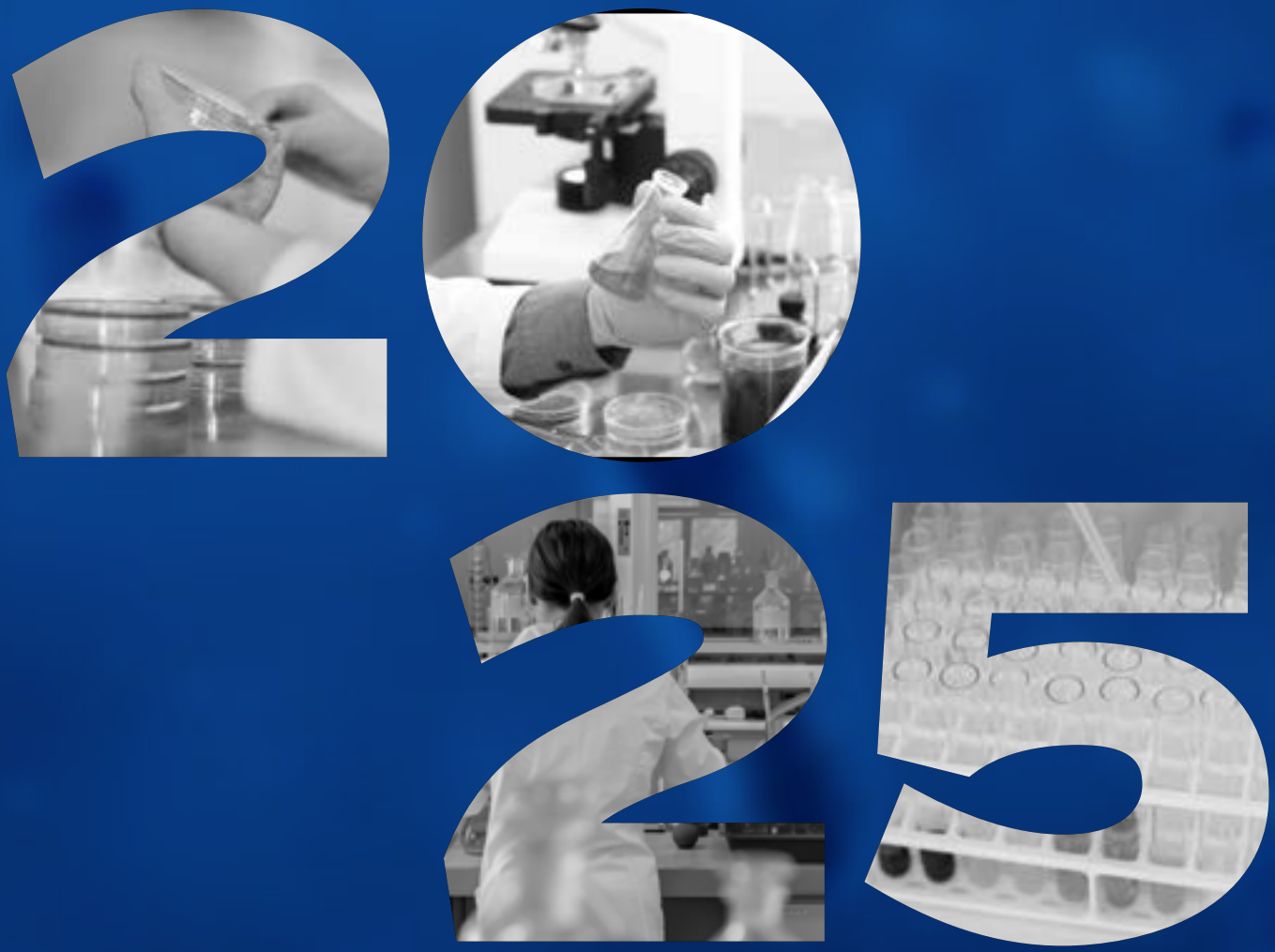
European Commission

The European Partnership
for Alternative Approaches to Animal Testing

ANNUAL REPORT

European Partnership for Alternative Approaches to Animal Testing (EPAA)



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1. Foreword

As EPAA celebrates its 20th year the value of the partnership and role we can play in advancing the regulatory use of 3R (replacement, reduction, refinement) methods feels as important as ever!

Our recent focus has been supporting the development of the Commission Roadmap to phase out animal testing for chemical safety assessment and in March we were honoured to support the organisation of the Animal-Free Chemical Safety Assessment (AF-CSA) conference where 250+ experts were brought together to develop short-, medium-, and long-term priorities for phasing out regulatory animal testing through maximising use of NAMs. The draft AF-CSA conference report is available on the website, and the final report and detailed breakout group proposals will be published in due course.

In addition, we've also had some notable highlights across our project platform activities:

Bridging the Research to Regulatory Use Gap

- Carcinogenicity project team have re-focussed their activities, starting with a January workshop, to develop a new Carcinogenicity Assessment framework.

Building Confidence in animal-free approaches

- Acute Toxicity project team have re-scoped their activities to focus on exploring the potential to waive acute dermal toxicity testing based on analysis of existing data.

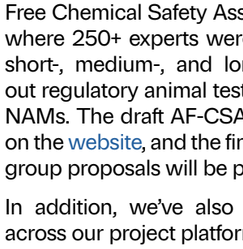
Transitioning to a new Global Regulatory Paradigm

- Environmental Safety Assessment project team have met several times in 2025 identifying short-, medium-, and long-term priorities and also starting to develop a new paradigm for environmental safety assessment.

Our communication activities this year will naturally culminate in our 20 th anniversary, two-day annual conference in November where we will invite EPAA partners and stakeholders to share their views on how EPAA can best support the implementation

of the Commission Roadmap and ensure the partnership continues to grow and thrive.

Finally, we welcome four new company members to EPAA this year (AstraZeneca, Clariant, Huntsman, Mane) and one new association member (IOFI – International Organization of the Flavor Industry) – we thank them and all our members and partners for their support this year!



Giulia DEL BRENNA

EPAA European Commission

Co-Chair

Gavin MAXWELL

EPAA Industry

Co-Chair / Unilever

2. EPAA Members

In 2025, the Partnership includes 5 Directorates-General of the European Commission, 41 companies, and 10 European industry federations, representing 9 industrial sectors. Further information is available at the EPAA website: https://ec.europa.eu/growth/sectors/chemicals/epaa/partners_en

NEW COMPANY MEMBERS: AstraZeneca, Clariant, Huntsman, Mane
NEW TRADE ASSOCIATION MEMBER: International Organization of the Flavor Industry (IOFI).

41 Companies (including 1 SME)

ADAMA
AstraZeneca
BASF We create chemistry
BAYER
Beiersdorf
Boehringer Ingelheim
CHANEL
CLARIANT
COLGATE-PALMOLIVE
CORTEVA agriscience
DOW
dsm-firmenich
essity
ESTĒE LAUDER
EVONIK Leading Beyond Chemistry
Gowan
GSK
Henkel
HUNTSMAN Enriching lives through innovation
Johnson&Johnson
Kanebo
L'ORÉAL GROUPE
LVMH
MERCK
MANE
NOVARTIS
novonesis
novo nordisk
P&G
reckitt
REVLON
sanofi
SHISEIDO
SOLVAY
stratiCell
SYENSQO
symrise
syngenta
Unilever
zoetis

10 Sectoral Associations

A·I·S·E since 1952
animalhealth europe
cefic
Cosmetics Europe the personal care association
CropLife EUROPE
efpia
FOODDRINK EUROPE
ifra
IOFI
sme united CRAFTS & SMEs IN EUROPE

5 DG's of the EC

European Commission

DG GROW
DG ENV
DG SANTE
DG JRC
DG RTD

Including Partner Agencies

ECHA EUROPEAN CHEMICALS AGENCY
efsa
EUROPEAN MEDICINES AGENCY SCIENCE MEDICINES HEALTH

Mirror Group (Advisory body)

Emma Grange (Chair), Emily McIvor, Christiane Hohensee, Helena Kandarova, Sirpa Pietikaïnen (MEP), Vera Rogiers, Julia Baines, Winfried Neuhaus, Monique Janssen

3. EPAA projects Overview

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 2025) on each individual project which are provided in green, 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 (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)(OECD TG 420) 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 were presented at international conferences in 2022/2023 and published in 2024 in Regulatory Pharmacology and Toxicology[2], The project findings together with additional information will also be published on the NC3Rs website.

NC3Rs continues to lead advocacy activities to increase the use of the OECD TG 420, including activities to revise the test guideline. A major step was taken in 2025 by organisation of two round-table discussions with various stakeholders to identify potential roadblocks and opportunities for communication and further research on acute oral and inhalation toxicity.

Supporting the development of the European Commission's Roadmap to Phase Out Animal Testing for Chemical Safety Assessment, a breakout group "Towards animal-free acute toxicity assessment" was held during EPAA's Animal-Free Chemical Safety Assessment Conference. Breakout group discussions focused on the identification of research needs short-, medium, and long-term, for acute oral, dermal and inhalation toxicity.

Building on promising recent scientific developments on in silico approaches to address acute oral toxicity testing, EPAA also supports an on-going BfR/ECHA-led pilot project to validate the CATMoS Consensus model[3] for potential replacement of acute oral toxicity in vivo testing in a regulatory context. Interim results were presented at the 13th World Congress on Alternatives and Animal Use in the Life Sciences, Rio de Janeiro and the 59th Eurotox Congress, Athens.

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 2025 continued to be mainly on the area of pyrogenicity testing. The group continued to develop the Frequently Asked Question including feedback from key stakeholders in the field to broaden input that could support the implementation of the Monocyte Activation Test (MAT) as replacement of the Rabbit Pyrogenicity Test (RPT) in Europe and globally. To further support the overall implementation effort, the group agreed to support the organisation of the EPAA and EDQM symposium" Pyrogen testing 2.0: Ethical, Evolving and Eco-friendly - Implementing safe, rapid, state-of-the-art and sustainable non-animal approaches worldwide", *that is going to take place on 25-26 February 2026 in*

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

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)

3 Mansouri et al., 2021, CATMoS: Collaborative Acute Toxicity Modeling Suite. Environmental Health Perspectives 129(4). doi: 10.1289/EHP8495

Brussels. The symposium is a follow up of the 2023 EDQM- EPAA conference "The future of pyrogenicity testing: phasing out the rabbit pyrogen test" (Cirefice at al, 2023.) The symposium aims to provide updates on the implementation of the RPT phase-out strategy across Europe, explore the challenges and solutions encountered during the transition to MAT; share insights into how other pharmacopoeias and regulatory frameworks outside Europe have adapted in response to the European Pharmacopoeia's initiative (link: https://www.edqm.eu/en/-/ph.-eur.-bids-adieu-to-rabbit-pyrogen-test-in-its-monographs); *and bring forward perspectives from regulators, industry, and international stakeholders on the future of animal-free pyrogenicity testing. The symposium will also offer an opportunity for participants to familiarise themselves with the current use of recombinant reagents, such as recombinant Factor C and recombinant Cascade Reagents for use in the bacterial endotoxins test (BET).*

The group has also started discussions and targeted follow up activities through its members to clarify the expectations from EMA and National Regulatory Agencies in Europe and outside on the implementation of the recombinant Factor C and recombinant Cascade Reagents for new and already registered human and veterinary biologicals.

Furthermore, 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 expected to bring a dedicated paragraph to be included in the Eur. Ph.'s 12th Edition. The group has been exploring additional dedicated activities to promote the implementation of the BINACLE (BINding And CLEavage) in vitro assay as a possible concrete replacement for Tetanus vaccine safety testing, currently performed in mice, and further promote the outcomes of the EDQM BSP136 that evaluated BINACLE's applicability to current products (link: https://www.edqm.eu/en/-/binacle-assay-for-tetanus-neurotoxin-outcomes-of-project-bsp136-2).

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[4]. 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.

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[5] and another on the workshop[6].

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.

The publication on the follow-up project was delayed due to unforeseen due to unexpected temporary unavailability of staff members of staff members at RIVM. Drafting of the manuscript is nearly completed and submission of the publication is planned for Autumn 2025. The publication will be accompanied by the databases developed in the last two EPAA-funded projects on this topic, to foster further analysis and research by other parties.

In addition, the project gave rise to a dedicated working group to develop input for the European Commission Roadmap on phasing out animal testing for chemical safety assessments. This working group kicked off late 2024 and convened for a hybrid meeting in Brussels in January 2025. The working group started with creating a list of similar efforts on carcinogenicity in different sectors and jurisdictions to foster understanding and create synergies. As a next step, discussions focused on the scope of the contribution to the roadmap, and the areas to cover. It was agreed to provide recommendations on policy, best practices, communication and science. The latter was considered to be covered quite well by the EPAA projects. Therefore, a brainstorm session held at the Animal-Free Chemical Safety Assessment conference focused on the other three areas. Based on the input collected and the knowledge gained in the EPAA projects, a chapter on implementing change for carcinogenicity assessments was drafted. The chapter provides background, an overview of related initiatives, a global carcinogenicity assessment framework and a series of recommendations, clustered according to the four areas defined. The chapter will be transformed into a publication for a scientific journal in Autumn 2025.

4 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

5 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

6 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. NAMs Designathon

This project focuses on addressing the gap between scientific research and regulatory use and explored frameworks such as the ECETOC Framework for chemical safety assessment incorporating NAMs within REACH[7] 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[8].

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[9], 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 for the challenge prototype solution. 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.

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, *the workshop flash report* and the recording of the dissemination webinar held on 8 May 2024, are available on the EPAA website[10].

Following the workshop, three key areas were identified for further exploration to build on the progress 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.

Three Working Groups (WG) *were established* to address these topics, *along* with an additional group overseeing the Designathon activity (Designathon Steering Team, *as the evolution of the original WG1), enabling the start of the co-creation phase in October 2024*. This new stage of the project was meant to closely align to progress with the EC roadmap for phasing out animal testing in chemical safety assessments.

On 1-2 July 2025, members of the Designathon Steering Team and the co-chairs of the three WGs convened at the JRC in Ispra, Italy, to review the progress made by the WGs. The chemical space WG focused on the creation of a Universal Set of chemicals that offers broad chemical space coverage, diverse use classes and good representation of regulatory databases. The group has begun exploring the chemical space of the Designathon Set (original 150 chemicals) by mapping it against the Universal Set. Ultimately, a Calibration Set that expands on the Designathon Set, representing a wider range of use classes and chemical properties will be created. They have compiled detailed physicochemical properties, use classes, and market availability for the Designathon Set,

7 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

8 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

9 NAMs Designathon challenge

10 EPAA launches Designathon for human systemic toxicity

and generated molecular identifiers and descriptors for both the Designathon and the Universal Sets, which are planned for the Calibration Set. Additionally, the group has established workflows for chemical space analysis.

The Biological Space WG focused on highlighting biological domains and collecting mechanistic information for the Designathon set (both in terms of toxicodynamic and toxicokinetics, as well as tools presented in the Designathon prototype solutions), to support a hypothesis-driven approach and serve as anchors for data integration. The group has begun reaching out the relevant projects from the ICCS (International Collaboration on Cosmetics Safety) to exchange information and explore collaboration.

The classification strategies WG concentrated on the design principles for the Designathon classification and started evaluating the relative value of relevant variables, or properties,for both toxicodynamics and toxicokinetics. They explored ways to combine these variables to define the boundaries of the three levels of concern (high, medium and low).

The meeting provided valuable insights and directions to streamline the work, prioritising key objectives and guide the Designathon participants toward achieving the goals of the co-creation phase. A new working model was discussed and approved, and specific activities planned for the upcoming months, focusing on co-creation and the future design of the testing phase. An initial framework or tiered strategy should be developed to ensure a straightforward, adaptable, and organised approach. Design principles will be further assessed and prioritised by connecting them with the tools, data, and methodologies of prototype NAM-based solutions from the pilot phase in a technology-neutral manner, evaluating the feasibility of each identified option. Additional crucial tasks include establishing minimum quality standards for key ADME (Absorption, Distribution, Metabolism, Excretion) and toxicokinetic properties, as well as the outputs of the Physiologically Based Kinetic (PBK) models. Key tasks for designing the testing phase include characterising and integrating uncertainty into the process, mapping the technology-specific datasets from the existing Designathon solution, and expanding the biological domain to enhance the chemical space, which is essential for defining the calibration dataset for testing.

The next steps involve establishing sub teams with volunteers to actively contribute to these activities.

e. NAMs User Forum

The initially formed 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 overall conclusion of this 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. A peer-reviewed publication reporting from the 2023 NAMs User Forum is available at: https://doi.org/10.1016/j.yrtph.2025.105796

A new EPAA NAMs User Forum took place on 30-31 October 2024, again hosted by ECHA in Helsinki, and followed a similar format to the kick-off meeting. The User Forum included a number of oral presentations and discussions related to the implementation of NAMs. Learnings from applying NAMs for regulatory use were presented by the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA). Experience from the development of the Developmental and Neurotoxicity in vitro battery (DNT IVB) and the ASPIS-initiated Safety Profiling Algorithm (ASPA) were shared. In addition, five case studies described the use of NAMs for chemical safety assessment. The presentations demonstrated the good progress in NAMs and highlighted how tiered testing strategies for non-

animal information can be organised to improve safety decision-making. A report from the 2024 NAMs User Forum is about to be finalised and will be submitted for publication at the end of 2025.

f. Environmental Safety Assessment (ESA)

The project is a follow-up of the EPAA Partners Forum (PF) held in November 2023[11] as the first PF to discuss alternatives to animal testing for environmental 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.

Discussions at this event concluded further that the long-term goal should be a system fully based on non-animal approaches, while in the short- and medium-terms the use of test methods based on invertebrates and vertebrate embryos should be part of the 3Rs approach. Achieving this goal could be facilitated by a revision of the current paradigm for environmental safety assessments, developed several decades ago based on the scientific knowledge and information available at that moment. In consequence, a cross-sector re-evaluation of the current scientific state-of-the-art as well as the protection goals and expectations of environmental safety assessments was identified as the way forward for moving towards a new paradigm that could ensure that animal testing is avoided or at least minimised.

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, running 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.

The timelines consider the feedback from EC, and each activity WG prepared by end Q1 2025 a document focusing on the key elements within each area. Each document starts with specific recommendations to be considered for the Roadmap, following by sections with the analysis of the current state of the science, summaries of the information sources, discussing the results, and justifying the proposed recommendations.

The recommendations were presented for discussion in the EC Environment Working Group and at the SETAC Europe meeting and will be further disseminated in other fora, including the WC13 on Alternative Methods and the Innovate EcoSafety Summit 2025.

To increase visibility and relevance the next milestone is to publish the documents in a peer-review journal as contributions to a Special Issue on recommendations for the EC Roadmap.

The next steps were discussed at a dedicated project meeting and will focus on supporting the EC Roadmap implementation, including the consideration for conducting cross-sector case studies.

g. Second non-rodent species for repeated-dose toxicity testing (new)

Aim of the project: to share experience in 3Rs opportunities in the use of NAMs for waiving and ultimately replacing non-rodent species toxicity testing.

Background: Regulations on human safety assessment may require testing in rodent and a second, non-rodent species for the development of new human and veterinary medicines, plant protection products, biocide products or feed additives in the EU and beyond. This testing is commonly conducted in dogs for repeated-dose toxicity (RDT) and rabbits for developmental toxicity in addition to rodent studies. The rationale for conducting non-rodent studies is to identify any potential health hazards that would not have been detected in rodent studies alone. However, the experimental design of non-rodent studies for RDT has several limitations, including low statistical power of the studies, leading to uncertainties in interpretation. As a consequence, the added value of these studies as part of a weight-of-evidence approach is questioned depending on the context of use. In addition, recent analyses have shown that, for agrochemicals at least, testing in the dog as the second species provided little added benefit to most human health risk assessments. Non-rodent species for repeated dose toxicity is not required by the EU REACH regulation and is banned in the cosmetic sector.

The project: The project aims to contribute to the development of the European Commission's Roadmap for phasing out animal testing for chemical safety assessments.

1. *A break-out session at the Animal free Chemical safety assessment conference (4-6th March Brussels) was held to allow industry and regulators to identify opportunities and ongoing efforts to waive the study completely for agrochemicals or, where this is not possible, further optimize study designs to reduce and refine animal use in the current studies. Representatives from various sectors met to provide cross-sectoral scientific awareness and exchange, priority setting and identification of 3R's opportunities towards replacing in vivo non-rodent species testing. The different industry sectors are starting from different states of requirements and readiness for phasing out non-rodent (e.g. dog) studies. It was highlighted that mapping the use of non-rodent species in the different sectors and the value of the studies depending on the different context of use could be a first step towards regulatory changes and roadmap implementation. Also, ensuring an equivalent or higher level of human health protection for chemicals and pharmaceuticals globally is key. The recommendations were organised into short, medium and long-term actions.*
2. *A poster for Eurotox 2025 was prepared as an output from the workshop and provides an overview of the current regulatory frameworks and health protection objectives of the second species used for RDT across different sectors.*
3. *A supporting document for the roadmap development is being prepared to describe the key ongoing initiatives and recommendations towards phasing out non-rodent species for repeated-dose toxicity.*

11 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. Communication

a. Refinement Prize 2025

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:

- Impact on animal welfare – evidence base
- Creativity and innovation
- Practicability, applicability and implementation potential to regulatory testing and/or quality control
- Potential for wider impact beyond immediate area
- Publication of data or potential for publication

In 2025, the refinement prize was won by 3R's Champion Group/Lee Robinson, Pawlowic Lab and their case study which focuses on Voluntary Administration of Substances. The University of Dundee, as part of its development of an institutional 3Rs strategy, has put in place a 3Rs Champions group (3RCG). This group comprises animal technicians from our facilities who work with research groups and the designated veterinarian to refine experimental and husbandry procedures.

> "The Dundee University 3R Champions Group is extremely proud of the refinement we have implemented in our facility and the recognition it has received. We hope this refinement is adopted by other facilities and is beneficial to their research"
>
> Lee Robinson

Quotes from the Evaluation Committee

"High impact on animal welfare"

"Simple method with large application"

"Clear welfare benefits by reducing restraint stress."

"Well-validated refinement with clear dual benefits for animals and staff."

b. 3Rs Student Grants 2025

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 2 full grant of €1000 were available for each of the eligible events: SETAC 2025, MPS World Summit 2025 and EUROTOX 2025.

Andrea Bezze, MPS World Summit 2025.

SETAC 2025 winners

1. Vanessa Srebny, "Comprehensive Toxicity Profiling of 26 BPA Alternatives Based on Eight Cell-Based in vitro assays and Abiotic CYP Oxidation Test"
2. Donghyeon Kim, "Ecotoxicity prediction of plastic additives and their alternatives using ToxCast/Tox21 data-based machine learning models within a cross-species adverse outcome pathway framework"

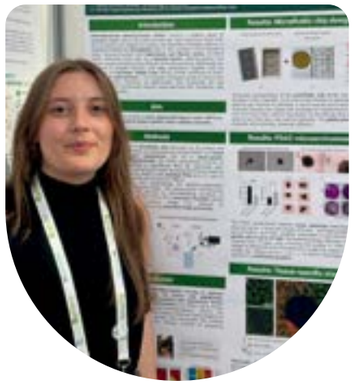
MPS World Summit 2025 winners

1. Andrea Bezze, "Engineering human-relevant Glioblastoma Microenvironment models for the optimisation of advanced drug delivery systems"
2. Sofia Tartaro, "Micro-physio/pathological system as a preclinical model to replicate pancreatic tumor microenvironment"

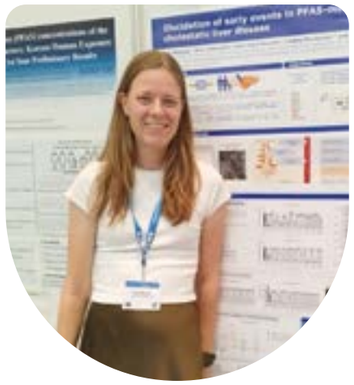
EUROTOX 2025 winners

1. Cloe Wray," Chemical-microbiome interactions in larval zebrafish: Unraveling the interactive effects of microbiome induced biotransformation of PAHs and host behavior"
2. Amy Maerten, "ELUCIDATION OF EARLY EVENTS IN PFAS - MEDIATED CHOLESTATIC LIVER DISEASE"

Donghyeon Kim, SETAC 2025.



Sofia Tartaro, MPS World Summit 2025.



Amy Maerten, EUROTOX 2025.

c. EPAA events

1. EPAA Team on a new paradigm for carcinogenicity safety assessment, 29 January 2025, Brussels
2. Animal Free Chemical Safety Assessment Conference, 4-6 March 2025, Brussels

3. EPAA NAM Designathon Workshop, 1-2 July 2025, Ispra

4. EPAA lunch debate in the European Parliament, 7 October 2025, Strasbourg

5. EPAA 20th Anniversary Conference, 5-6 November 2025, Brussels

d. External events

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

1. EFPIA workshop on the Pharma 3 basket approach to 3Rs, 16 January 2025, Brussels
2. Policy conference 'European Test Method & Validation Strategy', 22-23 January 2025, Amsterdam
3. EMA 3Rs Working Party (3RsWP), 2 April 2025, online
4. SETAC Europe Annual Meeting, 11-15 May 2025, Vienna

5. FELASA Congress, 2-5 June 2025, Athens

6. 13th World Congress on Alternatives and Animal Use in the Life Sciences, August 31 - September 4, 2025, Rio de Janeiro

7. EcoSafety Summit, 7-9 October 2025, Reykjavik

e. Publications

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 (2025).

Use of alternatives to animal testing for Environmental Safety Assessment (ESA): Report from the 2023 EPAA Partners' Forum,

Regulatory Toxicology and Pharmacology, Vol. 156. https://doi.org/10.1016/j.yrtph.2025.105774

Mark T.D. Cronina, Maria T. Baltazar, Tara S. Barton-Maclaren, Ofelia Bercaru, K. Nadira De Abrew, Christian Desaintes, Sylvia E. Escher, Petra Kern, Gavin Maxwell, Vera Rogiers, Katrin Schutte, Tomasz Sobanski (2025).

Report on the European partnership for alternative approaches to animal

testing (EPAA) "New approach Methodologies (NAMs) user Forum Kick-off Workshop"

Regulatory Toxicology and Pharmacology, Volume 159. https://doi.org/10.1016/j.yrtph.2025.105796

Mark T.D. Cronin, Elisabet Berggren, Sofia Camorani, Christian Desaintes, Marco Fabbri, Julia Fabrega, Matthias Herzler, Jay D.E. Ingram, Katia Lacasse, Gavin Maxwell, Katrin Schutte, Tomasz Sobanski, Georg Streck, Andrea Terron, Andrew P. Worth (2025)

Report of the European Commission Workshop on "The Roadmap Towards Phasing Out Animal Testing for Chemical Safety Assessments", Brussels, 11-12 December 2023

Regulatory Toxicology and Pharmacology, Volume 161. https://doi.org/10.1016/j.yrtph.2025.105818

5. Future prospects

Looking ahead to 2026, we welcome Dr Katia Lacasse (CEFIC) as EPAA industry co-chair and wish her the best of luck. Katia has played an active role in the day-to- day running of EPAA for the last year and has worked with the EPAA staff, co-chairs, and steering committee to develop our 2026-2030 Action Programme.

We also look forward to the publication of the Commission Roadmap to phase out animal testing for chemical safety assessment in the first quarter of 2026 and supporting the implementation of the roadmap across the rest of the the year. Developed in response to the 1.2 million signatures on the Save Cruelty Free Cosmetics European Citizens Initiative (ECI), the Roadmap underscores the European Union's long-standing policy on animal welfare and clearly represents a landmark achievement, and we must now work together to ensure it is successful.

To ensure success of phasing out animal testing, EPAA will continue to support the development of the short-, medium- and long-term roadmap priorities and address them through our projects. Furthermore, through our activities, we will strengthen and expand the space for cross-sector, multi- stakeholder discussions and communicating the outcomes. The roadmap represents an opportunity for Europe to inspire the world through leading the global transition to animal-free safety assessment – let's move forward together.

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

ED: Endocrine Disruptors

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

EFPIA: European Federation of Pharmaceutical Industries and Associations

ELISA: Enzyme Linked Immunosorbent Assay

EMA: European Medicines Agency

EP: European Parliament

EPAA: European Partnership for Alternative Approaches to Animal Testing

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

EUROTOX: Association of European Toxicologists and European Societies of Toxicology

EUSAAT: European Society For Alternatives To Animal Testing

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

IATA: Integrated Approaches to Testing and Assessment

IMI: Innovative Medicines Initiative

ITS: Integrated testing strategies

JEG 3Rs: Joint Expert Group on 3Rs

MGEN: Model Equation Generator software

MEB: Medicines Evaluation Board

NAMs: New Approach Methodologies

NC3Rs: National Centre for 3Rs (UK)

OECD: Organisation for Economic Co-operation and Development

PBTK: Physiologically-Based Toxicokinetic

REACh: Registration, Evaluation, Authorisation and Restriction of Chemicals

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

SEURAT-1: Safety Evaluation Ultimately Replacing Animal Testing

WC11: 11th World Congress on Alternatives and Animal Use in Life Sciences

WHO: World Health Organisation

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