ANNUAL REPORT

European Partnership for Alternative Approaches to Animal Testing (EPAA)

TABLE OF CONTENTS

1 Foreword

The last year has been a transitional time for the European Partnership for Alternative Approaches to Animal Testing (EPAA). In April we wished Giacomo Mattinò (outgoing European Commission Co-chair) well as he started his retirement and welcomed Giulia Del Brenna into the partnership as the new European Commission Co-chair. Then in September we welcomed Katia Lacasse (CEFIC) as Industry Vice Chair in anticipation of her role as Industry Co-Chair in 2026. Thank you, Giacomo, for all that you have done for EPAA over the last two years and welcome Giulia and Katia!

In parallel EPAA has continued to evolve our strategic and project platform activities to align with and inform the European Commission activities to accelerate the phasing out of animal testing and to develop the 'Roadmap towards phasing out animal testing for chemical safety assessment'. The 2024 EPAA annual report captures the collective achievements of the partnership with the following representing key scientific challenges that EPAA is now actively addressing:

- Use of New Approach Methods (NAMs) for Human health Systemic Safety Assessment via EPAA NAM Designathon Challenge and EPAA NAM User Forum activities
- Use of NAMs for Environmental Safety Assessment, following a successful EPAA Partners Forum last year, via new EPAA Environmental Safety Assessment project
- Use of NAMs for the Assessment of Endocrine Disruption, via our forthcoming 2024 EPAA Partners Forum

We have sought to collaborate beyond the partnership to address these challenges, in particular stepping up exchanges with the Partnership for the Assessment of Risks from Chemicals (PARC) and ASPIS cluster of European Horizon 2020 projects (ONTOX, RISK-HUNT3R, PrecisionTox).

Finally, we continue to engage in outreach activities to disseminate our findings (e.g. organizing a poster exhibition at Helsinki Chemical Forum 2024 in April) and sense-check our progress with stakeholders (e.g. Lunch Debate in European Parliament in September).

We would like to take this opportunity as EPAA co-chairs to thank all of our EPAA partners and our Mirror Group members for their contributions, help, and support that made 2024 such a successful and productive year for EPAA.

Gavin Maxwell,
EPAA industry Co-Chair

Giulia Del Brenna,
EPAA European Commission Co-Chair

2 Membership update

In 2024, the Partnership includes 5 Directorates-General of the European Commission, 39 companies, and 9 European industry federations, representing 8 industrial sectors. Further information is available at the EPAA website: https://ec.europa.eu/growth/sectors/chemicals/epaa/partners_en

39 Companies

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, Emma Grange, Julia Baines, Winfried Neuhaus, Monique Janssens

Partner EU Agencies

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

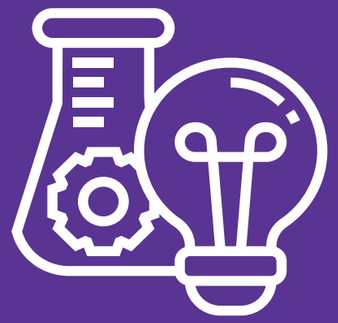
9 Sectoral Associations

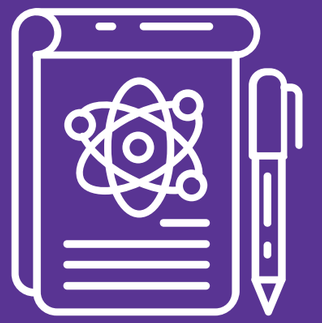
A·I·S·E

cefic

sme united
CRAFTS & SMEs IN EUROPE

3 Overview of the Project Platform in 2024





The EPAA aims to replace 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 those 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), including future approaches to hazard classification and engage and communicate with stakeholders in EU and globally.

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 2024, the PP has supported five project teams which synergistically combine the expertise and collaboration available across industry sectors, academia, NGOs and regulatory agencies.

This year, knowledge exchange on use of NAMs for hazard and risk assessment through user forum sessions has also been extended to address use of NAMs in priority regulatory testing requirements for chemicals, such as for systemic toxicity.

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 Carcinogenicity of Agrochemicals and Acute Toxicity are providing evidence to enable reductions and refinements of animal use in regulatory studies.

In the area of vaccines, the PP has so far supported the international collaborative study to validate the transferability and robustness of the selected ELISA for the replacement of the current in vivo potency test for the release of human rabies vaccines coordinated by EDQM and it will continue to follow the project progress in the next phase of routine testing, without being directly implicated.

Among the five ongoing projects, the environmental safety assessment project has newly been established following the recommendations from the related forum held in November 2023. This represents a major first step from EPAA towards support of non-animal methods for environmental safety assessment. Other projects (e.g., the Carcinogenicity of Agrochemicals and Acute Toxicity) are in the dissemination phase approaching completion.

Lastly, the PP is strategically re-orienting several of these projects towards the development of action plans in different areas of toxicology to directly contribute to the EC roadmap for phasing out animal testing in chemical safety assessments.

Projects in 2024

a. Acute Toxicity

b. Harmonisation of 3Rs in Biologicals

c. Carcinogenicity of Agrochemicals

d. Skin Sensitisation Dissemination (User Forum on use of NAMs)

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

f. Environmental Safety Assessment (ESA)

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 2024) on each individual project which are provided in blue, italicised text.*

a. Acute Toxicity

Identification of clinical signs predictive of mortality

Mammalian acute toxicity testing remains a requirement for chemicals, agrochemicals and biocides 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.)[1] were revised in waiving of acute toxicity testing via the dermal route under certain circumstances. Similarly, waiving criteria are included for dermal and inhalation toxicity in Regulation 283/2013 on active products used in plant protection products and in Regulation 528/2012 on biocidal products.

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

[1] 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

(NC3Rs) and its objective is to determine whether 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 project results are being widely disseminated. A peer-reviewed paper has been accepted for publication in Regulatory Pharmacology and Toxicology [2], and this work has been presented at international conferences including Society of Toxicology (San Diego 2022), ICT-Eurotox (Maastricht, September 2022), the European Society of Toxicology, Ljubljana 2023), and WC12 (12thWorld 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.

The projects’ findings are now being 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 according to OECD TG 420. This test 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 within OECD TG 420 (e.g. list of clinical signs that, if present, may be predictive of mortality at higher dose levels).*

Also, recommendations on a 3Rs-based classification & labelling decision framework to include replacement of death as an endpoint (for example using “evident toxicity”), and potentially other approaches (as in silico prediction, or harmonisation of route and/or low absorption based waiving criteria) are now being discussed. The potential for a 2025 workshop is being discussed. Outcome and recommendations will be reviewed by regulatory authorities and may contribute to development of the European Commission or EC Roadmap to ultimately phase out animal testing for chemical safety assessments.

[2] Sewell S. et al (2024) New supporting data to guide the use of evident toxicity in acute oral toxicity studies (OECD TG 420). Regulatory Toxicology and Pharmacology 146:105517 (https://doi.org/10.1016/j.yrtph.2023.105517)

b. Harmonisation of 3Rs in Biologicals

Deleting international regulatory requirements for in vivo general safety tests

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. This is because international divergence of testing requirements continues to be a challenge in the field of biological products. Therefore, 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 and manufacturing costs, and may delay patient access to essential vaccines and medicines.

The focus of the EPAA Biologicals project in 2024 remained on the area of pyrogenicity testing. Despite the fundamental shift on pyrogenicity testing taken by the European Pharmacopoeia which announced in June 2024 the complete stop to the rabbit-based test by July 1st 2025 (link: https://www.edqm.eu/en/-/ph.-eur.-bids-adieu-to-rabbit-pyrogen-test-in-its-monographs), and its openness towards a strategy to reach this key goal, other regions of the world still continue to require this assay and consider it as a gold standard. Furthermore, the uptake of the Monocyte Activation Test (MAT), which is a validated superior alternative, is rather slow by the authorities and manufacturers within and outside of Europe. The ALURES database of the European Commission shows a decrease of rabbits used for the pyrogenicity test from 2018 to 2022 (2020 statistics reported 23855 uses of rabbits for pyrogenicity, while 30277 were reported for 2019 and a similar number was reported for 2018). The statistics reports of 2021 and 2022 data document a further decrease (23 695 uses in 2021 and 19 168 in 2022), which amounts to a decrease of -35,7% from 2018 to 2022. This was underlined in the February 2023 three-day workshop, jointly organised by EDQM and EPAA that was held in Brussels (https://www.edqm.eu/en/-/joint-edqm-epaa-event-the-future-of-pyrogenicity-testing-phasing-out-the-rabbit-pyrogen-test). Authorities inside and outside the EU have ensured that there is a high interest regarding the use of MAT, however there is lack of experience and knowledge on the practical side.

To provide guidance to the authorities on the use of the MAT alternative method, the EPAA working group has agreed to draft answers

to Frequently Asked Questions. Those aim to provide guidance on how to implement the MAT and reduce potential concerns. Dissemination is foreseen via direct approach of authorities and conferences in 2024 and 2025, such as the AFSA/HSI workshop in September 2024, Pharmalab 2024, the 13th World Congress on Alternatives and Animal Use in the Life Sciences in 2025 and the AFSA/IABS conference at the end of 2025 (TBC).

Furthermore, the discussion on advocating for including the Vac2Vac project outcome to replace the in vivo potency assay of Tetanus, Diphtheria and acellular Pertussis containing vaccines with the developed in vitro assays in the pharmacopeia is ongoing. A proposal to EDQM has been submitted in September 2024 via the HELPDESK workflow where the group recommended the inclusion of a dedicated paragraph in the three relevant monographs.

c. Carcinogenicity of Agrochemicals

Waiving of two-year carcinogenicity studies

According to the current regulations 2-year carcinogenicity studies in rats and/or mice are performed to assess the potential for a non-genotoxic compound (i.e., a compound not inducing DNA damage) to increase the risk of cancer in humans. This is a regulatory requirement for pharmaceuticals, additives and chemicals (mainly agrochemicals and biocidal products). 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.

Previously, a successful EPAA project was conducted on the prediction of carcinogenicity of pharmaceuticals. The outcomes of the project provided evidence that in many cases a 2-year carcinogenicity study in rats could be waived without compromising human safety[3]. 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 project was followed-up with 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.

[3] 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

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 follow-up 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 most 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 [4] and another on the workshop [5].

A follow-up project was begun in March 2020 with the objectives of (i) identification of "known unknowns" and consolidation of MOAs, and (ii) 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 detailed review, since a substantial number of substances induced different types of tumours in different organs, with different combinations of unknown as well as known MOAs. Intermediate results were presented (orally plus poster) at ICT / EUROTOX (Maastricht, 2022). *The review has resulted in relevant information and data collected per organ and per tumour type, including potentially useful information to derive a MOA. The collected info were and are being discussed in a series of dedicated meetings with the project team. After these discussions are completed, the results for the known unknowns will be described in a scientific manuscript, to be submitted for publication in January 2025 at the latest. This publication will also entail the database that was developed for the project. 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". This will also contribute to a plan for alternative approaches to carcinogenicity assessment to support the development of the commission roadmap to ultimately phase out animal testing for chemical safety assessments.*

[4] 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

[5] 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

d. 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

Skin sensitisation to chemicals is a potential risk to human health and therefore reliable hazard evaluation and risk assessments need to be performed to ensure safe use of potentially sensitizing ingredients. 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 [6]. Recommendations from a previous Workshop [7] 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.

After a successful round of Skin Sensitisation User Forum sessions (covering amongst others cosmetics, fragrances, chemicals), each featuring a case study presentation followed by Q&A with more than ten EPAA member organizations, a second round started in 2023. The aim was to cover additional industry sectors, and case studies on assessments for pharmaceuticals and for agrochemicals were shared with new learnings for the user group. The potential to share case studies from the medical devices sector is being explored.

The needs for training sessions, publication and potentially merging with the systemic endpoint EPAA user forums are currently being evaluated.

[6] 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

[7] 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

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

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.

New approach methodologies (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) and the Scientific Committee of Consumer Safety has already uptake the NGRA approach in its Notes of Guidance[8].

In addition, The European Food Safety Authority (EFSA) has published a roadmap on the use

[8] SCCS (Scientific Committee on Consumer Safety), SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation 12th revision, 15 May 2023, corrigendum 1 on 26 October 2023, corrigendum 2 on 21 December 2023, SCCS/1647/22. https://health.ec.europa.eu/publications/sccs-notes-guidance-testing-cosmetic-ingredients-and-their-safety-evaluation-12th-revision_en

of NAMs in risk assessment [9] with a goal to routinely use NAMs to address data gaps by 2027. Furthermore, the European Commission, in its reply to the European Citizens' Initiative 'Save cruelty-free cosmetics – Commit to a Europe without animal testing', is developing a roadmap to ultimately phase out animal testing for chemical safety assessments [10].

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 topic is very relevant to the reduction of animal usage in REACH and other relevant regulations, the implementation of the EU Chemical Strategy for Sustainability, *and in the context of the European Commission's roadmap*. The EPAA is well placed to coordinate this work due to the cross-sectorial experience with use of NAMs in regulatory decision making.

The project held a "deep-dive" workshop in 2021 that identified several areas where progress could be made to increase the use and uptake of NAMs in regulatory decisions for chemical safety [11]. In addition, two EPAA Partners Forums (Brussels, May and November 2022) on "Exposure Considerations for Human Safety assessments" highlighted the importance of exposure-based approaches in facilitating the use and acceptance of NAMs approaches [12].

Since the workshops, two initial working groups (WG) were established to progress the NAMs related follow-up activities:

WG1 focussed on addressing the gap between scientific research and regulatory use and explored frameworks that could be used for regulatory purposes. This included the ECETOC Framework for chemical safety assessment incorporating NAMs within REACH [13] and the EC-JRC vison for a "Chemical 2.0" framework" centred on a classification matrix in which NAMs for toxicodynamics and toxicokinetics are used to classify chemicals according to their level of concern [14].

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 the use of NAMs in a specific area identified as currently being poorly addressed: the use of NAMs for classification of chemical-induced systemic human health effects. To facilitate this, EPAA launched the pilot phase of this work, the 'NAM Designathon 2023' Challenge for human systemic toxicity[15] which seeks to identify chemical classification systems capable of categorising chemicals based on their bioactivity (intrinsic toxicodynamic properties) and their potential systemic availability (intrinsic toxicokinetic properties). It was conceived as a collaborative approach which would involve any scientists interested working together to co-create a new NAM-based approach to hazard classification that does not need to predict the outcome of the animal studies, nor reproduce existing classifications but should reflect levels of concern associated with chemicals within the current GHS classification addressing systemic toxicity. EPAA hosted an information webinar, provided a list of 150 chemicals reflecting three levels of concern and a reporting template. *By the closing date of 31 December 2023, twenty-three teams submitted prototype solutions to the Designathon challenge. The aim of this pilot phase was to compare and contrast the different NAM-based solutions suggested by the participants and to co-create, rather than having a winning solution. Therefore, in March 2024 representatives from these teams met at the JRC in Ispra for a workshop with a subteam of the EPAA Project Team.*

[9] European Food Safety Authority (EFSA) has published a roadmap on the use of NAMs in risk assessment

[10] Commission acts to accelerate phasing out of animal testing in response to a European Citizens' Initiative

[11] 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

[12] 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

[13] 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

[14] 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

[15] NAMs Designathon challenge

Workshop participants explored the technical aspects of the NAMs used for bioactivity and bioavailability and discussed areas of commonality and differences in the approaches taken. Break-out sessions also explored approaches to classification of chemicals, integration of bioactivity and bioavailability information and what would be needed in the future to make the Designathon a success. Posters summarising each of the solutions, materials from the workshop and the recording of the dissemination webinar held on 8 May 2024, are available on the EPAA website [16].

Following the workshop, the team identified three key areas to be explored in greater depth to build on the work so far and to maintain the spirit of co-creation that defines the Designathon:

- *CHEMICAL SPACE: Explore chemical space and chemical uses, enrich the existing output with additional chemical information and further explore the results/classifications obtained with the reference chemicals to date.*

- *BIOLOGICAL SPACE: Focus on biological space by modes of action (including, but not limited to, AOP networks) and mechanistic relevance assessment of the TK and TD methods proposed to date.*

- *CLASSIFICATION STRATEGIES: Through the workshop, a common theme from the proposals was that a future NAM-based approach to classification would be tiered, though several different types of tiered strategies were described. The focus now will be to propose 2-3 classification approaches of human systemic toxicity and to understand how these different approaches could be further evaluated.*

Next steps involve the establishment of specific Working Groups to address these topics with an additional group overseeing the Designathon activity (Designathon Steering Team). This new stage of the project will be closely aligned to progress with the EC roadmap for phasing out animal testing in chemical safety assessments.

WG2 is focussed on building cross-sector, scientific consensus on regulatory use of NAMs for chemical safety assessment that was identified as a priority area in the EPAA NAMs deep-dive workshop. This team is extending the 'NAMs User Forum' format of scientific, case study-led discussions on NAM use that were developed under the EPAA Skin Sensitisation Dissemination activities to address other priority regulatory testing requirements for chemicals.

The kick-off meeting of the EPAA NAMs User Forum took place on 7-8 December 2023 in Helsinki hosted by ECHA. The two-day, hybrid event was attended by over 50 participants and started with introductory presentations sharing learnings and insights from the European Commission's Scientific Committee on Consumer Safety (SCCS) and EPAA's Skin Sensitisation Dissemination activities. The participants then heard and discussed, at length, five case studies that illustrated different regulatory uses of NAMs to address Systemic toxicity including Developmental and Reproductive Toxicity (DART) endpoints. The presentation titles and presenters were as follows:

- *EPAA workshops and other activities relating to the Development of Alternatives to Skin Sensitisation – Dr Petra Kern (Procter and Gamble) and Dr Katrin Schutte (DG Environment, European Commission)*

- *Use of NAMs in submissions to the EU SCCS: Personal insights and opinions – Prof. Em. Vera Rogiers (Vrije Universiteit, Brussels)*

- *Next Generation Risk Assessment (NGRA) using New Approach Methods (NAMs) to evaluate Systemic Safety for Consumers using Benzophenone-4 (BP-4) as a UV-filter in a sunscreen – Dr Maria Baltazar (Unilever)*

- *Integrating NAMs to prioritise and assess data poor Alternatives to Bisphenol A – Dr Tara Barton-Maclaren (Health Canada)*

- *A Connectivity Mapping (CMap) based assessment of Butylated Hydroxytoluene (BHT) for Endocrine Disruption (ED) potential – Dr Nadira De Abrew (Procter and Gamble)*

- *A Read-Across Case Study on Branched Carboxylic Acids for Repeated Dose Toxicity – Dr Sylvia Escher (Fraunhofer ITEM)*

- *Use of NAMs to refine and strengthen Structure-Activity Relationship (SAR) Read-Across for the Developmental and Reproductive Toxicity Effects of Branched-Alkyl Carboxylic Acids – Dr Petra Kern (Procter and Gamble)*

The overall conclusion of the kick-off meeting was that the EPAA User Forum was a valuable, open platform for regulators and stakeholders to share learnings and experiences of applying NAMs to fulfil systemic toxicity regulatory requirements. More generally, the consensus was that the User Fora provide a valuable platform for ongoing, cross-sector knowledge exchange that can help integrate efforts to standardise and build confidence in regulatory use of NAMs. The next EPAA NAM User Forum will take place on 30-31 October 2024, again hosted by ECHA in Helsinki, and will follow a similar format to the kick-off meeting.

[16] EPAA launches Designathon for human systemic toxicity

f. Environmental Safety Assessment (ESA)

The project is a follow-up of the EPAA Partners Forum (PF) held in November 2023 [16] *as the first PF to discuss alternatives to animal testing for environmntal assessments. During the PF, it became clear that the main priority in terms of high demand regarding the use of vertebrates and cross-sector relevance for environmental safety assessments is fish testing, covering two complementary elements: toxicity assessment (acute and chronic) and bioaccumulation potential. Such information is required for regulatory purposes such as the registration of chemicals, for classification and labelling and chemical safety assessments. In addition, an area of emerging interest is the use of amphibians for assessing the potential for endocrine disruption. Alternatives for toxicity testing on birds are very relevant in the case of agrochemicals. Mammals are currently mainly covered through the re-evaluation, in terms of ecological relevance, of toxicity studies conducted for the assessment of human health. Furthermore, discussions at the PF showed that the increase in knowledge of evolutionary biology and conservation of targets and processes across species could make it worth to foster dialogue and collaboration between the established fields of human health and environmental assessments when developing and implementing alternative approaches.*

In line with these discussions, the ESA project is structured as a set of activities , handled through specific Activity Working Groups, one for each action. It should be noted that all activities have been started from the very beginning of the project and are run in parallel. Coordination among related activities is ensured inter alia via the regular meetings of the whole project as well as through overlapping participation in the Working Groups. Activities are sub-divided into short-, medium- or long-term activities and are expected to give main results within two, five, or more than five years, respectively. The following list gives an overview of the activities:

Short-term: Integration of recent/draft OECD TGs in the regulatory frameworks:

- *Activity S1: WoE for fish acute toxicity*
- *Activity S2: Bioaccumulation*

Short- and medium-term:

- *Activity M1: Weight of evidence for waiving chronic fish testing: also covering acute fish testing when current OECD NAM TGs are not suitable.*
- *Activity M2: New approaches for validation of new methods, including recommendations for other groups.*
- *Activity M3: Alternative methods for endocrine disruption.*
- *Activity M4: Alternative methods for birds and mammals in ESA.*

Medium- and long-term:

- *Activity L1: Envisioning a new ESA paradigm, including replacement of all animal testing methods including invertebrates. Following the envisioning phase, specific activities may be identified.*

During the project kick-off meeting, following a presentation from the European Commission (EC), it was agreed to focus the project efforts for the first year in preparing proposals for supporting the EC roadmap to ultimately phase out animal testing for chemical safety assessments. Therefore, each action WG is preparing a document focusing on elements within each area, which could be relevant for supporting the roadmap development, such as:

- *What are the current information requirements (covering all EPAA sectors and the needs for both risk assessment and classification and labelling)?*
- *What are the current alternatives for addressing the information requirements?*
- *What is their state of standardisation and validation?*
- *What are the current efforts for integrating these alternatives in the EU regulatory frameworks?*
- *What are the main obstacles?*

The content will be adapted to each specific theme and drafted in the format of short Executive documents, complemented with Annexes if needed.

In line with the EC roadmap timeline, a first draft paper should be ready for discussion at a face-to-face meeting in November 2024 and the document (or the part relevant for the EC roadmap development) finalised latest by summer 2025.

Some activities are long-term and are expected to continue during the roadmap implementation, this is obvious in the case of the medium-term activities and the long-term activity for envisioning a new ESA paradigm.

16 EPAA Partners Forum (Nov, 2023): Use of Alternatives to Animal Testing for Environmental Safety Assessment. Flash report. https://single-market-economy.ec.europa.eu/system/files/2023-11/Partners%20Forum%202023%20-%20flash%20report.pdf

4 Dissemination and Communication

3Rs Science Prize 2024

The EPAA is dedicated to advancing the development, validation, and regulatory acceptance of 3Rs alternative approaches—Replacement, Reduction, and Refinement of animal testing. Every two years, the 3Rs Science Prize is awarded to a scientist whose work makes an outstanding contribution to the 3Rs principles. Through this prize, EPAA aims to highlight and encourage significant contributions from industry or academia, motivating more researchers to focus their efforts on the 3Rs goals.

Scientists working on innovative methods relevant to regulatory testing (e.g., safety, efficacy, batch testing) that demonstrate a significant impact on the 3Rs are invited to apply for the €10,000 prize.

Assessment is conducted over 6 selection criteria defined by the EPAA Steering Committee:

1. Impact on the 3Rs (reduction, refinement, replacement of animal uses)

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)

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 2024, a total of 6 high-caliber applications were submitted to the EPAA secretariat and evaluated by the selection committee.

The Prize was awarded to **Dr Jakub Tomek** and his case study **“ToR-ORd: A computational model of human ventricular cardiomyocyte for arrhythmia research and drug safety assessment”**. Dr Tomek works at the Department of Physiology, Anatomy, and Genetics, University of Oxford.

Quotes from the Evaluation Committee:

“Certain to reduce numbers of animals i.e. via prescreening of candidates prior to animal testing.”

“Applicable for each area requiring an assessment of cardiotoxicity”

“Good validation approach for in silico model”

“Demonstrated to be of interest for industry and regulators.”

3Rs Student Grants 2024

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.

This year, a full grant of €1000 and a half grant of €500 were available for ESTIV 2024, EUSAAT 2024 and EUROTOX 2024.

EUSAAT 2024 winners

A full grant: **Mariana Guedes**
"Establishing a lentiviral reporter platform for screening 3D human lung organoids: a proof-of-concept approach"

A half grant: **Emma Rowan**
"Multi-behavioral fingerprints in larval zebrafish can identify neuroactive environmental chemicals and underlying mechanisms"

EUROTOX 2024 winners

A full grant: **Peter Pôbiš**
"Novel *In Vitro* Protocol for Evaluation of Safety of Intraoral Medical Devices"

A full grant: **Nadia Katherine Herold**
"Multi-behavioral fingerprints in larval zebrafish can identify neuroactive environmental chemicals and underlying mechanisms"

ESTIV 2024 winners

A full grant: **Mariam Saleh**
"*In vitro* based testing strategy for the identification of non-genotoxic carcinogens (NGTxC)"

A half grant: **Francesca Carlotta Passoni**
"An Integrated Human and *In Vitro* Approach to Investigate the Role of miRNAs in Allergic Asthma"

EPAA EVENTS

1 - EPAA NAM Designathon Challenge

On 22-24 March, Ispra, Italy

At the European Commission's Joint research centre (JRC) in Ispra Italy, 44 scientists met for the first workshop to discuss the solutions submitted for the EPAA Designathon. The Designathon was launched in May 2023 and asked for solutions to be submitted that could allow an alternative, non-animal approach to hazard classification, based solely on the use of New Approach Methodologies (NAMs) for systemic toxicity. The aim of the Designathon is to design a potential future classification scheme to ensure equivalent protection, by capturing substances that are currently classified, but that could also increase the overall protection level, by assessing substances not currently classified due to a lack of information.

2 - EPAA Designathon Webinar

8 May, Online

EPAA Designathon Webinar was organised on 8 May (online) to report on the outcome of the Designathon workshop and next steps. The recording is available.

3 - EPAA lunch debate in the European Parliament

17 September, Strasbourg, France

Hosted by MEP Tilly Metz, this event focused on EPAA's efforts to promote the 3Rs principles and its role in advancing the European Commission's Roadmap for phasing out animal testing in chemical safety assessments. Key discussions centered around the regulatory use of non-animal methods (NAMs), with presentations from EPAA's leadership and MEPs emphasizing the urgency of collaboration between regulators, industry, and stakeholders to accelerate the transition to animal-free, sustainable innovation. The debate underscored EPAA's pivotal role in driving progress towards this goal.

4 - NAM User Forum: Integrated Approaches for Testing and Assessment (IATA) of Systemic Toxicity

30-31 October, Helsinki, Finland

This two-day workshop will focus on best practices and minimum requirements for regulatory use of New Approach Methods (NAMs) for systemic toxicity. The agenda includes lessons from ECHA and EFSA, an overview of the ASPA workflow from the ASPIS cluster, and five case studies on NAM-supported read-across, toxicokinetics/bioavailability, and toxicodynamics/bioactivity characterizations.

5 - EPAA Annual Conference 2024: Maximising NAM uptake under existing EU regulations

13 November, Brussels, Belgium

The annual event provides participants with insights into EPAA's key achievements. The event includes the announcement of the winners of the EPAA 3Rs Science Prize. Additionally, discussions take place across two panels on the potential to maximize the uptake of New Approach Methodologies (NAMs) under existing EU chemical and pharmaceutical regulations.

6 - EPAA Partners Forum: Use of NAMs for the assessment of Endocrine Disruption (ED) within EU regulatory frameworks (human health and environment)

14-15 November, Brussels, Belgium

A hybrid event organized annually as an opportunity for the EPAA members to meet and share knowledge and experience around a 3Rs-related topic of cross-sector interest. In 2024, it focuses on a strategic review of the use of NAMs for assessing Endocrine Disruption (ED) in EU regulatory frameworks, featuring over 20 presentations and round-table discussions aimed at generating scientific recommendations for a peer-reviewed publication.

EXTERNAL EVENTS

EPAA projects and achievements were presented at the folowing scientific events:

1 - EPAA at the Helsinki Chemicals Forum 2024

10-11 April, Helsinki, Finland

EPAA sponsored the event and showcased a poster exhibition highlighting the collaborative efforts of EPAA partners and stakeholders, to advance the use of New Approach Methods (NAMs) for chemical regulatory decision-making. A testimonial video featuring influential figures from the European Parliament, ECHA, EFSA, DG GROW, and industry also emphasized EPAA's impact in promoting non-animal testing methodologies across the EU.

2 - EMA 3Rs Working Party Stakeholders meeting

20 March, Online participation

3 - Multi-stakeholder Roundtable on the Commission Roadmap towards phasing out animal testing for chemical safety assessments

18 June, Brussels, Belgium

4 - 2nd Workshop to Commission's Roadmap towards phasing out animal testing for chemical safety assessments

25 October, Brussels, Belgium

PUBLICATIONS

C.V. Thompson; S.D. Webb; J. A. Leedale; P.E. Penson; A. Paini; D. Ebbrell; J.C. Madden (2024). "Using read-across to build physiologically-based kinetic models: Part 1. Development of a KNIME workflow to assist analogue selection for PBK modelling". Computational Toxicology https://doi.org/10.1016/j.comtox.2023.100292

C.V. Thompson; A. Paini; S.D. Webb; D. Ebbrell; J.A. Leedale; J.C. Madden (2024). "Using Read-Across to build Physiologically-Based Kinetic models: Part 2. Case studies for atenolol and flumioxazin". Computational Toxicology https://doi.org/10.1016/j.comtox.2023.100293

F. Sewell; I. Ragan; G. Horgan; D. Andrew; T. Holmes; I. Manou; B.P. Müller; T. Rowan; B.G. Schmitt; M. Corvaro (2024). "New supporting data to guide the use of evident toxicity in acute oral toxicity studies (OECD TG 420)". Regulatory Toxicology and Pharmacology https://doi.org/10.1016/j.yrtph.2023.105517

J.V. Tarazona; A. Fernandez-Agudo; O. Adamovsky; M. Baccaro; N. Burden; B. Campos; B. Hidding; K. Jenner; D. John; K. Lacasse; A. Lillicrap; D. Lyon; S.K. Maynard; A. Ott; V. Poulsen; M. Rasenberg; K. Schutte; M. Sobanska; J.R. Wheeler (2024). "Use of Alternatives to Animal Testing for Environmental Safety Assessment (ESA): Report from the 2023 EPAA Partners' Forum". [Manuscript in preparation].

M.T.D. Cronin; M. Baltazar; T. Barton-Maclaren; O. Bercaru; N. De Abrew; C. Desaintes; S. Escher; P. Kern; G. Maxwell; V. Rogiers; K. Schutte; T. Sobanski (2024). "Report on the European Partnership for Alternative Approaches to Animal Testing (EPAA) "New Approach Methodologies (NAMs) User Forum Kick-off Workshop". [Manuscript in preparation].

5 Future prospects

As mentioned in the Foreword, EPAA has evolved our strategic and project platform activities to inform the scoping of the European Commission 'Roadmap towards phasing out animal testing for chemical safety assessments'. The need for EPAA-EC Roadmap alignment continues to be a major driver for our 2025 workplan and we have sought to scope new strategic and project platform activities to address three main strategic goals:

1. BRIDGING THE RESEARCH TO REGULATORY USE GAP

a. Disseminating and acting upon the output of the EPAA 2024 Partners Forum 'NAMs for the assessment of endocrine disruption'

b.Broadening the scope of EPAA Carcinogenicity project to develop an Animal-Free Safety Assessment framework suitable for all chemicals

2. BUILDING CONFIDENCE IN NON-ANIMAL APPROACHES

a. EPAA NAM User Forum (Systemic Safety Assessment and Skin Sensitisation) to co-develop best practice for regulatory use through case study-led scientific dialogue

b. Broadening the EPAA Acute Toxicity project to review NAMs for Regulatory Use

c. EPAA Harmonisation of 3Rs in Biologicals project

3. TRANSITIONING TO A NEW GLOBAL REGULATORY PARADIGM

a. EPAA NAM Designathon (Systemic Safety Assessment) to co-develop a new NAM-based approach to chemical classification

b. EPAA Environmental Safety Assessment project to develop an Animal-Free Safety Assessment framework suitable to assess hazards and risks to the environment

All these activities will help inform the European Commission Roadmap discussions via EPAA representation on the European Commission working groups and through EPAA coordination of an Animal-Free Chemical Safety Assessment workshop in early 2025 (date tbc) that will seek to bring EPAA partners together with other collaborators to develop consensus proposals on short, medium, and long-term Roadmap priorities and challenges for EPAA to address via our 2026-2030 strategy.

Finally, 2025 represents an important milestone for EPAA as we will celebrate the twentieth anniversary of the partnership. To mark this occasion we will host a high level workshop ahead of our 2025 annual meeting to reflect upon the origins of EPAA, last two decades of progress, and debate how to position the partnership to accelerate the transition to animal-free sustainable innovation.

6 Acronyms and Abbreviations

3Rs: Replacement, Reduction and Refinement of Animal Testing

3T3 NRU PT: Neutral Red Uptake Photo-toxicity assay using the 3T3 mouse fibroblast cell line

AAT: Alternatives to Animal Testing

ANSES: Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, FR

BCOP: Bovine Corneal Opacity & Permeability Assay

BfR: Bundesinstitut fur Risikobewertung, DE

BSP: Biologicals Standardisation Programme

CEFIC: European Chemical Industry Council

CLP: Classification and Labelling of Products

CMR: substances that are carcinogenic, mutagenic or toxic to reproduction

CSS: Chemical Strategy for Sustainability

DG: Directorate General (of the European Commission)

DG ENV: European Commission Directorate-General for Environment

DG GROW: European Commission Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs

DG JRC: European Commission Directorate-General Joint Research Centre

DG RTD: European Commission Directorate-General for Research and Innovation

DG SANTE: European Commission Directorate-General for Health and Food Safety

EC: European Commission

ECHA: European Chemicals Agency

EDQM: European Directorate for the Quality of Medicines & HealthCare (Council of Europe)

EFPIA: European Federation of Pharmaceutical Industries and Associations

ELISA: Enzyme Linked Immunosorbent Assay

EMA: European Medicines Agency

EP: European Parliament

EPAA: European Partnership for Alternative Approaches to Animal Testing

EURL ECVAM: The European Union Reference Laboratory for Alternatives to Animal Testing

EUROTOX: Association of European Toxicologists and European Societies of Toxicology

EUSAAT: European Society For Alternatives To Animal Testing

EUToxRisk: An Integrated European 'Flagship' Programme Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st century

IATA: Integrated Approaches to Testing and Assessment

IMI: Innovative Medicines Initiative

ITS: Integrated testing strategies

JEG 3Rs: Joint Expert Group on 3Rs

MGEN: Model Equation Generator software

MEB: Medicines Evaluation Board

NAMs: New Approach Methodologies

NC3Rs: National Centre for 3Rs (UK)

OECD: Organisation for Economic Co-operation and Development

PBTK: Physiologically-Based Toxicokinetic

REACh: Registration, Evaluation, Authorisation and Restriction of Chemicals

RVis: R Visual; a prototype for the analysis of structure and performance of PBPK, and other models, written in the free, open source syntax R or C++

SEURAT-1: Safety Evaluation Ultimately Replacing Animal Testing

WC12: 12th World Congress on Alternatives and Animal Use in Life Sciences

WHO: World Health Organisation

Notes

EPAA website

LinkedIn

European Partnership for Alternative Approaches to Animal Testing (EPAA)