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Non-Animal Methods in Science and Regulation

EURL ECVAM Status Report 2025

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Non-Animal Methods in Science and Regulation

EURL ECVAM Status Report 2025

Contents

Abstract

The 2025 EURL ECVAM Status Report presents a comprehensive overview of recent advancements of the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) in the development, validation, and regulatory integration of non-animal methods, as well as progress made elsewhere. Anchored in the EU's Three Rs framework of Replacement, Reduction, and Refinement, this report highlights innovative scientific breakthroughs including organ-on-chip technologies, artificial intelligence, and multi-omics approaches that collectively enhance the predictivity, efficiency, and ethical standards of safety assessment.

EURL ECVAM's active participation in Horizon Europe initiatives, international standardisation efforts, and strategic partnerships reinforces its pivotal role in fostering a robust, innovation-driven ecosystem.

With the European Commission preparing to publish in 2026 a roadmap for phasing out animal testing, this status report serves as a critical resource documenting scientific, regulatory, societal, and economic progress. It underlines EURL ECVAM's commitment to evolving animal-free approaches that not only uphold EU ethical values but also promote sustainable growth, competitiveness, and public health outcomes in Europe and beyond.

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Executive summary

The 2025 European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) Status Report outlines significant EU-driven progress in developing, validating and promoting non-animal methods and approaches for chemical safety assessment, through innovative clusters, regulatory standardisation, and international collaborations. It also details efforts in knowledge sharing, including reduced animal use trends, educational programs, and databases to accelerate the adoption of humane and advanced science across research and policy.

Research and Innovation

Chapter 2 of the 2025 Status Report details progress in research and innovation for non-animal methods, emphasising EU-funded projects and collaborations that advance the Three Rs principles and broader policies like the European Life Sciences Strategy. EURL ECVAM contributes expertise to facilitate method progression toward regulatory acceptance and commercialisation. The chapter highlights key clusters and initiatives driving chemical safety assessment without animals.

For example, the ASPIS cluster unites three Horizon 2020 projects - PrecisionTox, ONTOX, and RISK-HUNT3R - involving over 70 organisations across 16 countries to develop New Approach Methodologies (NAMs) using genomics, *in vitro*/*in silico* tools, and artificial intelligence (AI) for faster chemical risk assessment. PrecisionTox advances phylotoxicology, building a database with omics data from multiple species and addressing NAM uptake barriers, including policy consensus on validation routes. ONTOX updates physiological maps, develops quantitative Adverse Outcome pathways (qAOPs) for liver injury, Quantitative Structure-Activity Relationship (QSAR) models, Physiologically Based Kinetic (PBK) modelling, and *in vitro* batteries for liver, kidney, and neurotoxicity, while launching the ONTOX Probabilistic Risk Assessment (OPRA). RISK-HUNT3R finalises case studies for OECD[1] review, refines the ASPIS Safety Profiling Algorithm (ASPA) for Next Generation Risk Assessment (NGRA) workflows with an online dashboard, and enhances uncertainty frameworks and training. ASPIS restructured into Task Forces on Impact, Sustainability, and ASPA-OPRA, hosting the 5th Open Symposium in Athens.

The ENKORE cluster (launched 2024) studies endocrine disruptors across five projects, with EURL ECVAM advising on NAMs via its International Advisory Panel. PARC, a €400M Horizon Europe partnership, prioritises NAMs for NGRA, contributes to the EC roadmap towards phasing out animal testing for chemical safety assessments, and develops PARCopedia for stakeholder collaboration. VHP4Safety integrates human physiology data into a Virtual Human Platform prototype with tools like QSAR models and ToxTempAssistant. Additional efforts include PANORAMIX on chemical mixtures, EURL ECVAM Open Lab access, *in vitro* biotechnology ecosystem analysis, and studies on the use of computational models in healthcare.

These activities align with EU goals for innovation in biotech and chemical safety, addressing barriers like scalability while promoting FAIR data (i.e. research data that are Findable, Accessible, Interoperable, and Reusable) and regulatory uptake. EURL ECVAM's role fosters synergies, ensuring NAMs support sustainable and relevant assessments.

Regulatory Science

Chapter 3 details EURL ECVAM's efforts to advance non-animal methodologies for chemical safety assessment through validation, standardisation, and regulatory integration. The chapter emphasises modernising processes to

1 Organisation for Economic Cooperation and Development

build trust in diverse NAMs while aligning with EU and international goals to phase out animal testing.

Validation remains central to establishing regulatory confidence in advanced non-animal testing methods. EURL ECVAM has modernised its submission process to handle both single methods and integrated approaches, making its Test Pre-submission Form (TPF) and Test Submission Template (TST) publicly available. The strategic aim is to integrate NAMs into international test guidelines and regulatory practices for hazard and risk assessment, engaging partners like OECD, UN GHS[2], ECHA[3], EFSA[4] and EMA[5].

EURL ECVAM assessed several innovative methods in 2025. The Blood-brain barrier-on-a-chip (BBB-oC) pre-submission models human BBB permeability using microfluidic devices with human cells, showing potential for pharmaceutical qualification and chemical neurotoxicity evaluation, although full Standard Operating Procedures (SOPs) and reproducibility data are needed. GARDskin Dose-Response, an extension of OECD TG 442E, quantifies skin sensitisation potency via gene expression in dendritic-like cells; modules on definition and transferability were sufficiently addressed, but within-laboratory reproducibility and SOP development are recommended. The InFiniteLungDT pre-submission uses live-cell imaging and digital twins to predict lung inflammation over extended periods, aligning with the timeframe within OECD Test Guidelines (TGs) 403, 412, 413 and 452, but requires clarifications on regulatory relevance and biology.

Ongoing internal validation involves the transfer of the United States Environmental Protection Agency's (US EPA) AR2 assay for androgen disruption to the EURL ECVAM laboratory, testing of 56 substances in agonist mode and pursuing the replacement of the Hershberger rat bioassay through computational modelling approaches. A major OECD project, co-led by EURL ECVAM and the US EPA, develops TGs for *in vitro* hepatic clearance and plasma protein binding, integrated into a Defined Approach (DA) for human toxicokinetics, supporting prioritisation and physiologically based toxicokinetic (PBTK) assessments.

External support covers PEPPER[6] and NTP NICEATM[7] endocrine disruptor validation studies and EDNA[8] transcriptomics screening for identification of endocrine disruptors using NAMs, as well as VALNAM funding evaluations for the validation of new approach methods in a regulatory context.

Revision of OECD Guidance Document (GD) 34 on the validation and international acceptance of new or updated test methods for hazard assessment, led by EURL ECVAM, US, and the Netherlands, progressed in 2025 with the drafting of chapters on essential elements of validation, design and conduct of validation studies and peer review; readiness criteria templates for *in vitro*, *in silico*, and DAs were developed for inclusion in the revised GD 34 and tested within different PARC work packages.

Omics standardisation features a review of transcriptomics/metabolomics standards, OECD guidance on sample collection, and the endorsed Omics2AOPs project linking omics to AOPs. Stem cell work promotes best practices via publications

2 United Nations Globally Harmonised System of Classification and Labelling of Chemicals
3 European Chemicals Agency
4 European Food Safety Authority
5 European Medicines Agency
6 Public-private Platform for the Validation of Endocrine Disruptors Characterisation Methods
7 National Toxicology Programme Interagency Centre for the Evaluation of Alternative Toxicological Methods (US)
8 Endocrine Disruptors using New Approach Methodologies

and collaboration with the CorEuStem COST Action for assessing the adoption of best practices and standards within stem cell core facility communities. EURL ECVAM also contributed to a recent publication addressing genetic variant impacts in human Pluripotent Stem Cells (hPSC)-based therapies.

The 14th meeting of EURL ECVAM's Preliminary Assessment of Regulatory Relevance (PARERE) network reviewed regulatory relevant topics of the 2024 Status Report and discussed its potential role at national level, highlighting challenges like resource limits and validation complexity. Initiatives of the European Partnership on Alternative Approaches to Animal Testing (EPAA) include the Animal-Free Chemical Safety Assessment (AFCSA) Conference action plans, the Designathon for systemic toxicity classification using NAMs, carcinogenicity frameworks, environmental safety assessments, and endocrine disruptor forums. Support for EMA's 3Rs Working Party revises guidelines on local tolerance and 3Rs acceptance, emphasising *in vitro*/NAMs. An EU Test Method Development and Validation Strategy proposes prioritisation and governance for validation, aligning with EU legislation on "One substance, one assessment (OSOA)" and the EC roadmap towards phasing out animal testing for chemical safety assessments.

At the 37th annual meeting of the Working Party of National Coordinators of the OECD Test Guidelines Programme (WNT), TG updates, like TG 497 for skin sensitisation and TG 467 on defined approaches on eye irritation for surfactants, as well as new TGs incorporating NAMs, like TG on IL-2 LTT assay for immunosuppression, were approved. Additional topics covered developmental neurotoxicity (DNT) batteries, non-genotoxic carcinogenicity Integrated Approaches to Testing and Assessment (IATA) and thyroid methods. Updates to the UN GHS, to which EURL ECVAM contributes, address skin sensitisation, endocrine disruptors, germ cell mutagenicity, and inclusion of non-animal methods.

EURL ECVAM has issued guidance to enhance the quantitative assessment of health impacts from chemicals in EU policy evaluations and impact assessments, stressing best practices for handling epidemiological data amid challenges like data gaps, multifactorial diseases, and low-level exposures. Recommendations focus on improving transparency through clear scoping, structured evidence reviews, expert disclosure, uncertainty reporting, and equity considerations for vulnerable groups. This approach aims to produce more reliable, comparable assessments for evidence-based policymaking.

A EURL ECVAM scoping review mapped epidemiological evidence linking chemical exposures—primarily metals and per- and polyfluoroalkyl substances (PFAS)—to heightened risks of respiratory infections, especially in infants and children, revealing a fragmented evidence base skewed toward certain chemicals and populations while underscoring gaps for adolescents and the elderly. This work aligns with the EU One Health initiatives in legislation and agency collaborations, advocating harmonised reporting, AOP frameworks to connect exposures to immunotoxicity and infection dynamics, and targeted prevention for vulnerable groups.

Chemical pollution significantly contributes to biodiversity decline, yet causal evidence linking exposures to biodiversity metrics like taxonomic richness remains limited due to mismatches between ecotoxicological data at individual/sub-organismal levels and population-level outcomes, as detailed in a 2025 JRC report analysing impacts across Essential Biodiversity Variables (EBVs). EURL ECVAM leads a synthesis of field and ecotoxicological studies (datasets due in 2026) to identify affected species, chemicals, and methods, while joining ECETOC's task force for

regulatory integration, with a 2026 workshop planned.

Regarding data and knowledge management, AOP-Wiki grew to 556 AOPs, with FAIR enhancements, Methods2AOP, and AI4AOP advances. The European Commission's Information Platform for Chemical Monitoring (IPCHEM) expanded monitoring data access. A JRC/EURL ECVAM-led new OECD guidance document covering the research data lifecycle and promoting harmonised and structured approaches to support the use of scientifically reliable and relevant data in decision-making across jurisdictions and policy areas, was published.

These multitude of activities underscores EURL ECVAM's pivotal role in 2025, driving NAM adoption through European and international collaborations and innovation to achieve regulatory adoption of animal-free safety assessments.

Knowledge sharing

Chapter 4 details EURL ECVAM's key contributions to advancing the Three Rs principles in animal use for scientific purposes across the EU and beyond. The report highlights significant progress in reducing animal testing, innovative research initiatives, education efforts, and collaborative projects supporting the transition to non-animal methodologies.

EURL ECVAM compiled the 2023 statistical data report, showing a decline in first-time animal uses to 7.97 million procedures (-4.9% vs. 2022, -9.6% vs. 2018), with regulatory testing down 35.1% since 2018. Non-human primate uses dropped 25% from 2022, severe procedures fell below 9% of total uses and reuses modestly increased. The data are accessible via the European Commission's ALURES database.

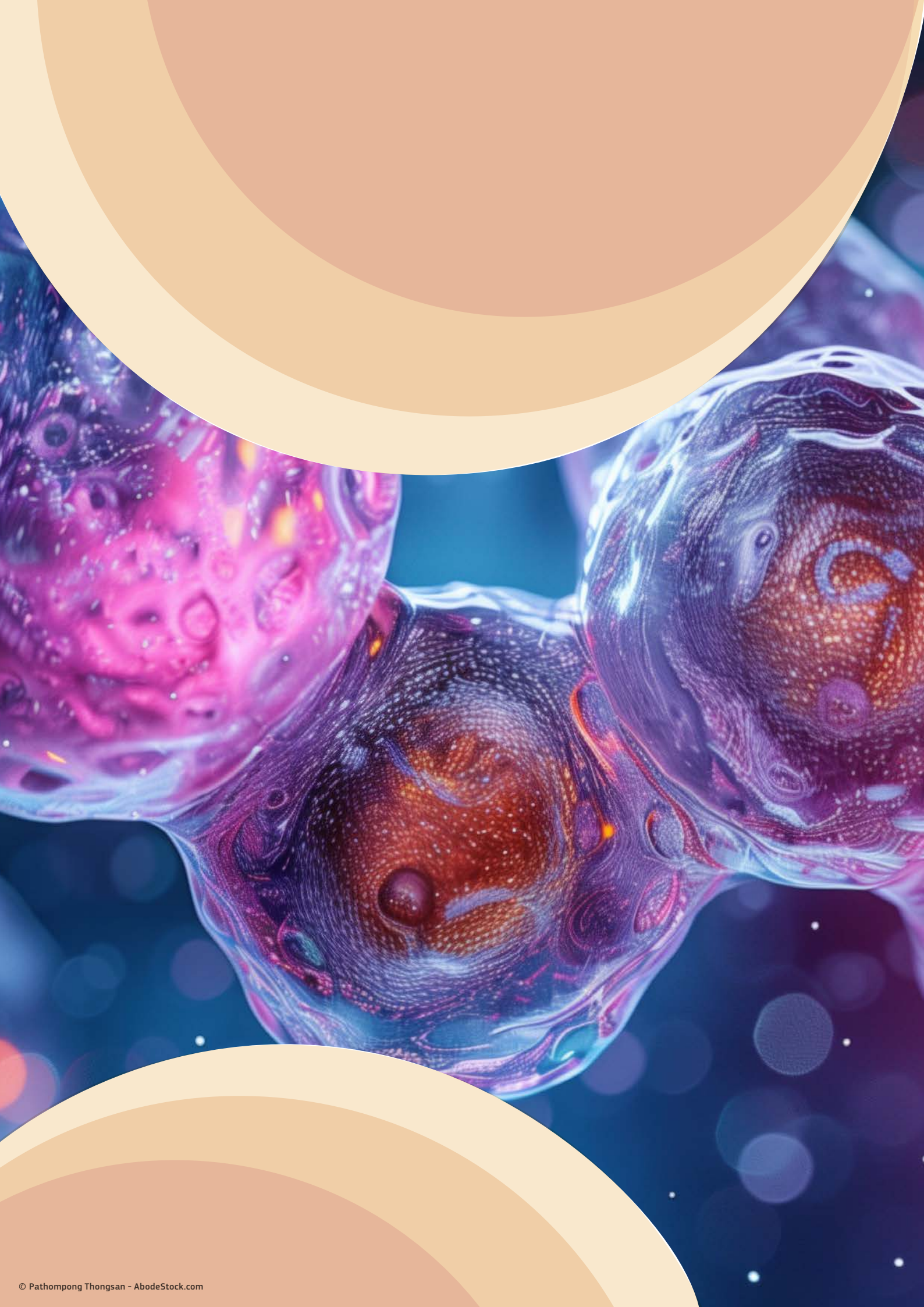
In September 2025, EURL ECVAM launched the first thematic review under Directive 2010/63/EU on Three Rs implementation in cardiovascular research, promoting *in silico*, *in vitro*, and *ex vivo* models to address Europe's leading cause of mortality affecting over 60 million people. Additionally, the BimmoH database, launched December 2025 and developed under a European Parliament pilot project, aggregates AI-analysed publications on human biology-based models like organ-on-chips, accelerating NAM adoption in biomedical research, drug development and safety assessment.

The fifth EURL ECVAM Summer School held in May 2025 in Ispra, Italy, trained postgraduate students and early career scientists on NAMs including induced Pluripotent Stem Cells (iPSCs), organ-on-chips, and AI, fostering sustainable practices with a greenhouse gas emissions report. The Student Ambassador Project engaged eight European students to promote NAMs in universities across multiple countries, gaining traction at international congresses; EURL ECVAM also supports the Global Education Hub (GEH) for animal-free innovation and Virtual Reality-based learning scenarios for secondary schools.

The SAFE[9] Consortium advances animal-free safety in pharma and chemicals via transdisciplinary action research, addressing barriers through publications and dialogues. The CHANGE project, funded by EFSA, maps regulatory toxicology system factors via workshops (Explore, Reflect, Design phases through 2026) to enhance NAM uptake, with EURL ECVAM participation.

Overall, 2025 marked robust strides toward ethical and relevant science, aligning with EU strategies for innovation and animal welfare.

9 Safety Assessment through Animal-Free Evolution



1. Introduction

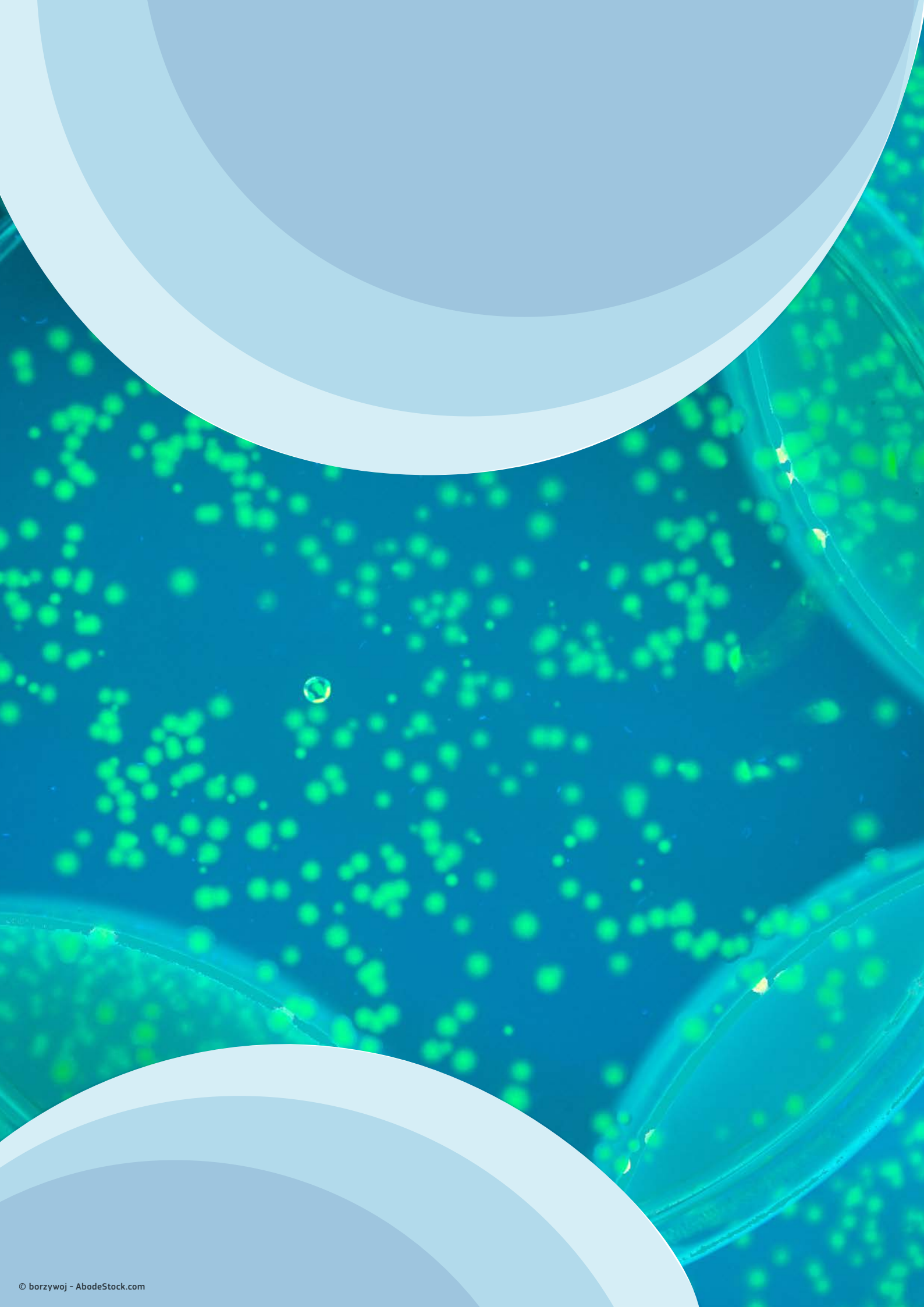
The 2025 EURL ECVAM Status Report continues the tradition of providing a comprehensive overview of EURL ECVAM activities aimed at advancing non-animal methods in scientific research and regulatory safety assessments. Building on decades of commitment to the Three Rs principles of Replacement, Reduction, and Refinement of animal testing, this report highlights recent progress in developing, validating, and promoting innovative methodologies that reduce reliance on animal testing across diverse sectors including chemicals, pharmaceuticals, cosmetics, and biomedical research.

The integration of cutting-edge technologies such as organ-on-chip platforms, advanced *in vitro* and *in silico* methods, artificial intelligence, and multi-omics approaches is reshaping the landscape of chemical and biomedical safety evaluation. Beyond their profound ethical advantages, which align with societal expectations and EU legislation aimed at phasing out animal testing, these methodologies deliver substantial economic impact by accelerating testing timelines, reducing costs, and improving the predictivity and relevance of safety data. For regulators and industry alike, this translates into more efficient development pipelines, smarter risk assessment strategies, and enhanced competitiveness on a global scale.

This report underscores EURL ECVAM's collaborative engagements across Horizon Europe projects, international standards organisations, and public-private partnerships, which collectively drive the validation, standardisation, and regulatory acceptance of new approach methodologies (NAMs). In tandem, EURL ECVAM's initiatives in education and knowledge dissemination empower researchers and policymakers to adopt these transformative approaches, ensuring the sustainability and growth of the non-animal testing ecosystem.

As the European Commission prepares to launch its comprehensive roadmap to phase out animal testing for chemical safety assessments in 2026, this Status Report serves as an essential resource documenting significant scientific advancements, regulatory milestones, and the tangible societal and economic value generated by moving towards an innovative, animal-friendly future. Through sustained investment and strategic collaboration, EURL ECVAM is not only advancing humane science but actively contributing to a robust, efficient, and economically sustainable safety assessment paradigm that benefits human health, the environment, and the European economy.

EURL ECVAM is an integral part of the European Commission's Joint Research Centre.



2. Research and Innovation

Through a range of EU-funded projects and research partnerships, considerable scientific and technical progress continues to be made on the development of non-animal methods. In addition to advancing the Three Rs in line with Directive 2010/63/EU (EU, 2010), research and development projects are contributing to the wider policy agenda. For example, the European Life Sciences Strategy[10] aims to boost innovation and make Europe more competitive by driving progress in areas such as healthcare, agriculture, food and biotechnology - areas in which the development of non-animal methodologies will contribute to the safe and sustainable use of chemicals. Furthermore, the EU Startup and Scaleup Strategy[11] and future European Biotech Act[12] aim to facilitate spin-offs, start-ups and scale-ups in bringing biotechnologies from the laboratory to the factory and onto the market.

EURL ECVAM contributes its in-house expertise to such activities in a variety of ways, such as by offering feedback and advice, and sharing best practices on method characterisation and standardisation. The overall aim is to identify promising methods and approaches and facilitate their progression to practical application, commercialisation and (where appropriate) regulatory acceptance.

2.1. ASPIS projects

With a view to developing NAMs for chemical safety assessment, three research projects funded under Horizon 2020 started their activities in 2021, led respectively by the Leiden University (RISK-HUNT3R), University of Birmingham (PrecisionTox), and the Vrije Universiteit Brussel (ONTOX). The three projects have joined forces in the collaborative group ASPIS ("aspis" means "shield" in ancient Greek), which gathers more than 70 scientific organisations across 16 countries of the European Union, the United Kingdom and the United States. Its mission is to use all available knowledge across disciplines to improve the accuracy, speed, and affordability of chemical safety testing without the use of traditional laboratory animals. Building on advances in (i) comparative genomics, transcriptomics and metabolomics, (ii) robust *in vitro* and *in silico* methodologies and (iii) artificial intelligence, ASPIS provides NAMs to rapidly accelerate and improve chemical risk assessment in the

10 https://research-and-innovation.ec.europa.eu/strategy/strategy-research-and-innovation/jobs-and-economy/strategy-european-life-sciences_en
11 https://research-and-innovation.ec.europa.eu/strategy/strategy-research-and-innovation/jobs-and-economy/eu-startup-and-scaleup-strategy_en
12 https://www.europarl.europa.eu/RegData/etudes/BRIE/2025/772866/EPRS_BRI%282025%29772866_EN.pdf

EU and beyond. The three, 5-year projects are complementary to each other and share common elements that form the basis of collaboration at a cluster level. Recent highlights of the three projects are given in **Box 2.1**, **Box 2.2** and **Box 2.3**.

The JRC (through EURL ECVAM) has set up a formal collaboration with each of the three projects individually and contributes to activities in ASPIS.

Website: https://aspis-cluster.eu/

Box 2.1. PrecisionTox

The main goal of PrecisionTox is to develop a new integrative assessment framework that safeguards human health and the environment from chemical exposure. This approach is based on observable mechanistic processes that lead to toxicity in distantly related species. Emphasis is on identifying the root causes of toxicity stemming from disruptions in key biological processes shared broadly among animals, including humans, due to evolutionary descent, while also considering genetic variation in individual susceptibility. The PrecisionTox project continues to advance our mechanistic understanding of chemical toxicity from a unique evolutionary perspective. Additionally, the project continues to address the socio-technical barriers and solutions related to the uptake of New Approach Methodologies (NAMs) in regulation, which have played a significant role in shaping the European Commission's roadmap to phase out animal testing for chemical safety assessments.

Phylotoxicology

Ongoing investigations provide evidence for the feasibility of understanding the health impacts of chemicals across all animals, including ecological keystone species and humans, based on the evolutionary conservation of pathways to toxicity. Recent achievements include:

- An increasing volume and diversity of comparative phenotypic data on cellular and systemic toxicity outcomes and pathways are emerging in non-sentient organism models and human cells. Species sensitivities vary. However, the relative toxicity of chemicals remains largely consistent among species.
- The PrecisionTox database is growing beyond the pilot study phase, supported by the PTX Data Explorer with training on navigating transcriptomics and metabolomics data from the controlled exposures of six organisms to multiple substances; range-finding data for nearly 200 compounds; phenotype data for a majority of PrecisionTox compounds; and extensive annotations for all omics features and substances. We have also included data from numerous external and internal sources to augment compound, metabolite and gene/protein annotations. The PTX Data Explorer is the central tool in PrecisionTox for comparative molecular toxicology, enabling cross-species and cross-chemical extrapolation and encouraging a weight-of-evidence approach to identify toxicity pathways and molecular signatures.
- Computational, data-driven, and knowledge-driven integration of omics data that uncovers putative, shared, and condition-dependent biomolecular responses across species.
- Early-stage discoveries suggest that distantly related species provide a conservative estimate of mammalian toxicity.

PrecisionTox Chemical Library

- ECVAM contributed directly to the design and implementation of the PrecisionTox chemical selection strategy and co-authored The PrecisionTox Chemical Library (Martinez *et al.*, 2025). This work is based on a rigorous and transparent approach for selecting 200 well-characterised chemicals from more than 1,500 candidates, ensuring that only compounds suitable for NAM-based testing—across physicochemical, toxicological and logistical criteria—were included.

Variation in Susceptibility

Ongoing investigations aim to determine whether the genetic basis of an organism's susceptibility to toxicity is also shared among distantly related species and whether target cells, tissues, or organs of toxicity are conserved. Recent achievements included:

- Comparative results of cell- and tissue-specific biomolecular toxicity responses by single-cell transcriptomics for alternative model species and human organoids.
- Discovering that sex-specific sensitivities to toxic substances are pervasive and determined by interactions among genes that are shared between Drosophila (a biomedical model species) and humans.
- The screening of the toxicological effects of 13 chemicals across the Human Genome Diversity Panel (HGDP) of 282 lymphoblastoid cell lines (from 1,050 individuals in 52 world populations) reveals significant genetic interactions that affect individual-level variation in susceptibility, which depend on population, sex, and concentration.

Embedded translation

The work aims to accelerate the uptake of new approach methodologies in regulatory and commercial applications by directly involving regulatory bodies, industry, and civil society in the project design and execution. Recent achievements included:

- Publication of an Action Plan on the solutions to previously identified roadblocks for the usage of a NAMs. The study determined the level of consensus among key stakeholders in the European Union regarding policy options. The study found broad consensus for specific policy solutions, including the regulatory use of data, education and training strategies and routes towards validation. The report is accessible for download here[13].
- Publication of the report identifying key gaps and opportunities for integrating NAMs into EU regulation. The report is accessible for download here[14].
- Progress on three case studies applying multi-omics (RNA-seq and hybrid LC-MS/MS) and comparative analysis on the grouping of three classes of substances (imidazoles, methacrylamides, acrylamides) according to their potential modes of action using Danio embryos and human cell lines as test systems. This work aligns with the PrecisionTox contributions to drafting of OECD guidance on performing and reporting omics-based grouping of data-poor substances, including the plausible toxicological interpretation of biomolecular profiles.

The results of PrecisionTox work provide scientific evidence supporting the benefits of an integrated approach to policymaking and interventions, including better utilisation of relevant information to assess the risks posed by chemicals to humans, other animals, and ecosystems through regulations. A transition to NAMs (assessments based on comparative mechanistic data) offers the best opportunity to effectively and scientifically extrapolate toxicological information from one species to another, including humans.

Website: https://precisiontox.org
Coordinator: John Colbourne, University of Birmingham, Centre for Environmental Research and Justice

13 https://precisiontox.org/wp-content/uploads/2024/11/0411-D6.3-Report-on-Solutions.pdf
14 https://precisiontox.org/wp-content/uploads/2025/02/D6.5-Regulatory-framework-report.pdf

Box 2.2. ONTOX

The vision of the highly interdisciplinary and intersectoral ONTOX consortium is to provide a viable and sustainable solution for advancing human risk assessment of chemicals without the use of animals in line with the principles of 21st century toxicity testing and next generation risk assessment.

Recent highlights include:

- WP1 continued updating all five physiological maps (PMs). All maps are accessible via permanent MINERVA links, with versions stored on BioStudies and GitHub. Updates included standardisation and documentation, and the development and publication of further training material. Furthermore, an ONTOX WikiPathways collection was built, and progress was made on establishing INDRA (Integrated Network and dynamical Reasoning Assembler) scripts to extract causal mechanistic information from a large number of full-text papers.
- WP2 developed two bibliographic databases compiling published clinical data on drug-induced cholestasis and steatosis, and constructed predictive models integrating clinical, physicochemical, and pharmacokinetic parameters to identify high-risk drugs and hazardous chemical features. A quantitative AOP (qAOP) Drug Induced Liver Injury (DILI) platform has been established, integrating a library of "assay digital twin" models with a systems-based human DILI model and a clinical data-informed virtual population, supporting cholestasis and steatosis use cases and enabling linkage between *in vitro*, *in silico*, and patient-specific data across tested compounds.
- WP3 developed computational models (QSAR and read-across) to predict the capability of chemicals to interact with endogenous protein targets (enzymes, receptors) relevant for the MIEs of the project's AOPs. Models were implemented in the last release of VEGA suite of models (v1.2.5). DockTox, a new docking server[15] was developed and implemented to simulate the binding of ligand with proteins relevant for MIEs. An *in silico* tiered testing strategy was developed integrating the output of various models.
- WP4 develop the QIVIVE framework for DNT (published as a collaboration with INOTIV and NIH) and is currently refining it with more tailored case-studies. WP4 also identified some applicability domains gaps for PBK modelling and accordingly is focused on testing the high-throughput PBPK framework for environmental chemicals and refining the kidney compartment to better predict kidney excretion. WP4 has developed the PBK model for PFOA using OSP tools and is currently working on making this a probabilistic model for integrating exposure data and the *in vitro* benchmark concentrations for the different organs and modes of action. In parallel, several *in vitro* distribution (mathematical) models to refine *in vitro* nominal to free concentration were also applied.
- ONTOX developed the largest chemical property database world-wide with 250 million data points on 120 million chemicals, based on import functions of public databases called BioBricks. Based on this, WP5 constructed a transformer model to predict 4,000 properties with about 90% accuracy for a 10% hold-out dataset, which is currently pre-validated for liver, kidney and developing brain toxicity by the consortium.
- WP6 has initiated a case study on PFOA, which is involving WP1 to WP9, following a protocol for probabilistic risk assessment (PRA) reported in 2024. WP6 maintained collaboration with a range of stakeholders to facilitate end-user acceptance of ONTOX PRA approach. The theoretical AI supported PRA approach – named OPRA is now in process to be operationalised in close cooperation with WP5 and with support from all partners in ONTOX. An internal assessment of the compliance of ONTOX partners with OECD standards (GLP-like) is ongoing, based on the recommendations provided by the EC reviewers.

15 https://chemopredictionsuite.com/DockTox

- WP7 further developed the two batteries of *in vitro* assays based on the updated AOP networks on chemical-induced cholestasis and steatosis. A weight-of-evidence approach to increase the reliability and applicability of the results was also included. Standard operating procedures have been generated for each assay, and the battery has been integrated in a decision tree for potential application in risk assessment of chemicals.
- WP8 developed a mechanistically anchored *in vitro* assay battery to quantify and validate key events central to kidney adverse outcome pathways (AOPs), in particular acute tubular necrosis. Using human conditionally immortalised proximal tubule epithelial cells (ciPTECs), mitochondrial depolarisation, reactive oxygen species (ROS) generation, lysosomal enzyme release, membrane integrity, and cell viability were assessed in response to exposure to toxicants. Furthermore, by integrating transcriptomic data from 1,261 differentially expressed genes shared across low-dose cisplatin, carboplatin, and oxaliplatin exposures in human kidney proximal tubule cells, WP8 delineated nine pathways and defined five novel, quantifiable key events that expanded the current AOP framework for nephrotoxicity testing.
- WP9 further characterised the developed human *in vitro* assays to study key neurodevelopmental processes, performed epigenomic analyses, tested several compounds, and contributed to the development of a decision tree for developmental neurotoxicity risk assessment of chemicals. A computational model was developed to predict the probability of neural tube closure defects based on chemical-induced gene perturbations.
- WP10 continued to steer the ONTOX research and non-research activities, monitored the implementation of EC reviewer feedback to the second periodic report, and organised the 4th annual ONTOX meeting in Valencia, Spain. ONTOX maintained collaborations with VHP4Safety, PARC, and the University of Seoul, initiated a new partnership with the VICT3R project, and continue to coordinate the ASPIS cluster, hosting the ASPIS Open Symposium 2025 in Athens, Greece.
- WP11 expanded the ONTOX data collection in BioStudies and contains now 67 private accessions and 10 publicly available ones. In more detail, the accessions comprise 20 transcriptomics data from WP2 and WP8, 3 ToxTemps from WP7-9, 14 NAMs data from WP7, 21 NAMs data from WP7 and 8 physiological maps from WP1. An additional 10 ToxTemps from WP7 will be added soon.
- WP12 released a third tutorial on MINERVA, a visualisation tool for physiological maps, accessible on the ONTOX YouTube channel (April 2025). In addition, 18 videos were produced either presenting the ONTOX consortium (SAB Insights, 3 videos; Work Package in Action, 2 videos, throughout 2025) and/or reflecting on scientific events such as the SOT 2025 (7 videos, March 2025), the 4th ONTOX annual meeting (3 videos, April 2025), the ASPIS Summer School 2025 (1 video, April 2025) and the ASPIS Open Symposium (2 videos, September 2025).
- WP13 established the ONTOX Hub and marketplace as a platform to offer computational, lab-based *in vitro*, and consultancy services from the project partners as well as external services for exploitation, developed an exploitation strategy and business plan using the EU Horizon Results Booster service, organized and performed several meetings, workshops, and surveys to support the partners to manage and integrate their IP, and to create the OPRA approach (ONTOX Probabilistic Risk Assessment). In addition, cross-project integration work of software applications within the ASPIS cluster was performed.

- WP14 further strengthened communication of the ONTOX and ASPIS cluster achievements. The ONTOX website was expanded with engaging descriptions and video content for all 14 work packages[16], while the Impact section was enriched with publicly available ONTOX webinars[17]. WP14 continued to actively engage audiences across social media platforms, reaching more than 3,000 followers. In 2025, WP14 released 4 newsletters[18] and 4 ONTOX Insights editions that showcased ONTOX-related publications. The team also co-organised the ASPIS Open Symposium 2025 and ASPIS Academy sessions and continued to manage the ASPIS website.

Website: https://ontox-project.eu
Coordinator: Matthieu Vinken, Vrije Universiteit Brussel

Box 2.3. RISK-HUNT3R

RISK-HUNT3R aims to develop, validate and implement integrated approaches to lead the way toward next generation risk assessment (NGRA). The proposed approach is based on mechanism-based human-relevant *in vitro* and *in silico* systems (new approach methodologies). Through systematic and iterative evaluation of its NAM toolbox, the project will optimise a strategy to assess chemical exposure, toxicokinetics, and toxicodynamics.

Recent highlights include:

- With RISK-HUNT3R entering its later project phase, the main focus has been on the finalisation of the project case studies, the further buildout of the ASPA NGRA workflow, and the development of its ASPA-assist digital interface.
- Over 10 large case studies are run project wide. Three case studies have been fully finalised and were submitted to the OECD IATA Case Study Project for expert review: i) on ECHA prioritization of highly toxic industrial chemicals, ii) on the use of IATA for systemic toxicity of conazoles, and iii) on NGRA of propylparaben from multiple sources. The remainder of the case studies are being finalised for OECD submission into the 2026 IATA review round.
- The ASPA workflow for NGRA is a major and impactful project outcome. Its development is incremental, and it has seen increasing international traction and recognition with stakeholders. With ASPIS partners, we refined the ASPA workflow via interaction with stakeholders (regulators, industry, academia, NGOs) at multiple international events. A major innovation that has started to bring ASPA to the end-users is the ASPA-assist online dashboard to transparently operate on the workflow whilst integrating and documenting the safety assessment procedure.
- RISK-HUNT3R brought together international scientific, industrial, and regulatory experts to its pivotal ASPA NGRA workshop (May 2025, Berlin). Co-organized with EFSA, ECHA, OECD, JRC, ASPIS, EPA, and NIH/NIEHS, the ASPA workflow was scrutinized in detail based on real-world data and case studies covering all the risk assessment pillars. Recommendations on how ASPA can best support regulatory decision-making and advance the transition towards animal-free chemical safety assessment are being deployed for a major update to both the ASPA workflow (to v3.0) as well as its ASPA-assist digital interface.
- To test if a chemical will enter the human body upon exposure, *in vitro* tests were implemented for uptake via the lung or gut. Moreover, our biokinetic models can predict concentrations in our tests while accounting for metabolism in the ADME pillar of ASPA, as well as for biological variability.

16 https://ontox-project.eu/project/
17 https://ontox-project.eu/webinars/
18 https://ontox-project.eu/#Newsletter-subscription

- To predict toxicological hazards that might occur when chemicals are taken up by the body, computational pipelines were established based on chemical structures of compounds to predict to which targets they may bind for various tissue types. We also generated a panel of pluripotent stem cells with stress response reporters for high-throughput hazard screening in different target organ lineages, like hepatocytes, renal proximal tubular cells and cardiomyocytes. High-throughput data for over 100 compounds include phenotypic, MIE (molecular initiating event) and KE (key event) assay data.
- For transcriptomics, showing changes in all cellular processes at the same time, comprehensive toxicogenomics prediction platforms were set up for kidney and liver cells, including stem cell-based models for these cell types, as well as for mature peripheral neurons. State-of-the-art combined transcriptome and metabolome studies were also set up to determine cell fate. Kidney and liver organoids were further developed, used for transcriptome mapping, and combined in a two-organ chip. Challenging compounds were assembled and tested to show how to avoid false negatives.
- Network mapping was used to delineate gene and protein networks and compounds associated with kidney toxicity, as well as putative new kidney biomarkers. Transcriptomic and morphological data were connected with clinical information, and a database for all project compounds based on existing human exposome knowledge has been constructed. AOPs were quantified, all the way from MIE to late KEs, and a framework to evaluate such qAOPs has been developed.
- RISK-HUNT3R further developed and implemented the uncertainty characterisation framework for NGRA. This includes the appraisal of separate sources of uncertainty and the evaluation of their combined impact within an assessment workflow, options to refine uncertainty, and the harmonisation of means to express and communicate uncertainties.
- RISK-HUNT3R further enriched its data and knowledge infrastructure, and its harmonised data template and compound database, all respecting FAIR data criteria.
- Over 25 NAM innovations have been identified as project outcomes to date. RISK-HUNT3R has been following up to evolve their readiness levels for relevant applications, and we delineated which innovations can be prioritized for seeking commercialization inroads.

RISK-HUNT3R has continued to provide highly rated training on NGRA to the new generation of scientists on risk assessment, including the practical context of linking lab research and regulatory reality.

Website: https://www.risk-hunt3r.eu
Coordinator: Bob van de Water, Leiden University

2.2. ASPIS Working groups and Task Forces

ASPIS has initially operated through nine Working Groups (WGs), composed of investigators from all three consortia, including early-stage researchers, who specialise in research domains central to the cluster's mission.

In the fifth year, ASPIS underwent a strategic restructuring to enhance impact, sustainability, and alignment with long-term goals. This shift was initiated during the 4th annual ASPIS Open Symposium, where the WG chairs and the cluster coordinators evaluated the relevance and future role of the WGs. As a result, the original nine WGs transitioned into a more streamlined structure comprising three Task Forces: 1) Impact, 2) Sustainability, and 3) ASPA-OPRA, alongside two continuing WGs: Communication and Dissemination and the ASPIS Academy.

The newly established Task Forces are designed to address key priorities outlined in the original H2020 call, including:

- Scientifically sound, practicable non-animal solutions for chemical safety assessment.
- Engagement with regulatory bodies to translate methods into practice.
- Uptake and commercial exploitation of developed approaches.
- Contribution to the 3Rs principles, with a particular emphasis on 'Replacement'.

In 2025, ASPIS partnered with PARC to identify synergies, continued collaboration with the VICT3R[19] project across five working groups, and co-hosted the ASPIS Academy webinar series with VHP4Safety. A key highlight was the 5th ASPIS Open Symposium on 17 to 18 September 2025 in Athens, Greece, organised by the current chairing project ONTOX, providing a platform for scientific dialogue, strategic planning, and stakeholder engagement.

Recent highlights of the ASPIS Task Forces and WGs are given in **Box 2.4**.

Box 2.4. Highlights from the ASPIS Working Groups and Task Forces

Task Force Impact

The Task Force Impact focuses on impact at the research, regulatory, industrial and societal level. The goal is to maximize ASPIS visibility and to leverage impact in view of generating sustainability and a tangible legacy. The Task Force intends to generate a general paper on the overall ASPIS impact as well as a visionary paper on the future of the use of NAMs for chemical safety evaluation and their impact.

Task Force Sustainability

The Task Force Sustainability looks closely on the legacy of ASPIS considering the Cluster's research contributions alongside ongoing initiatives that include the Partnership for the Assessment of Risks from Chemicals (PARC) and the EC Roadmap for phasing out animal testing in chemical safety assessments in Europe, the NIH Complement-ARIE programme in the USA and the United Kingdom's ambitions to progress NAMs as part of its nationalised Chemicals Strategy. The goal is to ensure that the Cluster's data are stably archived, publicly accessible and made useful to all NAMs stakeholders following the FAIR principles. Emphasis is placed on the completion and integration of project-specific databases. Moreover, the ASPIS NAM safety assessment tools will be made commercially available when possible. These scientific activities are being integrated with work being completed on the legislative and regulatory mapping of NAMs so that progress continues to be made at operationalising NAMs via guidance documents (e.g., OECD) and by nudging changes to regulatory statutes. The primary ambition of this Task Force is for ASPIS to lay a foundation on which the private sector may invest to accelerate NAMs development.

Task Force ASPA-OPRA

The main goal of the ASPA-OPRA Task Force is to follow up on the development, optimization and operationalization of the two next generation chemical risk assessment strategies generated in ASPIS, namely the Alternative Safety Profiling Algorithm (ASPA) and the ONTOX Probabilistic Risk Assessment (OPRA) approach. Furthermore, focus is put on finding synergies between ASPA, OPRA and the discoveries on the genetic basis of susceptibility by PrecisionTox by mutual application of a number of AI-based tools and probabilistic approaches for both exposure and hazard modelling. Most importantly, the ASPA-OPRA Task Force supports the demonstration of the relevance and reliability of both ASPA and OPRA in the ASPIS projects through a number of real-life case studies in collaboration with end-users in industrial and regulatory settings.

Working Group Communication and Dissemination

This WG harmonises dissemination activities and maximises the impact of ASPIS. Its objective is to effectively

19 https://www.vict3r.eu/

communicate scientific advances through a clear unified channel to regulatory stakeholders, policymakers, non-governmental organisations and the public. Taking in consideration these target audience objectives.
Recent highlights include:

- Strengthened stakeholder engagement by hosting booths at the 2025 SOT ToxExpo, promoting ASPIS achievements to regulators and industry.
- Co-organised the ASPIS Open Symposium 2025, fostering dialogue among regulators, industry, and academia on advancing animal-free chemical safety assessment.
- Represented ASPIS at major policy events, including the EU Roadmaps for Animal-Free Innovations (Brussels) and the 3rd EC Workshop on Phasing Out Animal Testing (Helsinki).
- Expanded the ASPIS Academy online presence with new content—interviews, videos, and news—supporting early-stage researchers and maintaining active social media outreach.
- Enhanced the ASPIS website with a new section highlighting cluster publications and key outputs.
- Produced impactful communication materials, including event recaps, feature stories, and video content from the ASPIS Open Symposium 2025.

ASPIS Academy

The ASPIS Academy is a network of Early-Stage Researchers (ESRs) focused on the development and use of NAMs in the safety assessment of chemicals. It promotes the careers of ESRs through specialised training, championing equal opportunity, and creating a platform devoted to the voices and aspirations of a new generation of regulatory scientists.
Recent highlights include:

- Opening to external ESRs since April 2024.
- Establishing the "Manual for the ASPIS Twinning Program" to provide opportunities for students from all three ASPIS projects to visit and collaborate with laboratories across the network.
- Organising a bimonthly webinar series with VHP4Safety project in which ESRs give scientific presentations on different topics based on the collaboration groups.
- Organising the in-person training "ASPIS Academy Summer School: Let's talk about data!" as a satellite meeting to the ONTOX annual meeting.
- Launching the ASPIS Academy: Back to School programme to bring NAMs to classrooms and inspire the next generation of scientists.
- Maintaining the mentoring activity through a 2nd edition across the three consortia.
- Organising a networking event at the ASPIS Open Symposium 2025 in Athens, Greece.
- Collaborating with other ESR communities:
  - Strengthened stakeholder engagement by hosting booths at the 2025 SOT ToxExpo, promoting ASPIS achievements to regulators and industry.
  - Co-organised the ASPIS Open Symposium 2025, fostering dialogue among regulators, industry, and academia on advancing animal-free chemical safety assessment.
  - Represented ASPIS at major policy events, including the EU Roadmaps for Animal-Free Innovations (Brussels) and the 3rd EC Workshop on Phasing Out Animal Testing (Helsinki).
  - Expanded the ASPIS Academy online presence with new content—interviews, videos, and news—supporting early-stage researchers and maintaining active social media outreach.
  - Enhanced the ASPIS website with a new section highlighting cluster publications and key outputs.
  - Produced impactful communication materials, including event recaps, feature stories, and video content from the ASPIS Open Symposium 2025.
- Further communicating and disseminating the activities of ASPIS Academy such as poster sessions, networking opportunities, interviewing of scientists, and social media management.

Website: https://aspis-cluster.eu/aspis-academy/

2.3. ENKORE cluster

ENKORE is a cluster of five research projects studying the health impacts of endocrine disruptors (EDs) and exploring ways to reduce exposure to these chemicals. It began its work in January 2024 and has a duration of five years. The projects are funded under the call HORIZON-HLTH-2023-ENVHLTH-02-03, focusing on the "Health impacts of EDCs: bridging science-policy gaps by addressing persistent scientific uncertainties". The cluster aims to develop policy briefs and other communications where they compile and integrate research findings of the cluster projects into clear and actionable policy recommendations.

The projects in the ENKORE cluster are the following:

- EDC-MASLD explores the role of EDs in the progression of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)[20].
- ENDOMIX investigates how EDs target the immune system to cause disease, aiming to deliver new knowledge and recommendations[21].
- HYPIEND studies the effects of EDs on the hypothalamus-pituitary axis during development stages, focusing on pregnant and breastfeeding women and children[22].
- MERLON improves knowledge on how ED exposure impacts reproductive health during critical life stages, and develops tools to better identify and regulate EDs[23].
- NEMESIS investigates how EDCs disrupt normal metabolic processes and lead to metabolic diseases[24].

EURL ECVAM is a member of the ENKORE International Advisory Panel (IAP) and provides advice, particularly with regard to regulatory requirements, to facilitate the uptake of relevant NAMs for ED identification. The first annual meeting of the ENKORE cluster took place at the Helmholtz Centre for Environmental Research (UFZ) in Leipzig in September 2025, where the IAP was introduced to the cluster members. Discussions focused on the cluster's working groups, strategy and deliverables.

20 https://edc-masld.eu/
21 https://endomix.eu/
22 https://hypiend.eu/
23 https://merlon.dtu.dk/
24 https://www.nemesis-project.eu/

Figure 2.1. First annual meeting of the ENKORE cluster (Leipzig, September 2025)

Source: ENKORE cluster coordinators

Website: https://enkore-cluster.eu/
Coordinators 2025: Ana Zenclussen, Helmholtz Centre for Environmental Research, Germany (ENDOMIX) and Chiara Baudracco, Eurecat Technology Centre, Spain (HYPIEND).

2.4. PARC

The European Partnership for the Assessment of Risks from Chemicals (PARC) supports the development and implementation of a research and innovation programme to meet current and future needs in chemical risk assessment. Formally launched on 1 May 2022, PARC is a seven-year Horizon Europe public-public partnership, co-funded by the European Commission and Member States with a €400 million budget. ANSES, the French Agency for Food Safety, Environmental Protection and Occupational Health, coordinates the partnership.

As a multinational European project, PARC engages nearly 200 institutions focused on environment or public health from 28 countries, plus three EU authorities: ECHA), EFSA, and the European Environment Agency (EEA). Five European Commission Directorates-General (DG RTD, DG GROW, DG ENV, DG SANTE, and JRC) and relevant national ministries contribute to PARC's governance and monitor its activities. The JRC has also established a formal collaboration agreement with PARC, enabling JRC staff to participate in work aligned with the JRC Work Programme.

PARC's success will be measured by its tangible positive impact on regulatory processes and decision-making. Many PARC projects aim to integrate new approaches into current regulatory practice.

PARC advances (among other topics) a NAM-based Next Generation Risk Assessment (NGRA, Herzler *et al.*, 2025). Within this effort, PARC WP2 ("a common science-policy agenda") strengthens the science-regulatory interface by:

- Prioritising PARC projects on NAMs, tools, NGRA concepts, and frameworks to address regulatory needs identified by EU and Member State authorities.
- Contributing to the European Commission's roadmap for phasing out animal testing in chemical safety assessments under the "NGRAroute"[25] activity, through roadmap working groups on human health and environmental safety assessment. This involves cooperation with the European Commission, European agencies, the European Partnership for Alternatives to Animal Testing (EPAA), and researchers from ASPIS cluster projects such as RISK-HUNT3R, Ontox, and PrecisionTox.
- Developing PARCopedia[26], a knowledge management and community platform for capacity building, knowledge exchange, and collaboration among chemical risk assessment stakeholders—including academia, authorities, industry, NGOs, and others—within and beyond PARC.

A key challenge for NAM-based NGRA is the scarcity of validated methods and limited validation capacities at both EU and Member State levels.

In 2024, PARC's management board issued a "scoping paper on validation-related activities within PARC to progress new methods and approaches for hazard and risk assessment of chemicals." This document defines PARC's validation scope: compiling suitable NAMs into IATAs but not conducting ring trials. To advance this, an ongoing project pursues these objectives:

- Expanding ReadEDTest for self-assessment of WP5-prioritised endpoints.
- Selecting NAMs to fill regulatory gaps.
- Organising independent peer-review of readiness assessments of NAMs used and/or developed in WPs 5 and 6.
- Supporting validation activities for selected NAMs among willing partners.
- Optionally, drafting a manuscript based on the scoping document.

EURL ECVAM provides input and support to these efforts, ensuring expertise transfer and readiness to engage EU-NETVAL for prioritised validations.

Additionally, WP5 promotes NAM regulatory uptake through 'beta testing.' For instance, when addressing data gaps for contaminants like mycotoxins from the Enniatin or Alternaria toxin groups, PARC applies omics-enhanced test guideline studies alongside NAMs to directly compare results in regulatory decision-making. PARC has delivered its first report on investigated AOPs.

Website: https://www.eu-parc.eu/
Parcopedia: https://parcopedia.eu
Coordinator: Pascal Sanders, ANSES.

25 https://www.parcopedia.eu/wp-content/uploads/2024/10/PARC_AD2.1_submitted_approval_pending.pdf
26 https://parcopedia.eu

2.5. Virtual Human Platform for Safety Assessment (VHP4Safety)

The Virtual Human Platform for Safety Assessment (VHP4Safety) is a five-year research project funded by the Dutch Research Council (NWO) programme 'Dutch Research Agenda: Research on Routes by Consortia' (NWA-ORC). The VHP4Safety project started in June 2021, with the mission to improve the prediction of the potential harmful effects of chemicals and pharmaceuticals based on a holistic, interdisciplinary definition of human health, thereby accelerating the transition from animal-based testing to innovative safety assessment. VHP4Safety will integrate data on human physiology, chemical characteristics and perturbations of biological pathways, in order to incorporate: 1) human-relevant scenarios to discriminate vulnerable groups such as disease state, life course exposure, sex and age; 2) chemicals from different sectors: pharma, consumer products and chemical industry; and 3) different regulatory and stakeholder needs.

Recent highlights include:

- Integration of Scrum[27] methodology as a collaborative approach into the build of the Virtual Human Platform.
- Integration of three case studies from the VHP4Safety project and their tools on the platform, demonstrating how the platform can be used to carry out risk assessments.
- Development of different tools and models (to be found at https://cloud.vhp4safety.nl), including:
  - QSAR models for Parkinson's disease and the thyroid case study, accessible via the QSPRpred service.
  - A generalisable docking tool for molecular interaction prediction.
  - A generic PBK model to perform Quantitative I*n Vitro* to *In Vivo* Extrapolation (QIVIVE) for organophosphate pesticides induced acetylcholine esterase inhibition in humans.
  - The ToxTempAssistant, an LLM-based tool to facilitate researchers to fill in harmonised method descriptions of their cell-based models.
  - The OECD QSAR Toolbox AI Assistant (O-QT) to analyse chemicals, assess hazards, and obtain read-across recommendations using a powerful multi-agent AI system connected to the OECD QSAR Toolbox.
- Launch of a running prototype of the platform[28] with a front-end user interface with three sections (Tools, Case Studies and Data).
- Specification of performance criteria based on user engagement and interviews, ensuring the platform meets end users' needs.
- A sustainability plan is under development to ensure the platform's long-term value for users.
- Development of new learning modules and programmes for interdisciplinary education and training in non-animal methods.

Coordinators: Anne Kienhuis, National Institute for Public Health and the Environment (RIVM); Cyrille Krul, HU University of Applied Sciences Utrecht; and Juliette Legler, Utrecht University
Project Manager: Esmeralda Krop, Utrecht University
Website: https://vhp4safety.nl/

27 https://scrumguides.org/scrum-guide.html
28 https://platform.vhp4safety.nl/

2.6. Virtual Physiological Human (VPH) – The Society for In Silico Medicine

THE SOCIETY FOR IN SILICO MEDICINE

Virtual Physiological Human (VPH) – The Society for In Silico Medicine is a Belgian-based international non-profit organisation, aims to advance the development and implementation of computational models and the Virtual Human Twin technology in biomedical research and clinical settings. This innovative approach, known as *in silico* medicine, utilises computer simulations of human pathophysiology and artificial intelligence to enhance healthcare and medical research. With support from the EC, the VPH Society facilitates collaboration and advocacy among stakeholders in computational medicine, including researchers, clinicians, industry leaders, policymakers, and patient organisations, to promote awareness, connection, and progress in the field.

Recent highlights of VPH Society's activities include:

- Publication of the EDITH's Roadmap (EDITH consortium, 2025) for building the European Virtual Human Twin: EDITH was a Coordination and Support Action funded by the EC, aiming at building a European ecosystem around the Virtual Human Twin (VHT) technology and at drafting a detailed roadmap towards its implementation. The VPH Society had a prominent role in the EDITH consortium, with its Executive Director Liesbet Geris being EDITH's project coordinator. The vision for the VHT is to use multi-scale and multi-organ *in silico* models to represent human health or disease states at different anatomy levels (e.g. cells, tissues, organs) to represent and predict disease evolution and responses to treatments. The EDITH's Roadmap is a comprehensive analysis of the current ecosystem around this technology and outlines a detailed strategy for its deployment, considering technical, regulatory, ethical, legal and social aspects for a successful implementation of the VHT. The EDITH Roadmap is the foundation for the recently launched European Virtual Human Twins Initiative[29], designed to put the VHT vision into practice.
- The annual VPH Summer School[30] took place in Barcelona with a programme focused on Integrated Medicine. The event brought together more than 60 participants, including junior engineers, early researchers and medical doctors, and spanned topics including AI applications in clinical settings, *in silico* approaches in cancer research and subject-specific computational models for surgical planning.

Coordinator: Liesbet Geris, University of Liège and KU – Leuven (Belgium)
Website: https://vph-society.org/

29 https://digital-strategy.ec.europa.eu/en/policies/virtual-human-twins
30 https://www.vph-institute.org/news/news-from-the-8th-vph-summer-school-in-barcelona.html

2.7. Computational model lifecycle: from academic research to impact in healthcare

The integration of computational modelling and artificial intelligence in medicine holds great promise for transforming the healthcare landscape. By leveraging advanced simulations and data analytics, clinicians can enhance diagnostic accuracy, tailor treatment strategies to individual patients, and accelerate the development of novel therapies.

EURL ECVAM conducted a study (Bridio *et al.*, 2025) to map the applications of computational medicine, identifying opportunities and barriers to its adoption, and spotlight key European initiatives advancing the field. The peer reviewed paper provides examples of sophisticated computational models of human pathophysiology in diverse areas, such as cardiovascular medicine, orthopaedics, and oncology. Despite the development of advanced applications, the full potential of *in silico* models remains largely underutilised beyond academic research, hindered by a range of technological, regulatory, logistical, and cultural barriers.

To facilitate the identification of these challenges, the paper introduces the concept of the "computational model lifecycle", which describes the iterative process of model development, refinement, and translation from academic research to industrial research and development, and to pre-clinical and clinical applications. At each stage of the lifecycle, the study identifies potential uses of computational tools as well as bottlenecks to the translation to next stages. These include data management, model validation, ethical considerations and regulatory compliance. In this context, the paper analyses European regulations and guidelines, and highlights European initiatives, such as the European Health Data Space[31] and the Virtual Human Twins Initiative[32], which aim to foster the development and integration of computational medicine in healthcare.

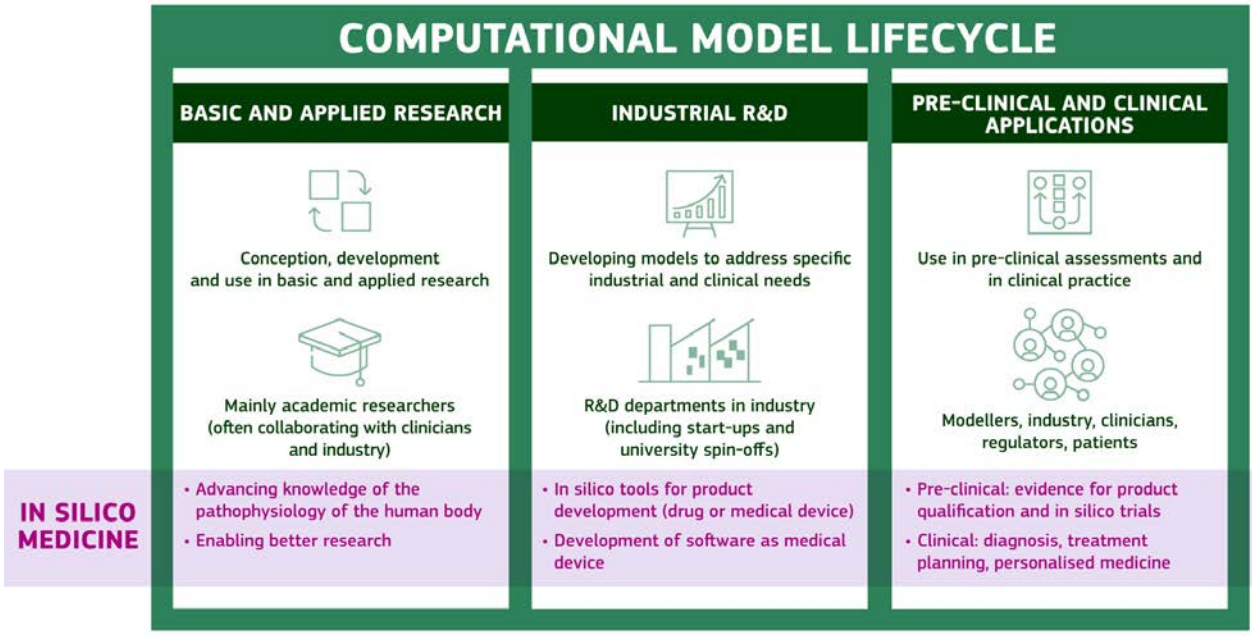
EURL ECVAM uses the computational model lifecycle framework as a reference to plan and shape further research activities in the field. Ongoing efforts include the analysis of how computational models can be used to run *in silico* trials to derisk and accelerate the development of new treatments, and an in-depth analysis of the factors enabling the uptake of Digital Twins as clinical decision support systems.

"Computational modelling and Digital Twins have the potential to revolutionise healthcare, by streamlining the development of new therapies and enabling a true personalisation of clinical care. At EURL ECVAM we investigate translation pathways to bring this technology from academic research to impactful applications that will benefit the healthcare ecosystem"

Sara Bridio
Project Officer
European Commission - Joint Research Centre

31 https://health.ec.europa.eu/ehealth-digital-health-and-care/european-health-data-space-regulation-ehds_en
32 https://digital-strategy.ec.europa.eu/en/policies/virtual-human-twins

Figure 2.2. The computational model lifecycle framework.



Source: Bridio et al. (2025), 'The computational model lifecycle: Opportunities and challenges for computational medicine in the healthcare ecosystem' published in Science Progress under CC BY 4.0

2.8. Panoramix

The overarching goal of PANORAMIX (2021–2026), a H2020 project, is to develop a new assessment framework for protecting human health from exposure to chemical mixtures. The project places particular emphasis on estimating the reproductive and developmental neurotoxicity of environmental, food, and human samples through whole-mixture bioassays, and on characterising and comparing the chemical exposome across these samples.

A central focus is the chemical and toxicity profiling of 750 human cord blood samples, integrated with extensive health data on reproductive and neuropsychological function in the corresponding children. The aim is to identify chemical mixture drivers of adverse health outcomes by combining multiple state-of-the-art methodologies, including whole-mixture testing linked to adverse outcome pathways, suspect screening for chemical profiling, and effect-directed analysis (EDA). In addition, PANORAMIX employs theoretical mixture risk assessment approaches based on chemical profiling results and is developing a web-based tool for estimating mixture effects.

Recent highlights include:

- Identification of chemical mixtures in environmental (wastewater, effluents, surface waters), food (fish, milk, drinking water), and human matrices (adult and cord blood, breast milk), representing an average European exposure scenario.
- Experimental confirmation of the concentration-additivity principle for a large number of real-life chemical mixtures tested at low concentrations in a diverse panel of bioassays linked to reproductive and developmental neurotoxicity outcomes. The tested mixtures were based on chemicals identified in environmental, food, and human samples.

- Effect-directed analysis (EDA) applied to environmental, food, and human blood samples, combining micro-fractionation, bioassay testing, and chemical profiling of active fractions. This approach reduces analytical complexity by focusing on chemicals contributing to bioassay-observed hazards.
- Analysis of 750 human cord blood samples in five bioassays, combined with suspect screening of selected samples showing "low" or "high" adverse outcomes, and comparison with reproductive and neuropsychological health indicators in the children.
- Mixture risk assessment of chemicals detected in human breast milk based on real-life exposure data, revealing an excessively high Hazard Index, with bisphenol A identified as a major risk driver.
- Development of a beta version of the "Chemical Mixture Calculator", a software tool that enables preliminary screening of potential mixture effects based on bioassay results from the ICE platform, incorporating both reproductive and neurotoxic activity data and toxicokinetic-corrected human exposure levels.

Coordinator: Anne Marie Vinggaard, Technical University of Denmark
Website: https://panoramix-h2020.eu/

2.9. EURL ECVAM Open Lab

In 2025, the EURL ECVAM experimental facility joined the JRC Open Laboratory programme opening its doors to host external users. In line with the EC's commitment to fostering collaboration, innovation, and transparency in scientific research, the JRC continues to advance its Open Laboratory initiative, which grants access to selected JRC facilities for users from both the public and private sectors. The Open Lab framework defines eligibility criteria, safety protocols, and access procedures, with free access provided under specific relevance-driven conditions. Proposals are selected through open calls and a rigorous peer-review process conducted by an independent User Selection Committee.

Following the first call for proposals, two projects have been successfully selected for access to the EURL ECVAM High Throughput Testing (HTT) laboratory. The first project aims to implement a systemic toxicity assay potentially capable of providing mechanistic insights while assessing *in vitro* cell viability. The second project aims to identify the steps required to automate the use of an innovative organ-on-a-chip device, with the ultimate goal of exploring its potential broader application in drug screening.

Through this initiative, EURL ECVAM seeks to enhance the development, validation, and dissemination of non-animal testing approaches, foster scientific exchange, and support the transferability of innovative methods relevant to regulatory applications and the biotech sector.

More information on the JRC Open lab framework: https://joint-research-centre.ec.europa.eu/open-access-jrc-research-infrastructures_en

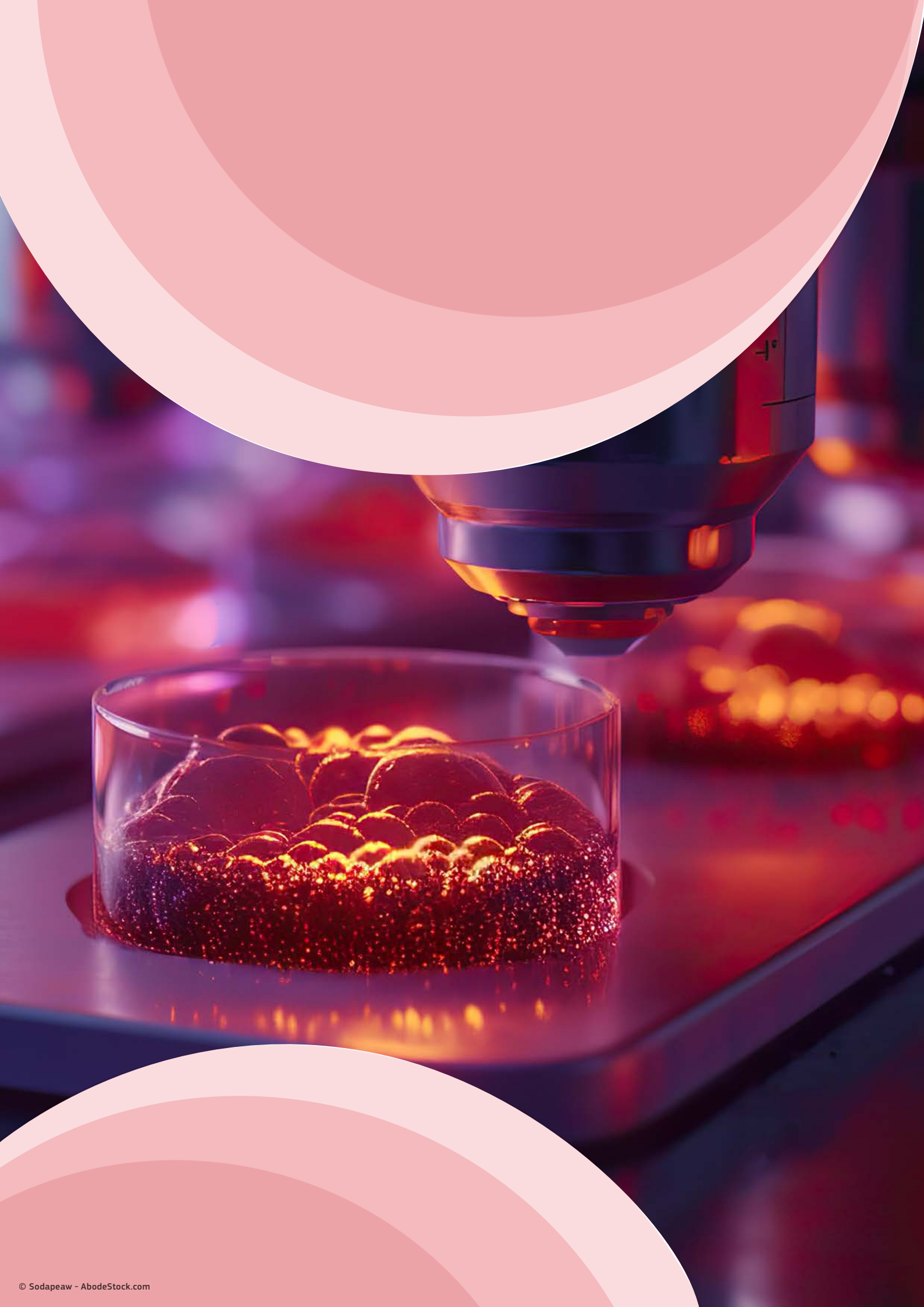
2.10. Analysis of the industrial ecosystem around *in vitro* biotechnologies

Biotechnology plays a crucial role in enhancing the competitiveness and modernisation of European industries. It offers significant potential for innovation, growth, and productivity. Recent EU policy initiatives recognise biotechnology as essential to European autonomy and aim to support the scale-up and commercialisation of innovative biotechnologies.

Within this context, *In Vitro* Biotechnology (IVB) is a rapidly growing sector. It involves biological materials, devices, consumables, instruments, software, and specialised services. IVB enables the creation of advanced *in vitro* models (e.g. organ-on-chip, stem cells, organoids) and methods for a wide variety of applications. These technologies are widely adopted in biomedical research, pharmaceutical development, and food safety.

The segmentation, market analysis, and value chain of the IVB sector, along with a SWOT analysis of its competitiveness, were detailed in the publication "Strengthening the competitiveness of EU *in vitro* biotechnologies" (Mennecozzi *et al.*, 2025a) and the complementary JRC report (Mennecozzi *et al.*, 2025b). Together, these reports outline the current landscape and strategic opportunities for Europe in this rapidly evolving domain. In 2023, Europe led in the 3D cell-based IVB sector and held the second largest share of the global IVB market. The projected compound annual growth rate (CAGR) for 2023 to 2028 remains positive. However, Asia is emerging as a strong competitor with the fastest rate of growth in the IVB sector.

Despite its potential, the EU IVB faces challenges. These include high implementation costs, limited technology transfer and scalability, and low confidence in novel IVB technologies. Addressing these issues requires targeted interventions and coordinated policies. Key actions include dedicated funding for technology transfer and implementation of standards, improved data sharing, and investment in centralised IVB infrastructures. Strengthening the IVB industrial ecosystem will boost EU competitiveness and deliver products and services with higher economic and societal impact. In turn, this will accelerate the transition towards more sustainable and human-relevant research and development.



3. Regulatory Science

Validation is central for establishing trust in cutting-edge non-animal testing methods. EURL ECVAM is actively working to modernise and simplify this process to keep pace with the increasing diversity and complexity of NAMs, catering to the distinct needs of industry, regulators, and academia. Serving as a key scientific and technical facilitator, EURL ECVAM promotes standardisation and guidance revision to advance NAMs as internationally recognised OECD Test Guidelines. Furthermore, EURL ECVAM plays a significant and collaborative role in both EU and international initiatives. By actively engaging with major global partners, such as the OECD, the UN Globally Harmonized System (UN GHS), and various EU regulatory agencies, EURL ECVAM is accelerating the advancement of non-animal sciences and technologies. The ultimate strategic objective is to secure the smooth and effective integration of NAMs into standard regulatory practices for chemical hazard and risk assessment, a commitment evidenced by its substantial ongoing efforts across multiple platforms throughout 2025.

3.1. Test submissions

The EURL ECVAM test method submission process[33] has been updated. It now accepts submissions not only for single methods but also for combinations of methods that together form an integrated approach for chemical safety testing.

In addition to the Test Pre-submission Form (TPF), the first step in the process, the Test Submission Template (TST) is now available. Method developers and test submitters should complete the TST only after a positive TPF evaluation.

However, they are encouraged to review the TST early on to understand the requirements for validation and use of the method in chemical safety assessments.

The Tracking System for Alternative methods towards Regulatory acceptance (TSAR[34]), maintained by EURL ECVAM on behalf of all members of the International Cooperation on Alternative Test Methods (ICATM), provides information on test submissions received.

3.1.1. Blood-brain barrier-on-a-chip

EURL ECVAM received a pre-submission on the Blood-brain barrier-on-a-chip (BBB-oC) for drug testing of brain-targeting delivery. The BBB-oC is a microfluidic device designed to model the human blood-brain barrier by culturing a combination of human cells (brain microvascular endothelial cells, pericytes, astrocytes and/or neurons) in an organ-on-chip device. Barrier characterisation and permeability are measured by immunofluorescence assays, gene expression analysis (RT qPCR) and transendothelial electrical resistance (TEER) measurement.

33 https://joint-research-centre.ec.europa.eu/projects-and-activities/reference-and-measurement/european-union-reference-laboratories/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/alternative-methods-toxicity-testing/validation-and-submission-process/eurl-ecvam-test-method_en

34 https://tsar.jrc.ec.europa.eu/

The method appears to be mechanistically relevant: the proposed endpoint informs on the disturbance of the BBB after exposure to test items. For a full assessment, complete SOPs would be required, including the description of all procedural steps (e.g. generation/banking of the test system, complete details of the experimental phase, test item preparation, data analysis) and of quality acceptance criteria. Moreover, EURL ECVAM suggested to generate additional information on the within-laboratory reproducibility, the design of which should take into due account all factors that can affect variability (e.g. cellular components, culture reagents, device material).

Based on the provided information, EURL ECVAM recognises the method's potential regulatory application in the pharmaceutical sector. Therefore, pursuing a qualification procedure or an Innovation Task Force (ITF) meeting, an early, informal dialogue between medicine developers and EMA regulatory experts on innovative products and technologies, may be appropriate.

Of note, the method's assessment of BBB permeability also holds scientific relevance for the chemical sector, the submitter was thus invited to consider its use for evaluating chemical uptake in the brain and neurotoxicity.

3.1.2. GARDskin Dose-Response

EURL ECVAM received a full submission on the GARDskin Dose-Response (DR) test method, an amendment to the validated and internationally adopted GARDskin assay (OECD, 2024b), suitable for supporting the classification of skin sensitisers and non-sensitisers under the United Nations Globally Harmonised System (GHS).

Both methods employ the NanoString nCounter technology to quantify the mRNA transcripts of the 196 genes comprising the GARDskin Genomic Prediction Signature (GPS) in a human surrogate dendritic cell-like cell line, thus monitoring mechanistic events aligned with Key Event 3 of the skin sensitisation AOP. Gene expression data is analysed by a Support Vector Machine (SVM) to generate a Decision Value used to assist classification.

The foundational GARDskin method was evaluated in a validation study coordinated by SenzaGen AB (Johansson *et al.*, 2019) and subsequently peer reviewed by the EURL ECVAM Scientific Advisory Committee (ESAC) prior to its OECD adoption. The GARDskin DR maintains the established protocol but introduces two critical elements for determining potency: the assessment of a concentration-response series and an expanded analysis pipeline to discriminate between GHS sub-category 1A sensitisers and non-sub-category 1A (NC/1B) chemicals.

EURL ECVAM's assessment of the information provided in the TST found that the modules on Test Definition and Transferability were satisfied, owing to the test method's similarity to the validated GARDskin assay. Nevertheless, the test submitter was advised to develop a dedicated SOP for the GARDskin DR.

To complete the validation process, EURL ECVAM recommended generating data on between-laboratory reproducibility and further evidence of the test method's predictive capacity for identifying GHS sub-category 1A chemicals.

3.1.3. InfiniteLungDT

EURL ECVAM received a pre-submission for the InFiniteLungDT method[35], an *in vitro* learned-digital twin designed to predict lung hazards and the evolution of inflammation, with a specific focus on the influx of neutrophils into the lung. Clusters of the murine epithelial lung tissue cell line (LA-4) and the murine alveolar lung macrophages (MH-S) are monitored by live cell

35 Quantitative prediction of inflammation from acute to chronic condition associated with inhalation and delivered ahead of time by lung animal-free in-vitro-learned digital twin (InFiniteLungDT) https://tsar.jrc.ec.europa.eu/test-method/tm2025-02

microscopy over a 24-hour period. The dynamics of their responses are then input into a set of differential equations (digital twin) to extend the 24-hour *in vitro* observations over longer periods (e.g., 14 days to 12 months), aligning with the timeframe required by OECD, 2018b, OECD, 2018c, OECD, 2024a.

Live microscopy observations target predefined, mechanistically relevant key events associated with inflammation, such as pro-inflammatory mediator secretion or membrane dysfunction. The observed cell dynamics are used to construct the mathematical model that predicts neutrophil influx, an event not directly observable *in vitro*. Regulatory-relevant concentration levels, such as the Least Observable Adverse Effect Level (LOAEL) and No Observable Adverse Effect Level (NOAEL) for sub-acute, sub-chronic and chronic conditions, are derived from the *in vitro* concentration-response relationships.

The test submitter claimed potential applications for pre-screening and selecting safer materials early in development, as well as for grouping and read-across of materials, such as different nano-forms.

EURL ECVAM considered the information provided in the test pre-submission insufficient for a proper preliminary assessment. Following a teleconference, the test submitter was advised to review and resubmit the TPF with clarifications on the regulatory application of the test method, the biological relevance of the cell model and the mechanistic relevance of the measured endpoints (including the relationship to inflammatory key events).

3.2. Validation studies

3.2.1. Validation of a high-throughput in vitro assay to identify androgen-disrupting chemicals

EURL ECVAM is continuing the transfer of the androgen receptor dimerization (AR2) assay to its laboratory that hosts high throughput testing platforms. The cell-based assay developed by the US EPA measures a ligand-dependant homodimerization of the Androgen Receptor (AR) to facilitate identification of chemicals with (anti) androgenic properties (Brown *et al.*, 2023). To date, AR2 was tested against a library of 56 test items in three biological runs to comprehensively characterise its performance in agonist mode. The next phase involves optimisation under antagonist conditions and assessment of its reproducibility. It is envisioned that this activity will support revision of a computational model to predict AR activity, thereby promoting the replacement of the Hershberger Bioassay in Rat (OECD, 2009) used for screening (anti)androgenic properties of chemicals across different regulatory jurisdictions.

3.2.2. Validation of in vitro ADME methods and a defined approach for human toxicokinetics

Chemical safety assessments increasingly require toxicokinetic (TK) data, traditionally obtained through *in vivo* Absorption, Distribution, Metabolism and Excretion (ADME) studies (e.g., OECD, 2010). However, *in vitro* methods are gaining prominence for measuring key ADME parameters and extrapolating to *in vivo* TK data through *In Vitro* to *In Vivo* Extrapolation (IVIVE) approaches. These methods enable human TK characterisation without human *in vivo* studies and are widely used to measure plasma protein binding and intrinsic hepatic clearance, critical parameters for chemical distribution and elimination, respectively. Despite their routine application, standardised OECD test guidelines for these *in vitro* approaches currently do not exist, creating a gap in international harmonisation of human-relevant ADME measurements.

A publication led by EURL ECVAM established a framework for characterising *in vitro* hepatic metabolic clearance methods (Gouliarmou *et al.*, 2018), providing a foundation for the eventual harmonisation efforts.

In April 2025, the Working Party of National Coordinators of the OECD Test Guidelines Programme (WNT) approved the project to develop test guidelines for measuring human intrinsic hepatic clearance and plasma protein binding using *in vitro* methods and incorporating these two experimental toxicokinetic parameters into a defined approach (DA) that provides important human toxicokinetic summary characteristics including chemical half-life, average concentration in the blood at steady state (Css), and area-under-the-curve (AUC) concentration profile in the blood.

The project, co-led by the EU (JRC/EURL ECVAM) and the United States (US EPA), aims to:

- Standardise and validate the *in vitro* methods for measuring human hepatic clearance using pooled primary hepatocytes and plasma protein binding, including the definition of applicability domains and development of acceptance criteria for the results.
- Integrate the parameters into a Defined Approach (DA) for predicting human TK outcomes.
- Ensure regulatory relevance through engagement with OECD experts and relevant regulatory agencies.

Successful development of the TGs and DA is anticipated to yield directly applicable data for various regulatory purposes, including chemical prioritisation, screening-level human health risk assessments, and PBT/vPvB evaluations to assess human bioaccumulation potential. The resulting data will also support weight-of-evidence analyses.

3.2.3. EURL ECVAM's support to external validation studies

EURL ECVAM continued to support the validation management teams for several studies organised by the public-private partnership PEPPER and NTP NICEATM to progress *in vitro* methods for endocrine disruption to regulatory acceptance. Furthermore, EURL ECVAM contributed to VALNAM, a joint initiative co-funded by the German Federal Ministry of Research, Technology and Space (BMFTR) and the Dutch organisation for Knowledge and Innovation in Health, Healthcare and Well-being (ZonMw). VALNAM aims for validation of new approach methods in a regulatory context, with the ultimate goal of implementing new animal-free, human relevant methods as OECD guidelines or establishing qualified models to be used in efficacy testing for new pharmaceuticals. EURL ECVAM evaluated ten project proposals and participated in meetings of the peer review panel to select the most promising proposals for funding. The outcome of the selection procedure is foreseen in April 2026.

Finally, EURL ECVAM will be part of the external advisory board of the newly launched "Towards transcriptomics-based screening and identification of Endocrine Disruptors using New Approach Methodologies" (EDNA) project[36] by the Karolinska Institute. This project aims to build a cross-sectoral collaboration that can support the development and validation of a novel method for identifying endocrine disrupting chemicals (EDs) and ensure its relevance and applicability to meet the needs of regulatory agencies and industry.

36 https://ki.se/en/research/research-areas-centres-and-networks/research-groups/health-risk-assessment-methodology-anna-beronius-group#tab-edna

3.3. Annual meeting of the EURL ECVAM Network for Preliminary Assessment of Regulatory Relevance (PARERE)

The 14th meeting of the Preliminary Assessment of Regulatory Relevance network (PARERE) was held online on 3 June 2025. The meeting gathered representatives from 14 EU Member States, EU Commission services, EU agencies such as ECHA and EFSA, and scientific committees SCCS and SCHEER.

The meeting focused on two main agenda points. First, the 2024 EURL ECVAM Status Report on non-animal methods in science and regulation was overviewed. This was followed by updates from EURL ECVAM representatives on a few selected regulatory relevant activities within the report, such as standardisation of complex test systems and technologies (stem cells; see **Section 3.6.2**) and organ-on-chip;); OECD GD34 revision (see **Section 3.4**); progress made on thyroid hormone disruption methods (see **Section 3.7.4**); revision of UN GHS classification criteria for germ cell mutagenicity (see **Section 3.11.2**) and the informal group on non-animal methods (see **Section 3.11.3**), and the EPAA Designathon (see **Section 3.9.2**). The updates were followed by a "Question and Answer" session on the whole report.

Discussions highlighted the complementary roles of ISO and OECD standards for method standardisation, and challenges with confidence building in non-validated NAMs for regulatory application.

Second, there was a detailed update on the EC roadmap towards phasing out animal testing for chemical safety assessments (see **Section 3.8**).

The PARERE network's role was discussed with participants sharing experiences on national-level function. Common challenges include limited resources and expertise to evaluate and adopt alternative methods, the inertia of established toxicological practices, and the complexity of validating integrated approaches. Some countries highlighted the value of scientific and technical collaboration with 3Rs Centres and the need for enhanced training and two-way communication within the network. The network is seen as a crucial platform to support the roadmap's implementation, but its effectiveness depends on increased policy attention, resources, and inclusive engagement.

Overall, the meeting underscored the EU's commitment to advancing alternatives to animal testing via science, regulation, and stakeholder collaboration, while recognising practical and organisational hurdles to overcome. Further roadmap developments and network enhancements are planned to accelerate progress towards animal-free safety assessment aligned with regulatory and societal goals.

Box 3.1. Preliminary Assessment of Regulatory Relevance (PARERE) network

The Preliminary Assessment of Regulatory Relevance (PARERE) network constitutes a trans-sectoral group comprising regulatory authorities from EU Member States, representatives of EU agencies, and pertinent policy services of the European Commission. Established by EURL ECVAM pursuant to Directive 2010/63/EU (EU, 2010), the network's mandate is to provide expert advice concerning the regulatory relevance and appropriateness of alternative methodologies presented for validation.

PARERE members are engaged periodically throughout the year to deliberate on the regulatory relevance of individual methods or approaches submitted to EURL ECVAM for validation, peer review, or assessment. Additionally, their expertise is sought on matters including EURL ECVAM Recommendations and on methodologies and approaches developed within research initiatives funded under the EU Framework Programme for Research and Innovation.

The PARERE network thereby ensures that alternative approaches to animal testing align with regulatory requirements and facilitates their acceptance for application within regulatory testing frameworks.

Additional information regarding the PARERE network can be accessed via the following link: https://joint-research-centre.ec.europa.eu/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/alternative-methods-toxicity-testing/advisory-and-consultation-bodies/parere-eurl-ecvam-network-preliminary-assessment-regulatory-relevance_en

3.4. Revision of OECD Guidance Document 34 on the validation and international acceptance of new or updated test methods for hazard assessments

Since its publication in 2005, OECD Guidance Document 34 (OECD, 2005) has served as a cornerstone reference for the validation and international acceptance of test methods used in hazard and risk assessment. This document was originally developed based on principles agreed upon during an OECD workshop in 1996. Since then, toxicological sciences have evolved considerably, along with the range of methodologies available to assess the hazardous properties of chemicals. While the fundamental validation principles outlined in GD 34, that methods should be both reproducible and relevant for their intended purpose, continue to hold true, certain aspects of the validation process no longer align with current scientific advancements and technological innovations.

There is a widespread consensus on the need to update the validation and acceptance processes to better support the adoption of modern testing technologies. Such updates are essential to ensure that GD 34 continues to offer practical and comprehensive guidance for validating a diverse array of methods and approaches.

The revision project is jointly led by EURL ECVAM, the United States, and the Netherlands. A project group (PG) has been established to support the efforts, holding six meetings to date, both virtual and face-to-face. This includes the initial virtual meeting in December 2023 (see Zuang *et al.*, 2024), two face-to-face meetings in 2024 (see Zuang *et al.*, 2025), and three meetings in 2025 (two virtual and one face-to-face).

The PG meetings held in 2023 and 2024 focused on establishing the foundation and direction for the GD 34 update. Key activities included developing validation workflows for individual methods and defined approaches, developing two templates for readiness criteria applicable to individual *in vitro*, *in chemico*, and *ex vivo* methods, and to defined approaches, alongside proposing a new document outline for GD 34, simplification of terms, integration of language around protected elements and associated conditions, discussions on transferability and

reproducibility, and importance of training prior to transfer. In 2025, the work progressed into the drafting phase.

During the virtual PG meeting on 12 May 2025, a revised version of chapter 2, covering the essential elements of validation, was presented and discussed. Responses to comments on the new GD 34 outline related to this chapter were also provided.

At the virtual PG meeting on 19 September 2025, discussions focused on test readiness criteria for *in silico* methods and a redraft of chapter 5 concerning peer reviews.

At the face-to-face meeting on 4 to 5 December 2025, chapters 4 and 6, addressing the design and conduct of validation studies and the international regulatory acceptance of validated methods, respectively, were presented and discussed. Additionally, revised versions of chapters 2 and 5 were also discussed. A comprehensive review of the project's overall progress and the planning of deliverables for 2026 were also undertaken. Two face-to-face meetings are scheduled for 2026 for finalising the revision with an aim to submit the revised GD34 for WNT approval in April 2027.

3.5. Readiness criteria

To promote public dissemination and standardise the assessment of readiness of methods and approaches at an international level, EURL ECVAM has developed readiness criteria in the framework of the revision of GD 34, considering its existing submission process as well as other existing resources (DNT criteria by Bal-Price *et al.*, 2018; ToxTemp template by Krebs *et al.*, 2019; ReadED test by Crouzet *et al.*, 2023; Holzer *et al.*, 2023; Petersen *et al.*, 2023; van der Zalm *et al.*, 2022; OECD, 2018a). The developed readiness criteria evaluate the readiness of individual *in vitro*, *in chemico* and *ex vivo* methods and include criteria to assess test method definition and description, data management, evaluation and interpretation, test method reliability, test method relevance and data integrity and quality assurance. It is specified if the criteria must be fulfilled either before a method enters validation, before transferability to other laboratories or before peer review for test guideline development.

EURL ECVAM's readiness criteria were proposed as an annex to the updated OECD GD34 (see **Section 3.4**) and were well received by the OECD GD34 project group. Currently, the review is ongoing, alongside draft readiness criteria templates for *in silico* methods and defined approaches (DAs).

The draft GD34 readiness criteria template for an individual method is already used by the OECD expert group (TDM-EG) to evaluate thyroid hormone disruption methods and feedback received from two laboratories that used the template will be used to improve it. Additionally, the template is proposed for testing within ongoing EU-funded projects given that self-assessment of test readiness for validation has become a critical deliverable for these initiatives. The template has been presented and received positive feedback at various international meetings e.g. the Scientific Committee on Consumer Safety (SCCS) Methodology Workshop on Next Generation Risk Assessment in Brussels in December 2024 (SCCS, 2026). It was also discussed with EFSA and EMA 3RWP for considering using the readiness criteria in their respective qualification programmes.

3.6. Standardisation of complex test systems and technologies

3.6.1. Omics-based methods

EURL ECVAM has been engaged in standardisation of omics-based methods (specifically, transcriptomics and metabolomics) to facilitate their application in regulatory toxicology. A recently published manuscript (Malinowska *et al.*, 2025) evaluated existing documentary standards and reference materials for transcriptomics- and metabolomics-based *in vitro* methods. The review demonstrated that for transcriptomics-based methods, formal standardisation bodies produced some documentary standards, whilst for metabolomics-based methods, efforts have been primarily community-driven. The paper also examined how the application of standards could make test guidelines incorporating omics-based *in vitro* methods (i) reliable, (ii) accessible across jurisdictions, and (iii) sustainable over time. These are crucial attributes of new test methods that enter the OECD test guideline programme.

Additionally, EURL ECVAM contributed to drafting the new OECD Guidance Document on good practices and standardisation of sample collection for omics analysis (OECD, 2025b), coordinated by the ECHA. The guidance is anticipated to increase reliability of the omics data generated for regulatory purposes and focuses on the pre-analytical phase of the omics-based workflow, from study exit through sample collection, processing, storage and its transportation. This new guidance has been drafted for three omics technologies: transcriptomics, proteomics, and metabolomics, and focuses on the collection of three sample types: *in vitro*, *in vivo*, and alternative test species. Examples of SOPs for collection of different sample types are also included in the document, including one developed by EURL ECVAM in collaboration with the University of Birmingham, UK ("sampling HepaRG cells for metabolites").

Lastly, a new project "Omics2AOPs", led by EURL ECVAM and Tampere University, was endorsed by the OECD Working Party on Hazard Assessment (WPHA) in June 2025. Omics2AOPs supports contextualisation of omics data by linking sets of genes, proteins and metabolites to key events in AOPs, and will use established knowledgebases and ontologies commonly employed to interpret omics data.

In late October 2025, the plenary Omics2AOPs workshop and kick-off meeting took place at the JRC in Ispra, Italy, which brought together cross-disciplinary experts from areas such as AOPs, toxicology, omics and Natural Language Processing (see **Figure 3.1**). The meeting offered opportunities to review and discuss work from complementary activities, align the project with ongoing efforts, and inspire novel approaches. Using this information, the meeting facilitated key decision making on structure and direction, cementing its direction and formally launching the project.

Figure 3.1. Participants of the Omics2AOPs workshop and kick-off meeting at the European Commission – Joint Research Centre in Ispra, Italy

Source: European Commission – Joint Research Centre, Ispra, Italy

3.6.2. Stem cells

Human pluripotent stem cell (hPSC) research has the potential to revolutionise preclinical and regulatory safety testing of drugs and chemicals. To facilitate the uptake of human stem cell-based models and the utilisation of the data generated with them, quality standards and reporting best practices should be promoted and implemented. In this context, EURL ECVAM published in 2025 a perspective article titled "Promoting the adoption of best practices and standards to enhance quality and reproducibility of stem cell research" (Selfa Aspiroz *et al.*, 2025). The aim of this paper was to provide an overview of current best practices and standards for cell culture methods and highlight strategies to promote their implementation early in stem cell research. The conclusions of this paper highlight the need for a cultural shift that prioritises quality and reproducibility to enable a broad adoption of stem cell standards. This should be supported by collective actions across stakeholder levels, from journal publishers and research funders to core facilities and the research community.

As a follow up, EURL ECVAM has established a collaboration with the CorEuStem COST Action[37] to assess the adoption of best practices and standards within stem cell core facility communities. This COST Action promotes the standardisation and harmonisation of quality control protocols and assays to enhance reproducibility, rigor, and comparability across Europe. To evaluate standards adherence and conduct a gap analysis, a comprehensive survey was prepared and distributed to COST Action members in November 2025.

Human stem cells are increasingly used to develop transformative cell therapies for a wide range of diseases. A key remaining challenge is assessing the impact of genetic variants on the safety and efficacy of hPSC-based therapies. This topic is addressed in the recent publication "A call to action for deciphering genetic variants in human pluripotent stem cells for cell therapy", to which EURL ECVAM contributed. The paper emphasises the need for centralised data

37 https://coreustem.eu/

repositories to share genetic variant information and for *in vitro* assays to assess and predict variant tumorigenicity (Benvenisty *et al.*, 2025).

3.7. Regulatory application of test methods and integrated approaches to testing and assessment

3.7.1. Developmental neurotoxicity

Developmental neurotoxicity (DNT) is a pressing public health and socioeconomic concern because of its potential to cause a wide range of cognitive disabilities, including impairments in learning, memory, and behavioural development.

Driven by the limitations of traditional animal tests, there is growing interest in developing innovative and reliable non-animal methods for DNT evaluation. This collaborative effort has culminated in the DNT *in vitro* battery (DNT-IVB), a collection of assays based on animal and human cell culture models (OECD, 2023).

The main regulatory challenge is demonstrating the transferability and reproducibility of the DNT-IVB methods. EFSA is funding the DNT RAP2 project (conducted by IUF – Leibniz Institute for Environmental Medicine) to optimise the SOPs and assess transferability and preliminary reproducibility. The project, started in May 2024 and is foreseen to last until the end of 2027. EURL ECVAM is continuing to assist EFSA and IUF by providing expert advice on various aspects of the project.

Within the PARC project P5.2.1.e "Neurotoxicity" (Work Package 5), the development of the DNT-IVB v2.0 relies on a well-defined, high-quality chemical library to ensure consistent evaluation of assay performance and applicability domains. EURL ECVAM played an essential role in this process, by contributing to the selection, design, curation and provision of the DNT 2.0 chemical library (consisting of 94 commercially available reference chemicals), as a contribution to the PARC project P5.2.1.e. The goal of this project is to develop new NAMs for DNT to address uncertainties in the DNT-IVB (Tal *et al.*, 2024).

The material was delivered to 14 PARC participating institutions (16 partner laboratories) to support the joint project's objective of assessing, refining, and optimising a set of *in vitro* or alternative DNT methods. By enabling partners to work with a harmonised and scientifically selected chemical set, this coordinated effort will generate comparable datasets essential for evaluating the reliability and relevance of the methods and for expanding the mechanistic domain of the existing DNT-IVB.

This initiative represents a substantial contribution to the PARC community, establishing the potential added value of new DNT NAMs, supporting future regulatory applicability, and fostering transparent data sharing. In accordance with the agreement, resulting datasets will be disseminated in the public domain, including through the JRC Data Catalogue.

3.7.2. Developmental and reproductive toxicity

In 2025, EURL ECVAM joined as a Challenge Partner of the NCR3s CRACK IT Challenge #45[38]. The project, led by Toxys in collaboration with the University of Antwerpen and ESQlabs, aims to develop and qualify multi-species (human, rabbit and rat) *in vitro* approaches that, together with computational models, can reliably predict the teratogenicity of new drug candidates. The project is supported by in-kind contributions from eight international sponsors, including pharmaceutical and not-for-profit organisations. Through this collaboration, the team will qualify the *in vitro* assays using positive and negative reference compounds and compare assay

38 https://nc3rs.org.uk/crackit/crossdart

predictions across species. EURL ECVAM will contribute its expertise in the validation and qualification of alternative methods for safety assessment, and as a partner, will provide key expert advice and input to help bridge the gap between innovation and market adoption.

3.7.3. Integrated approach to testing and assessment for non-genotoxic carcinogenicity

The evaluation of non-genotoxic carcinogenicity (NGTxC) has been identified as a critical regulatory gap. To address this, EURL ECVAM supports the OECD expert group in developing an IATA for non-genotoxic carcinogenicity.

The group has already established an overarching IATA framework based on mechanistic insights into carcinogenicity (Jacobs *et al.*, 2020) and evaluated key assays covering different stages of the carcinogenic process[39,40]. Recently, discussions have focused on the more downstream key events, particularly proliferation and morphological changes, where adaptive (sustained) proliferation and hyperplasia become maladaptive.

Additionally, the group is exploring the role of immune dysfunction (e.g., immunotoxicity, immune evasion, suppression, and inflammation-driven responses) as a critical carcinogenic event, aiming to integrate it into the NGTxC IATA (Colacci *et al.*, 2025).

Ongoing efforts also include developing guidance for the use of a Modular Strategy of Testing and Assessment (Louekari *et al.*, 2024) helping users to fill in the various modules and to conclude the assessments using a weight of evidence approach.

3.7.4. Progress made for methods relevant to the thyroid hormone system

Central to knowledge exchange, tracking ongoing activities, and developing testing strategies is the OECD Thyroid Disruption Methods Expert Group (TDM-EG), established by the OECD WNT, which in 2025, met once in October. For regulatory approaches to thyroid hormone system disruption (THSD), additional methods, data and insights into the most common or critical mechanisms are needed. As a result, progress in the development of IATAs has slowed for now.

A key initiative aiming to advance testing strategies is the PARC project 6.1.1e, which integrates *in silico*, *in vitro* and *in vivo* data from fish and amphibians. This project will clarify which THSD modes of action lead to specific adverse *in vivo* effects and identify cases where further *in vivo* testing is unnecessary.

Progress also hinges on validation activities initiated by various stakeholders following the EURL ECVAM / EU-NETVAL validation study of a thyroid-relevant method battery.

- The US-EPA completed the validation of its human thyroid microtissue assay (Foley *et al.*, 2025), which addresses multiple mechanistic modes of action. The validation management team concluded that it reliably measures changes in thyroxine (T4) production in the thyroid tissue.
- PEPPER-funded projects are validating a deiodinase-1 inhibition method based on the Sandell Kolthoff (SK) reaction and a T4 displacement method from transthyretin (TTR), both are ongoing. Validation of a third method for TPO-catalysed tyrosine iodination inhibition awaits successful cell line deposition by supplier Charité.
- Germany is funding validation of methods for NIS inhibition (SK reaction-based), deiodinase 1,2,3 inhibition (SK reaction-based), and iTPO inhibition (using Amplex UltraRed).

39 https://www.mdpi.com/journal/ijms/special_issues/NGTxC

40 https://www.mdpi.com/journal/ijms/special_issues/348DJPZT0M

The TDM-EG conducted an informal assessment of several methods from contract research organisations (CROs), using test readiness criteria (see **Section 3.5**). It recommended further validation of an SK reaction-based NIS inhibition method and an analytical method for deiodinase 1, 2, 3 inhibition, though funding is required to proceed.

No validation activities are underway for other prioritised modes of action, such as MCT8 inhibition and dehalogenase inhibition.

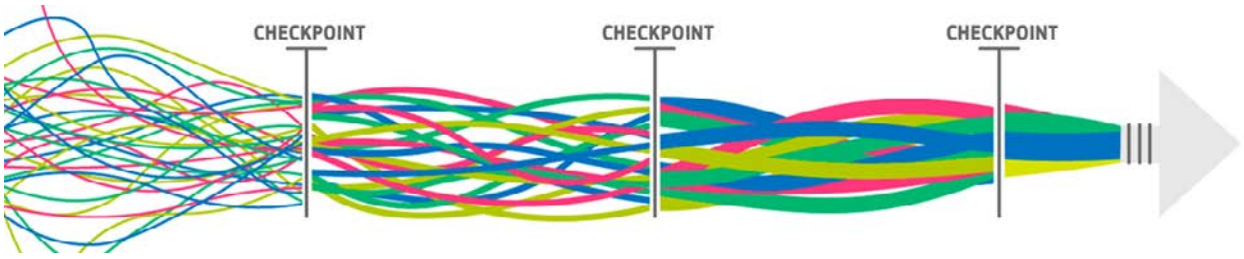
Beyond NAMs, THSD-relevant endpoints are being incorporated into existing *in vivo* test guidelines TG 236 (FET) and TG 210 (FELS). Proposed additions, thyroid histopathology, swim bladder inflation, eye development, and hormone measurements, are under validation in OECD project 2.64, overseen by the OECD VMG-eco.

3.8. EC roadmap towards phasing out animal testing for chemical safety assessments - Activities of the Change Management Working Group

EURL ECVAM co-chaired the Change Management Working Group together with DG GROW supporting the development of the roadmap towards phasing out of animal testing in chemical safety assessments, focussing on the establishment of the transitional initiatives, defining indicators and get inspired and informed through bilateral meetings with stakeholders.

Transitional initiatives can be considered as "units of change" towards the ultimate goal of the roadmap, and they include any initiative contributing directly or indirectly to the replacement or reduction of the use of animals in regulatory assessment of chemicals. EURL ECVAM has launched an open call[41] to notify any initiative intended to achieve an outcome that might contribute to the roadmap. EURL ECVAM published a catalogue[42] of transitional initiatives and keeps it updated with new notified initiatives, also during the implementation of the roadmap. In addition, EURL ECVAM is considering supplementing the catalogue with transitional initiatives that were not notified but are nonetheless recognised as relevant.

Figure 3.2. Transitional initiatives, illustrated as threads, contributing directly or indirectly to the phasing out of animals in chemical regulatory assessment. The checkpoints are planned along the roadmap for further strategic planning and coordination of the initiatives, assisting in interweaving the threads, and more efficiently progress towards the ultimate goal.



Source: European Commission – Joint Research Centre, Ispra, Italy

At present the transitional initiatives appear as mostly uncoordinated threads. A better overview would enable strategic planning and coordination, the interweaving of threads leading to faster and sustainable progress. Such a mapping would further clarify what is still missing to reach the final goal and help to reinforce ongoing efforts while also avoiding duplicative efforts. Therefore,

41 https://joint-research-centre.ec.europa.eu/projects-and-activities/reference-and-measurement/european-union-reference-laboratories/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/transitional-initiatives_en

42 https://data.jrc.ec.europa.eu/dataset/a8517c2c-dec8-42f8-b906-709ca9db748d

transitional initiatives provide a means of tracking and guiding progress towards the final goal.

To measure change and progress along the roadmap there is a need to define indicators that can be used to develop both holistic (cross-sector) and sector-specific narratives, recognising that there will be methodological differences between sectors in terms of data availability and accessibility. To solicit ideas from the multitude of stakeholders involved in the roadmap development, a crowd-sourcing approach[43] led to a list of over 50 candidate indicators. Not all these indicators will be applied. EURL ECVAM will develop a few and report on them during the implementation phase. Also other Commission services, European agencies, Member State Authorities and other stakeholders are encouraged to host indicators for which they have a specific interest and also access to the necessary underpinning data.

The summary report on the learnings from the bilateral discussions with stakeholders is publicly available[44], and the insights gained have informed some of the actions in the Commission roadmap.

3.9. EPAA promotion of the regulatory acceptance of alternatives to animal testing

The European Partnership for Alternative Approaches to Animal Testing (EPAA), a collaboration between 40 companies from 9 industry sectors, 5 Directorates-General of the European Commission and 3 EU Agencies, is continuing its activities with a vision to replace, reduce and refine (3Rs) animal use for meeting the regulatory requirements through better and more predictive science.

The EPAA projects 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 the EU and globally. EURL ECVAM is active in and co-chairs a number of individual projects described below. The status of the ongoing projects is summarised in the EPAA Annual Report. This year the EPAA has been very active in directly contributing to the EC roadmap towards phasing out animal testing for chemical safety assessments through the development of action plans in different areas of toxicology. In this context, the EPAA organised in March 2025 “The Animal-Free Chemical Safety Assessment (AFCSA) Conference” that involved more than 250 experts working in breakout groups to develop animal-free safety assessment strategies capable of addressing European chemical regulatory information requirements without animal testing. Each breakout group also proposed short-, medium- and long-term actions for potential inclusion in the Commission Roadmap. EURL ECVAM was involved in the coordination of several of these breakout groups.

Finally, the EPAA promotes its activities through knowledge sharing, its annual conference and stakeholder dialogue including the continued and valuable input received from the mirror group. This year the EPAA celebrated its 20th anniversary in an event hosted in the European Parliament by MEPs Tilly Metz, Sirpa Pietikainen and Niels Fuglsang and inspiringly opened by President Roberta Metsola (see **Figure 3.3**). High-level keynote speeches reflected on the EPAA’s 20-year journey and the role EPAA can play accelerating the transition to animal-free approaches through informing policy initiatives and facilitating regulatory acceptance. This was followed by the EPAA annual conference entitled “From Roadmap to Roadtrip: The role of stakeholders in supporting the Commission Roadmap”. The conference was also the opportunity to announce the EPAA 3Rs Refinement Prize winner.

43 https://webgate.ec.europa.eu/circabc-ewpp/d/d/workspace/SpacesStore/4015d661-04da-440e-96d9-a937e955d341/file.bin

44 https://single-market-economy.ec.europa.eu/document/download/5f00f9ee-ab79-422e-a4ed-9f9d372748c5_en?filename=Summary%20of%20bilateral%20discussions%20-%20Change%20Management%20WG.pdf

Figure 3.3. EPAA celebration of its 20th anniversary at the European Parliament. From left to right J. Fentem (Executive Vice President, Safety, Environment and Regulatory Science, Unilever), T. Metz (MEP), R. Metzola (EP President), S. McGuinness (ECHA Executive Director), V. Moutarlier (Deputy Director-General DG GROW), S. Pietikainen (MEP)

Source: European Union 2025 – European Parliament

Read more:
EPAA Annual report: https://webgate.ec.europa.eu/circabc-ewpp/d/d/workspace/SpacesStore/4fecbb5f-9e5c-4b85-ab79-f73586a5e0f6/download
Brochure about the achievements of 20 years of the EPAA: https://webgate.ec.europa.eu/circabc-ewpp/d/d/workspace/SpacesStore/ca929d64-4312-426e-9b5e-ba31f1c46710/download
Animal-Free Chemical Safety Assessment (AF-CSA) Conference flash report: https://webgate.ec.europa.eu/circabc-ewpp/d/d/workspace/SpacesStore/4015d661-04da-440e-96d9-a937e955d341/download

3.9.1. Implementing change for carcinogenicity assessment

The Commission Roadmap highlights the new approach to carcinogenicity assessment as a prime example of integrating NAMs into regulatory practice. This shift translates innovative methods into practical solutions that maintain or exceed current human protection levels. To facilitate this transition, the EPAA has established a working group with representatives from key initiatives. The group is developing a framework for carcinogenicity assessment, using weight of evidence-based strategies that incorporate *in silico*, *in chemico*, *in vitro*, and short-term *in vivo* tests that is applicable to different sectors.

A review of the ongoing initiatives uncovered strong commonalities: all emphasise mechanistic approaches to detect potential carcinogenicity earlier in the process, underpinned by a shared rationale for alternative approaches. This informed an overarching assessment framework, with key components, for experts

and stakeholders to evaluate opportunities for a paradigm change. Harmonisation across sectors, at both European and global levels, is vital for success, but it requires consensus on best practices, resolution of policy differences, regulatory adaptations and transparent communication with governments, stakeholders, and the public.

A stepwise action plan guides this evolution, detailing short-, medium-, and long-term actions to address scientific gaps and enact changes. This plan will be summarised in the staff working document accompanying the Commission Communication on the roadmap towards phasing out animal testing for chemicals safety assessments.

Genotoxicity assessment is considered an integral part of the carcinogenicity assessment framework warranting more efforts to move away from animal testing. The EPAA, therefore, recently established a new working group to focus on issues related to harmonisation across sectors, understanding the reasons for divergencies and learn about available non-animal genotoxicity strategies and how these can be applied to other sectors.

3.9.2. EPAA Designathon for human systemic toxicity

The Designathon is a classification challenge focused on human systemic toxicity[45], aiming to develop innovative classification approaches that categorise chemicals into three levels of concern (high, medium and low) using bioactivity and systemic bioavailability data derived exclusively from non-animal methods (Berggren *et al.*, 2023, Worth *et al.*, 2025). This new non-animal methods-based classification seeks to provide the same level of protection as the current system, ensuring equivalent risk management outcomes for classified chemicals[46].

This challenge is being approached iteratively through various phases. The co-creation phase started in November 2024, building on the pilot phase, which saw 23 prototype NAM-based solutions submitted using 150 reference chemicals provided by EPAA. Initially, the effort was organised into three working groups (see **Figure 3.4**), focusing on exploring chemical space, biological space, and design principles for the classification strategy. Regular feedback from the Steering Team was complemented by a face-to-face meeting between Steering Team members and working group co-chairs in July 2025 at JRC. This meeting offered valuable insights and guidance to help Designathon participants achieve the co-creation phase goals[47].

For the remainder of the co-creation phase, participants will concentrate on six targeted activities outlined in July 2025 (see **Figure 3.5**). These include constructing a streamlined workflow, defining essential questions enabling classification, establishing minimum relevant and quality criteria for key ADME and TK properties, characterising and integrating uncertainty into the process, defining the chemical calibration set for the testing phase that combines TK, ADME and biology information, and mapping existing tools and technology-based datasets from the pilot phase to refine the initial workflow and design the testing phase.

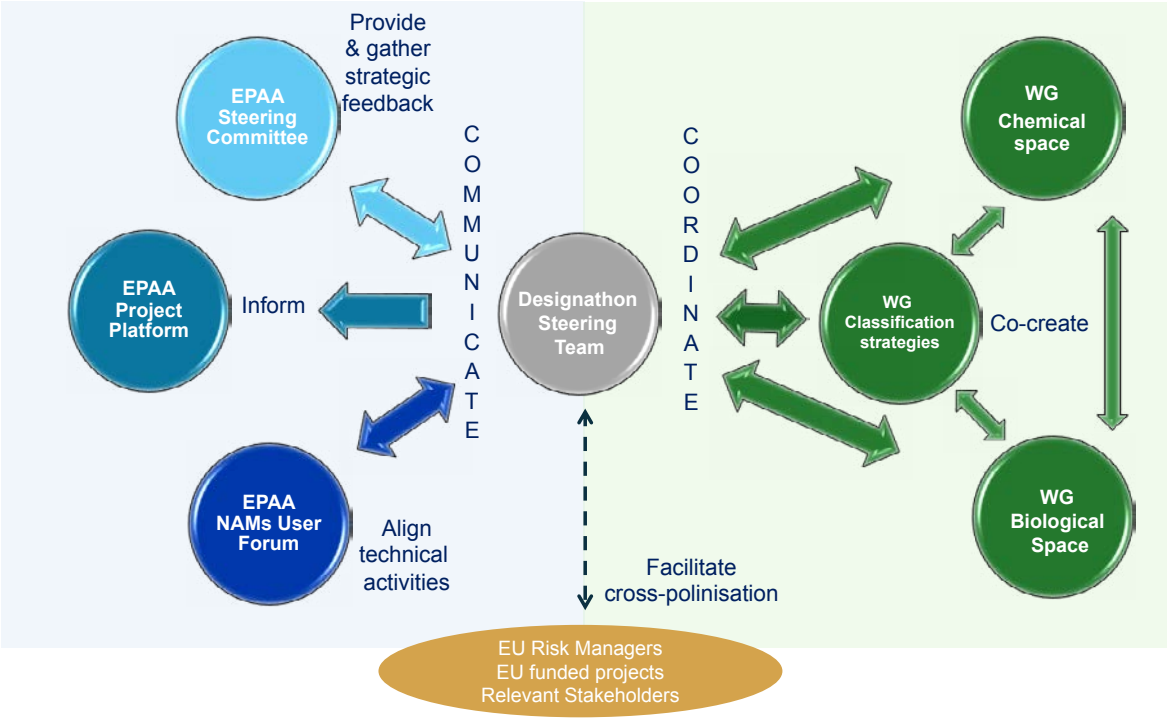
The ultimate aim is to develop co-created solution(s) and a plan for the testing phase, ready for discussion with all participants and stakeholders at a workshop in 2026.

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

46 https://data.jrc.ec.europa.eu/dataset/a8517c2c-dec8-42f8-b906-709ca9db748d

47 https://ec.europa.eu/docsroom/documents/61054

Figure 3.4. EPAA NAM Designathon project implementation – overview of the Communication and Co-creation strategy



Source: EPAA Designathon team

Figure 3.5. Six sub-teams and targeted activities for co-creation and future design of the testing phase

Source: EPAA Designathon team

3.9.3. Environmental safety assessment

Environmental safety assessment (ESA) is a priority for JRC. EURL ECVAM collaborates with research institutions, EU agencies, and international partners, including OECD within the Validation Management Group for Ecotoxicity Testing (VMG-Eco) and the EPAA Environmental Safety Assessment (ESA) project team, to progress towards a next-generation ESA. The new concept of ESA involves leveraging NAMs to minimise animal testing with the overall goal of enhancing environmental protection.

The EPAA ESA project team assessed the current state of non-animal methodologies related to areas such as acute and chronic fish toxicity, bioaccumulation testing, endocrine disruptors, assessments related to birds and mammals, and new approaches for validation. For each of these areas, the EPAA ESA team provided expert recommendations for short-, medium-, and long-term strategies that align with the EC roadmap towards phasing out animal testing for chemical safety assessments.

Specifically, the expert group for endocrine disruption coordinated by EURL ECVAM, identified several key action points focused on maximising the effort to demonstrate the performance of new methods, on disentangling endocrine and non-endocrine biological pathways and on building up knowledge on *in silico* cross-species extrapolation.

Additionally, the EPAA ESA team is envisioning a new paradigm that emphasises a shift from traditional testing methods, often limited to single endpoints and species, to a more holistic and integrative approach. This transformation is essential for facilitating the replacement of animal testing with innovative methodologies that can better assess environmental safety.

Follow-up activities are currently being identified and planned.

3.9.4. EPAA and endocrine disruptors for human health

EPAA organised for the first time a Partners' Forum on endocrine disruptors that was held on 14 to 15 November 2024 in Brussels.

A manuscript titled "Use of New Approach Methodologies for the assessment of Endocrine Disrupting Chemicals within European Union regulatory frameworks: Report from the 2024 European Partnership for Alternative Approaches (EPAA) Partners' Forum" is close to publication (Tarazona *et al.*, 2026). Excerpts of this manuscript, especially discussions on the challenges and the opportunities for cross-cutting collaborations, will inform the staff working document of the EC roadmap towards phasing out animal testing. The report describes the main discussions and conclusions from the EPAA Partners' Forum. The EPAA Partners' Forum 2024 aimed to deliver a strategic, cross-sector review of NAM-based frameworks within the European regulatory context, capturing lessons learned, identifying scientific gaps and research challenges, and outlining opportunities for cross-sector industry collaboration. The event gathered 77 participants, both in person and online, representing regulatory agencies, the Scientific Committee on Consumer Safety (SCCS), the EC, eight industry sectors, EU-funded research projects (EURION, ENKORE, ASPIS), and other relevant organisations (e.g., OECD). The forum opened with brief sector-specific presentations on the current status of NAM development and application, challenges and priorities, followed by a roundtable discussion on opportunities for cross-sector collaboration to protect human health and the environment. EURL ECVAM chaired, in collaboration with EPAA, the human health discussion. The manuscript details the contributions of each sector, the identified scientific gaps and research challenges, and the opportunities for collaboration that emerged from the discussions. In addition, the ED Breakout

Group of EPAA's Animal-Free Chemical Safety Assessment (AF-CSA) Conference, held in March 2025 and chaired by the EURL ECVAM, GROW, EPAA and the International Collaboration on Cosmetics Safety (ICCS), further explored opportunities and challenges in ED identification across contexts and highlighted persistent uncertainties that must be addressed before transitioning to animal-free ED assessment.

3.10. EU test method development and validation strategy

The Ministry of Infrastructure and Water Management of the Netherlands, in collaboration with the French Ministry for Ecological Transition, Biodiversity, Forests, the Sea and Fisheries, and a Task Force comprising ECHA, EFSA, OECD, the Netherlands Food and Consumer Product Safety Authority (NVWA), and the National Institute for Public Health and the Environment (RIVM), has developed a concept paper outlining a governance model that proposes a coordinated EU strategy for the development, validation, and funding of test methods to assess chemical safety. This initiative stems from a joint German and Dutch Caracal thought-starter aimed to accelerate the availability of regulatory-accepted test methods[48].

They propose establishing an "EU Test Method Development and Validation Strategy" that includes coordination, prioritisation, and funding for test method development and validation.

This strategy is meant to address the urgent need to accelerate the development and regulatory acceptance of modern test methods for chemical safety assessment. Current innovation in substances and materials outpaces the development and validation of test methods necessary to ensure their safety. The lack of alignment between test method development, validation, and regulatory implementation, as well as insufficient coordination, prioritisation, and financial support, causes delays and high costs for industry and regulators. There is also a societal demand for animal-free test methods, which are insufficiently available and validated.

The EU Strategy for Test Method Development and Validation aims to establish within six years a system that significantly enhances regulatory hazard and risk assessment of chemicals on the European market. This system will first outline and periodically prioritise regulatory needs for new validated test methods, inclusive of societal needs such as animal-free approaches. This prioritisation guides the selection of test methods for validation and informs science and funding bodies about key development challenges, fostering demand-driven innovation.

Secondly, new promising test methods will be prioritised based on their regulatory need and a coordinated mechanism for their validation will be established. This involves defining roles and responsibilities, decision-making processes, and financial arrangements within the validation activities.

This strategy aligns with the European Commission's 'One Substance One Assessment' (OSOA) initiative, promoting cooperation among EU regulatory agencies like ECHA, EFSA, EMA, and others for data sharing and methodology development. The strategy also supports the EC Roadmap towards phasing out animal testing, linking prioritised test methods and validation recommendations within that framework.

48 https://circabc.europa.eu/ui/group/a0b483a2-4c05-4058-addf-2a4de71b9a98/library/616824f8-fafb-4962-b14c-a37029049992/details

Two mandates underpin the strategy's realisation:

1. Mandate 1 focuses on identifying and prioritising EU regulatory needs for test methods to be adopted in the OECD Test Guidelines Programme. Activities include stocktaking of toxicological endpoints, existing guidelines, proposed methods, validated methods, and development gaps. This mandate also proposes priorities for new method development, validation, and OECD inclusion.
2. Mandate 2 develops a governance model for validation activities of prioritised test methods from Mandate 1. It entails defining roles, coordination, financial resources, and mechanisms, assessing validation costs, exploring funding models such as PEPPER and EU-NETVAL laboratories, and coordinating with OECD processes.

Governance models involve key EU agencies, member states and stakeholders and require European Commission approval. This comprehensive approach targets improving chemical safety testing efficiency, reducing animal use, and better protecting health and the environment.

Figure 3.6. Task Force responsible for the development of the concept paper on an EU Test Method Development and Validation Strategy

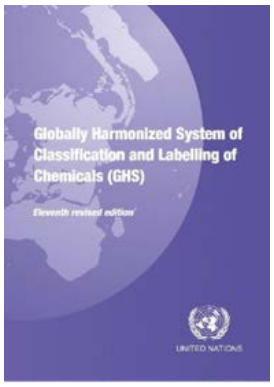
Source: RIVM and the Ministry of Infrastructure and Water Management of the Netherlands

"A coordinated and collaborative EU strategy is essential to accelerate targeted test method development and validation."

3.11. Classification and Labelling

In 2025, the 11th edition of the United Nations Globally Harmonised System of Classification and Labelling of Chemicals (GHS) was published (UN, 2025a), including amongst other revisions the updated chapter 3.4 on the classification of skin sensitisation regarding mixtures. The Sub-Committee of experts on GHS is making the technical recommendations and updates to the GHS, resulting in a proposal for revision every second year. The GHS is implemented within the EU through the CLP Regulation (EC, 2008b).

The Sub-Committee of Experts on the GHS collaborates in parallel with the Sub-Committee of Experts on the Transport of Dangerous Goods (TDG), with both groups sharing updates on their ongoing discussions. They report to a parent committee that coordinates strategic and policy directions, approves work programmes and provides formal endorsement for the sub-committees' recommendations. For TDG purposes, the GHS is implemented via the UN Recommendations on the Transport of Dangerous Goods - Model Regulations (UN, 2025b, UN, 2025c), which are updated biennially, in line with the GHS revision cycle.



The Purple Book

The Orange Book

3.11.1. Inclusion of new hazard classes for endocrine disruptors in UN GHS

The EU is working through the UN GHS Sub-Committee to introduce the new hazard classes for EDs for Human Health (EDs-HH) and EDs for the Environment (EDs-ENV) in the GHS in alignment with the EU CLP Regulation. However, no consensus has yet been reached on how to address the current gap in the GHS with regard to the proper identification, classification, and communication of EDs. Some experts dispute whether such a gap exists, while others debate whether a new hazard class is needed or if existing GHS hazard classes should be modified to also account for the ED hazard.

From 2023 to 2024, the GHS Sub-Committee mandated the OECD to review the state of the science on EDs and to undertake a gap analysis in current GHS hazard classes regarding the identification of chemicals as EDs according to the WHO/IPCS definition, i.e. identify substances that are active through an endocrine mechanism that can be plausibly linked to an adverse effect. The review concluded that the GHS, which focuses on adverse effects rather than underlying mechanisms (except mutagenicity), cannot fully identify EDs based on the WHO/IPCS definition. This confirms a clear gap in the system.

However, some delegations opposed OECD's interpretation. It was therefore decided that additional analysis would be carried out by the EU and US, starting from HH. The US and the EU

have started this additional analysis. Both agree that the current GHS only partially identifies EDs for human health but does not consistently label them as such, creating communication gaps. US supports expanding the Specific Target Organ Toxicity (STOT) guidance to better address EDs, while the EU advocates for a new stand-alone hazard class for EDs.

It was generally acknowledged that further work is needed on how the GHS should be amended. The EU and US are working toward reaching a consensus on how to address the gap. The next step is to submit an informal document to the GHS-50 session in July 2026. In Q3 2026, the Potential Hazard Issues - Informal Working Group (PHI-IWG) will discuss potential approaches to addressing the gap. Finally, an informal document detailing the work plan for 2027–2028 will be submitted to the GHS-51 session in December 2026.

3.11.2. Revision of the UN GHS classification criteria for germ cell mutagenicity

Members of the informal working group struggled to reach consensus on the sub-criterion for substances deemed capable of inducing heritable germ cell mutations in humans and classified in Category 1B. The current wording "the ability of a substance or its metabolites to interact with the genetic material of germ cells", drew objections from several EU Member States. They proposed revising it to "the ability of a substance or its metabolites to reach the gonads" citing difficulties in classifying substances as Category 1 and concerns that it may not be sufficiently protective. This is because most chemicals in Category 2 are proven mutagenic to somatic cells, posing high concern for human health. Consequently, the informal working group is working on a compromise solution that will include minor changes to the current wording of the criterion and a detailed description of the supporting evidence that would be needed to fulfil the criterion.

The JRC through EURL ECVAM, representing the EU delegation, leads this working group on germ cell mutagenicity, established by the GHS Sub-Committee in 2021. The group's tasks include reconsidering the criteria, updating chapter 3.5 on germ cell mutagenicity (incorporating non-animal evidence where possible), finalising the chapter, concluding work within the current biennium, and integrating the revised text into the 12th revision of the GHS.

3.11.3. Work of the informal working group on non-animal methods

Chapter 1.3 of the GHS, which addresses general classification issues applicable to all GHS classes, is currently under revision. An informal working group is updating the existing text on animal welfare and weight-of-evidence considerations, while adding new recommendations on non-animal test methods, non-test methods (computational methods) and defined approaches. This work is nearly finalised, with the updated chapter proposed for inclusion in the 12th revision of the GHS. The informal working group on non-animal methods is led by the Netherlands and the UK, with the JRC invited by the leads to provide support.

3.12. Highlights of the 37th meeting of the Working Party of National Coordinators of the OECD Test Guidelines Programme and the 51st EU meeting of the National Coordinators of Testing Methods

The OECD TGs include internationally harmonised methods for chemical safety testing, used by governments, industry, research labs, and academia. They support regulatory safety testing, chemical registration, and are regularly updated to reflect scientific progress and member countries' needs. The Test Guidelines Programme and the Mutual Acceptance of Data (MAD) agreement ensure global harmonisation, facilitating trade and protecting workers, consumers, and the environment. The MAD system saves governments and industry around 309 million euro each year (OECD, 2019). OECD promotes animal welfare by applying the 3Rs principles and by preventing duplicate tests through MAD. Once approved, OECD TGs become official EU test methods under the EU Test Method Regulation (EC, 2008a). The programme is managed by the Working Party of National Coordinators (WNT), with the JRC (through EURL ECVAM) representing the EU and European Commission as a national coordinator.

The 37th meeting of the Working Party of National Coordinators of the OECD Test Guidelines Programme (WNT) was held in Paris on 1 to 4 April 2025. The following new and updated TGs and Guidance Documents (GD) were approved:

- Updated Test Guideline 239 on water-sediment Myriophyllum spicatum toxicity test.
- New Test Guideline on the Mason bees (Osmia sp.), acute contact toxicity test.
- Updated Guidance Document 122 on the determination of the toxicity of a test chemical to the dung beetle Aphodius constans and Onthophagus taurus.
- Updated Guidance Document on sediment and aquatic toxicity testing of nanomaterials.
- New Guidance Document on accumulation potential of nanomaterials.
- New Test Guideline on IL-2 LTT assay for immunosuppression.
- Updated Test Guideline 443 on the extended one-generation reproduction toxicity study.
- Updated Test Guidelines 470 and 488 on *in vivo* gene mutation assays (revision of *in vivo* genotoxicity test guidelines' "evaluation and interpretation of results" and "test report" language).
- Updated Test Guideline 467 on defined approaches on eye irritation for surfactants.
- Updated Test Guideline 497 on defined approaches on skin sensitisation:
  - Inclusion of the SARA-ICE model for the determination of a point of departure for skin sensitisation potential.
  - Inclusion of alternate *in vitro* and *in chemico* methods to methods in the original DAs 2 out of 3 and ITS.
- Updated Test Guideline 437 on BCOP for eye hazard potential including additional guidance on the utility of eye histopathology.
- Corrections to TGs 111, 307, 308 and 316 using radioactive labelling of substances.

In addition, the following TGs and GDs including concise amendments were approved:

- Targeted revision of OECD TGs 407, 408, 422, 421 (toxicology) as well as TGs 203, 210 and 236 (ecotoxicology): Incorporating optional sample cryopreservation.
- Revision of TG 431 and TG 439 to remove the EpiSkin™ model following discontinuation of production.
- Revision of TG 442B to include the GHS subcategorisation criterion for the LLNA: BrdU-ELISA.
- Adaptation of TG 456: H295R steroidogenesis assay to the recent change in the data interpretation procedure.
- Revision of the TG 491 applicability domain for the short time exposure test method (STE0.5) with respect to the implementation of a defined approach for the ocular toxicity predictions of surfactants.
- Addition of a human materials checklist to the GD on Good *In Vitro* Methods Practices (GIVIMP).

Fifteen new project proposals were added to the TGP workplan. The meeting also covered the link between OECD Test Guidelines and other standards, updates on OECD GD 34 revision, IATA for non-genotoxic carcinogens and related initiatives within EPAA and PARC. Discussions included next steps for the DNT *in vitro* battery, PEPPER project reviews, the TGP work plan, and selected OECD Working Party on Hazard Assessment (WPHA) activities.

More information on OECD TGs and the OECD Test Guideline Programme: https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/test-guidelines.html

On 31 March 2025, the 51st EU meeting of National Coordinators of Testing Methods, led by the EC's JRC, preceded the WNT 37 meeting. The EU meeting aims for a harmonised and coordinated EU position to the approval of OECD TGs and GDs. Key WNT 37 agenda topics were addressed, alongside updates on EU Test Method and CLP regulations (EC, 2023), REACH annex revisions, and the EC roadmap towards phasing out animal testing for chemical safety assessments.

3.13. Quantification of health impacts in EU chemical policy evaluations

EURL ECVAM has completed its work offering recommendations to support the quantitative characterisation of health impacts in EU policy evaluations related to chemicals (Chinchio *et al.*, 2025b). The guidance emphasises best practices for collecting, evaluating, and reporting relevant evidence, especially epidemiological data, to improve the methodological quality and transparency of EU chemical policy evaluations and impact assessments. Policy evaluations and impact assessments are core to the EU policy cycle, ensuring evidence-based and effective law-making[49]. However, assessing the health impacts of chemicals is complex due to the multitude of substances, the multifactorial nature of diseases, and the difficulty in characterising low-level, long-term exposures.

The analysis of recent case studies revealed that studies supporting EU chemical policy evaluations often face data gaps, prompting reliance on expert judgment and the extrapolation of data from single Member States to EU level. This introduces considerable uncertainty, making transparency in the assessment process crucial.

The recommendations emphasise the need for greater transparency and rigour in defining the scope, selecting the evidence base, and reporting results. They also stress the necessity for clear disclosure of all assumptions, limitations, and uncertainties, including their potential influence on the resulting estimates. Key proposals include defining clear and manageable assessment questions and systematically selecting substances and health effects, using structured methods for evidence reviews, and transparently disclosing the affiliations of contributing experts. For epidemiological data, the consistent use of established frameworks to identify and integrate evidence is advised to ensure reliability. Furthermore, future assessments must incorporate considerations of equity and vulnerable populations, as certain groups may be disproportionally affected by chemical exposure.

This guidance will support policy officers and external contractors in conducting methodologically sound and more comparable quantitative health impact assessments.

49 https://commission.europa.eu/law/law-making-process/planning-and-proposing-law/better-regulation_en#objectives-of-the-better-regulation-agenda

"Achieving reliable health impact estimates demands greater transparency and rigour in defining the assessment's scope, systematically selecting the evidence base, and clearly disclosing all assumptions and uncertainties throughout the process."

Eleonora Chinchio
Project Officer
European Commission - Joint Research Centre

3.14. Assessing the impact of chemicals on biodiversity

Biodiversity is clearly in decline, and chemical pollution plays a major role in driving this loss. The uncertainty surrounding the magnitude of this threat often stems from a lack of a well-defined, causal body of evidence linking chemical exposure to biodiversity outcomes in both retrospective and prospective evaluations. This gap arises from a mismatch between ecotoxicological metrics and biodiversity metrics: ecotoxicology tends to report chemical effects across various biological levels, primarily at the individual and sub-organismal scales, whereas biodiversity is commonly assessed in terms of taxonomic richness and population abundance. A recent JRC report (Baccaro *et al.*, 2025) addresses the existing evidence (the obvious and the subtle ones) of the impact of chemicals on each sub-class of descriptors proposed by the list of Essential Biodiversity Variables (EBVs). For each biological level of organisation, examples of ecotoxicological studies are reported that highlight strengths and weaknesses in describing the effects of chemical pollution on the specific biodiversity identifiers. In the last part, different modelling approaches are flagged as important tools and are described according to their potential to answer such a complex issue.

Furthermore, EURL ECVAM is leading a study to compile and synthesise essential information from both field studies directly assessing the impact on biodiversity, and ecotoxicological studies which indirectly estimate the effects of chemicals across different biological organisation levels using extrapolation methods. The resulting datasets (foreseen to be available in 2026) will be used to provide a detailed analysis of the evidence, including the most affected species, the most studied chemical classes and regions, and the most effective and promising methodologies.

Amongst other external initiatives, EURL ECVAM is part of a task force formed by ECETOC which aims to evaluate the state of biodiversity research and its integration within EU chemical regulation. A workshop is scheduled for May 2026 to further advance these efforts and consolidate findings from ongoing research and collaborations.

3.15. Chemicals exposure and infectious disease risk: an epidemiological perspective

The growing recognition that human, animal, and plant health are tightly linked to the environment—embodied in the One Health concept—calls for an integrated view to bolster public health preparedness against emerging threats. This imperative for close cooperation has been incorporated into European legislation (EU, 2021, EU, 2022) and has been adopted by

European agencies (Bronzwaer *et al.*, 2022) to strengthen the EU's ability to prevent, predict, detect, and respond to health threats.

To explore this policy context, EURL ECVAM conducted a scoping review to systematically map the epidemiological evidence on the relationship between chemical exposures and human infectious disease susceptibility or severity. The review draws on 94 primary analytical studies published from 1980 through August 2023 (Chinchio *et al.*, 2025a).

The evidence base is fragmented but points to a trend of increased infection risk associated with chemical exposure. Research is heavily skewed toward metals (34 studies) and PFAS (20 studies). Most studies focus on respiratory infections (72 studies), chiefly in infants (18 studies) and children (28 studies). For example, exposure to metals such as arsenic, cadmium, and lead has been linked to higher risks of respiratory infections, as have PFAS like PFOA and PFOS. Notably, adolescents (3 studies) and the elderly (1 study) are markedly underrepresented, highlighting a critical knowledge gap.

The substantial heterogeneity across studies underscores the need for more harmonised reporting standards in epidemiology. Where findings diverge, there is an opportunity to apply the AOP framework to connect chemical exposures to immunotoxicity mechanisms and, ultimately, to infection dynamics. This framework can support targeted prevention strategies against environmental immunotoxicants, with particular emphasis on protecting vulnerable populations.

3.16. Support of the 3Rs in the pharmaceutical sector – EMA 3Rs Working Party

EURL ECVAM continues to support the European Medicines Agency (EMA) 3Rs Working Party (3RsWP), a joint committee that advises the Committee for Medicinal Products for Human Use (CHMP) and the Committee for Veterinary Medicinal Products (CVMP) on pursuing the Replacement, Reduction, and Refinement (3Rs) of animal use in regulatory testing of medicines. EURL ECVAM is actively involved in revising two guidelines: the "Guideline on non-clinical local tolerance testing" and the "Guideline on the principles of regulatory acceptance of 3Rs testing approaches".

As part of this work, the Guideline on non-clinical local tolerance testing is being updated to reflect current 3Rs best practices, including:

- A greater emphasis on *in vitro* testing for assessments such as skin and eye irritation.
- The integration of NAMs to evaluate the skin sensitisation potential of topical pharmaceuticals, with reference to OECD test guidelines (e.g., 497) that combine *in silico*, *in chemico*, and *in vitro* data.
- A further elaboration of the weight-of-evidence approach to determine whether *in vivo* local tolerance studies are necessary.

Progress continues on the revision of the Guideline on the principles of regulatory acceptance of 3Rs testing approaches, with a new section on terminology nearing finalisation. In addition, an EMA working group has begun drafting an annex on complex *in vitro* models for cardiac safety pharmacology.

EMA has revised two "Reflection papers on the current regulatory testing requirements for human and veterinary medicinal products and opportunities for implementation of the 3Rs" (EMA, 2024, EMA, 2025). These revisions are expected to be published by mid-2026 and aim to incorporate new developments and acceptable 3Rs approaches into regulatory decision-making during veterinary medicinal product assessments.

3.17. Data and knowledge management

3.17.1. The Adverse Outcome Pathway Knowledge Base

The Adverse Outcome Pathway (AOP) Framework provides a structured means to link molecular initiating events to adverse outcomes of regulatory relevance for human health and the environment. The AOP-Wiki, which is part of the AOP Knowledge Base (AOP-KB), is the main online platform where scientists collaboratively describe AOPs consisting of Key Events (KEs) and Key Event Relationships (KERs). It serves as both a scientific resource and a practical link between mechanistic data and regulatory use.

Governance of the AOP-Wiki is coordinated through the AOP Knowledge Base Coordination Group (AOP-KB CG), which consists of representatives from the organisations that fund or have funded the system's development and maintenance - namely EURL ECVAM, the US EPA, Environment and Climate Change Canada (ECCC), and the OECD. The group reports to these funding bodies and ensures that resources are used efficiently and in accordance with jointly defined strategic objectives. Scientific and community input is provided through the OECD Advisory Group on Emerging Science in Chemicals Assessment (ESCA) and the Society for the Advancement of AOPs (SAAOP). Their feedback and recommendations are formally acknowledged and, where relevant, taken forward by the AOP-KB CG to guide prioritisation and implementation of system improvements.

As of November 2025, the AOP-Wiki contained 556 AOPs (+69 compared to the same month 2024), 1538 KEs (+192), and 1538 KERs (+82), illustrating its continued growth as a global collaborative resource.

The SAAOP Knowledgebase Interest Group (SKIG) has expanded to around 70 members from 20 countries. Meeting every six weeks, it serves as a key forum for community exchange and coordination. The SKIG Report 2023-2024 (Wittwehr *et al.*, 2025) summarised discussions on ontology harmonisation, quality curation, umbrella KEs, AI tools for AOP development, and integration of quantitative and temporal information. In 2025, the group further addressed semantic consistency across ontologies, quantitative modelling approaches, automated AOP-Wiki data extraction, visualisation of AOP networks, community training modules, and outreach to early-career scientists. These discussions feed directly into AOP-Wiki priorities and the design of upcoming functionalities. A new SKIG Report 2025 compiling these outcomes will be published in early 2026.

The FAIR AOP Cluster released a roadmap report (Mortensen *et al.*, 2025) that sets practical milestones to make AOP data Findable, Accessible, Interoperable and Reusable. The roadmap outlines measurable FAIRness indicators, harmonised metadata fields, and improved versioning procedures, ensuring that AOP data can be efficiently integrated with other mechanistic and regulatory databases.

The Methods2AOP (Karmaus *et al.*, 2025) collaboration concluded its work in 2025 and has release its final report (Karmaus *et al.*, 2025) . This international initiative proposes structured, ontology-based documentation of test methods, particularly NAMs, linking them directly to KEs. This approach will enhance transparency, comparability, and regulatory confidence in mechanistic data.

Within AI4AOP (Wittwehr, 2026), the JRC-led initiative exploring artificial intelligence for AOPs, a public challenge invited researchers and students to test AI tools for AOP development, while the KEC2KE activity investigates how AI can translate narrative Key Events into ontology term triplets (process-object-action). Results from both are expected in 2026. Ontology-related issues were presented to a wider audience in a poster (Filipovska *et al.*, 2025) at Eurotox 2025.

The Omics2AOP project explores ways to link genes to AOP Key Events and is described in **Section 3.6.1**.

In terms of ICT development and maintenance of the AOP-Wiki, several new features have been under development in 2025, driven by community feedback. These include enhanced OECD and user-facing features, major backend infrastructure modernisation, and incremental stability and usability improvements (versions 2.7.2–2.7.4). The upcoming release, version 2.8, will make the AOP-Wiki ready for the next big step: AOP-Wiki 3.0.

EURL ECVAM continues to fund AOP-Wiki maintenance and incremental feature additions, ensuring stability while preparing for future system enhancements driven by community needs.

"The AOP-Wiki is growing not only in size but in maturity. By sharing not just data but ways of thinking, the global AOP community is building a more open, creative, and connected foundation for tomorrow's toxicology."

Clemens Wittwehr
Senior Expert
European Commission - Joint Research Centre

3.17.2. Chemical monitoring data in IPCHEM

Chemical monitoring data can support chemical, environmental and health policies in several ways. They are used for example to look at effectiveness of chemical management measures, to explore temporal and spatial trends in chemical exposure and related risks, or to investigate exposure sources. IPCHEM[50], the European Commission's Information Platform for Chemical Monitoring, is a single access point to chemical monitoring data across relevant media. It allows exploring chemical concentrations in environmental media, food and animal feed, indoor air and in humans.

Monitoring data are used more and more by EU agencies in chemical safety assessment activities. For example, EFSA (EFSA, 2025) was tasked to explore possible reasons for the occurrence of 21 pesticide active substances not authorised for use but detected more frequently in organic food. Monitoring data for water and soil from IPCHEM were used to assess potential exposure routes through irrigation water or plant uptake from residues in soil.

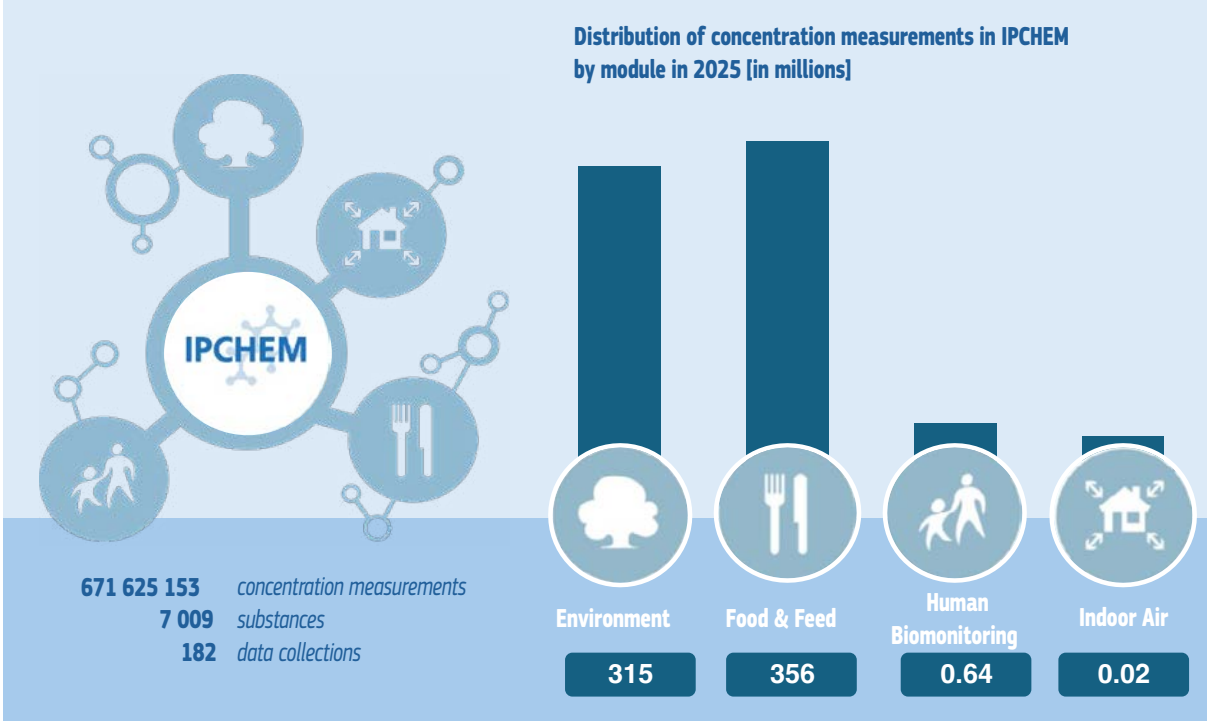
In addition to the recent focus on facilitating data use, the platform continues to expand its coverage by including additional data, e.g. in

50 https://ipchem.jrc.ec.europa.eu/

the food and feed area (see **Figure 3.7**). New data on indoor air monitoring are expected to be provided soon in collaboration with the IDEAL research cluster[51].

In the future, IPCHEM will be even better connected to other chemical data. With the adoption of the "one substance, one assessment package" in November 2025, work is starting on establishing a Common Data Platform for Chemicals (CDPC) to provide access to chemicals data compiled under EU legislation on hazards, physicochemical properties, presence in the environment, emissions, uses, and environmental sustainability. IPCHEM will be integrated into the CDPC over the next three years in close collaboration with EU agencies (EU, 2025).

Figure 3.7. Overview of monitoring data integrated into IPCHEM (status December 2025)



Source: European Commission – Joint Research Centre, Ispra, Italy

3.17.3. Generation, reporting and use of research data for regulatory assessment

Within the OECD Working Party on Hazard Assessment (WPHA), JRC/EURL ECVAM led a project to develop a Guidance Document on the Generation, Reporting and Use of Research data for Regulatory Assessments (OECD, 2025a). It aims to enhance the utility and regulatory uptake of research data, focusing on hazard, exposure and risk assessment data generated outside formal regulatory testing frameworks. It promotes harmonised and structured approaches to support the use of scientifically reliable and relevant data in decision-making across jurisdictions and policy areas.

The document is structured around the research data lifecycle, from generation and reporting to identification, evaluation and integration into regulatory assessments. It provides practical considerations and specific recommendations for key stakeholder groups, including funders, researchers, publishers, repository managers, assessors and risk managers. Additional sections of the document offer tools, references, and case studies to support stakeholders in implementing the guidance.

51 https://www.idealcluster.eu/

Related to the guidance development and recognising that there is currently no standardised reporting template for research data, the OECD Harmonised Template for Research Data (OHTR) project was initiated. The OHTR aims to standardise data reporting requirements for research data, targeting use by all stakeholder groups who can use the OHTR as specifications for data entry screens in data management systems including IUCLID. The OHTR project was led by US EPA and EU JRC.

More information: OECD webinar on 14 November 2025 (recorded): https://www.oecd.org/en/events/2025/11/webinar-on-the-oecd-guidance-document-on-the-generation-reporting-and-use-of-research-data-for-regulatory-assessments.html

4. Knowledge sharing

4.1. Statistics on the use of animals for scientific purposes in the EU and Norway in 2023

To support the implementation of Directive 2010/63/EU (EU, 2010), EURL ECVAM is responsible for compiling and analysing the data on the use of animals for scientific purposes reported annually by the EU Member States and Norway. In 2025, EURL ECVAM prepared the latest report (expected in early 2026) covering the year 2023, in line with the reporting requirements introduced under Commission Implementing Decision 2020/569/EU (EU, 2020). The 2023 data show continued meaningful progress under the Directive, with positive trends in reducing animal use, particularly for regulatory purposes and basic research. The total number of animals used for the first time fell to around 7.97 million: -4.9% compared to 2022, -9.6% compared to 2018. Uses for regulatory testing continued their long-term decline, reaching a total reduction of 35.1% since 2018, with substantial decreases in areas such as human medicinal products, food legislation, and batch potency testing. Notably, first uses of non-human primates dropped sharply (-25% compared to 2022), although this does not yet indicate a stable long-term trend. Overall, the total number of all animal uses (including first uses and any subsequent reuse) decreased to 8.08 million in 2023 (-4.7% compared to 2022), accompanied by a continued fall in the proportion of severe procedures, now below 9% of all uses. The report also highlights modest increases in reuses and ongoing fluctuations in the maintenance of genetically altered animal colonies. All underlying data are publicly accessible through the ALURES Statistical EU database[52], enabling users to explore detailed information directly.

4.2. Biomedical research

4.2.1. Launch of the thematic review on the state of Three Rs implementation in cardiovascular research

n September 2025, EURL ECVAM officially launched the first thematic review under Directive 210/63/EU on the protection of animals used for scientific purposes[53]. Article 58 states that *"the Commission shall, where appropriate, and in consultation with the Member States and stakeholders, conduct periodic thematic reviews of the replacement, reduction and refinement of the use of animals in procedures, paying specific attention to non-human primates, technological developments, and new scientific and animal-welfare knowledge."* Led by EURL ECVAM, this thematic review marks the first such initiative by the Commission under the provisions of Directive 2010/63/EU. The effort is a collaboration between EURL ECVAM and the Commission department responsible for safeguarding animals in scientific endeavours (DG ENV), supporting the

52 https://webgate.ec.europa.eu/envdataportal/content/alures/section1_number-of-animals.html

53 https://joint-research-centre.ec.europa.eu/jrc-news-and-updates/jrc-launches-review-models-and-methods-used-cardiovascular-research-2025-09-11_en

European Research Area's action on non-animal approaches as outlined in the European Life Science Strategy (EC, 2025b). Practically, the review is a targeted, evidence-driven initiative to evaluate the current state of scientific models and methodologies (e.g., computational, experimental, clinical) in cardiovascular research, with the aim of enhancing research outcomes by shifting toward innovative non-animal methodologies and thus, reducing reliance on animal experiments. Cardiovascular diseases affect more than 60 million people in Europe and are the leading cause of mortality, underscoring the urgency of this review. This thematic review aligns with the European Cardiovascular Health Plan (EC, 2025a) and responds to the Council's call for intensified prevention, early detection, treatment, and rehabilitation efforts. It promotes advanced techniques such as computer modelling (*in silico*), laboratory-cultured cells and tissues (*in vitro*), and explanted cells or tissues (*ex vivo*) to better replicate human biological systems, while ensuring compliance with legal requirements governing animal use.

4.2.2. European Parliament pilot project on the development and use of artificial intelligence/machine learning approaches for biomedical models review

On 1 December 2025, EURL ECVAM introduced BimmoH, the largest public database dedicated to scientific publications using human biology-based models in biomedical research. This launch represents a major step in EU's commitment to advancing human-centric and ethical science, in line with the European Life Science Strategy, which promotes innovation in biotechnology while progressively reducing reliance on animal testing.

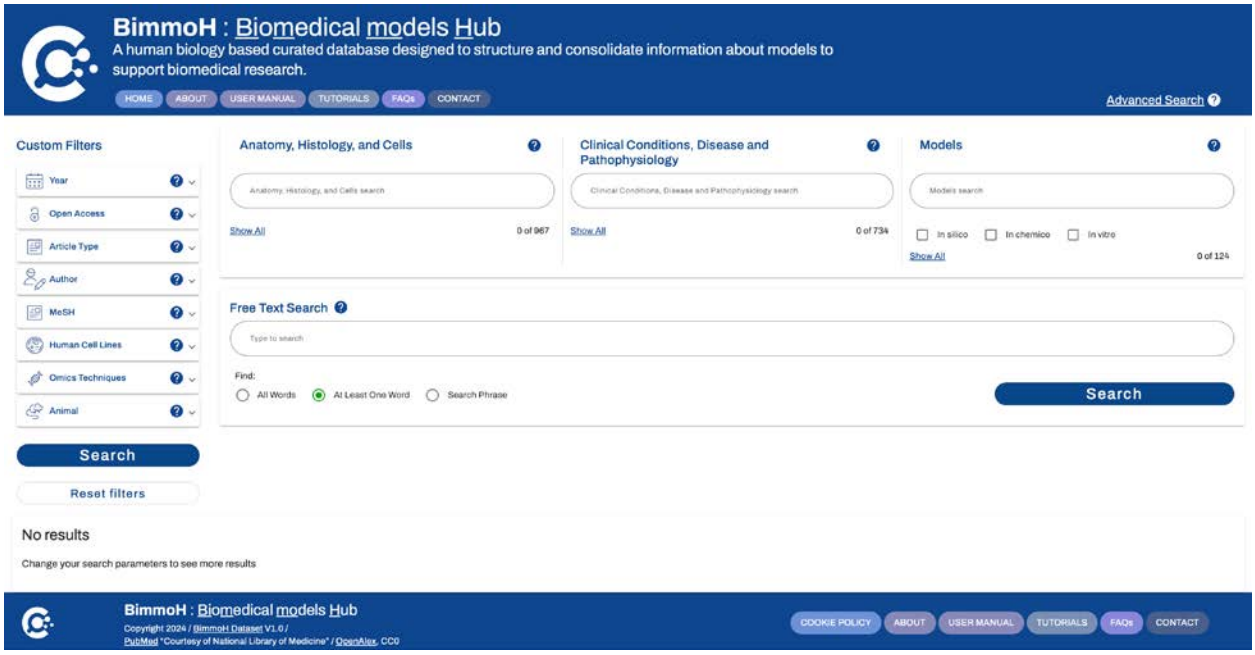
BimmoH consolidates information on a wide range of models based on human biology, including organ-on-chip technologies, 3D cell cultures, and computational models. By bringing together these resources, it enables researchers to design studies that are more relevant and translatable to human health. The platform makes these cutting-edge tools more accessible, fostering their wider adoption across the scientific community.

Developed under a European Parliament Pilot Project, BimmoH uses artificial intelligence to analyse millions of scientific papers, automatically identifying and organising references to human biology-based research methods. The result is a comprehensive, curated collection of hundreds of thousands of resources that can significantly reduce research time and accelerate innovation. Regular updates will ensure the database remains current with the latest scientific advances[54] (Deceuninck *et al.*, 2026).

Beyond academia, BimmoH offers value to regulators, policymakers, funders, and industry stakeholders. It can inform evidence-based decisions across areas such as drug development, safety assessment, and early-stage biomedical exploration. The initiative also supports EU's Three Rs principle, reinforcing European leadership in animal welfare and sustainable innovation.

54 https://data.jrc.ec.europa.eu/dataset/ba511666-1c31-4ac0-a4a4-97567e480aba

Figure 4.1. Screenshot of BimmoH web page (https://bimmoh.eu)



Source: BimmoH web page

4.3. Education and training: fostering a community of young scientists in non-animal approaches

4.3.1. EURL ECVAM Summer School experience

Part of EURL ECVAM's mandate, as established by EU legislation on the protection of animals used for scientific purposes, is to promote uptake of alternative methods and the Three Rs in science. Education and training activities are essential for advancing non-animal methodologies. Held from 19 to 23 May 2025 at the Joint Research Centre in Ispra, Italy, EURL ECVAM organised the fifth edition of its international biennial Summer School on Non-Animal Approaches in Science, titled "Changing the paradigm", (Berggren *et al.*, 2025). The Summer School is tailored for post-graduate students and early-career scientists, offering these young researchers a chance to learn from field experts, exchange knowledge with peers, and build professional networks. The programme aims to disseminate knowledge and experience on the latest non-animal approaches in science and to promote their use in biomedical research, regulatory applications, and drug development, thereby shaping the next generation of scientists and supporting ethical, sustainable, and innovative research. Since its inception in 2017, the Summer School has trained over 500 students worldwide. The fifth edition featured sessions on cutting-edge technologies such as induced pluripotent stem cells, organ-on-chip, imaging, omics technologies, computational modelling, and artificial intelligence, technologies that drive world-class science while advancing sustainability, societal benefit, and technological progress. The event also explored the current and future role of the Three Rs and included dedicated time for discussions about the Commission's ongoing commitment to a roadmap for phasing out animal testing in chemical safety assessments. Students led debate sessions and presented their own research in flash presentations, while also examining potential career paths in the expanding field of non-animal approaches.

EURL ECVAM remains deeply committed to reducing its environmental footprint, ensuring the Summer School is organised with a genuinely green approach. Measures to minimise food waste, single-use plastics, paper printing, and

local transport emissions are in place, including a fully green vegan menu and encouragement of sustainable travel. To monitor and improve environmental performance, the JRC conducted an impact study resulting in a Greenhouse Gas Emissions (GHG) Report for the 2025 Summer School (Nasi *et al.*, 2025).

In line with these ongoing efforts, the JRC Summer School has twice received the European Commission's internal prize for sustainable conferences and events.

4.3.2. The Student Ambassador Project

The Summer School's significant impact underscored the need for a continuous, engaging community of young scientists to shape the future of biomedical research and regulatory testing. To meet this demand, EURL ECVAM launched the Student Ambassador Project[55], first engaging master's students in toxicology from the Karolinska Institute and participants in the EUROoCS Summer School in 2024. These efforts formed the inaugural group of eight student ambassadors from across Europe. Since then, these master's and PhD students have delivered presentations to peers in Portugal, the Netherlands, Italy, Romania, Finland, Germany, and Sweden, raising awareness of *in vitro*, *ex vivo*, and *in silico* techniques. Their outreach has emphasised the scientific and ethical value of human-relevant, animal-free research, inspiring many students to pursue thesis projects on non-animal methodologies.

The project gained prominence at key 2025 international events, including EURL ECVAM's Summer School, the 13th World Congress on Alternatives to Animal Use in the Life Sciences (WC13) in Brazil, the 3Rs Genova Summer School in Italy, and the Young Transition to Animal-free Innovations (TPI) Annual Meeting in the Netherlands, where it drew widespread acclaim and interest. Ultimately, the Ambassador Project seeks to empower new generations to develop, adopt, and advance alternatives to animal testing. It promotes a paradigm shift in the university settings, by embedding NAMs into curricula, advocating for updated course content, and fostering training programs that equip future scientists with the skills to drive NAMs forward.

Figure 4.2. The first cohort of Ambassadors at the latest JRC Summer School on Non-Animal Approaches in Science held in May 2025 in Ispra (Italy)

Source: European Commission – Joint Research Centre, Ispra, Italy

55 https://joint-research-centre.ec.europa.eu/projects-and-activities/reference-and-measurement/european-union-reference-laboratories/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/education-and-training/eurl-ecvam-student-ambassador-project_en

4.3.3. Developing a Global Education Hub for animal-free innovation

EURL ECVAM has been actively involved in the development of a global education hub (GEH) (Janssens *et al.*, 2025) for animal-free innovation, an initiative started by the collaboration between the interdisciplinary group TPI Utrecht and PETA UK established in 2024. GEH is envisioned as a scientific community focused on replacing the use of animals in research, testing, teaching, and training, while promoting a safer, healthier, and more sustainable world. Its mission is to accelerate the transition to animal-free innovation by centring education, training, and communication in its activities. The hub aims to connect and inspire individuals and organisations by co-creating, sharing, and disseminating educational tools, resources, and materials about non-animal methods for life sciences research and regulatory testing. It embraces principles of sharing, trust, reciprocity, and solidarity, encouraging collaboration among diverse stakeholders including academia, industry, regulators, and NGOs. Since the values and objectives of GEH are very much aligned with the student ambassador project (see **Section 4.3.2**), the first cohort of ambassadors has become actively involved in the hub and is helping to develop a webinar series launching in spring 2026.

By providing free access to educational resources and fostering community engagement, the hub, with the support of EURL ECVAM and its ambassadors, seeks to advance scientific excellence and ethical practices globally.

4.3.4. Development of learning scenarios for the EURL ECVAM Virtual Lab

To accompany the open-access virtual reality (VR) application that educates students aged 14-18 about alternatives to traditional animal testing in scientific research, EURL ECVAM partnered with the European Schoolnet (EUN) within the Scientix framework. The goal is to expand the existing suite of Science, Technology, Engineering and Mathematics (STEM) resources that promote the Three Rs in the context of animal use in science. The Learning Scenario (LS) employs innovative pedagogical approaches to integrate the EURL ECVAM Lab VR into secondary biology curricula across Europe. It emphasises collaborative and inquiry-based learning strategies to help students and educators develop competencies in ethical research practices using *in vitro* methods. This initiative supports both high-quality scientific and ethical education and the EU's vision of a sustainable, digitally empowered society.

4.4. Understanding the transition to non-animal safety assessment

4.4.1. Towards animal-free SAFEty assessment: the contributions of the SAFE consortium

The Safety Assessment through Animal-Free Evolution (SAFE) Consortium is a 5-year transdisciplinary research project funded by the Dutch Research Council (NWO). Through its unique composition of members from academia, industry, NGOs, and regulatory bodies, the consortium aims to accelerate the transition to animal-free safety assessment.

Since its launch in 2023, SAFE has applied an action research approach and the latest social-scientific theories on transition governance. This framework allows the consortium to actively participate in the transition while simultaneously studying it. By engaging in iterative research cycles, SAFE observes, analyses, and acts to provide insights on governing this shift,

publishing its findings in both academic and popular outlets.

The consortium's work focuses on the pharmaceutical and chemical sectors in the EU and US, incorporating lessons from other major transitions. Through direct engagement with key stakeholders, SAFE identifies and overcomes barriers to accelerate the transition to animal-free innovation.

Recent highlights include:

- Advocating for the importance of social science and transformative governance in the European Commission's roadmap towards phasing out animal testing for chemical safety assessments (Policy Labs, 2024).
- Insights into the alternatives to the Botulinum neurotoxin in pharmaceutical batch testing in mice (Watkins *et al.*, 2025).
- Theoretical contributions on how to analyse social and institutional barriers in animal free safety assessment.
- Organising transdisciplinary and cross-sectoral platforms for dialogues on accelerating the transition to animal-free innovation.
- Ethnographic study into the USA transition and the institutional barriers at play in both the EU and USA (Policy Labs, 2025).

Coordinator: Prof. Dr. I.J. Visseren-Hamakers, Institute for Management Research, Radboud University.

Website: https://www.nwo.nl/en/projects/nwa139520004

LinkedIn: https://www.linkedin.com/company/safe-accelerating-the-transition-to-animal-free-safety-assessment/?viewAsMember=true

4.4.2. The Collaboration to Harmonise the Assessment of Next Generation Evidence - the contributions of the CHANGE project

The Collaboration to Harmonise the Assessment of Next Generation Evidence (CHANGE)[56] project aims at contributing to the effective use of NAMs in the regulatory toxicology system by addressing the broader system-level factors that shape their uptake. CHANGE is led by the Norwegian Scientific Committee for Food and Environment (VKM), supported by the Evidence-Based Toxicology Collaboration (EBTC), and funded by the European Food Safety Authority (EFSA). The goal of CHANGE is to design system-level interventions that will increase the effectiveness of use of NAMs in regulatory decision-making. Regulatory toxicology is a system of many parts. Parts can be tangible (e.g. infrastructure, resources) and intangible (norms, incentives, power dynamics). Interactions between these parts determine how well the system functions in relation to its goals. By identifying the parts and describing how they interact, a model of system function can be developed and used to design interventions which make the system function better. To achieve this, VKM organised one in-person and several online workshops in 2024 (Bearth *et al.*, 2025), a second in-person workshop in 2025, and will organise a third in-person workshop in 2026, corresponding to the three phases of the project: Explore, Reflect and Design. EURL ECVAM has been supporting the project by actively participating in in-person and online workshops.

56 https://vkm.no/english/change/collaborationtoharmonisetheassessmentofnextgenerationevidence.4.46e970eb18cbde223fe711f2.html

System factors are not directly observable because nobody experiences “the system” as a whole. However, people have contact with and experience parts of the system. Therefore, during the first phase workshop in 2024 (Explore), the project leads collected anecdotes of experience from various stakeholders working in or around the regulatory toxicology system, from which they mapped the full process from R&D to use of a method/data in a regulatory assessment, and derived an initial theory of factors influencing system function. In the second phase workshop (Reflect), organised in June 2025, the initial process and system factor hypothesis derived from the anecdotes of phase 1 were presented to workshop participants for challenge and improvement. The main goal of the workshop was therefore to collect reflections and feedback on these initial hypotheses of process and system-level factors that facilitate or inhibit a well-functioning regulatory toxicology system around NAMs. The workshop counted with about 60 attendees from NGOs, national and international authorities and agencies, industry, and academia. The project leads will now update the initial hypothesis based on the feedback received during this second workshop and organise a third workshop in 2026 in the final phase of CHANGE (Design) to design interventions to improve system function using the refined theories from phase 2.

5. Conclusions

The 2025 EURL ECVAM Status Report underscores significant developments in advancing a humane, human-relevant, and sustainable framework for chemicals safety assessment across the EU. It highlights the EC's roadmap to progressively phase out animal testing, prioritising validated NAMs, computational modelling, and integrated data strategies that align with international guidelines while upholding rigorous protection for human health and the environment. This report reinforces the EU's ethical leadership in regulatory science, seamlessly integrating with broader European policies to foster responsible and sustainable innovation.

The Commission's roadmap for phasing out animal testing will set clear milestones for reducing animal use in chemicals safety assessments through accelerated validation of NAMs, expanded non-animal data integration, and integrated testing strategies. Key advancements include organ-on-chip technologies, *in silico* predictions, and read-across approaches, enabling regulatory acceptance without compromising safety outcomes. These efforts build on enhanced data sharing and transparency, ensuring a phased transition that minimises animal testing where alternatives prove protective equivalence or superiority.

The European Life Sciences Strategy bolsters this transition by promoting digitalisation, real-world data use, and tiered non-animal testing, with the recently proposed Biotech Act poised to catalyse investments in scalable NAM platforms and ethical data stewardship. This synergy drives high-throughput screening and advanced modelling, streamlining regulatory pathways and supporting sustainable chemical innovation. By embedding NAMs into core policy instruments, the EU positions itself as a global leader in biotech-driven risk assessment.

As detailed in Chapter 4, NAMs are increasingly central to biomedical research, offering precise, human-specific insights that surpass traditional models in relevance and efficiency. These methodologies, encompassing microphysiological systems and AI-enhanced predictions, are reshaping research paradigms, reducing ethical concerns while accelerating therapeutic development. Concurrently, EURL ECVAM's educational initiatives are equipping the next generation of scientists with expertise in NAM validation, data integration, and regulatory application, fostering a skilled workforce committed to animal-free science.

Looking ahead, sustained collaboration among stakeholders will ensure the roadmap's full realisation, with transparent metrics tracking progress toward zero animal testing. Recommendations include bolstering funding for NAM infrastructure, harmonising international standards, and expanding training programs to embed these approaches in routine practice. Through these measures, the EU will deliver safer chemicals, ethical science, and innovative leadership aligned with societal values.

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List of abbreviations and definitions

3Rs	Replacement, Reduction, Refinement
ADME	Absorption, distribution, metabolism and excretion
AFCSA	Animal-Free Chemical Safety Assessment
AI	Artificial intelligence
ALURES	Animal Use Reporting EU System (EU statistics database on the use of animals for scientific purposes under directive 2010/63/EU)
ANSES	French National Agency for Food, Environmental and Occupational Health and Safety
AOP	Adverse Outcome Pathway
AOP-KB	Adverse Outcome Pathway Knowledge Base
AR2	Androgen receptor dimerization assay
ASPA	ASPIS Safety Profiling Algorithm
ASPIS	Animal-free Safety assessment of chemicals: Project cluster for Implementation of novel Strategies (H2020)
AUC	Area-under-the-curve
BBB	Blood-Brain Barrier
BCOP	Bovine Corneal Opacity and Permeability
BimmoH	BioMedical Models Hub
BMFTR	German Federal Ministry of Research, Technology and Space
BrdU	Bromodeoxyuridine
CAGR	Compound Annual Growth Rate
CDPC	Common Data Platform for Chemicals
CG	Coordination Group
CHANGE	Collaboration to Harmonise the Assessment of Next Generation Evidence
CHMP	Committee for Medicinal Products for Human Use
ciPTECs	Conditionally Immortalised Proximal Tubule Epithelial Cells
CLP	Classification, Labelling and Packaging
CorEuStem	European Network for Stem Cell Core Facilities
CROs	Contract Research Organisations
Css	Steady-state concentration
CVMP	Committee for Veterinary Medicinal Products
DA	Defined approach
DG ENV	Directorate-General for Environment (EC)
DG GROW	Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs (EC)
DG RTD	Directorate-General for Research and Innovation (EC)
DG SANTE	Directorate-General for Health and Food Safety (EC)
DILI	Drug Induced Liver Injury
DNT	Developmental neurotoxicity
DR	Dose-Response
EBTC	Evidence-Based Toxicology Collaboration
EBV	Essential Biodiversity Variable
EC	European Commission
ECCC	Environment and Climate Change Canada
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
ED	Endocrine disruptor

EDA	Effect-Directed Analysis
EDNA	Project entitled 'Towards transcriptomics-based screening and identification of Endocrine Disruptors using New Approach Methodologies'
EEA	European Environment Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ENKORE	ENdocrine disrupting chemicals and Knowledge On health-Related Effects' cluster of five research projects from the call HORIZON-HLTH-2023-ENVHLTH-02-03 'Health impacts of endocrine-disrupting chemicals: bridging science-policy gaps by addressing persistent scientific uncertainties'
ENV	Environment
EP	European Parliament
EPA	Environmental Protection Agency
EPAA	European Partnership for Alternative to Animal Testing
ESA	Environmental safety assessment
ESAC	EURL ECVAM Scientific Advisory Committee
ESCA	Advisory Group on Emerging Science in Chemicals Assessment
ESR	Early Stage Researchers (MSCA-ITN)
EU	European Union
EUN	European Schoolnet
EURION	European Cluster to Improve Identification of Endocrine Disruptors
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
EUROoCS	European Society of OoC
FAIR	Findability, accessibility, interoperability, and reusability (of data)
GD	Guidance Document
GEH	Global Education Hub
GHG	Greenhouse Gas Emissions
GHS	Globally Harmonized System of classification and labelling of chemicals
GIVIMP	Good *In Vitro* Methods Practices
GPS	Genomic Prediction Signature
H2020	Horizon 2020
HGDP	Human Genome Diversity Panel
HH	Human health
hPSC	Human Pluripotent Stem Cell
HTT	High Throughput Testing
IAP	International Advisory Panel
IATA	Integrated Approach to Testing and Assessment
ICCA	International Council of Chemical Associations
ICT	Information and Communication Technology
IL-2 LTT	Interleukin-2 Luciferase Lymphocyte Toxicity Test
INDRA	Integrated Network and dynamical Reasoning Assembler
IPCHEM	Information Platform for Chemical Monitoring
IPCS	International Programme on Chemical Safety
iPSCs	Induced Pluripotent Stem Cells
ISO	International Organization for Standardization

ITF	Innovation Task Force
IUF	Leibniz Institute for Environmental Medicine
IVB	*In Vitro* Biotechnology
IVIVE	*In Vitro* to *in Vivo* Extrapolation
IWG	Informal Working Group
JRC	Joint Research Centre (EC)
KE	Key Event
KER	Key Event Relationship
LA-4	Murine epithelial lung tissue cell line
LC-MS	Liquid Chromatography - Mass Spectrometry
LLM	Large Language Model
LLNA	Local Lymph Node Assay
LOAEL	Least Observable Adverse Effect Level
LS	Learning Scenario
MAD	Mutual Acceptance of Data
MASLD	Metabolic Dysfunction-Associated Steatotic Liver Disease
MEP	Member of European Parliament
MH-S	Murine alveolar lung macrophages
MIE	Molecular Initiating Event
MS	Mass Spectrometry
NAM	New Approach Methodology
NGO	Non-Governmental Organisation
NGRA	Next Generation Risk Assessment
NGTxC	Non-genotoxic Carcinogenicity
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences (US)
NIH	National Institutes of Health (US)
NIS	Sodium/Iodide Symporter
NOAEL	No Observable Adverse Effect Level
NTP	National Toxicology Programme (US)
NVWA	the Netherlands Food and Consumer Product Safety Authority
NWA-ORC	Dutch Research Agenda: Research on Routes by Consortia
NWO	Dutch Research Council
OECD	Organisation for Economic Co-operation and Development
OHT	OECD Harmonised Templates
OHTR	OECD Harmonised Template for Research Data
ONTOX	Ontology-driven and artificial intelligence-based repeated dose toxicity testing of chemicals for next-generation risk assessment (ASPIS cluster)
OoC	Organ-on-Chip
OPRA	ONTOX AI-supported PRA approach
O-QT	OECD QSAR Toolbox AI Assistant
OSOA	One Substance, One Assessment
PARC	European Partnership for the Assessment of Risks from Chemicals
PARERE	Preliminary Assessment of Regulatory Relevance network
PBK	Physiologically-Based Kinetics (also PBPK, PBBK, PBTK)

PBT	Persistent, Bioaccumulative, Toxic
PEPPER	Public-private platform for the validation of endocrine disruptors characterization methods
PF	Partners' Forum
PFA	Per- and Polyfluoroalkyl Substance
PFOA	PerFluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
PG	Project group
PhD	Doctor of Philosophy
PHI	Potential Hazard Issues
PMs	Physiological Maps
PP	Project Platform
PRA	Probabilistic Risk Assessment
PrecisionTox	Toward Precision Toxicology: New Approach Methodologies for Chemical Safety (ASPIS cluster)
PRO-MaP	Promoting Reusable and Open Methods and Protocols
qAOP	Quantitative AOP
QIVIVE	Quantitative *in Vitro* to *in Vivo* Extrapolation
QSAR	Quantitative Structure Activity Relationship
R&D	Research and development
RC	Readiness criteria
REACH	European Regulation (EC) No. 1907/2006 Registration, Evaluation, Authorisation and Restriction of Chemicals
RISK-HUNT3R	RISK assessment of chemicals integrating HUman centric Next generation Testing strategies promoting the 3Rs (ASPIS cluster)
RIVM	National Institute for Public Health and the Environment (NL)
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SAAOP	Society for the Advancement of AOPs
SAFE	Safety Assessment through Animal-Free Evolution
SARA-ICE	Skin Allergy Risk Assessment – Integrated Chemical Environment
SCCS	Scientific Committee on Consumer Safety
SCHEER	Scientific Committee on Health, Environmental and Emerging Risks (EU)
SK	Sandell Kolthoff
SKIG	SAAOP Knowledgebase Interest Group
SOP	Standard Operating Procedure
STEM	Science, Technology, Engineering and Mathematics
STOT	Specific Target Organ Toxicity
SVM	Support Vector Machine
T4	Thyroxine
TDG	Transport of Dangerous Goods
TDM-EG	Thyroid Disruption Methods Expert Group
TEER	Transendothelial electrical resistance
TG	Test Guideline (OECD)
TGP	Test Guidelines Programme (OECD)

THSD	Thyroid hormone system disruption
TK	Toxicokinetic
TPF	Test Pre-submission Form
TPI	Transition Programme for Innovation without the use of animals
TPO	Thyroperoxidase
TST	Test Submission Template
TTR	Transthyretin
UFZ	Helmholtz Centre for Environmental Research
UK	United Kingdom
UN	United Nations
US	United States (of America)
VHP4Safety	Virtual Human Platform for Safety Assessment
VHT	Virtual Human Twin
VICT3R	VIrtual Control groups To reducE animal use in toxicology Research
VKM	Norwegian Scientific Committee for Food and Environment
VPH	Virtual Physiological Human
vPvB	very Persistent, very Bioaccumulative
VR	Virtual reality
WG	Working Group
WHO	World Health Organization
WNT	Working Party of National Coordinators of the OECD Test Guidelines Programme
WoE	Weight of Evidence
WP	Working Party
WPHA	OECD Working Party on Hazard Assessment
ZonMw	Dutch organisation for knowledge and innovation in health, healthcare and

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