Pyrogenicity testing workshop (14th-16th Feb 2023)
- EPAA Exposure considerations in Human Safety Assessment Partners Forum publication
- EPAA contributions to ECHA 'Towards an Animal-free Regulatory System for Industrial Chemicals' workshop (31st May-1st June 2023)

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 2023 a very important year to boost the activities for the partnership.

Giacomo MATTINÒ
EPAA European Commission
Co-Chair

Gavin MAXWELL
EPAA Industry Co-Chair

2. EPAA Members

In 2023, the Partnership includes 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)

5 DG's of the EC

DG GROW

DG ENV

DG SANTE

DG JRC

DG RTD

Mirror Group (Advisory body)

Emily McIvor (Chair), Tuula Heinonen, Christiane Hohensee, Helena Kandarova, Sirpa Pietikaïnen (MEP), Vera Rogiers

New: Emma Grange, Julia Baines, Winfried Neuhaus, Monique Janssen

Including Partner Agencies

ECHA EUROPEAN CHEMICALS AGENCY

efsa EUROPEAN FOOD SAFETY AUTHORITY

EUROPEAN MEDICINES AGENCY SCIENCE MEDICINES HEALTH

8 Sectoral Associations

A·I·S·E

animalhealth europe

cefic

Cosmetics Europe the personal care association

ifra THE INTERNATIONAL FRAGRANCE ASSOCIATION

efpia European Federation of Pharmaceutical Industries and Associations

CropLife EUROPE

sme united CRAFTS & SMEs IN EUROPE

3. EPAA Project Platform

The EPAA aims to reduce and replace regulatory animal testing by innovative, non-animal methods / New Approach Methodologies (NAMs), 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 at national, European and global levels. Replacement methods embrace increasing knowledge of toxicity mechanisms 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 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 2023, the PP has supported eight project teams which synergistically combine the expertise and collaboration available across industry sectors, academia, NGOs and regulatory agencies. Of the eight projects, it is pleasing to see, after considerable efforts and excellent work by the project teams that the Clostridial Vaccines (for veterinary use) and Monoclonal Antibody Safety projects have been successfully completed, while the Carcinogenicity of Agrochemicals and Acute Toxicity ones are in the dissemination phase approaching completion.

Projects in 2023

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) Non-animal science (NAMs) in regulatory decisions for chemical safety

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 2023) 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

This project has been completed successfully. All laboratory work for the project has been finished with the development of novel in-vitro methods suitable for replacement of older animal-based methods for evaluation of the toxicity and antigenicity of Clostridium (C.) septicum veterinary vaccines. The project has resulted in appropriate and important revisions of three European Pharmacopoeia (Ph. Eur.) monographs and wide dissemination of the results. 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.

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 thus allow more accurate vaccine blending.

Therefore, a project was undertaken on *C. septicum* vaccines 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 completed. 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, a 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. The results 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

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

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. Fourteen laboratories, including vaccine manufacturers and official control laboratories in Europe, USA, Morocco and Mexico, participated in the study. The experimental work was successfully completed in 2018 and the results demonstrated that the optimised *in vitro* tests were very consistent, with intra- and inter-laboratory variations far lower than those for the analogous *in vivo* tests. This indicated that the non-animal, cell line-based assays3 for in-process toxicity and antigenicity testing of *C. septicum* vaccines outperformed the animal-based methods. The superior sensitivity and accuracy of the *in vitro* MLD and TCP tests will become apparent when implemented by the manufacturers as in-process controls.

In light of the results of the project, the 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 1 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)4. The regulatory consequences of the study were presented at 11th World Congress on Alternatives and Animal Use in the Life Sciences (Maastricht, 2021)5. 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 March 2021 in webinar format with over 200 registered participants from more than 40 countries. Presentations and session summaries of the webinar have been published online[6] together with the webinar proceedings[7].

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 [NIH] 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 this test will have a high impact on animal use and it is therefore a priority for the implementation of the 3R principles[8]. The aim of this project is therefore the replacement of the NIH *in vivo* test with an *in vitro* antigen (G glycoprotein, GP) quantification assay using an ELISA technology. A specific GP 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.

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.

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

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

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

6 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

7 https://freepub.edqm.eu/publications/PUBSD-168/detail

8 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

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 assessment[23] with a goal to routinely use NAMs to address data gaps by 2027.

This project aims to provide a cross Industry/EC environment for creative appraisal of current use of NAMs / non-animal science for decision-making and to define the needs to increase the confidence for routine use of NAMs more routinely in 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 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 available[24], and a full report of the workshop has been published[25]. In addition, a poster summarising the workshop conclusions was presented at 'One – health, environment, society' (EU Conference, Brussels, June 2022)[26] together with an OpEd article in Euractiv (2022)[27].

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

23 Escher SE. et al. (2022) Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment. EFSA Supporting Publications 19 https://doi:10.2903/sp.efsa.2022.EN-7341

24 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

25 Westmoreland C. et al. (2022) Use of New Approach Methodologies (NAMs) in regulatory decisions for chemical safety: Report from an EPAA Deep Dive Workshop. Regulatory Toxicology and Pharmacology 135 https://doi.org/10.1016/j.yrtph.2022.105261

26 https://www.efsa.europa.eu/en/events/one-conference-2022

27 https://www.euractiv.com/section/health-consumers/opinion/accelerating-uptake-of-non-animal-safety-science-into-european-chemical-legislation/

Phase 1 of the study, the transfer of the assay and protocol from the developing laboratory to the French OMCL (ANSM, France) 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 was conducted in 2022 and 2023; an inter-laboratory comparison of different vaccines, statistical analyses and a Phase 2 report have been completed. An international technical workshop was held in June 2023 to discuss the results. 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. Statistical analysis showed very good transferability of the ELISA method between laboratories, good assay precision (all confidence limits within 80-125%) and good repeatability despite the limited proficiency of most participants for the GP ELISA. A report on Phase 2 has been issued and distributed to study participants as well as the Ph. Eur. group of Experts 15 (human vaccines) in 2023. Phase 3 was launched in 2023 to collect data on the applicability of the GP ELISA to the testing of routine production batches. Participants will be allowed approximately 1 year to report data. Phase 3 which includes testing in production will continue through 2023 and the first half of 2024.

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 2023-24.

A new project proposal is being discussed to evaluate the applicability of the ELISA method to veterinary inactivated rabies vaccines, which use virus strains similar or identical to those for the human vaccines. Several veterinary manufacturers and official medicines control laboratories (OMCLs) are supportive.

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.)[9] 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.

The project is being conducted in collaboration with the UK National Centre for the 3Rs (NC3Rs) and its

9 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

objective 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. Unfortunately, the subjective nature of "evident toxicity" based on clinical signs (in contrast to mortality) appears to be preventing wider uptake of the OECD Test Guideline (TG) 420 and it is not currently the test of choice.

The project has collected data (including mortality, clinical signs and body weight) from previous acute oral toxicity studies which was then mined and statistically analysed in collaboration with the NC3Rs, the UK Chemicals Regulation Directorate and EPAA member companies. This has delivered data on approximately 90 studies (from an initial 200) 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 may 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 Fixed Dose Procedure (FDP) over other currently accepted methods and has the potential to reduce the suffering and numbers of animals used when in vivo acute oral toxicity studies are required.

The projects' findings are now being applied to develop guidance on use of evident toxicity as an endpoint and to support use of the FDP for acute oral toxicity studies according to 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. In this regard, it is noteworthy that the project advocacy plan includes further liaison with OECD regarding guidance in TG 420. Also, recommendations on a 3Rs-based classification & labelling decision framework to include replacement of death as an endpoint are now being developed and will be reviewed with the regulatory authorities.

The project results are being widely disseminated. A peer-reviewed paper has been accepted for publication in Regulatory Pharmacology and Toxicology[10], and presentations / posters have been given at international conferences including Society of Toxicology (San Diego 2022), ICT-Eurotox (Maastricht, September 2022), the European Society of Toxicology, Ljubljana 2023), and WC12 (12th World Congress on Alternatives and Animal Use in the Life Sciences, Niagara Falls 2023). The project findings together with additional information will also be published on the NC3Rs website.

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.

10 Sewell S. et al (2023) New supporting data to guide the use of evident toxicity in acute oral toxicity studies (OECD TG 420). Regulatory Toxicology and Pharmacology (accepted)

The Biologicals project continues to (a) encourage deletion of deletion of in vivo tests of innocuity of biologicals 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 Humane Society International (HSI), and (c) coordinate dissemination activities on deletion of in vivo tests of innocuity by EPAA, industry and HSI. An overview of progress achieved in the harmonisation of 3Rs in Biologicals project was presented at WC11 (Maastricht, 2021). *In 2023, the Project Team has also considered further 3Rs opportunities in the area of human vaccines, (e.g., in vitro potency assays for diphtheria, tetanus and pertussis (DTaP)), together with opportunities arising from the VAC2VAC project outcomes.*

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 2023:*

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 was previously 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 a large number of Ph. Eur. monographs in the years to 2026, removing the rabbit test[11]. A new general chapter 2.6.40 MAT for vaccines containing inherently pyrogenic components (Pharmeuropa 33.3) was adopted in March 2023. A revised chapter on MAT (2.6.30) was published for comments (Pharmeuropa 34.2) in April 2022 and comments are under review. A new general chapter on Pyrogenicity (5.1.13) together with all revised Ph. Eur. texts omitting the rabbit pyrogen test was published for comments (Pharmeuropa 35.1) in January 2023.

A dedicated three-day workshop, jointly organised by EDQM and EPAA was held (Brussels, February 2023); two days of presentations and discussion on deletion of RPT and the third day for a training event on MAT. The event was public, open to all stakeholders impacted by RPT deletion (e.g., health authorities, industry users, service providers) and sought international perspectives to support global alignment by promoting alternative assays such as MAT and the bacterial endotoxin test (BET). Presentations and a report from the workshop are available[12] and a peer reviewed article has been published[13]. Follow-up activities are planned, including dedicated training on MAT superiority to EU and non-EU regulators, and promotion of inter-pharmacopoeias dialogue. In collaboration with EFPIA and EDQM, the potential application of next generation sequencing in viral safety and promoting animal-free technologies for detection of endotoxin is being discussed (e.g., use of recombinant factor C and other options).

There are also developments in non-EU countries including China and South Korea to prepare for future use of the MAT alternative.

e) Monoclonal Antibody Safety

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

This project has been completed successfully in 2023. All analyses and interpretation of data from regulatory agencies and industry have been finished with the development of an iterative, weight-of-evidence (WoE) model which considers factors that influence the overall risk for a mAb to cause toxicity. 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. In addition, analysis of the projects' data led by NC3Rs, found that recovery animals (required for assessment of reversibility of adverse findings) are included in a high number of toxicity studies with mAbs and often in multiple studies across the mAb development programme.

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

12 https://www.edqm.eu/en/-/joint-edqm-epaa-event-the-future-of-pyrogenicity-testing-phasing-out-the-rabbit-pyrogen-test?p_l_back_url=%2Fen%2Fweb%2Fedqm%2Fsearch%3Fq%3Dpyrogenicity

13 Cirefice G. et al. (2023) The future of pyrogenicity testing: Phasing out the rabbit pyrogen test. A meeting report. Biologicals 84 https://doi.org/10.1016/j.biologicals.2023.101702

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 results have been widely disseminated in 2022-23.

Briefly, this project aimed 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). The specific objectives of this project were to: 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; 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 initiate discussions to document these new criteria in EMA guidance. This EPAA project was led by the Dutch MEB (Medicines Evaluation Board) with strong support from EC DG ENV, 14 pharmaceutical companies, industry experts and the UK National Centre for the 3Rs *(NC3Rs)*.

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 enabled 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.

A paper based on the workshop and the WoE approach to chronic toxicity studies for human therapeutic monoclonal antibodies has been published[14]. A second paper on recovery data, prepared by NC3Rs was also published[15]. Project outcomes were presented in conjunction with NC3Rs in a webinar[16]. 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), the American College of Toxicology (ACT, Denver, November 2022) and WC12 (Niagara Falls, 2023).

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). 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[17]. The waiver could be granted based upon

14 Chien,H-T. et al. (2023) Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach. Regulatory Toxicology and Pharmacology 138 https://doi.org/10.1016/j.yrtph.2022.105329

15 Prior H. et al. (2023) The use of recovery animals in nonclinical safety assessment studies with monoclonal antibodies: further 3Rs opportunities remain. Regulatory Toxicology and Pharmacology 138 https://doi.org/10.1016/j.yrtph.2023.105339

16 https://nc3rs.org.uk/our-portfolio/re-evaluating-need-mab-chronic-toxicity-studies

17 van der Laan JW. et al. (2016) Prediction of the Carcinogenic

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 project[18] and another on the workshop[19].

A second 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, that is to establish a virtual waiver for the two-year rodent carcinogenicity assay. *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. Consensus on criteria for filtering of high dose findings has been reached within the project team. These criteria were applied to the set of 114 tumour cases, related to 72 substances, for which the MOA involved was unknown. Next, in order to discriminate between non-genotoxic carcinogens for which a MOA can be hypothesized versus true unknowns a stepwise approach was developed, to be applied per organ system. In total, 19 different organs were reviewed. This work was complex, requiring a very careful and detail review. This was due to the fact that a substantial number of substances induced different types of tumours in different organs, with different combinations of unknown as well as known MOAs. Potentially useful information to derive a MOA was available for tumours occurring in lungs and adrenal glands. For brain and the hematopoietic system, it turned out to be more challenging to identify a MOA. Analogous to the MOAs already known, the MOAs for lung and adrenal glands were described using the Adverse Outcome Pathway (AOP) concept, in order to better understand the key events underlying the tumour formation. The project team is considering how to best publish the database and to define the*

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

18 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

19 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

scope of a manuscript to be submitted to a peer-reviewed journal in 2024. A poster and a presentation on the project were given at ICT / Eurotox (Maastricht, 2022). Next steps involve predicting carcinogenic potential based on defining a WoE approach without the need for rodent carcinogenicity studies together with disseminating the results for "known unknowns".

g) Skin Sensitisation Dissemination (NAM User Forum)

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

Sensitisation of human skin to chemicals is a potential danger to human health and therefore reliable hazard evaluation and risk assessments need to be performed to ensure potentially sensitizing 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 development and assessment of non-animal testing methods and as a result, a number of validated New Approach Methods (NAMs) and Defined Approaches (DA) are now accepted as OECD Test Guidelines (TGs). These and other approaches are being increasingly used for hazard identification as well as to inform a Next Generation Risk Assessment (NGRA) approach for skin sensitization.

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[20]. Recommendations from a previous Workshop[21] and the Partners Forum have been followed-up in 2020-23 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.

EPAA has since provided a forum to discuss use of NAMs for Skin Sensitisation regulatory testing by running a series of knowledge sharing workshops that have evolved into the ongoing Skin Sensitisation NAM User Forum.

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 IATA22. *To maximise the impact of the User Forum approach, the team is inviting other interested parties to share knowledge between sectors and regulators, to review successes and failures around NAMs and defined approaches to skin sensitisation (DASS; e.g., OECD GL 497). A second round of User Forum sessions is being conducted 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 assessments for pharmaceuticals and for agrochemicals will be held in 2023, and the potential to share case studies from the medical devices sector is being explored. The need for training sessions or a workshop in 2024 are being evaluated.*

h. 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

20 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

21 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 109 https://doi.org/10.1016/j.yrtph.2019.104477

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

discussions aimed to identify major challenges faced by policy makers and NAM users. The following key areas for further development of NAMs were identified:

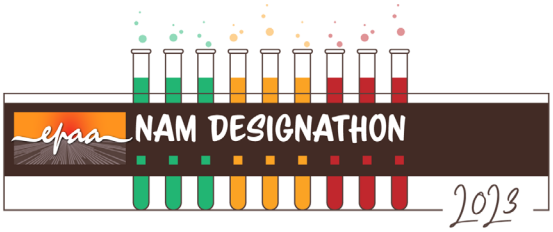
1. **Building trust through defining criteria for robust, reliable and reproducible use of NAMs, and level of acceptable variability. (Scientific)**
2. **Existing regulation could be revised to further explore tiered schemes that include exposure and NAMs without seeing animal studies as the gold standard. (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 initial working groups (WG) have been established to progress the NAMs related follow-up activities:

WG1 is focussed on addressing the gap between scientific research and regulatory use and is exploring frameworks that could be used for regulatory purposes. This includes the ECETOC Framework for chemical safety assessment incorporating NAMs within REACH (based on Ball et al., 2022)[28] and the EC-JRC vison for a "Chemical 2.0" framework" (a long-term objective for chemical safety assessment) centred on a classification matrix in which NAMs for toxicodynamics and toxicokinetics are used to classify chemicals according to their level of concern[29].

From the WG1 discussions, three areas were suggested for follow up: (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. Given the expertise within the group and current priorities, the group agreed to focus on the classification of hazards and to explore a new concept of future use of NAMs for hazard classification.

To facilitate this, EPAA launched a 'NAM Designathon 2023' Challenge for human systemic toxicity which seeks to identify chemical classification systems, using systemic toxicity as a case study, capable of categorising chemicals based on their bioactivity (intrinsic toxicodynamic properties) and their potential systemic availability (intrinsic toxicokinetic properties). The Designathon is seeking scientific solutions from the global scientific community. EPAA has hosted an information webinar, provided a list of 150 chemicals reflecting three levels of concern and a reporting template. In this initial prototype phase there will be no winning solution, rather the aim is to compare and contrast different NAM-based solutions suggested by the participants and to co-create. The deadline for submission of solutions is the end of 2023 and will be followed by a workshop in early 2024 to discuss the solutions with the submitters. All relevant documents can be downloaded from the EPAA website ('EPAA launches designathon for human systemic toxicity')[30]. The 2023 Designathon challenge was described and further promoted during three presentations given by members of the EPAA WG at the WC12 (12th World Congress on Alternatives and Animal Use in the Life Sciences, Niagara Falls 2023).



WG2 is focussed on addressing the lack of cross-sector, scientific consensus on NAMs use for chemical regulatory testing that was identified in the EPAA NAMs deep-dive workshop. It is progressing the implementation of the NAMs User Forum to allow scientific, case study-led discussions on the use of NAMs to address priority regulatory testing requirements for chemicals. The kick-off meeting of the EPAA NAMs User Forum is planned for 7-8 December 2023 (Helsinki) hosted by ECHA and will include discussion of different toxicological endpoints and NAMs.

In addition to the project activities, two *recent EPAA Partners Forums (Brussels, May and November 2022) on "Exposure Considerations for Human Safety assessments" included discussion on opportunities to harmonise and standardise approaches to*

28 Ball N. et al. (2022) A framework for chemical safety assessment incorporating new approach methodologies within REACH https://doi.org/10.1007/s00204-021-03215-9

29 Berggren and Worth (2023) Towards a future regulatory framework for chemicals in the European Union – Chemicals 2.0. Regulatory Toxicology and Pharmacology 142 https://doi.org/10.1016/j.yrtph.2023.105431

30 https://single-market-economy.ec.europa.eu/calls-expression-interest/epaa-launches-designathon-human-systemic-toxicity_en

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 each of the Partners Forums were reported in flash reports[31] [32] and have recently been published as a full, peer-reviewed publication[33]. This year EPAA's Partners Forum will discuss the "Use of Alternatives to

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Exposure considerations in human safety assessment: Report from an EPAA Partners' Forum

Mark T.D. Cronin [a], Nicholas Ball [b], Sonja Beken [c], Hans Bender [d], Ofelia Bercaru [e], Laura Caneva [f], Marco Corvaro [g], Richard A. Currie [h], Jeffrey L. Dawson [i], Paul Desert [j], Sylvia E. Escher [k], Antonio Franco [l], Amaia Irizar [m], Jyotigna M. Mehta [n], Vera Rogiers [o], Raphaël T. Tremblay [p], Carl Westmoreland [q], Gavin Maxwell [q]

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Animal Testing for Environmental Safety Assessment" (13-14 Nov 2023) to identify where EPAA can help to accelerate the adoption of Environmental NAMs. The Forum will be hosted by CEFIC and organised in partnership with ECETOC & ICCS.

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

32 EPAA Partners Forum (Nov, 2022) Exposure Considerations in Human Safety Assessment» Partners' Forum, Part II. Flash Report: https://single-market-economy.ec.europa.eu/document/e47059e3-634d-44b2-be81-e6d574c93141_en

33 Cronin MTD. et al. (2023) Exposure considerations in human safety assessment: Report from an EPAA Partners' Forum. Regulatory Toxicology and Pharmacology 144 https://doi.org/10.1016/j.yrtph.2023.105483

4. Dissemination and Communication

a) Refinement Prize 2023

Refinement PRIZE 2023

epaa The European Partnership for Alternative Approaches to Animal Testing

Refinement Prize of €6,000 is granted to a laboratory technician, animal caretaker or technologist who has demonstrated outstanding achievements in new, novel approaches to advance the implementation and/or awareness raising of refinement of animal testing.

Laboratory technicians, technologists and animal caretakers carry out much of the work using animals for regulatory safety and efficacy testing purposes. They are thus closely involved in efforts to apply refinement strategies in such studies. Refinement is one of the 3Rs (replacement, reduction and refinement of testing on animals). It involves the modification of any procedure, husbandry and care practices with laboratory animals along their entire lifetime, so as to minimise pain and distress and enhance their well-being.

The purpose of this prize is to target those actually implementing alternative approaches to animal testing and/or raising awareness of their role, in particular for the day to day application and innovation of the refinement principles.

The selection criteria are:

a) Impact on animal welfare – evidence base

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 2023, the highest score was attributed to Dana Matzek and her case study which focuses on Mobile-R-Pen housing system. Dana Matzek is a part of the management team of the Core Facility Animal Models at the Biomedical Center of the University of Munich with focus on animal care, education and training coordination. Member of the animal welfare office with the tasks of implementation, monitoring and improvement of in vivo projects as well as 3Rs measures.

Quotes:

„ Great improvement in animal welfare “

„ Concrete impact on wellbeing of animal”

„ Easy to apply and extend to different legislation “

„ Significant improvement of the wellbeing of the animals “

b) 3Rs Student Grants 2023

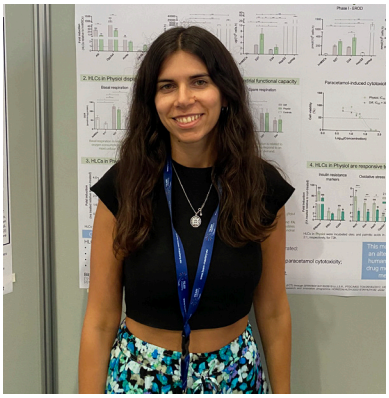
Every year, several high-profile international meetings bring together world-class scientists working on the development and acceptance of 3R alternatives to animal testing (Replacement, Reduction or Refinement). Costs linked to participation may prevent students with promising work or young scientists 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 2023, two full grants and one super grant were given in total.

EUROTOX 2023

A full grant: **Anouck Thienpont** *"Unlocking the power of transcriptomic biomarkers in qualitative and quantitative genotoxicity assessment of chemicals"*

A full grant: **Joana Saraiva Rodrigues** *"The role of glucose homeostasis and glucocorticoid signalling for thedevelopment of relevant hepatic in vitro models for toxicology, drugmetabolism and energy metabolism studies"*



12th World Congress on Alternatives and Animal Use in the Life Sciences (WC12)

Super grant: **Prem Chand** *"Characterization of Hepatic 3D Spheroids Using Multiphoton Microscopy and OMICS"*

c) EPAA events

- Joint EDQM-EPAA Event on "The future of pyrogenicity testing: phasing out the rabbit pyrogen test" (14 – 16 February, European Commission Meeting Centre A. Borschette, Brussels and hybrid)

- EPAA-NC3Rs webinar "Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies: weight of evidence and further 3Rs approaches" (17 April)

- EPAA "NAM Designathon for human systemic toxicity" webinar (13 July)

- EPAA lunch-debate in the European Parliament (12 September, EP, Strasbourg)

- Partners Forum on "Use of Alternatives to Animal Testing for Environmental Safety Assessment" (13-14 November, Cefic offices, Brussels and hybrid)

- Annual Conference 2023 "Protection of people and our environment through NAMs" (15 November, European Commission Meeting Centre A. Borschette, Brussels and hybrid)

d) External events

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

- ECHA's "New approach methodologies workshop: Towards an animal-free regulatory system for industrial chemicals", 31 May - 1 June 2023, ECHA, Helsinki; presentation by Gavin Maxwell (EPAA Industry Co-Chair)

- The 20th International Workshop on Quantitative Structure-Activity Relationships in Environmental and Health Sciences (QSAR 2023), 5-9 June, Copenhagen, Denmark, "Developing New Physiologically-Based Kinetic Models Using Data from Existing Models in a Read-Across Approach"; presentation by Judith Madden, Professor of in Silico Chemical Assessment, Liverpool John Moores University.

- 12th World Surfactant Congress (CESIO 2023) (5-7 June, Rome, Italy), presentation by Gavin Maxwell

- 12th World Congress on Alternatives and Animal Use in the Life Sciences (27-31 August, Niagara Falls, Canada), EPAA activities were presented through a number of posters and presentations (https://single-market-economy.ec.europa.eu/news/epaa-12th-world-congress-2023-08-02_en)

e) Publications

- *"New approach methodologies in human regulatory toxicology – Not if, but how and when!" (2023).*
  S. Schmeisser; A. Miccoli; M. von Bergen; E. Berggren; A. Braeuning; W. Busch; C. Desaintes; A. Gourmelon; R. Grafstrom; J. Harrill; T. Hartung; M. Herzler; G.E.N. Kass; N.Kleinstreuer; M. Leist; M. Luijten; P. Marx-Stoelting; O. Poetz; B. van Ravenzwaay; R. Roggeband; V. Rogiers; A. Roth; P. Sanders; R.S. Thomas; AM. Vinggaard; M. Vinken; B. van de Water; A. Luch; T. Tralau
  https://doi.org/10.1016/j.envint.2023.108082

- *"Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach" (2023).*
  H.-T.Chien; H. Prior; L.Andrews; L. van Aerts; A. Cauvin; D. O. Clarke; K. Datta; M.Dempster; N. Dybdal; W. Freebern; L. de Haan; D. Herzyk; A. Hey; T. Kissner; S. Kronenberg; M.W. Leach; D. Lee; K. Schutte; F. Sewell; K. Trouba; P. van Meer
  https://doi.org/10.1016/j.yrtph.2022.105329

- *"Exposure considerations in human safety assessment: Report from an EPAA Partners' Forum" (2023).*
  M.T.D. Cronin; N. Ball; S. Beken; H. Bender; O. Bercaru; L. Caneva; M. Corvaro; R. A. Currie; J.L. Dawson; P. Desert; S.E. Escher; A. Franco; A. Irizar; J.M. Mehta; V. Rogiers; R.T. Tremblay; C. Westmoreland; G. Maxwell
  https://doi.org/10.1016/j.yrtph.2023.105483

- *"The future of pyrogenicity testing: Phasing out the rabbit pyrogen test. A meeting report" (2023).*
  G. Cirefice; K. Schütte; I. Spreitzer; E. Charton; S. Shaid; L. Viviani; M. Rubbrecht; I. Manou
  https://doi.org/10.1016/j.biologicals.2023.101702

- *New supporting data to guide the use of evident toxicity in acute oral toxicity studies (OECD TG 420) (2023)*
  F. Sewell, I. Ragan, G. Horgan, D. Andrew, T. Holmes, I. Manou, B.P. Müller, T. Rowan, B.G. Schmitt and M. Corvaro (in press)

5. Future Prospects

EPAA 2024 activities will integrate, coherently with the EPAA mission, the important policy inputs received as a follow up to the European citizen's initiative – 'Save Cruelty Free Cosmetics – Commit to a Europe without Animal Testing' - and the subsequent European Commission commitment to prepare a roadmap defining milestones and concrete actions to reduce and replace animal testing, aiming for a transition to complete animal-free testing for chemicals legislation.

EPAA 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 2024:

1. Address science and technology gaps

- EPAA will seek to identify priority research challenges in collaboration with ASPIS, PARC, and other relevant research groups as input to the roadmap.

2. Improve intra and inter sectorial collaboration and coordination

- Building on the outcomes of the 2022 and 2023 EPAA Partners Fora, EPAA will seek to facilitate regulatory use of exposure science and NAMs for environmental safety.

3. Optimise translation from research to regulatory practice

- EPAA 'Use of NAMs in Regulatory Decisions for Chemical Safety' WG1 team will organise workshops to review the NAM Designathon Challenge entries and communicate next steps.

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

- EPAA 'Use of NAMs in Regulatory Decisions for Chemical Safety' WG2 team will organise a series of NAM User-fora to review regulatory use of NAMs for addressing priority regulatory information requirements.

5. Communicate scientific opportunities and challenges

- EPAA will help develop a roadmap capturing activities relevant to the EP Resolution on 'Accelerate a Transition to Innovation without the use of Animals in Research, Regulatory Testing and Education' (P9_TA(2021)0387) with an initial focus on use of Animals for Regulatory Testing of Chemicals.

6. Promote education and knowledge-sharing

- EPAA will organise an EU Parliament poster exhibition on 'Accelerating a Transition to Animal-Free, Sustainable Chemical Innovation' that will also be presented at Helsinki Chemicals Forum 2024.

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