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EURL ECVAM STATUS REPORT 2022

Non-animal methods in science and regulation

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Executive summary

The 2022 EURL ECVAM status report provides updates on activities and achievements related to research and development, validation and qualification, uptake and use of non-animal methods in the areas of basic, applied and translational research, as well as for regulatory testing. In addition, it informs about EURL ECVAM's education initiatives on the Three Rs (Replacement, Reduction and Refinement of animal use for scientific purposes).

The development of non-animal methods and approaches continued predominantly within projects funded under the EU Framework Programmes for Research and Innovation, Horizon 2020 and Horizon Europe, and in collaborative partnerships. These projects aim at developing new approach methodologies (NAMs), as well as new chemical assessment frameworks with a view to address more efficiently current and future needs in chemical risk assessment. The ultimate aim is to improve the protection of human health and the environment from chemical pollution as provided for in the EU Green Deal and its Chemicals Strategy for Sustainability (EC, 2020).

Under Horizon 2020, EURL ECVAM is collaborating with the ASPIS cluster and its three projects, PrecisionTox, ONTOX and RISK-HUNT3R. ASPIS works towards animal-free, reliable and sustainable chemical risk assessment fit for the modern age. Within EURION, the European cluster to improve scientific approaches for the identification of endocrine disruptors, EURL ECVAM provides guidance and training on the validation of *in vitro* methods, on the application of Good *In Vitro* Method Practices (GIVIMP) and on the introduction of test readiness criteria to assess the completeness of the methods developed within the project.

Another H2020 project to which EURL ECVAM contributes is the European Human Biomonitoring Initiative (HBM4EU) that aims at improving the collective understanding of human exposure to hazardous chemicals and mixtures and developing human biomonitoring (HBM) as an exposure assessment method.

The recently launched Horizon Europe project titled "European Partnership for the Assessment of Risks from Chemicals (PARC)" supports the development and implementation of a research and innovation programme to address current and future needs in relation to chemical risk assessment. PARC will develop innovative methodologies and tools, and generate and share data for exposure, hazard and risk assessment. The JRC will collaborate on various aspects of PARC's work and many of its projects.

In addition to these large EU-funded projects, EURL ECVAM also collaborates on various other research projects ultimately aiming to transition from animal-based testing to innovative, next generation risk assessment of chemicals.

In this endeavour, validation and standardisation remain cornerstones to enable the regulatory use and international adoption of NAMs. EURL ECVAM works with its European and international partners to make the validation process as efficient and effective as possible in order to keep pace with new and emerging technologies.

In 2022, methods to measure cytotoxicity, skin sensitisation and genotoxicity were submitted to EURL ECVAM for evaluation. Two methods for genotoxicity testing using reconstructed skin models will likely progress to peer review by the newly established EURL ECVAM Scientific Advisory Committee (ESAC).

The EURL ECVAM-coordinated study to validate a number of *in vitro* methods for different modes of action relevant for the thyroid endocrine system came to its final phase. Most of the fifteen laboratories from the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) submitted the validation data to EURL ECVAM.

Standard operating procedures (SOPs) and study reports are made available in EURL ECVAM's online tracking system (TSAR) along with links to related scientific publications. Ongoing work for some of the methods will be finalised in 2023 and the output will be included in TSAR when available.

The first study reports and SOPs for the methods investigating Deiodinase-1 activity, Thyroperoxidase inhibition and Tyrosine iodination were transferred to a newly-established OECD expert group on Thyroid Disruption Methods (TDM-EG). The TDM-EG's responsibility is to coordinate the development of test guidelines for these *in vitro* thyroid-related methods and other related projects expected within the next years.

As a follow-up to the "Putting Science into Standards" workshop on Organ-on-chip (OoC) organised by the JRC and CEN-CENELEC in 2021, a CEN-CENELEC Focus Group was launched in March 2022. Its goal is to map current initiatives, identify specific issues, draft a roadmap and define priorities for the next standardisation activities for OoC.

Furthermore, in partnership with the Regulatory Advisory Board (RAB) of the European Organ-on-Chip Society (EUROoCS), EURL ECVAM produced a catalogue of resources for developers and end-users to support the validation and qualification of OoC devices, in order to encourage the use of these novel approaches for regulatory applications.

EURL ECVAM led an action of the Chemicals Strategy for Sustainability to develop options for an impact assessment on extending the information requirements under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). The scope of the exercise included the better assessment of so-called critical hazards, the introduction of Chemical Safety Assessment at all tonnage levels and a more extensive use of NAMs. As next steps, preferred options for extending the REACH information requirements, including those for endocrine disrupting properties, will be outlined in a Commission Staff Working Document, and will form the basis of a legal proposal by the Commission.

In early 2022, EURL ECVAM convened its regulatory network PARERE (Preliminary Assessment of Regulatory Relevance) to inform and seek input on the extended information requirements under REACH and the potential to introduce NAMs into chemical hazard and risk assessment practices under the REACH regulation. In addition, since respiratory sensitisation has been identified as a critical hazard in the European Commission's Chemicals Strategy for Sustainability, EURL ECVAM started an exploratory discussion with PARERE on the short-, mid- and long-term needs for tackling the field of respiratory sensitisation and the use of NAMs to satisfy regulatory information requirements.

A draft proposal of hazard classes for endocrine disruptors to be introduced into the Classification Labelling and Packaging (CLP) Regulation ((EC) No. 1272/2008), was adopted on 19 December 2022 by the European Commission. It is expected to enter into force early 2023, after adoption by the European Parliament and Council. The endocrine disruptor hazard classes are based on the International Programme on Chemical Safety (IPCS) / World Health Organisation (WHO) definition, building on the existing criteria for pesticides and biocides. It is proposed to have categories for known or presumed endocrine disruptors (category 1), as well as suspected endocrine disruptors (category 2). A proposal for these new hazard classes was also submitted to the United Nation's Globally Harmonized System of Classification and Labelling of Chemicals (GHS) at the GHS Sub-Committee meeting that took place 7 to 9 December 2022.

At international level, EURL ECVAM continued to support the OECD Test Guidelines Programme by reviewing new project

proposals, commenting on new or updated test guidelines and guidance documents and contributing to the organisation of OECD webinars and a Working Party of National Coordinators of the OECD Test Guidelines Programme (WNT) workshop on "How to prepare for the uptake of emerging science and technologies within the Test Guidelines Programme?". In addition, the JRC / EURL ECVAM continued to lead the project on the development of a draft test guideline on relative metal / metalloid release using a simple simulated gastric fluid.

Within the OECD Working Party on Hazard Assessment (WPHA), EURL ECVAM proposed a new project to develop a guidance document for improving the use of academic data in regulatory assessments, another action of the Chemicals Strategy for Sustainability.

At the UN level, the work conducted by the Informal Working Group on the use of Non-Animal Test Methods for classification of health hazards in the biennium 2021-2022 focussed on the revision of chapter 3.4 on skin and respiratory sensitisation of the UN's GHS. The aim is to fully incorporate non-animal testing methods for skin sensitisation. EURL ECVAM is contributing to the work by providing scientific support.

The JRC / EURL ECVAM is furthermore leading an Informal Working Group that aims at revising the UN GHS germ cell mutagenicity chapter 3.5. Although this activity was initially proposed to clarify the classification criteria for germ cell mutagenicity in category 1B, the GHS subcommittee subsequently agreed to extend the revision to criteria for classification in category 2 and category 1A. So far, the IWG has revised the terminology related to germ cell mutagenicity and updated the list of methods in the main chapter. It also initiated the discussion on revisions of the criteria of germ cell mutagenicity, which will continue in the next biennium 2023-2024.

Complementary to all these activities is the parallel development of comprehensive and well-structured data and knowledge bases such as the Adverse Outcome Pathway (AOP)-Wiki, the Endocrine Active Substances Information System (EASIS), the Information Platform for Chemical Monitoring (IPCHEM) and the OECD's transcriptomics and metabolomics reporting framework which support policies on the safety of chemicals at EU and global level.

With more than six million uses of animals for research purposes in 2019 in the EU and Norway, research remains by far the main area using animals for scientific purposes. However, the replacement of animals in research has been particularly challenging. Research is about asking the right question and addressing it using the best model or combination of models, which in practice is not always taking place due to a variety of reasons, including the lack of awareness of the existence of appropriate non-animal models.

In order to support the uptake of non-animal models in research, EURL ECVAM continued its work on promoting knowledge sharing and cross-disciplinarity in biomedical research, identifying existing non-animal models, creating additional resources for education and training, and developing indicators to measure the uptake of non-animal methods in research. In 2022, EURL ECVAM published three more reviews on immunogenicity testing for advanced therapy medicinal products (ATMPs), cardiovascular disease and autoimmune disease, closing the series of studies to review available and emerging non-animal models being used for research in seven disease areas.

To keep the collection of models up-to-date, EURL ECVAM launched a pilot study, funded by an initiative from the European Parliament, to develop an automated database, based on Artificial Intelligence / Machine Learning approaches, that

collects, structures and shares information on NAMs used in biomedical research.

EURL ECVAM also gathered the main findings of its analysis on the outputs and societal impact of EU-funded biomedical research over the last 20 years in the fields of Alzheimer's disease, breast cancer and prostate cancer. As a follow-up, EURL ECVAM undertook a study for developing indicators that capture the societal impact of EU-funded projects in the same disease areas, which will be published early 2023.

Finally, EURL ECVAM added to its Three Rs education and training resources for primary and secondary school teachers, as well as for higher education.

Abstract

The 2022 EURL ECVAM Status Report describes research, dissemination and promotion activities undertaken recently by the Joint Research Centre's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) to further the uptake and use of non-animal methods and approaches in science and regulation. The principle of the Three Rs, i.e. Replacement, Reduction and Refinement of animal use in basic, applied and translational research, as well as for regulatory testing purposes, is firmly anchored in EU legislation, with complete phasing out of animal procedures being the ultimate goal. This is achievable through a transition to new approach methodologies and models based on innovative non-animal technologies and better understanding of biology and disease. The activities and results described in this report have only been possible through fruitful collaboration with EURL ECVAM's many committed partners and stakeholders.

1. Introduction

EU policies and legislation call for the replacement, reduction and refinement (the Three Rs[1] principle) of animal use in basic, and applied/translational research, as well as for regulatory purposes. A complete replacement of animal testing with innovative technologies not using living animals is the ultimate goal (EC, 2010). Where this is not yet possible, all animal studies must comply with EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Animals cannot be used for scientific purposes without prior authorisation, and authorities can only allow the use of animals when there are no alternative methods available. In addition, the use of the animals must be justified by the expected benefits, also taking into account ethical considerations.

The 2022 EURL ECVAM Status Report provides information on the progress being made in the development, validation and regulatory application of non-animal methods, as well as on their use and promotion for research purposes, and in education and training programmes.

EURL ECVAM's mandate is broad and described in Directive 2010/63/EU (Article 48 and Annex II). The duties include coordinating and promoting the development and use of alternatives to procedures including in the areas of basic and applied research and regulatory testing; coordinating the validation of alternative approaches at Union level; acting as a focal point for the exchange of information on the development of alternative approaches; setting up, maintaining and managing public databases and information systems on alternative approaches and their state of development and promoting dialogue between legislators, regulators, and all relevant stakeholders, in particular, industry, scientists, consumer organisations and animal-welfare groups, with a view to the development, validation, regulatory acceptance, international recognition, and application of alternative approaches.

In the regulatory field, EURL ECVAM supports a broad range of European policies on chemicals and products including industrial chemicals, plant protection and biocidal products, medicinal products for human and veterinary uses, toys, medical devices and cosmetic products. EURL ECVAM is an integral part of the European Commission's Joint Research Centre (JRC).

1 Three Rs and 3Rs are used interchangeably in this report.

2. Development

Through a range of EU-funded projects and research partnerships, considerable scientific and technical progress is being made on the development of non-animal methods. EURL ECVAM contributes to such activities in a variety of ways, such as by offering expertise and advice, sharing best practices on method characterisation and standardisation. The overall aim is to identify promising methods and facilitate their progression to practical application and regulatory acceptance.

2.1 Collaborative partnerships

2.1.1 ASPIS

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 University of Birmingham (PrecisionTox), the Vrije Universiteit Brussel (ONTOX) and Leiden University (RISK-HUNT3R). The three projects have joined forces in the so-called ASPIS cluster ("aspis" means "shield" in ancient Greek), which gathers 70 scientific organisations towards the replacement of animal testing. While the three 5-year projects are complementary to each other, they have common elements that form the basis of collaboration at cluster level. Recent highlights of the three projects are given in *Box 1*.

The JRC / EURL ECVAM has set up formal collaboration agreements with each of the three projects individually, and also contributes to activities at cluster level.

To encourage the regulatory relevance of the research, EURL ECVAM took the initiative to set up the ASPIS Regulatory Forum. The first forum meetings focused on the extended Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) information requirements and the inclusion of NAMs. Two additional meetings took place in spring 2022 discussing ideas from the different ASPIS projects on how to facilitate the regulatory implementation of NAMs. Currently, an initiative at cluster level aims to gain an overview and organise different activities within the cluster to understand their possible contribution in a next generation risk assessment. In 2023 the forum will focus on how to progress this while contributing to the long-term objective of developing a NAM-based chemical safety assessment paradigm.

2.1.2 EURION

The European Cluster to Improve Identification of Endocrine Disruptors, EURION, is a cluster of eight European research projects, aiming to develop new test methods for identification of endocrine disruptors. The cluster was established in order to optimise synergies and avoid overlaps between the projects selected for funding from the Horizon 2020 call SC1BHC-27-2018 'New testing and screening methods to identify endocrine disrupting chemicals'.

Each project focuses on different aspects of new testing and screening methods for identifying endocrine disruptors including thyroid hormone disruption, metabolic disruption, female reproduction and developmental neurotoxicity. Transversal working groups share best practice on common issues such as validation and the process of chemical selection, development of AOPs and Integrated Approaches to Testing and Assessment (IATA). The concept behind the cluster is to develop reliable and relevant methods within the context of IATA underpinned by AOPs. Efforts to validate the methods are an essential part of each project.

In this context, EURL ECVAM has been providing guidance on the process of validation and what could be done within the timeframe of the projects to demonstrate the reliability and relevance of the methods being developed. This year the focus was on the self-assessment of the maturity of the individual methods according to a set of 'Test Readiness Criteria' (TRC), which are

Box 1

ASPIS: research to accelerate the transition towards non-animal testing

PrecisionTox

The overarching goal of PrecisionTox is to propose a new assessment framework based on observable mechanistic processes leading to toxicity. Emphasis is placed on the use of an evolutionarily diverse suite of model organisms, taking into consideration genetic variation in individual susceptibility.

Recent highlights include:

- Implementation of a machine learning workflow to identify reliable biomarkers.
- Implementation of a chemicals selection strategy based upon stakeholder priorities.
- Creation and use of key standard operational procedures (SOPs) that harmonise the experimental conditions for testing the first 50 chemicals across the five model species plus cell lines.
- Implementation of novel automation procedures for gene expression and metabolomics data.
- Genomic and metabolomic resources of the model test species Daphnia (water flea) that will help unite human and eco-toxicology.

Coordinator: John Colbourne, University of Birmingham.

▸▸ Website: *https://precisiontox.org*

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:

- Systematic review of scientific literature to collect biological, toxicological, physico-chemical and kinetic data to populate ontologies.
- Establishment of physiological maps mechanistically describing relevant homeostatic functions in the liver, kidneys and brain.
- Development and optimisation of Adverse Outcome Pathway (AOP) networks focused on selected adversities in the liver, kidneys and brain.
- Selection of chemicals to set up *in vitro* test batteries to predict toxic effects of chemicals in the liver, kidneys and brain.
- Establishment of a group of stakeholders with industrial and regulatory background to create confidence and end-user acceptance.

Coordinator: Matthieu Vinken, Vrije Universiteit Brussel.

▸▸ Website: *https://ontox-project.eu*

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 (*Figure 1*). The project strategy was delineated by Pallocca *et al.*, 2022.

Recent highlights include:

- Virtual meeting in January 2022 ('Current and future regulatory needs for chemical risk assessment') followed up with virtual mini-symposium ("Orienteering for regulatory needs").
- Workflows to collate relevant data and parameterise physiologically-based kinetic (PBPK) models (Khalidi *et al.*, 2022).
- Tools to describe organ/tissue-specific interactomes and gene co-expression networks, allowing comparison of networks across conditions/species (van der Stel *et al.*, 2022).
- High-content imaging and high-throughput transcriptomics to address mitochondrial perturbation as a critical event in chemical-induced organ toxicities.
- *In vitro* models of the peripheral nervous system were developed to support studies on peripheral neuropathies (Holzer *et al.*, 2022a; Holzer *et al.*, 2022b).
- Framework document outlining the RISK-HUNT3R strategy for quantitative AOPs (qAOPs) and quantitative systems toxicology. Current case studies focus on the characterisation of qAOPs for fibrosis leading to liver injury, mitochondrial toxicity (developmental and neurotoxicity), and liver steatosis.

Coordinator: Bob van de Water, Leiden University.

▸▸ Website: *https://www.risk-hunt3r.eu*

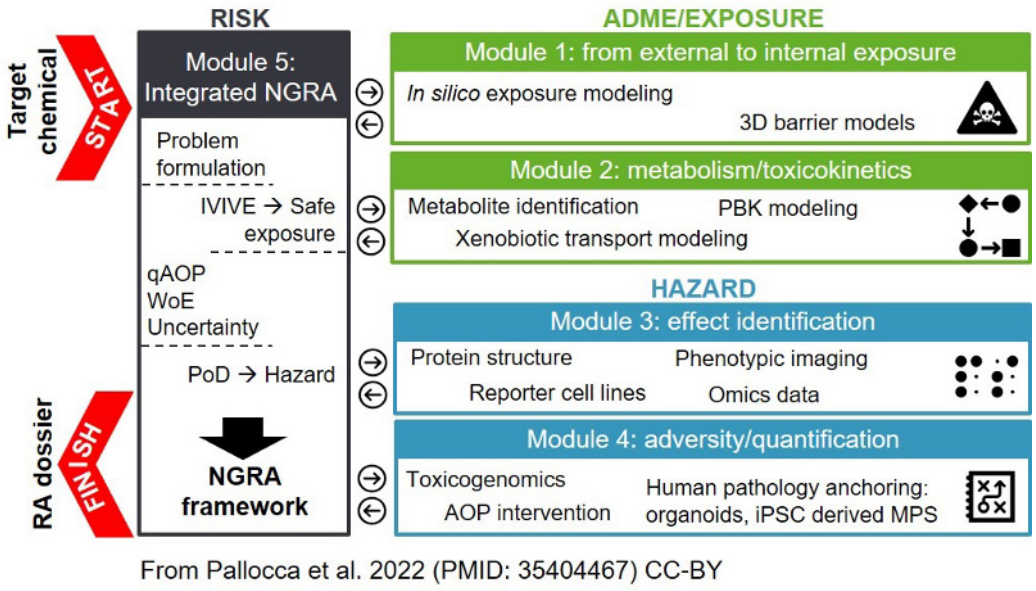

Figure 1: Overarching RISK-HUNT3R project strategy.

used to assess the completeness of an *in vitro* method to enter the validation process. EURION members evaluated their own methods by completing the TRC template provided to them before the training. The aim was to assess how complete their method is to proceed to validation and to increase self-awareness of method developers as to what is required for their method to be as complete as possible (see *section 3.7*).

In June 2022, a training was provided to EURION members on: a) key aspects on validation of *in vitro* methods; b) key messages on the application of GIVIMP; and c) introduction of TRC. The EURION cluster's penultimate annual meeting was held in Amsterdam, the Netherlands on 30 to 31 January 2023.

▸▸ Website: *https://eurion-cluster.eu/*

2.1.3 PARC

The European Partnership for the Assessment of Risks from Chemicals (PARC) was established to support the development and implementation of a research and innovation programme to address current and future needs in relation to chemical risk assessment. It is a Horizon Europe public-public partnership, co-funded by the European Commission and the Member States with a budget of 400 Million Euro. Three European Agencies (EEA, EFSA, ECHA) are partners. Some institutes from UK and Switzerland are also participating with their national funding.

The seven-year project formally started on 1 May 2022, and had its kick-off meeting in Paris on 12 to 13 May 2022, under the French Presidency of the Council of the European Union. There were 200 participants in Paris and a further 200 on-line (*Figure 2*).

Figure 2: Kick-off meeting of the PARC consortium. © Anses - Xavier SCHWEBEL.

With a common Strategic Research and Innovation Agenda (SRIA), PARC will develop innovative methodologies and tools, generate and share data for exposure, hazard and risk assessment. During the first year, several projects involving NAMs were launched (*Table 1*).

Table 1: PARC projects considering NAMs.

Work Package	*Brief description of projects*
Hazard assessment	Data gap filling concerning the Bisphenol A alternatives using *in silico* and modelling tools.
	Exploration of Nuclear Receptor-driven effects using stably transfected cell lines.
	Gaining mechanistic knowledge on thyroid hormone system disruption, including species differences.
	Human relevance assessment of *in vitro* 'omics data with the *in vivo* situation.
	Development of AOPs related to immunotoxicology, neurotoxicology, non-genotoxic carcinogenesis, endocrine disruption and metabolic disruption.
	Development of physiologically based kinetics (PBK) models for integration with AOP.

Innovation in regulatory risk assessment	Assessment of human relevance of AOPs and the relevance of NAMs related to the key events (KEs) or key event relationships (KERs).
	IATA development for the identification of endocrine disruptors (thyroid hormone system and anti-androgenicity).
	IATA development for genotoxicity.
	IATA development for specific target organ toxicity (STOT), with initial focus on liver toxicity, and at a later stage, adapted for respiratory toxicity.
	Assessing the value of NAMs for Estrogen, Androgen, Thyroid and Steroidogenesis (EATS) endpoints in the classification of chemicals either as Category 1 or 2 for ED properties.
	Comparing the use of NAMs with mixture risk assessment methods relying on component-based standard toxicity information.
	Promoting and facilitating the regulatory acceptance and practical use of NAMs for use in chemical risk assessment.
FAIR data	Ontology-based data curation and its automated maintenance using NLP (Natural language processing).
Concepts and Toolboxes	Integrating models related to near and far field exposure modelling, reconstruction of HBM data, internal exposure assessment accounting for co-exposure interaction and quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) for introducing NAMs in the risk assessment process.

The JRC is setting up a formal collaboration agreement with PARC, so that JRC staff can be involved in various aspects of the work consistent with the JRC Work Programme.

Coordinator: Pascal Sanders, Ineris.

Website: https://www.eu-parc.eu/

2.1.4 Virtual Human Platform for safety assessment (VHP4Safety)

The Virtual Human Platform for Safety Assessment (VHP4Safety) is a research project funded by the Dutch Research Council (NWO) programme 'Dutch Research Agenda: Research on Routes by Consortia (NWA-ORC). The mission of VHP4Safety is 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.

VHP4Safety is developing a platform to identify, collect, integrate and analyse relevant human data, using state-of-the-art technology for data integration to ensure the transparency and security of the process. AOPs are used to define and model the mechanisms underlying perturbations to human biological pathways. Artificial intelligence is employed to link these AOPs to chemical structures and the output of results from case studies to make safety estimates based on exposure. VHP4Safety will collect existing human relevant data and generate new human data for defined sets of chemicals and pharmaceuticals, to feed into the platform. This will include data on exposure, toxicokinetics and toxicological effects, generated with advanced *in vitro* human models. To accelerate the transition towards animal-free safety assessment, VHP4Safety investigates what is needed to gain confidence and trust in this new framework, in order to ensure a sustainable transition towards animal-free safety testing, while striving for improved reproducibility and predictability in science.

Coordinator: Juliette Legler, University of Utrecht.

▸▸ *Website: https://vhp4safety.nl/*

2.1.5 Mechanistic analysis of repeated dose toxicity studies

In a two-year EURL ECVAM-funded study, the Free University of Amsterdam (VU Amsterdam), together with Edelweiss Connect GmbH, have collected and analysed toxicological information provided by repeated dose systemic studies. The aim of the study, which kicked off in October 2020, is to "reverse engineer" repeated dose toxicity studies by describing how they capture the key characteristics (KCs) of repeated dose systemic toxicants. A key characteristic is considered as any molecular or cellular event triggered by a chemical that is implicated in the development of an adverse event after repeat exposure.

A key feature of the project was to refine and organise the mechanistic evidence in terms of KCs, and to create logical and plausible associations with specific organ toxicity. The identification of KCs was based on a combined literature and data driven approach.

During the last year, the work focused on collecting, merging and analysing oral rat data from various database sources and mapping the target organ systemic effects and KCs to the time of exposure. To that end, the list of KCs in the prioritised organs (liver, kidney, heart and lung) was linked to possible mechanisms of toxicity, gathered from *in vivo* data analysis combined with *in vitro*, clinical and 'omics data when available, for a selection of compounds. A set of four source chemicals (one per target organ) supplemented with toxicologically mechanistically-based similar compounds were evaluated to ensure that the KCs selected are not chemically specific to the main compound. It is expected to make the results of the project available in the public domain through a JRC technical report and peer reviewed papers.

2.2 Applications of NAMs and modelling

2.2.1 Physiologically based kinetic models

Physiologically based kinetic (PBK) models are playing an increasingly important role in *in vitro* to *in vivo* extrapolation and chemical risk assessment (Chang *et al.*, 2022). These models simulate what the body does to the chemical, in terms of how the chemical is absorbed, distributed, metabolised and eliminated, so called ADME processes. The human health risks of a chemical are directly linked to its levels in body tissues, which vary over time as the chemical is absorbed and removed.

To accelerate the acceptance and use of PBK models, the JRC and the US Environmental Protection Agency (EPA) led an international working group at the Organisation for Economic Cooperation and Development (OECD) to draft a guidance document on the characterisation, validation and reporting of these models (OECD, 2021). To promote the guidance document, a dedicated webinar[2] was hosted by the OECD on 10 May 2021. A further webinar[3] was hosted on 6 April 2022 to discuss the "acceptability" of selected case studies in next generation PBK modelling for regulatory assessment.

EURL ECVAM was also a "champion" for a project funded by the European Partnership for Alternative Approaches to Animal Testing (EPAA) and carried out by Liverpool John Moores University. The four main aims of this project were to: (i) conduct and publish a systematic review and collation of existing, published PBK models in rats and humans (and other mammals) to provide a readily updatable resource for PBK model developers and users; (ii) assess the chemical space coverage of existing PBK models in relation to food additives, drugs, cosmetics, pesticides and industrial chemicals; (iii) investigate similarity assessment metrics (e.g. chemical fingerprints) to determine the most appropriate for selecting analogues for PBK development; and (iv) develop a freely available software tool to assist the identification of appropriate analogues via an automated workflow. The results of the systematic review (Thompson *et al.*, 2021) have been included in a dataset[4] in the JRC data catalogue (EURL ECVAM collection). The project results are also elaborated in a PhD thesis[5].

2.2.2 Modelling AOPs and AOP networks

Quantitative AOPs (qAOPs) are toxicodynamic models based on adverse outcome pathways (Spînu, 2021). Combined with PBK models, qAOP models are expected to play an increasingly impactful role in next generation risk assessments using NAMs without the need to conduct animal studies. There is, however, limited experience and virtually no guidance on best practices for qAOP development. As a step forward, EURL ECVAM contributed to the organisation of an ECETOC workshop on Quantitative Response-Response Relationship, which was held as a hybrid meeting on 18 to 19 October 2022. The aims of the workshop were to: 1) bring together different stakeholders to

2 *https://www.youtube.com/watch?v=PT7w6PB97Ag&t=4252s*

3 *https://www.youtube.com/watch?v=3u_ghfQsH58*

4 *https://data.jrc.ec.europa.eu/dataset/f98e9abf-8435-4578-acd6-3c35b5d1e50c*

5 Thompson, C (2023). Development of Tools to Support the Application of Physiologically-Based Kinetic Modelling in Safety Assessment. Doctoral thesis, Liverpool John Moores University. Available at: *https://researchonline.ljmu.ac.uk/id/eprint/18629/*

build on the knowledge gained from existing experience of qAOP development; 2) make recommendations that will enable the design, interpretation and application of Quantitative Response-Response Relationships based on AOPs that are trusted to provide confidence in use in decision making; and 3) encourage the use of open standards to support the "FAIRification[6]" of qAOP models.

▸▸ *ECETOC workshop: https://www.ecetoc.org/event/workshop-on-quantitative-response-response-relationships-qaops/*

▸▸ *Interactive qAOP visualisation tool (developed by Liverpool John Moores University): https://public.flourish.studio/visualisation/11342208/*

2.2.3 From in silico medicines to in silico toxicology

The Universal Immune System Simulator (UISS) is an *in silico* modelling platform developed by the University of Catania and designed to simulate the human immune system. The UISS has been developed to simulate general immune system behaviour connected to multiple immune system responses, triggered by drugs, viruses, bacteria, tumors, and auto-immune diseases.

In a collaboration between EURL ECVAM, the University of Catania, and the University of Milan, the UISS is being explored for its applicability in a different context of use, i.e. the immunotoxicity risk assessment of chemicals. Initial work focused on the ability of the UISS to reproduce the immunotoxic effects of perfluorinated alkylated substances (PFAS) such as PFOA and PFOS (Pappalardo *et al.*, 2022). Further work demonstrated the extension of the modelling platform to the identification of skin sensitisers (Russo *et al.*, 2022).

2.2.4 Chemical mixtures in the European population

Humans are generally exposed to multiple chemicals from different sources and through various pathways. To gain a better understanding of the chemical exposure of the general population in Europe, the JRC and the H2020 project HBM4EU[7] investigated the presence of chemicals in humans and the possible related risks.

HBM4EU has gathered and harmonised data from previous human biomonitoring studies so that general risks of mixtures could be estimated based on data from 15 European countries[8]. By looking at typical compositions of chemical combinations at 50th percentile concentrations for adults and children, it was possible to draw an average picture of exposure, complemented by data on the 95th percentile exposure as a worst-case scenario. The derived mixture information indicates that co-exposure to many substances is very likely in the European population.

Based on human biomonitoring guidance values, the possible risks to humans were investigated. Due to the limited availability of such reference values, this was only possible for 20 chemicals in adults and 17 chemicals in children. Estimated mixture hazard indices range from 2.8 for children at the 50th percentile up to 9.2 for adults at the 95th percentile, which indicates that a risk to human

6 *https://www.go-fair.org/fair-principles/*

7 *https://www.hbm4eu.eu/*

8 *https://ipchem.jrc.ec.europa.eu/index.html#showmetadata/HBM4EUAGGREGATED*

health cannot be excluded. Six to seven substances were responsible for over 95% of the risk in all cases. Limitations of the study related to the narrow coverage of chemicals, as well as considering all substances as contributors to the combined risk argue for caution in the interpretation of results. Further details can be found in Socianu *et al.*, 2022.

3. Validation

Validation is at the interface between test method development and regulatory acceptance and ensures a science-based and rigorous evaluation of test methods and approaches by establishing their overall performance and fitness for a given purpose or context of use. EURL ECVAM directly contributes to assessing scientific validity of test methods through the EURL ECVAM validation process, as well as by continuing exploring and evolving validation approaches to keep pace with scientific progress.

This chapter describes some selected activities related to establishing and building confidence in new test methods. However, various aspects of validation are also evaluated in several of the projects presented throughout the report. In particular, they range from those aiming at developing and optimising new approach methodologies (chapter 2) to those contributing to international standards (chapter 4).

3.1 Test method submissions

A comprehensive list of test method presubmissions and submissions and their status can be found in TSAR[9]. Since December 2021, EURL ECVAM has received three presubmissions of methods for cytotoxicity, skin sensitisation and genotoxicity testing, as well as two full submissions on methods for genotoxicity testing using reconstructed skin models.

3.1.1 LUCS-VALITOX

The *in vitro* light-up cells system (LUCS) measures general cytotoxicity in cells of human origin. It is based on a photoinduction system that produces Reactive Oxygen Species (ROS) inside live cells and measures the change in fluorescence before and after applying light, informing on changes on cell homeostasis.

LUCS was proposed as a method to describe cytotoxicity and to inform on acute oral toxicity, in particular, to replace the 3T3 NRU assay for providing the starting dose for the *in vivo* acute oral toxicity test as described in OECD GD 129.

Cytotoxicity assays are usually evaluated in combination with other read-outs within a test system. Moreover, EURL ECVAM does not prioritise the evaluation of similar methods. Therefore, EURL ECVAM decided not to progress the submission further and suggested to the test submitter to approach directly the OECD for proposing its method for the purpose of providing the starting dose for *in vivo* acute oral toxicity tests.

3.1.2 ProtReact

ProtReact is an *in chemico* method proposed for skin sensitisation hazard assessment that addresses the covalent binding of electrophilic chemicals with nucleophilic sites of amino acids in skin proteins (i.e., haptenation) which represents the molecular initiating event of the skin sensitisation AOP. In the ProtReact, reactivity of a test chemical is evaluated by quantifying the depletion of lysine and cysteine peptides through fluorescence/absorbance in 96 well-plates. EURL ECVAM concluded that the test method is mechanistically relevant and that it may be more cost-effective and less time consuming than the Direct Peptide Reactivity Assay (DPRA) or the Amino acid Derivative Reactivity Assay (ADRA) currently described in OECD TG 442C. Future work on the method should focus on the evaluation of the reproducibility within and between laboratories and on the characterisation of the predictive capacity using curated reference data.

3.1.3 GENOMARK

GENOMARK is a transcriptomics-based signature or biomarker aiming at providing insights into the molecular events underlying the genotoxic mode of action of different types of test chemicals. The GENOMARK biomarker includes 84 genes which were selected based on whole genomics DNA microarray profiles of 24 (non-)genotoxic reference chemicals covering diverse modes of action (MoA) in metabolically competent HepaRG™ cells. A prediction model using a supervised machine learning algorithm is applied to classify the test

9 *https://tsar.jrc.ec.europa.eu/*

chemicals as genotoxic (GTX) or non-genotoxic (NGTX) based on the gene expression values for the 84 genes. Additional information needs to be provided by the submitter before the assessment can be finalised.

3.1.4 Genotoxicity in reconstructed skin models

Cosmetics Europe has led the validation of *in vitro* human skin-based genotoxicity assays for topically exposed substances, including cosmetics ingredients. These are the reconstructed skin comet assay and micronucleus test (Pfuhler *et al.*, 2021a; Pfuhler *et al.*, 2021b). EURL ECVAM is finalising the assessments of these methods. At the first meeting of the new ESAC (see *section 3.3.2*) scheduled in February 2023, their progression towards ESAC peer review will be discussed and a specific ESAC sub-group (see *section 3.3.3*) will be established. A proposal for the development of new OECD test guidelines for both assays has already been included in the OECD work plan.

3.2 Validation studies

EURL ECVAM continued the validation of 18 methods relevant for the thyroid signalling pathway.

3.2.1 Thyroid methods validation study

The multi-laboratory study, aiming at validating a number of *in vitro* methods (OECD, 2014) for different modes of action relevant for the thyroid hormone signalling pathway, has come to its final phase. Most of the fifteen laboratories from the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) have submitted the data generated to EURL ECVAM and a report describing the validation study of these methods is foreseen to be published Q1 of 2023. The report also includes among others, the list of the 30 chemicals tested blindly in the battery of methods covering the different molecular initiating and key events of the thyroid hormone signalling pathways.

All available standard operating procedures (SOPs) and study reports are being made available in EURL ECVAM's online tracking system (TSAR) along with links to related scientific publications. Ongoing work for some of the methods will be finalised in 2023 and the output added to TSAR when available.

The preparatory work, the development of SOPs, the experimental work and the reporting required substantial resources from the EU-NETVAL laboratories. Moreover, several EU-NETVAL laboratories made particular efforts to substitute some of the animal-derived reagents (Bartnicka *et al.*, 2021). There was a noticeable positive impact on the quality and speed of the validation work where method developers supported the EU-NETVAL laboratories with training and transfer of their scientific knowledge and where EU Member States provided the laboratories with financial support.

In Part 1, aiming to characterise the methods through the development of SOPs and the assessment of transferability and within-laboratory reproducibility, data were generated with the reference and control chemicals for most methods. Three methods did not proceed through the validation study because of lack of specificity (method 2b), requirement of further optimisation (method 4c) and further characterisation of test system (method 8b). The assessment

of two methods (TRH receptor activation method 1a and the angiogenisis / vasculogenisis method 8c) will stop after completion of Part 1 due to reductions in human resources at the EU-NETVAL laboratory. In Part 2, data were generated for up to 30 selected chemicals for most of the remaining methods with the aim to provide additional information on their biological relevance. All methods, EU-NETVAL laboratories and Method Developers (MD) involved in the validation of the different methods and the respective links in TSAR, are provided in *Table 2*.

Table 2. Methods assessed in the thyroid validation study, Method Developers (MD) and EU-NETVAL laboratories involved and links to the methods in TSAR.

Method #, title and MIE or KE	Method developers and EU-NETVAL laboratories	Test method # in TSAR
1a; PATHHUNTER® BETA-ARRESTIN thyrotropin-releasing hormone (TRH) receptor activation (beta-galactosidase) measuring agonist and antagonist activities.	MD: Eurofins Discover X corporation EU-NETVAL: Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IT)	*TM2019-02*
1b; Thyrotropin-stimulating hormone (TSH) receptor activation based on cAMP measurement.	MD: University of Pisa (IT) EU-NETVAL: National Reference Laboratory for Experimental Immunotoxicology / National Institute of Public Health (CZ)	*TM2019-03*
2a; Thyroid peroxidase (TPO) inhibition based on oxidation of Amplex UltraRed®.	MD: United States Environmental Protection Agency (USA) EU-NETVAL: RISE - Research Institutes of Sweden (SE)	*TM2019-04*
2b; Thyroid peroxidase (TPO) inhibition based on oxidation of Luminol.	MD: Wageningen Food Safety Research (NL) EU-NETVAL: VITO-ABS / Flemish Institute for Technological Research, Research team Applied Bio & molecular Systems (BE)	*TM2019-05*
2c; Tyrosine iodination using liquid chromatography: The TPO-catalyzed iodination assay	MD: National Center for Toxicological Research, U.S. Food and Drug Administration (USA) EU-NETVAL: Charles River Laboratories Den Bosch B.V. (NL)	*TM2019-06*
2d; Activation of the sodium iodide symporter (NIS) based on Sandell-Kolthoff reaction.	MD: United States Environmental Protection Agency (USA) EU-NETVAL: Labfit - HPRD Health Products Research and Development Lda (PT)	*TM2019-07*
3a; Thyroxine-binding prealbumin (TTR) / thyroxine-binding globulin (TBG) binding using fluorescence displacement (ANSA).	MD: Luxembourg Institute of Science and Technology (LU) EU-NETVAL: European Union Reference Laboratory for alternatives to animal testing / EURL ECVAM (IT)	*TM2019-08*
3b; Thyroxine-binding prealbumin (TTR) binding using fluorescence displacement (T4-FITC).	MD: Free University of Amsterdam (NL) EU-NETVAL: Wageningen Food Safety Research (NL)	*TM2019-09*

Method #, title and MIE or KE	Method developers and EU-NETVAL laboratories	Test method # in TSAR
4a; Colorimetric method for assessing deiodinases activity based on Sandell-Kolthoff reaction with human microsomes: DIO1-SK assay.	MD: Charité Universitaetsmedizin Berlin (DE) EU-NETVAL: BASF SE Experimental Toxicology and Ecology. Laboratory for Development of Alternative Methods (DE)	*TM2019-10*
4b; Inhibition of thyroid hormones (THs) glucuronidation using liquid chromatography/mass spectrometry (LC/MS).	MD: Accelera S.r.l. (IT) EU-NETVAL: Accelera S.r.l. (IT)	*TM2019-11*
4c; Inhibition of THs sulfation using liquid chromatography.	MD: Accelera S.r.l. (IT) EU-NETVAL: Accelera S.r.l. (IT)	*TM2019-12*
5a; Inhibition of monocarboxylate transporter 8 (MCT8) based on Sandell-Kolthoff reaction.	MD: Charité Universitaetsmedizin Berlin (DE) EU-NETVAL: Instituto de Salud Carlos III (ES)	*TM2019-13*
6a; Human thyroid hormone receptor alpha (TRα) and Human thyroid hormone receptor beta (TRβ) reporter gene transactivation measuring agonist and antagonist activities.	MD: Indigo Biosciences Inc. (USA) EU-NETVAL: Vitroscreen S.r.l. (IT)	*TM2019-14*
6b; TRCALUX human thyroid hormone receptor beta (TRβ) reporter gene transactivation measuring agonist and antagonist activities.	MD: BioDetection Systems (BDS) (NL) EU-NETVAL: Vitrox (IT)	*TM2019-15*
7a; Measurement of intrafollicular thyroxine (T4) using zebrafish eleutheroembryos.	MD: IDAEA CSIC, Department Environmental Chemistry (ES) EU-NETVAL: Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IT)	*TM2019-16*
8a; Measurement of proliferation of rat pituitary-derived cell line GH3.	MD: Luxembourg Institute of Science and Technology (LU) EU-NETVAL: Nofer Institute of Occupational Medicine (PL)	*TM2019-17*
8b; Proliferation, migration and oligodendrocyte maturation (including myelin formation) in mixed neuronal/glial culture (neurospheres) derived from human induced Pluripotent Stem Cells (hiPSC)	MD: University of Lausanne (CH), in collaboration with IUF – Leibniz Research Institute for Environmental Medicine (DE)	*TM2019-18*
8c; In vitro human adipose stromal cell-human umbilical vein endothelial cell (hASC-HUVEC) vasculogenesis / angiogenesis method.	MD: FHAIVE (FI) EU-NETVAL: FHAIVE (FI)	*TM2019-19*

3.3 EURL ECVAM Scientific Advisory Committee (ESAC) peer reviews and renewal of the ESAC members

The EURL ECVAM Scientific Advisory Committee (ESAC) is a formal Expert Group of the European Commission that is charged with providing EURL ECVAM with independent scientific advice. In particular, the ESAC acts as a scientific peer-review body by providing EURL ECVAM with its opinion on the adequacy and outcome of formal validation studies carried out to assess the reliability and relevance of non-animal methods/approaches, typically in the context of regulatory safety assessment. The ESAC may also provide scientific advice on other scientific issues of relevance to the work and mission of EURL ECVAM. ESAC's tasks are:

- to assess the scientific validity of non-animal methods/approaches intended for a given purpose;
- to advise the JRC on other scientific matters related to the work of EURL ECVAM and the protection; of animals used for scientific purposes;
- to share its knowledge and experience on non-animal methods/approaches used in science.

3.3.1 SENS-IS

The ESAC Working Group (WG) in charge of the peer-review of the SENS-IS test method completed the evaluation of the additional information on the method provided by the test submitter, including supplementary data in support of its reproducibility (see 3.3.2 in Zuang *et al.*, 2021). In March 2022, the ESAC WG communicated to EURL ECVAM that they were not in the position "to ensure a transparent, consistent and reliable peer review of the SENS-IS test method" due to the quality of the submitted SENS-IS data. As a consequence, EURL ECVAM decided to stop the ESAC peer review.

3.3.2 Renewal of the ESAC

On 25 March 2022, the JRC launched an open call for applications for the renewal of the members of the ESAC, with a closing date for submitting an application on 1 May 2022. Renewed every five years, ESAC is composed of external scientists who are appointed on the basis of their scientific expertise and who act independently and in the public interest. The call was published online on the Register of Commission Expert Groups and Other Similar Entities. Twenty-five candidates submitted an application by the deadline for nine available positions. A Selection Committee comprising JRC staff and representatives from other Commission services and Agencies (external members) with vast experience in this type of work was established to assess the candidates, as described in the open call for applications. All twenty-five applicants were deemed eligible by the Selection Committee on the basis of the eligibility criteria published in the open call for applications, and were therefore evaluated against the selection criteria described in the same document. In its selection process, the Selection Committee also considered representation from different geographic regions and gender balance. A separate Committee was established by the JRC to assess the declarations of interests (DoI) of all candidates, to identify potential conflicts of interests, to decide on the candidates' independence to serve on ESAC, and to establish a final list of candidates to be appointed as ESAC members by the Director of JRC.F. On the basis of these evaluations, nine experts were formally appointed on 11 October 2022 as the new members of the ESAC (see *Box 2*).

Box 2

New ESAC members as from 1 November 2022

The final selection of the ESAC members achieved a good gender balance (4 women and 5 men), acceptable geographical distribution in terms of nationality (3 from Germany, 2 from Italy, 2 from the USA, the Netherlands and Spain) and place of employment (2 in Germany, 2 in the USA, Italy, Finland, the Netherlands, the UK and Switzerland), and good cross-sectoral representation (3 members from regulatory authorities, 2 members from consultancy firms, and 4 members from academia several of the selected candidates also have experience working for/with industry). More details on the new ESAC including its membership, Terms of Reference and Rules of Procedure can be found on the Register of Commission Expert Groups and Other Similar Entities.

- Ms Rebecca CLEWELL
- Ms Emanuela CORSINI
- Mr Dario GRECO
- Mr Sebastian HOFFMANN
- Ms Anne KIENHUIS
- Mr David LEHMANN
- Ms Blanca RODRIGUEZ
- Mr Helmut SEGNER
- Mr Tewes TRALAU

⏩ *Register of Commission Expert Groups and Other Similar Entities: https://ec.europa.eu/transparency/expert-groups-register/screen/calls-application?lang=en*

⏩ *Call for applications for the selection of members of the EURL ECVAM Scientific Advisory Committee (expired): https://ec.europa.eu/transparency/expert-groups-register/core/api/front/calls-application/77960/download*

⏩ *Continuously open call for applications for the selection of members of ESAC sub-groups (open): https://ec.europa.eu/transparency/expert-groups-register/core/api/front/calls-application/88753/download*

⏩ *Terms of Reference and Rules of Procedure of ESAC: https://ec.europa.eu/transparency/expert-groups-register/screen/expert-groups/consult?lang=en&groupID=3602*

3.3.3 Continuously open call for applications for the selection of members of ESAC sub-groups

Peer reviews and other work of the ESAC are normally facilitated by specialised ESAC sub-groups set up by the JRC. On 25 October 2022, the JRC launched a continuously open call for applications for the selection of members of the sub-groups operating under the ESAC (*Box 2*).

In order to ensure the smooth functioning of ESAC sub-groups, the JRC will establish a list of suitable experts (expert pool) from applicants complying with the eligibility criteria referred to in this call. The members of a specific sub-group that are not members of the ESAC shall be selected by the JRC from the expert pool, on the basis of the selection criteria referred to in this call and of their qualifications/expertise related to the specific question(s) under review.

Each ESAC sub-group must comprise at least one ESAC member who shall chair it, but may be composed of any combination of ESAC members and sub-group members that are not members of the ESAC. ESAC sub-groups typically consist of up to 10 members in total. Members of ESAC sub-groups are individuals appointed in a personal capacity who shall act independently and in the public interest (Type A members).

3.4 EURL ECVAM Network for Preliminary Assessment of Regulatory Relevance (PARERE)

The 11th meeting of the Preliminary Assessment of Regulatory Relevance (PARERE) network (see *Box 3*) was held online on 17 February 2022. The purpose of the meeting was to provide updates on 3Rs activities undertaken

in EU Member States and by Commission services and relevant EU agencies; to inform and seek input on extended information requirements under REACH and the potential to introduce NAMs into chemical hazard and risk assessment practices under the REACH regulation, as foreseen by the EU Green Deal.

The session on a roadmap for respiratory sensitisation testing included presentations that introduced the topic, a summary of the feedback received from PARERE on various EURL ECVAM consultations on respiratory sensitisation and examples of approaches used for chemical risk assessment of respiratory sensitisers under various EU legislation. Since respiratory sensitisation has been identified as a critical hazard in the EC's Chemicals Strategy for Sustainability, the aim was to have an exploratory discussion on the short-, mid- and long-term needs for tackling the field of respiratory sensitisation and the use of NAMs to satisfy regulatory information requirements.

Box 3

Preliminary Assessment of Regulatory Relevance (PARERE) network

Directive 2010/63/EU requires that Member States nominate a single point of contact to provide advice on the regulatory relevance and suitability of alternative approaches proposed for validation. The PARERE network is trans-sectoral and includes regulators of the EU Member States, representatives from EU agencies such as the European Medicines Agency (EMA), the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA), and relevant policy services of the EC. Regulators operating within all sectors of relevance to alternative methods are involved as early as possible in providing a preliminary view on the potential regulatory relevance of methods and approaches submitted to EURL ECVAM for validation or peer review or evaluation. PARERE members are consulted on several occasions over the year, either on the regulatory relevance of individual methods or approaches that are submitted to EURL ECVAM or on other topics such as e.g. EURL ECVAM Recommendations, standardisation and validation frameworks for novel technologies developed within research projects funded by the EU Framework Programme for Research and Innovation.

More information on the PARERE network can be found here: 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

3.5 A framework for establishing scientific confidence in new approach methodologies

EURL ECVAM staff collaborated with three US federal agencies (Environmental Protection Agency – EPA, Consumer Product Safety Commission – CPSC, and National Institutes of Health – NIH), the OECD and PETA Science Consortium International to develop and publish a robust and flexible framework to establish scientific confidence in NAMs for regulatory use. The framework (van der Zalm *et al.*, 2022), addresses five essential elements: fitness for purpose, human biological relevance, technical characterisation, data integrity and transparency, and independent review (*Figure 3*). The framework is intended to be flexible because the order of assessment of these elements can vary and they can be addressed separately, in parallel or iteratively. While the paper focuses on NAMs for assessing human health effects, many of the suggested elements are expected to apply also to ecotoxicological effect assessments. Use of the framework will enable a confidence building process that allows for the timely uptake of fit for purpose and biologically relevant NAMs that

can be used for regulatory decision-making. Universal uptake of this framework would also facilitate the timely development and use of NAMs by the international community.

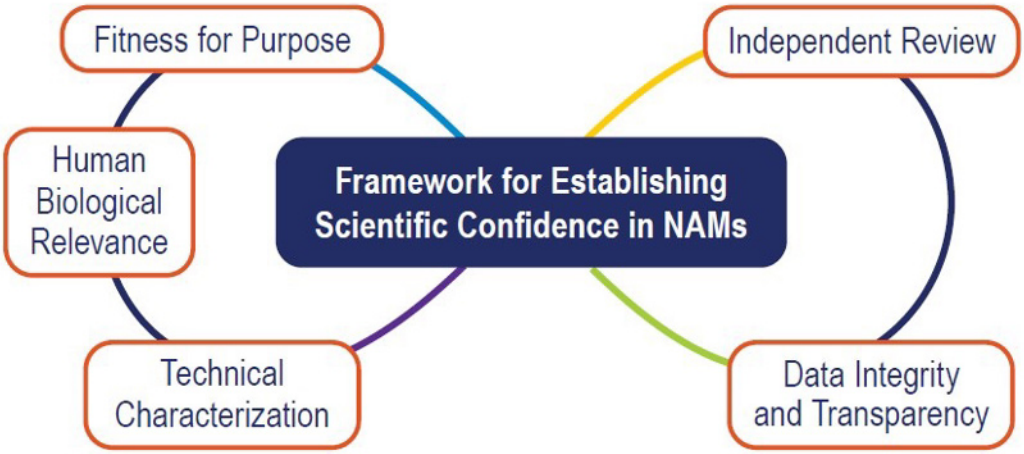

Figure 3: Schematic illustrating the interconnectedness of the five essential elements for establishing scientific confidence in NAMs for assessing human health effects. The framework is intended to be flexible because the order of assessment of these elements can vary and they can be addressed separately, in parallel or iteratively. (Reprinted from van der Zalm et al., 2022, Archives of Toxicology, © 2022, Barroso under CC BY 4.0 *http://creativecommons.org/licenses/by/4.0/*).

3.6 Supporting the scientific validity of Organ-on-chip

The scientific community is advocating for an increased use of Organ-on-chip (OoC) for internal decision-making and regulatory applications. By combining advanced cell and tissue cultures with micro-engineering, these methods can provide human-relevant data for biomedical research and risk assessment, among many other areas. To demonstrate the scientific validity of these very innovative tools, EURL ECVAM is actively contributing to standardisation activities at European and international level. In addition, EURL ECVAM also facilitates the communication of the regulatory needs to the developers' community.

3.6.1 Standardisation activities

As the main outcome of the "Putting Science into Standards" workshop on OoC (Piergiovanni *et al.*, 2021) organised by the JRC and CEN-CENELEC last year, a CEN-CENELEC Focus Group[10] was formally established and launched in March 2022. Its goal is to map current initiatives, identify specific issues, draft a roadmap and define priorities for the next standardisation activities for OoC. The group is chaired by Professor Andries van der Meer (University of Twente) and the Secretariat is provided by NEN (Nederlands Normalisatie Instituut). Interested experts are welcome to join.

10 *https://www.cencenelec.eu/areas-of-work/cen-cenelec-topics/organ-on-chip/*

At the international level, EURL ECVAM was also involved in defining the terminology related to OoC and microphysiological systems (MPS). As a result, the "Standard Terminology Relating to Microphysiological Systems" was published by the American Society for Testing and Materials (ASTM)[11].

3.6.2 EURL ECVAM validation and qualification resources

EURL ECVAM has compiled a collection of resources that can be useful tools in the validation and qualification (in the context of medicinal products) of novel test methods.

EURL ECVAM produced a catalogue of resources[12] for developers and end-users to promote the validation and qualification of OoC devices, in order to encourage the use of these novel approaches for regulatory applications. The collection incorporates two separate, but complementary set of resources:

a) a carefully-selected list of relevant reading documents, such as guidance documents, guidelines, reports, reflection papers published by a wide range of relevant organisations (ECHA, EFSA, EMA, EURL ECVAM, FDA, ICH and OECD), peer-reviewed publications, workshop reports, etc., and
b) a comprehensive set of Frequently Asked Questions (FAQ) in relation to the main regulatory areas of interest to the OoC community (chemical, pharma and food safety).

These resources were compiled in partnership with the Regulatory Advisory Board (RAB) of the European Organ-on-Chip Society (EUROoCS) and are expected to serve as a point of reference for supporting OoC approaches towards their regulatory acceptance.

This list is a dynamic, non-exhaustive database that will be periodically updated as additional relevant resources will become available.

> *The EUROoCS annual conference in July 2022 was the opportunity to raise awareness of the OoC research and development communities about regulatory aspects of relevance to OoC. The EUROoCS Regulatory Advisory Board (RAB) worked closely together with EURL ECVAM on a very original and relevant idea: to provide the OoC community with a catalogue of resources in the form of: i) a list of existing reference documents in connection with validation and qualification and ii) a list of FAQs on regulatory acceptance of OoC. This catalogue can be viewed as a reference point in supporting regulatory acceptance of innovative OoC approaches and is expected to be a valuable tool for the whole EUROoCS community, and especially developers and end-users.*
>
> *Nathalie Delrue*
> *Administrator, Test Guidelines Programme, Environment Health and Safety Division, Environment Directorate, OECD*

3.7 Training the research community

During recent years, EURL ECVAM has taken advantage of the popularity of the virtual meeting format to provide training in different aspects of validation. Of special relevance in 2022 was the training given on the 1st June to the EURION cluster (see *section 2.1.2*) focusing on the assessment of method readiness for validation. The objective of the workshop was to help method developers to assess whether their methods developed in the frame of EURION were complete enough to qualify for validation as a step towards regulatory application. The training focused

11 *https://www.astm.org/f3570-22.html*

12 *https://data.jrc.ec.europa.eu/dataset/7bcb1db5-5c7e-460b-b79e-ca5f642514a4#details*

on key aspects of validation of *in vitro* methods, the application of Good *In Vitro* Method Practices (GIVIMP), and the presentation of the individual Test Readiness Criteria (TRC) for assessment of completeness of *in vitro* methods to enter validation.

The TRC were adapted from the readiness criteria for regulatory use of NAMs developed for developmental neurotoxicity (DNT) methods published previously (Bal-Price *et al.*, 2018) and were amended to include terminology used in GIVIMP. A score was used to assign the grade of development of a method, i.e. from 'A' (test method is close to ready or ready for validation) to 'D' (not ready at all). The updated template was sent to the EURION members prior to the workshop, asking them to apply it to their methods with the purpose to get their feedback and discuss specific issues during the training. Overall, the purpose of the TRC template was to assess how complete an *in vitro* method is to proceed to validation and to increase self-awareness of method developers as to what is required for their method to be as complete as possible and enter into the validation process. It proved to be a very useful tool for the training.

Furthermore, other *ad hoc* short training sessions were given to collaborators from the US Food and Drug Administration (FDA) and the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ consortium) on the general principles of validation as a way to build trust in *in vitro* methods.

4. Regulatory application

In order to balance the benefits of using chemicals in industrial, agricultural and consumer products, against the potential risks they pose to people and our ecosystem, effective regulation of chemicals is crucial. Striking this balance is an overarching goal in the EC's Chemicals Strategy for Sustainability (EC, 2020) - a central element of the European Green Deal (EC, 2019) and its 'zero pollution' ambition. Standardised methods that can reliably measure parameters relevant to a chemical's hazardous properties along with smart knowledge management solutions are essential in order to deliver on this ambition. Efforts to increase and promote the development and uptake of NAMs and comprehensive knowledge bases on chemicals to support policies on the safety of chemicals at EU and global level are described in this chapter.

4.1 Test methods and integrated approaches to testing and assessment

4.1.1 Defined Approaches for skin sensitisation

Following the adoption of the OECD Guideline 497 on defined approaches for skin sensitisation in 2021, project proposals were included in the OECD work programme to update the Guideline in relation to: a) the use of similar (me-too) methods and b) the inclusion of a defined approach (DA) able to provide a quantitative measure of sensitisation potency.

a) The objective of this proposal is to apply the assessment framework in GL 497 on defined approaches for skin sensitisation to the same DAs (2 out of 3, Integrated Testing Strategy), but with alternative information sources. These "me-too" information sources would come from the corresponding key event-based test guidelines for the *in chemico* and *in vitro* assays (TG 442C, 442D and 442E) or from *in silico* models that demonstrate equivalent performance and reliability to those in the current version of the guideline.

b) The objective of this proposal is to determine the feasibility of adding the Skin Allergy Risk Assessment (SARA) model to GL 497 that would provide a quantitative result to establish a point of departure for quantitative risk assessment. This would be a novel addition to the GL, which currently contains rule-based hazard classification and UN GHS potency classification models. The SARA model (Reynolds *et al.*, 2019; Reynolds *et al.*, 2022) is a computational model that uses a Bayesian statistical approach to quantitatively predict human skin sensitisation potency based upon any combination of data from the human repeat insult patch test, direct peptide reactivity assay (DPRA; TG 442C), KeratinoSens™ (TG 442D), h-CLAT (TG 442E), or U-SENS™ (TG 442E).

4.1.2 Developmental neurotoxicity - in vitro battery

The OECD guidance document (GD) on evaluation of data from a developmental neurotoxicity *in vitro* battery (DNT-IVB) was presented to the WNT for commenting in February 2022. This GD brings to fruition a collaborative effort between EFSA, OECD, US EPA and Danish EPA and many other contributors, including the JRC / EURL ECVAM. In March 2022, the guidance was presented to an audience of European stakeholders (primarily European risk managers and regulatory authorities) in a workshop organised by EFSA, focusing not only on the guidance content, but also the potential next steps in deploying the battery within a regulatory risk assessment context.

Participants were requested to elaborate the following three main questions:

- What would be necessary for the inclusion of the DNT-IVB as data requirement in different EU legislation?
- What could be potential option(s), in the context of the IATA, for the use of DNT-IVB in DNT hazard and risk assessment?
- What would be a suitable tiered approach for using the DNT-IVB in the different regulatory legislation recognising that one approach may not fit to all regulatory frameworks?

The main outcomes from the presentations and discussions can be found in a summary report[13]. Overall it was considered that the use of the DNT-IVB is offering a unique opportunity to include NAMs in the regulatory process and can provide support in achieving the main goal to assess any chemical for DNT.

Following an additional OECD WNT commenting round, the GD is tabled for adoption at the forthcoming WNT meeting in April 2023. The Appendix E on a tiered testing approach was considered premature for adoption and thus a dedicated OECD DNT sub-group is currently developing the approach further so that it could be issued as an addendum at a later stage.

4.1.3 IATA for non-genotoxic carcinogens

An IATA for non-genotoxic carcinogens is under development by an OECD expert group. This work was prompted by the recognition that the assessment of non-genotoxic carcinogens is considered a regulatory gap (Jacobs *et al.*, 2016; Jacobs *et al.*, 2020).

Currently the expert group is finalising the evaluation of the assays that address the main key events in the development of cancer through non-genotoxic pathways. A related special issue is in preparation.

Other initiatives are ongoing that ultimately aim at defining guidance following a weight of evidence-based approach to predict carcinogenic potential without the rodent bioassay (Luijten *et al.*, 2020; Hilton *et al.*, 2022). For instance, an IATA case study has already been submitted to the OECD Working Party on Hazard Assessment. The idea is to then use the OECD feedback to inform the development of a cluster of new case studies.

"The workshop was a great opportunity to explain to our stakeholder community in detail the nature of the huge work undertaken to demonstrate the utility of an in vitro battery of 17 assays in probing the many ways in which chemicals may disturb neurodevelopmental processes"

Iris Mangas
Scientific Officer, PREV Unit, EFSA

4.1.4 Relative metal/metalloid release using a simple simulated gastric fluid

The EC (through EURL ECVAM) is leading the project for the development of an OECD test guideline that describes how to conduct a test using a simple simulated gastric fluid (0.032M HCl) to generate relative metal/metalloid release data for metals and metalloids in massive and powder forms. An OECD expert group composed of regulators, industry and academia is supporting the project.

The second WNT commenting round on the draft TG was launched in December 2021 and comments were received beginning of February 2022 together with a testing proposal from the Netherlands, aiming at

"Following on from the success of the workshop, additional testing and a plan for implementation of the laboratory transferability and reproducibility of the assays is expected to take place in 2023."

Andrea Terron,
Senior Scientific Officer, PREV Unit, EFSA

13 European stakeholders workshop on New Approach Methodologies (NAMs) for Developmental Neurotoxicity (DNT) and their use in the regulatory risk assessment of chemical. *https://doi.org/10.2903/sp.efsa.2022.EN-7402*

clarifying a possible role of the particle size on the metal/metalloid release in the simulated gastric fluid.

Two expert group meetings were organised in February and June 2022 to discuss several technical aspects related e.g., to hygroscopy and the need to dry samples before testing, pH controls and preparation of HCl solution, options for the physical repository for reference and proficiency materials, and the effect of particle size and surface area on metal/metalloid release. Bi-lateral discussions with experts from France, the Netherlands and Health Canada, helped to address the drying sample issue, the testing proposal, and the method to prepare the HCl solution and the pH adjustments, respectively.

An updated draft TG and replies to comments will be provided to WNT members for further commenting as soon as some remaining aspects from the above bi-lateral discussions will be resolved.

Regulatory and policy issues related to the use of the metal release data are discussed in parallel at a Competent Authorities for REACH and CLP (CARACAL) bioelution subgroup. No meeting of the subgroup took place in 2022.

4.1.5 OECD Thyroid Disruption Methods Expert Group

Testing methods for thyroid-related molecular initiating and key event effects including those within EURL ECVAM's thyroid validation study (see *section 3.2.1*) have been recognised by the OECD's Advisory Group on Testing and Assessment of Endocrine Disruptors (AG EDTA) as high priorities for TG development. As a consequence, the AG EDTA proposed to form an Expert Group to coordinate the development of test guidelines for these *in vitro* thyroid-related methods and other related projects expected to deliver within the next years (such as US thyroid projects and thyroid projects with the EURION cluster). The proposal was endorsed by the WNT at its 34th meeting and the Thyroid Disruption Methods Expert Group (TDM-EG), composed of experts in thyroid physiology and/or *in vitro* test validation, was created. The group has already met twice (in June and November 2022) to build on their terms of reference and discuss the foreseen work programme over the next five years.

The first study reports and SOPs submitted to EURL ECVAM from EU NETVAL for the methods investigating Deiodinase-1 activity, Thyroperoxidase inhibition and Tyrosine iodination have been transferred to the TDM-EG. Specific assessment groups have been set up within the EG to evaluate the performance of the methods, as well as available data from similar methods to assess if and what further information is needed in order to bring forward the methods as OECD TGs and to propose further experimental activities (if deemed necessary). Further assessment groups will be convened to assess the other EU-NETVAL methods as and when the study reports are provided to the OECD. Examples of additional activities could be the demonstration of transferability to another facility or between-laboratory reproducibility with a limited number of chemicals.

The work of the assessment groups will continue throughout the first half of 2023 and the plan is to hold the next meeting of the TDM-EG in May 2023.

4.1.6 Establishing a framework for hazard testing of microbial pesticides using new approach methodologies

The OECD organised a conference on *Innovating Microbial Pesticide Testing* on 13 to 16 September 2022. Microbial pesticides refer to microorganisms (e.g. viruses, bacteria, fungi, protozoa etc.) used against a broad spectrum of agricultural pests.

Hazard testing of microbial pesticides is a challenging task since the guidelines currently used have been developed with reference to chemical pesticides, and do not consider the special characteristics of microbial pesticides. Furthermore, NAMs are gaining ground for hazard testing, however they have been validated for single chemical substances / pesticides and the knowledge on application to microbial pesticides is very limited. EURL ECVAM was invited to participate in a panel discussion of experts in the field of NAMs.

The first day of the Conference was dedicated to future test guidelines involving innovative NAMs. More specifically, two separate sessions were organised on:

a) the regulatory requirements and currently used test guidelines for mammalian testing and the potential for the development and wider use of NAMs and
b) the current knowledge and ongoing research in the field of NAMs.

The contribution of EURL ECVAM focused on experiences gained from other fields (mostly from the bio-pharmaceuticals / Advanced Therapy Medicinal Products field) in relation to the validation and use of NAMs for hazard and risk assessment. The presentations and the discussion in the expert panel set the stage for the development of Action Plans to establish a framework for the evaluation of microbial pesticide effects on human health by using NAMs. The main aim is to incorporate NAMs into test guidelines for regulatory decision-making. As a follow up to the Conference, the recommendations will be used for the development of project proposals to be included in the OECD pesticides programme work plan. This activity will be coordinated by the OECD and overseen by the Working Party on Pesticides (WPP); furthermore, the OECD will publish the Conference proceedings and a paper summarising all discussions.

▸▸ *Conference link: Conference on Innovating Microbial Pesticide Testing - OECD*

4.1.7 OECD webinars on emerging technologies

At the 34th meeting of the WNT (see *Box 4*), it was agreed to hold an OECD WNT workshop to discuss any adaptations needed to the test guidelines programme for the uptake of emerging technologies.

For the purpose of the workshop, emerging technologies were defined as *in vitro*, *in chemico* and *in silico* methods with potential to be covered by a test guideline, either on their own or in combination. In other words, all non-animal methods and also technologies such as 'omics that are mature enough and show potential to address a regulatory need (on their own/in combination).

In order for these technologies to be amenable to TG development, they need to be relevant and reliable and address a regulatory need. The OECD test guidelines programme may also need to adapt certain processes and guidance, where appropriate, to reap the benefits of these technologies. The workshop, which was held on 1 to 2 December 2022 in the OECD headquarters, discussed what steps need to be taken to equip the test guidelines programme for emerging technologies and to develop relevant pieces of guidance. A series of preparatory webinars ranging over a variety of relevant topics took place between July and October 2022 in preparation for the workshop.

Box 4

Highlights from the 34th meeting of the WNT 2022

The OECD test guidelines (TG) for the testing of chemicals are a collection of internationally harmonised and agreed testing methods used by governments, industry, research or contract laboratories and academia to assess the safety of chemicals and products. They are primarily used in regulatory safety testing and subsequent chemical notification and registration. The set of test guidelines is updated on a regular basis to keep pace with scientific developments and Member Countries' regulatory needs. OECD-wide networks of national coordinators provide input from scientists in government, academia and industry.

The OECD test guidelines programme (TGP), with the Mutual Acceptance of Data (MAD) agreement is the main instrument to ensure a globally harmonised regulatory safety testing of chemicals. This supports an open global market, avoids creating non-tariff barriers to trade for the chemicals industry and supports protection of the safety of workers, consumers and the environment. OECD also considers animal welfare and is committed to the implementation of the 3Rs principles in the development of TGs. The MAD system saves governments and industry around 309 million euro each year (OECD, 2019) and thousands of animals by avoiding duplicate testing for different jurisdictions. The programme is overseen by the Working Party of National Coordinators of the Test Guidelines Programme (WNT). The JRC acts as a National Coordinator for the OECD Test Guidelines Programme, representing the European Commission and the EU, and as such, is a member of the WNT.

The 34th meeting of the WNT was held on 26 to 29 April 2022. The following new and updated TGs, as well as other types of documents, were approved:

- New TG on Volume-Specific Surface Area
- New TG on Nanomaterials Particle Size and Size Distribution
- New TG on Rapid Androgen Disruption Activity Reporter Assay (RADAR)
- New TG on Anaerobic Transformation of Chemicals in Liquid Manure
- New TG on Defined Approaches for Serious Eye Damage/Eye Irritation
- New TG on Mammalian Erythrocyte Pig-a Mutation Assay
- Updated TG 492B on Time-to-Toxicity using Reconstructed human Epithelium for Eye irritation
- Updated TG 442E including the GARD™ Skin method
- Updated TG 442C (updated ADRA method)
- Updated TG 488 on Rodent Somatic and Germ Cell Gene Mutation Assays
- Updated Guidance Notes 156 on Dermal Absorption
- Detailed Review Paper on the mini-Ames test
- Detailed Review Paper on *in vitro* immunotoxicity focused on immunosuppression
- Study report and preliminary guidance on adaptations of the micronucleus assay TG 487 for nanomaterials safety testing Performance Standards for the assessment of proposed similar or modified methods for Reconstructed human Epidermis for phototoxicity testing
- Corrections to TGs 117, 123, 406, 442D, 456 and 425.

In addition, 20 new project proposals were included into the TGP's workplan.

More information can be found on the OECD website of the TGP: www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm

4.2 Non-animal science in regulatory decisions for chemical safety in the EU

To support one of the actions under the Chemicals Strategy for Sustainability (EC, 2020), EURL ECVAM was tasked by DG ENV to carry out an exercise to develop options for increasing the information requirements under REACH. Aims of the exercise included the better assessment of so-called critical hazards including endocrine disruption, the introduction of Chemical Safety Assessment at all tonnage levels (1 t.p.a upwards), and a more extensive use of NAMs. As a preparatory step, EURL ECVAM conducted a survey to evaluate the state of play in the practical implementation of NAMs for chemical safety assessment. The 80 responses received from a range of stakeholders (*Figure 4*) show that NAMs for some endpoints (e.g. local toxicity, genotoxicity, aquatic toxicity) are mature and already being used by companies for in-house safety assessments, while for other endpoints (e.g. carcinogenicity, immunotoxicity, respiratory sensitisation) fewer methods were reported as being used.

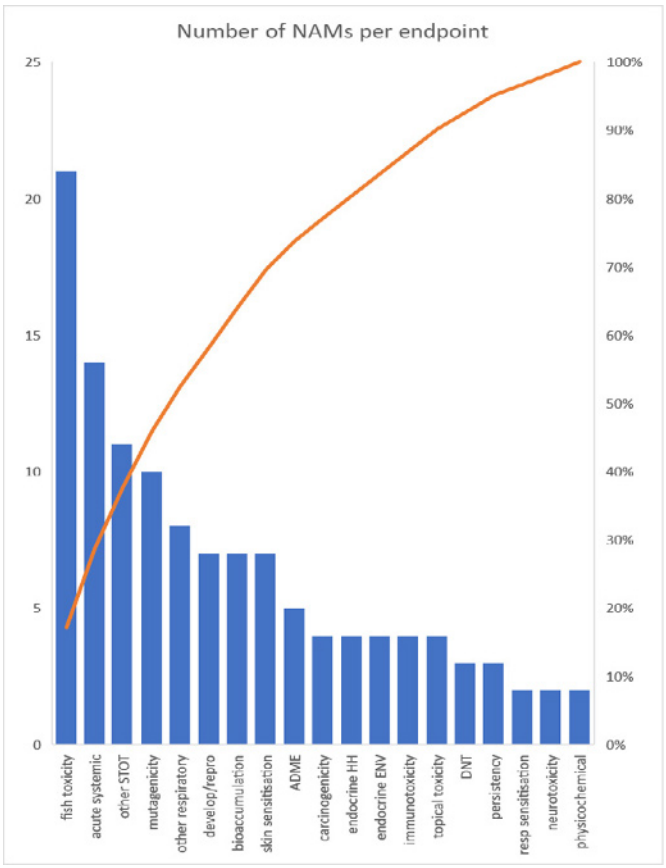

Figure 4: Results of a JRC survey on the regulatory use of NAMs (80 responses).

In addition to giving an indication of the state-of-play in the use of NAMs, respondents also provided information on costs of testing, some of which were used in an Impact Assessment of the costs and benefits of the different options. Based on the findings of the Impact Assessment, the Commission services are working towards a preferred option.

At the same time, in response to a further action under the Chemicals Strategy for Sustainability, and based on a separate Impact Assessment, the Commission is aiming to develop a preferred option for identifying the endocrine disrupting properties of chemicals. The information generated according to these requirements will be the basis for applying the criteria for hazard classes for endocrine disrupting chemicals to be introduced into the CLP Regulation (see *section 4.4.1*).

As next steps, preferred options for extending the REACH information requirements, including those for endocrine disrupting properties, will be outlined in a Staff Working Document, and will form the basis of a legal proposal by the Commission, expected to be adopted in Q4 2023.

Box 5

Highlights from the OECD Working Party on Hazard Assessment 2022

The 6th meeting of the OECD Working Party on Hazard Assessment (WPHA) was held as a hybrid meeting on 22 to 24 June 2022. Topics included country updates, progress on OECD-related IT Tools (IUCLID, eChemPortal, QSAR Toolbox, OHTs), an update on ECHA's Robust Study Summaries project, the IATA Case Studies Project (endorsement of Case Studies and Considerations Document from 8th Review Cycle), and finalisation of the Occupational Biomonitoring Guidance Document. The latter document provides recommendations on a limited number of approaches for deriving occupational biomonitoring levels, a system for assessing confidence in the values, and linking validated effects biomarkers to AOPs to improve interpretation. A follow-up project on AOPs development for effect biomarkers will also consider environmental biomonitoring. Progress on the QSAR Assessment Framework project, led by Italy, was also presented. This project, to which EURL ECVAM is contributing, is carrying out a targeted update of the 2007 QSAR Validation Guidance Document, with an emphasis on the identification and characterisation of uncertainties in QSAR predictions.

A new project to develop an application reporting module (ARM) for 'omics, for the sake of uniformity. to support chemical grouping, led by the UK, was added to the Hazard Assessment Programme of Work in Q2 2022. This project was proposed following the 2021 WPHA/EAGMST Joint Session on the Omics Reporting Frameworks, and the identified need for development of tools and guidance for using 'omics, for the sake of uniformity. to support chemical grouping (see *section 4.5.3*). This project will also feed into the update to the OECD Guidance Document 194 on Chemical Grouping that is underway.

The JRC (EURL ECVAM) proposed a new project to develop a Guidance Document to Improve the Use of Academic Data in Regulatory Assessments (see also *section 4.5.6*). The proposal was subsequently adopted by written procedure.

▸▸ *OECD IATA webpage: https://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm*

4.3 EPAA project on non-animal science in regulatory decisions for chemical safety in the EU

The European Partnership for Alternative Approaches to Animal Testing (EPAA) is a public-private collaboration between the European Commission, EU Agencies (ECHA, EFSA and EMA) and industry stakeholders from eight business sectors to progress the replacement, reduction and refinement (3Rs) of animal use for meeting regulatory requirements.

In addition to running a number of projects, the EPAA seeks to promote the 3Rs through international collaboration, knowledge-sharing and stakeholder dialogue including the valuable input received from the mirror group.

EURL ECVAM co-chairs the project platform and also a number of the individual projects. This year several projects are near to completion and are currently in the dissemination phase:

- Identification of clinical signs predicting mortality in acute oral toxicity testing;
- Collaborative study for the validation of cell line assays for in-process toxicity and antigenicity of *Clostridium septicum* vaccine antigens;
- Optimal duration studies to assess the safety of monoclonal antibodies;
- Physiologically-based kinetic modelling for safety assessment.

The remaining projects are continuing to progress. These include:

- Replacement of *in vivo* Rabies potency test by an *in vitro* method;
- Use of NAMs for skin sensitisation user forum;
- Harmonisation of 3Rs in biologicals;
- Prediction of carcinogenic potential of agrochemicals in humans using mechanistic information to waive the two-year bioassay;
- Project on "Non-animal science in regulatory decisions for chemical safety in the EU", for which more information is reported in *Box 6*.

Finally, EPAA is seeking to strengthen its networks by broadening its collaborations to include the ASPIS cluster and the new PARC consortium and creating more fora for scientific exchange between industry and regulatory NAM users. For instance, it recently ran two "Partners Fora" events on comparing how exposure information and approaches are used across the partnership and identifying opportunities for greater harmonisation, knowledge-sharing and investment.

This year's EPAA annual conference focused on "Accelerating the Transition to Animal-Free, Sustainable Innovation". As every year, it also aimed at presenting EPAA's achievements, as well as the 2022 Refinement Prize. EURL ECVAM gave an update on the activity exploring the possible introduction of NAMs into the REACH requirements (see *section 4.2*).

▸▸ *EPAA annual report: annual-report-2022-web (3).pdf (europa.eu)*

▸▸ *EPAA annual conference: https://single-market-economy.ec.europa.eu/events/epaa-annual-conference-2022-accelerating-transition-animal-free-sustainable-innovation-2022-11-15*

Box 6

EPAA project on non-animal science in regulatory decisions for chemical safety in the EU

The EPAA project on opportunities to use non-animal science in regulatory decisions for chemical safety in the EU builds on the actual experience of EPAA partners in the use of NAMs for decision-making and on the exchange of this experience between the industry sectors and Commission partners (see *section 4.3*).

The first exchange of information happened during the 'deep-dive' workshop on NAMs (November 2021). The report was published in September 2022 (Westmoreland *et al.*, 2022) and the highlights were presented in June 2022 at the EU ONE conference in Brussels. An OpEd Article, published in Euractiv (Pietikäinen, 2022) further promotes the outcome of this workshop.

Since the workshop, two working groups (WG) have been established to progress the NAM-related activities. WG1 is working on the regulatory frameworks and further to a first meeting in June 2022, three topics were proposed for follow up:

- to examine exposure-based approaches in the context of REACH, building on the concept of "classification of exposures" (based on Ball *et al.*, 2022).
- to survey existing weight of evidence approaches and evaluate their potential use to characterise chemical hazards, and
- to investigate a tiered approach as an alternative classification system for risk management/classification and labelling without using animal data.

A scientific working session held on 5 to 6 December 2022 at the JRC in Ispra elaborated further on future WG1 activities around these topics Follow-up meetings will be organised in 2023 to explore them more. WG2 is progressing the implementation of the *NAMs Users Forum* and held a first virtual planning meeting in November 2022 for a User Forum workshop in 2023.

The project steering team plans to instigate an exchange forum with other relevant projects such as PARC and ASPIS to share the EPAA NAMs work.

▸▸ *Sirpa Pietikäinen (2022) https://www.euractiv.com/section/health-consumers/opinion/accelerating-uptake-of-non-animal-safety-science-into-european-chemical-legislation/*

▸▸ *Poster Gallery | ONE Conference 2022 (one2022.eu): https://www.one2022.eu/posters/gallery?field_session_category_target_id=All&field_assigned_session_title=All&combine=westmoreland.*

Participants of the Scientific Working Session of the EPAA project on the Use of NAMs in Regulatory Frameworks at the JRC.

4.4 Classification and Labelling

4.4.1 Inclusion of endocrine disruptors in the CLP Regulation

Work on hazard classes for endocrine disruptors to be introduced into the CLP Regulation ((EC) No. 1272/2008) was progressed in 2022 with the conduct of an impact assessment. This led to the release of a draft proposal for public consultation on 20 September[14].

The endocrine disruptor hazard classes are based on the IPCS/WHO definition, building on the existing criteria for pesticides and biocides. It is proposed to have categories for known or presumed endocrine disruptors (category 1), as well as suspected endocrine disruptors (category 2). The criteria state that classification in categories 1 or 2 shall be largely based on evidence from human or animal data, or from both human and animal data. The basis for classification shall be the criteria and a weight of evidence determination of each of the criteria, where studies performed with adequately validated alternative test systems (*in vitro*, *in silico* studies) predictive of adverse effects in humans or animals are listed as relevant scientific data.

The value of introduction into the CLP regulation means that it can be applied across all sector-specific legislation, which supports the one substance, one assessment approach. With this in mind, it was decided to have separate hazard classes for human health and the environment to facilitate their implementation in specific pieces of legislation. The proposal to update the CLP regulation also contains hazard classes proposed for PBT, vPvB, PMT and vPvM[15] substances.

The European Commission adopted the proposal (delegated act) on 19 December 2022. It is expected to enter into force early next year, after scrutiny by the European Parliament and Council[16]. The new hazard classes were also put forward to the UN's Globally Harmonised System of Classification and Labelling of Chemicals (GHS) at the GHS Sub-Committee meeting on 7 to 9 December 2022, where it was agreed that the EU will chair a new UN informal working group to explore the development of global criteria for the newly adopted hazard classes[17].

4.4.2 Revision of the GHS chapter on skin sensitisation

The work conducted by the Informal Working Group on the use of Non-Animal Test Methods (NATM) for classification of health hazards in the biennium 2021-2022 focussed on the revision of Chapter 3.4 of the UN's GHS to fully incorporate non-animal testing methods for skin sensitisation. EURL ECVAM is contributing to the work by providing scientific support to the project leads, the Netherlands and the UK. The proposal is currently limited to the criteria for the classification of substances. Amendments to the classification of mixtures is still under discussion and a formal proposal is foreseen to be submitted to the Sub-Committee early in the 2023-2024 biennium. The resulting text will be included in the 10th revision of the GHS to be published in 2023.

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14 *https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13578-Introducing-new-hazard-classes-CLP-revision_en*

15 PBT – persistent, bioaccumulative and toxic; vPvB - very persistent, very bioaccumulative; PMT - persistent, mobile and toxic; vPvM - very persistent, very mobile

16 *https://ec.europa.eu/commission/presscorner/detail/en/IP_22_7775*

17 *https://unece.org/info/events/event/368936*

In the revised chapter (ST/SG/AC.10/C.4/2022/14)[18] the evaluation of available information follows a tiered approach. Tier 1 classification is based on human data, standard animal data, defined approaches or stand-alone *in chemico/in vitro* methods. In Tier 2, classification is based on inconclusive data from Tier 1, non-standalone *in vitro/in chemico* methods, or non-test methods. In Tier 3, classification is based on an overall weight-of-evidence, including additional indicators.

Within each Tier, a weight of evidence assessment can also be applied where the available information gives inconsistent and/or conflicting results.

The principle of test method neutrality described in paragraph 1.3.2.4.3 of the GHS was maintained and several validated methods/approaches other than OECD test guidelines are mentioned in the revised chapter as being potentially useful for classification purposes.

In addition, the IWG NATM is developing background guidance on the interpretation/use of *in vivo* (human and animal) and non-animal (*in chemico/in vitro* methods and non-standard methods) data, which is expected to be finalised early in the 2023-2024 biennium.

4.4.3 Revision of the GHS classification criteria for germ cell mutagenicity

The JRC is leading an Informal Working Group that aims at revising the GHS germ cell mutagenicity chapter 3.5. Although this activity was initially proposed to clarify the classification criteria for germ cell mutagenicity in category 1B, the GHS subcommittee subsequently agreed to extend the revision to criteria for classification in category 2 and category 1A.

The current criteria in category 1B have led to diverging opinions between experts during implementation, in particular, the requirement for "demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells". In fact, data proving the substance's molecular interaction with germ cell DNA are so far only occasionally available. Therefore, this wording is prone to different interpretations. To assist this scientific discussion, the Health and Environmental Science Institute's (HESI) Genetic Toxicology Technical Committee (GTTC) is collecting and analysing existing data with the aim of providing their opinion on what positive endpoints in somatic tissues and/or level of exposure in gonads would allow classification and labelling as a germ cell mutagen without germ cell testing.

The Informal Working Group has so far revised the terminology related to germ cell mutagenicity and updated the list of methods in the main chapter. It also initiated the discussion on revisions of the criteria of germ cell mutagenicity, and this will continue in the next biennium 2023-2024.

▸▸ *Informal document: https://unece.org/sites/default/files/2022-11/UN-SCEGHS-43-INF26e.pdf*

18 ST/SG/AC.10/C.4/2022/14 – Revision of Chapter 3.4 to fully incorporate non-animal testing methods for skin sensitisation (UK, the Netherlands): *https://unece.org/transport/documents/2022/09/working-documents/revision-chapter-34-fully-incorporate-non-animal*

4.5 Data and knowledge management

4.5.1 AOP Knowledge Base

The Adverse Outcome Pathway (AOP) Framework, which is steered by the OECD Extended Advisory Group for Molecular Screening and Toxicogenomics (EAGMST), aims at mechanistically describing toxicity as a series of Key Events that lead from an organism's first molecular interaction with a stressor towards an adverse outcome like cancer, reproductive problems, or death. The framework's central information and communication technology (ICT) repository, the AOP knowledge base (AOP-KB), is managed by the EAGMST AOP-KB subgroup with support from the Society for the Advancement of AOPs (SAAOP). This subgroup is co-chaired by EURL ECVAM, and its members include staff from the United States Environmental Protection Agency (US EPA), the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), Environment and Climate Change Canada (ECCC), the Organisation for Economic Cooperation and Development (OECD), the US Army Engineer Research and Development Center (ERDC), RTI International, Maastricht University, University Paris Cité, and others.

The AOP-KB is the central hub for real life application of the AOP Framework. AOP authors draft, finalise and publish their AOPs in the KB, reviewers apply their comments here, and knowledge consumers use the contents of the KB to find the AOPs or AOP elements they need.

Within the AOP-KB architecture (see *Figure 5*), the AOP-Wiki[19] is the central web-based application for capturing, disseminating and reviewing AOP knowledge, both for AOPs on the official OECD work plan and AOPs spontaneously suggested and inserted by the scientific community.

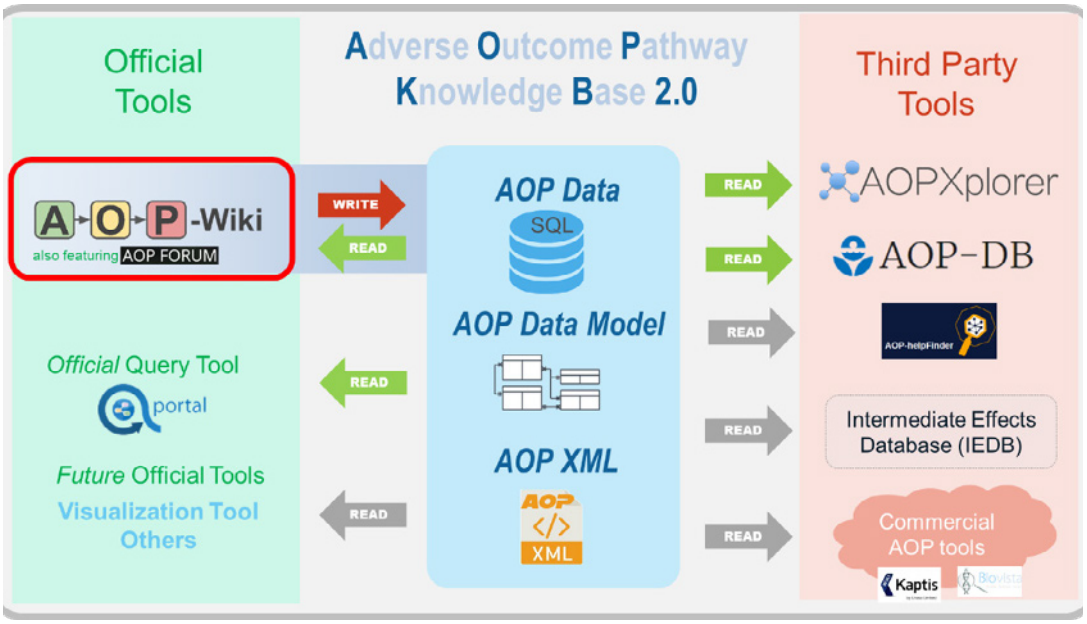

Figure 5: Modules and third party tools of the AOP Knowledge Base.

19 *https://aopwiki.org/*

AOP-Wiki release 2.5, launched on 16 July 2022, came with a series of added functionalities, provides better access to background information, and rethinks the role of stressors:

1. Improved and seamless integration of OECD AOP guidance into the Wiki: The official "AOP Developers' Handbook"[20] is now an integral part of the AOP-Wiki, and context sensitive help is never far away. It is always possible to check under which version of the guidance a particular version of an AOP was developed, which is essential when endorsement and validity of an AOP are discussed.
2. Information about available AOP(-Wiki) training material is at the users' fingertips: A new AOP Training page provides a summary of the online training options with links to the appropriate material.
3. The AOP-Wiki has opened its doors to third party tools: As the Wiki cannot possibly cover all functionalities that the stakeholder community might request, a series of pilot collaborations with outside software providers was initiated. Connections to their systems are now integrated into the AOP-Wiki: Novel ways of graphical AOP depictions, and support in identifying literature that strengthens the evidence behind AOP elements are now provided by third party tools. Based on these pilot experiences, more possibilities for such collaborations will be identified in the future.
4. The AOP-Wiki now reflects the fact that the AOP Framework is "stressor-agnostic": AOPs are triggered by the interaction of a "stressor" (e.g. chemical, nanomaterial, radiation, virus) with the organism on molecular level, leading to the Molecular Initiating Event (MIE). The scope of the AOP framework starts with the MIE and does not explicitly include the stressor - in other words, AOPs are "stressor-agnostic". However, in the AOP-Wiki, it is possible to identify any stressors that were used when the AOP evidence was collected. By emphasising the fact that these stressors are just "prototypical" examples, the false impression that the Wiki provides any kind of risk assessment concerning them is now avoided.
5. More structured information about the strategy with which an AOP was authored: Transparency about the way an AOP was assembled is of utmost importance. Stakeholders will trust, and ultimately apply AOP knowledge in a regulatory context, only when they can fully comprehend why, how and by whom an AOP was put together. This need for traceability is now implemented with dedicated "strategy fields" in the Wiki, in which AOP authors can provide information about their motivations when working on the AOP.
6. More structured information about Modulating Factors: Modulating factors are extrinsic or intrinsic variables that, while not a direct causal element of an AOP, influence the response to the perturbation(s) represented in the AOP. They can be factors that convey greater or lesser sensitivity, can result in more, or less, severe effects, can alter time to effect(s) etc. In the new AOP-Wiki, information on modulating factors is now highlighted in tabular form allowing authors to give a precise overview of how, when and to what extent an AOP is influenced by such factors.
7. New features improve the user experience: Tables (AOPs, KEs, etc.) can now be sorted and filtered, which helps users to always have control of the aspects they want to see in the AOP-Wiki at any given moment.

20 *https://aopwiki.org/handbooks/3*

8. An improved licensing model for AOP-Wiki content helps authors to protect their content while they are in the process of peer-reviewed publication.

Earlier, EURL ECVAM had commissioned an "AOP Framework Study", the results of which became available as a Science for Policy report in 2021 (Carusi *et al.*, 2021) The study strongly indicated that in the chemicals sector, there is a trust crisis between the regulators and the regulated community, and so the AOP-Wiki was used as one means to remedy this gap. Among the new features mentioned above, feature 5 was a direct reaction to the report.

Among the features foreseen for later (2023 to 2024), many are based on the report's findings, like better integration of test method information in the Wiki (see *section 4.5.2*), better traceability of who exactly contributed which element to a particular AOP, and improved AOP visualisation. Based on the new strategy areas, systematic approaches will be incorporated into the AOP development process. For example, the role of ontologies when naming AOP elements will be strengthened in order to better link the AOP Framework to neighbouring domains.

The AOP-Wiki currently features 390 AOPs, and it has 800 registered users, 280 of which with AOP authoring privileges. These numbers are constantly increasing, and the AOP-Wiki's scalability allows for significant growth in the future.

Link to the AOP-Wiki: https://aopwiki.org/

4.5.2 Methods2AOP

One of the fundamental rules in the AOP Framework is that AOP Key Events must be measurable and detectable in order to be accepted. This is also the reason why there is a free text section "*How it is measured or detected*" on each KE page, where AOP authors can give various methods-related information (see *Figure 6*).

Figure 6: Test methods are described in unstructured free text in the current AOP-Wiki.

However, this loose and verbal description does not reflect the importance of the link between a specific key event and the test method(s) it can be measured with (and the other way around). In addition, the role of the "stressors" as triggers of key events was downplayed in the recent AOP-Wiki version 2.5 to that of "prototypical stressors", i.e. ones that e.g. a test method developer can use as positive control.

This means that the proper linkage between possible real-life stressors (beyond prototypical stressors) that can trigger a KE and that particular KE must be established via the test method:

- A stressor is tested using a certain test method.
- In the AOP-Wiki, this test method is stored and earmarked as relevant for a certain KE.
- Thereby the link between the stressor and the KE is established: Depending on the result of the test. the stressor is either implicated in the KE or not.

This will result in the desired situation illustrated in *Figure 7*.

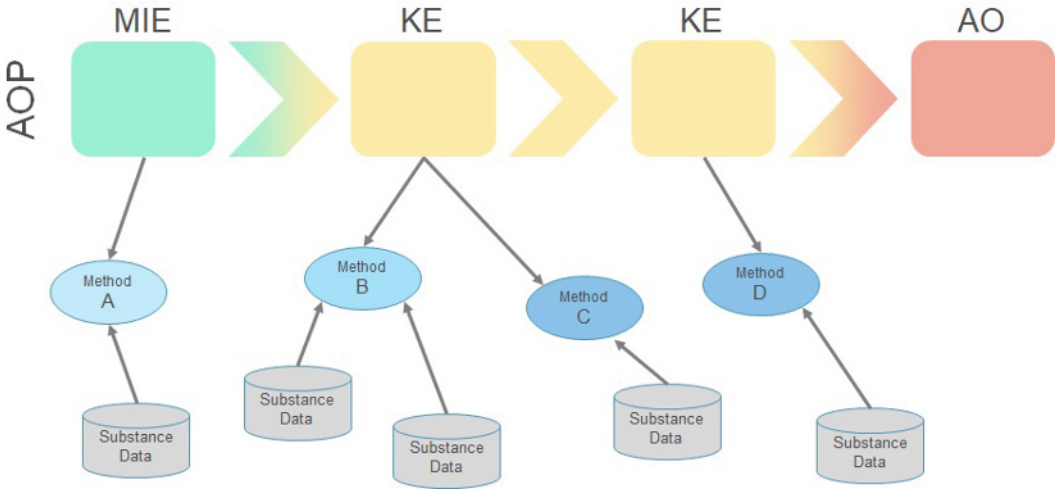

Figure 7: Desired outcome of the Methods2AOP initiative: Test method descriptions become more structured.

The management of test methods in the AOP-Wiki must therefore be enhanced, and the Methods2AOP initiative will explore ways in which this can be achieved.

The initiative, which was started in 2022, is a collaboration between European (currently EURL ECVAM) and North American (US EPA, Environment and Climate Change Canada, NIH and others) organisations, and is facilitated by EURL ECVAM.

The goal of the Methods2AOP initiative is to deliver:

- a series of actionable test method related recommendations (target audience: OECD EAGMST, and other affected stakeholders)
- a collection of implementable ICT requirements (target audience: OECD EAGMST AOP-KB subgroup)

The initiative is ongoing and will produce tangible results in 2023 and beyond.

4.5.3 Transcriptomics and metabolomics reporting framework

Increased transparency in the reporting of 'omics data is a crucial step to improve their use in regulatory toxicology.

In the context of a project launched by the EAGMST, an international team of experts, including representatives from EURL ECVAM, developed the harmonised "Transcriptomics reporting framework" and "Metabolomics reporting framework".

After testing the framework in several relevant scenarios, the final version of the reporting templates and supporting guidance for completing the templates has been published.

The frameworks focused on describing the generation, processing and analysis of 'omics data, without linking to any specific regulatory application.

To extend the OECD 'omics Reporting Frameworks, EURL ECVAM is now involved, with other experts, in a new OECD project, aimed at developing the Chemical Grouping (CG) Application Reporting Module (ARM) to advise on how to report molecular mechanistic data for grouping chemicals based on mode of action similarity (see also *Box 5*). In fact, it has been shown that the classification of structurally-similar substances in chemical categories can be supported by experimental evidence describing similar mechanisms of toxicity (i.e. mode of action similarity).

CG-ARM will focus on the use of metabolomics and transcriptomics to generate mechanistic data, although the reporting framework will aim to be compatible with all types of 'omics data and, more broadly, panels of molecular biomarkers.

4.5.4 Endocrine Active Substances Information System (EASIS)

The JRC launched a new version of the Endocrine Active Substances Information System (EASIS) this year in support to the Chemicals Strategy for Sustainability (EC, 2020), a key element of the EU's Green Deal.

This new tool provides information on endocrine active properties of chemical substances.

These chemicals can interact or interfere with our hormonal (endocrine) system that regulates and controls the release of hormones in our body. Its disruption can lead to hormone-related cancers.

EASIS[21] is a web-based application that gives access to studies on chemicals with endocrine active properties. It will allow collection of information on endocrine active substances from peer-reviewed studies and databases which are often hard to compare because they are stored in different formats. EASIS is based on the OECD harmonised template, which ensures compatibility with other international data collection undertakings.

These data can be freely used by authorities in the assessment of chemicals suspected to be endocrine disruptors and by scientists to understand more about how chemicals can interfere with the endocrine system of living organisms. EASIS can be used for data retrieval or as a data repository to support screening and assessment of potential endocrine disruptors.

EASIS is fully supporting the Chemicals Strategy for Sustainability launched by the EC in 2020, and more specifically the move to more efficient "One Substance One Assessment" processes and the better use of scientific data in regulatory assessments.

21 *https://easis.jrc.ec.europa.eu/*

The new EASIS was officially launched at the Commission's Forum on Endocrine Disruptors in May 2022, with a hands-on opportunity for attendees to explore the new features and content in the second part of the Forum held in September (see *Box 7* and *Figure 8*).

Figure 8: Launch of EASIS at the 4th Forum on Endocrine Disruptors.

Box 7

Highlights from the 4th Annual Forum on Endocrine Disruptors 2022

The European Commission's Communication of 7 November 2018, "Towards a comprehensive European Union framework on endocrine disruptors", committed the Commission to organising an Annual Forum on Endocrine Disruptors, to bring together all interested parties to exchange knowledge, identify challenges and build synergies.

The 4th Annual Forum, co-organised by DG ENV and ANSES (the French National Agency for Food, Environmental and Occupational Health and Safety), was held in two parts. The first part was held in Paris on 12 May 2022 as part of the French presidency's ministerial conference on chemical products, where the Deputy Director General of DG ENV, Patrick Child, gave an update on the regulatory activities underway to improve identification of endocrine disruptors (including launching EASIS). This was followed by a roundtable on Member States' activities to control endocrine disrupting chemicals (EDCs), including awareness raising campaigns on the risks of exposure to EDCs.

The second part, held in Brussels on 21 to 22 September 2022, had a more scientific flavour. The Commissioner for Environment, Oceans and Fisheries (Virginijus Sinkevicius) together with the General Director of ANSES (Matthieu Schuler) opened the proceedings.

The Forum was organised in four sessions with panel discussions and Q&A:

It started with updates from DG ENV on the progress since May on regulatory activities relevant to EDCs, specifically the expansion of the REACH annexes to include data requirements to facilitate ED identification; the application of the generic risk approach to EDs in consumer products and professional use products; and the proposal for ED hazard classes (see *section 4.4.1*). DG SANTE described the progress in evaluating endocrine disrupting properties of biocide and pesticide active substances. Feedback on the outcome of the ED session at the EU ONE conference held in Brussels in June 2022 was provided by EFSA, where the take home message was to focus on mechanism-based approaches to extrapolate between species, specifically highlighting the need to

do so with respect to extrapolation between human health assessment and eco-toxicological assessment.

This was followed by a session entitled 'From bench to validated test guidelines' raising the importance of validation in demonstrating the performance of a method with respect to reliability and relevance in the process of developing a method for regulatory use. Work carried out under a DG ENV–funded project by the Universities of Heidelberg, Antwerp and the Danish Technical University was presented, demonstrating the feasibility of introducing thyroid endpoints into a fish assay at the same time as merging two existing OECD test guidelines in fish (TG 229 and 234).

The next step towards an OECD TG will require additional validation, which was the cue for a lively panel discussion. The panel agreed that the validation process is essential to confirm reliability and relevance of a test method and the time and resources needed for this should be acknowledged and funded sufficiently.

There was a specific session focusing on thyroid system disrupting chemicals and the lack of reliable methods to investigate thyroid-disrupting modes of action. The JRC presented the progress towards the finalisation of EURL ECVAM's multi-laboratory validation study on a wide range of *in vitro* methods being conducted to address this gap (see *section 3.2.1* & *4.1.5*).

The session also included presentations on the ongoing research to develop additional methods and testing strategies to identify thyroid system disruptors under the H2020 thyroid-focused EURION projects, ATHENA and SCREENED. The need for reliable methods for the measurement of thyroid hormones (T3 and T4) in serum and in tissues such as the brain was highlighted as a particular concern, which is being addressed by a EURION cross-cluster working group.

- *French Ministerial Conference on Chemical Products, 11 & 12 May, 2022: https://wayback.archive-it.org/12090/20221120144656/https://presidence-francaise.consilium.europa.eu/en/news/press-release-ministerial-conference-on-chemical-products-better-protection-of-health-and-the-environment/*
- *4th ED Forum: https://environment.ec.europa.eu/events/fourth-annual-forum-endocrine-disruptors-2022-09-21_en*
- *EU ONE conference: https://www.one2022.eu/programme/many-ways/endocrine-disruptors-exploring-present-challenges-and-future-developments*

4.5.5 EU Common data platform on chemicals, including IPCHEM

Chemical safety assessments are performed under various pieces of legislation, by various actors and at different points in time, creating potential inefficiencies and discrepancies in the assessments. The EC Chemicals Strategy for Sustainability (EC, 2020) therefore proposed to introduce a 'one substance, one assessment' approach aimed at improving the coordination and transparency of chemical safety assessments.

One element of the 'one substance, one assessment' approach is the sharing and reuse of data. The Commission will develop a common open data platform on chemicals to facilitate the sharing, access and re-use of information on chemicals coming from all sources. Data will be made available in appropriate formats and tools to ensure interoperability, i.e. IUCLID[22] for hazard data and IPCHEM[23] for chemical monitoring data.

IPCHEM, the Information Platform for Chemical Monitoring, is a single access point where EU authorities, national and regional authorities, and researchers can find and share information about where chemicals are found in the environment and at which concentrations. IPCHEM allows the user to discover, access and retrieve information on chemical concentrations throughout Europe

22 *https://echa.europa.eu/support/registration/creating-your-registration-dossier/what-is-iuclid-*

23 *https://ipchem.jrc.ec.europa.eu/_*

in air, water and soil, food and animal feed and in humans. As scientific and technical lead of the platform, the JRC has until now integrated data from more than 180 studies.

Several additional datasets are now publicly available in IPCHEM (*Figure 9*). Thanks to collaboration with Health Canada via the OECD, IPCHEM now hosts data of the Canadian Health Measures Survey in its Human Biomonitoring module. Through a collaboration with EFSA, recent data have been added to the food and feed module on pesticide residues, veterinary pharmaceutical residues and contaminants. The environmental module has also grown substantially, including updated data from European Environment Agency (EEA) on air and water quality, as well as further data on emerging pollutants from the Environmental Monitoring of POllutants DATabase (EMPODAT) of the Norman Network[24].

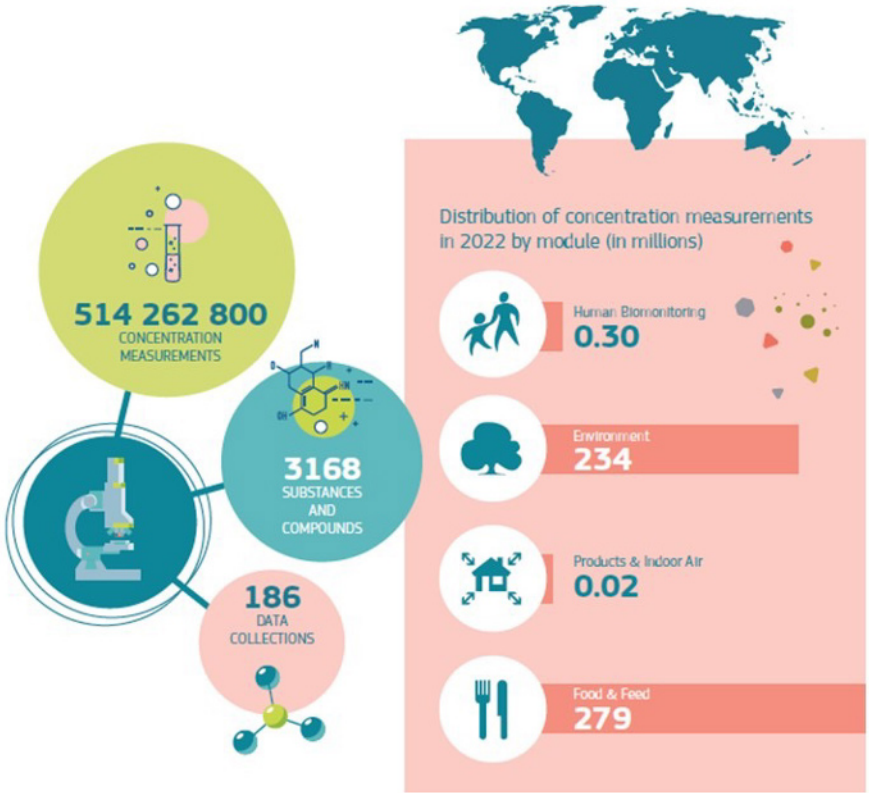

Figure 9: Overview of data integration status in IPCHEM in 2022.

4.5.6 Improving the use of academic data in risk assessment

Building on the commitment to improve the use of academic data in regulatory assessments (EC, 2020), the JRC / EURL ECVAM proposed a new project to the OECD Working Party for Hazard Assessment (see also *Box 5*). An expert group with 28 representatives of member countries and affiliated institutions was created in October 2022 to scope and support the project.

24 *https://www.norman-network.com/nds/empodat/*

Academic data feed into assessment processes, among other data sources. Although commonly not conducted according to standardised test guidelines, they may be relevant and complementary to more standardised information requirements. The challenge of screening for relevant and reliable data from scientific sources is common across jurisdictions. However, regulatory bodies have taken different approaches to the challenge. Differences exist also within the EU, between different policy areas. The situation points to potential efficiency gains.

In support of this initiative, the JRC hosted on 25 to 26 October 2022 the workshop "Improving the use of academic data in regulatory assessments" (see *Figure 10*). The event brought together 35 experts from the European Commission, EU-funded research consortia (PARC, ASPIS, EURION), ECHA, EFSA, and other international regulatory bodies (Canada, US).

The discussion provided a shared understanding of the different perspectives and outlined possible solutions to improve current practice. This may include:

- guidance on setting minimum quality and reporting requirements for regulatory consideration of academic data,
- a search guide to help assessors to find, access and evaluate data from non-standard peer reviewed studies,
- harmonisation of data collection activities within scientific or regulatory systematic reviews.

Figure 10: Participants at the JRC workshop on improving the use of academic data in regulatory assessments.

5. Alternatives in research and education

With more than six million of animal uses taking place for research purposes in 2019 in the EU and Norway, research is by far the main sector using animals for scientific purposes (see *Figure 11*).

Yet, implementing the Three Rs in research represents a challenging task, requiring specific approaches. According to Directive EU/2010/63, scientific projects must be evaluated, taking into account the 3Rs before being approved[25]. Replacement remains a particularly difficult exercise given the nature of scientific experimentation itself, i.e. developing new methods, testing hypothesis and confronting new ideas.

Changing mind-sets to include more often non-animal approaches in basic, applied and translational research is essential to succeed in this direction.

25 *https://ec.europa.eu/environment/chemicals/lab_animals/alures_nts_en.htm*

In 2022, EURL ECVAM continued its work initiated during the past few years in biosciences with the promotion of knowledge sharing and cross-disciplinarity in biomedical research, the identification of existing non-animal models, the creation of additional resources for education and training, and the development of indicators to measure the uptake of non-animal methods in research[26].

26 *https://joint-research-centre.ec.europa.eu/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam_en*

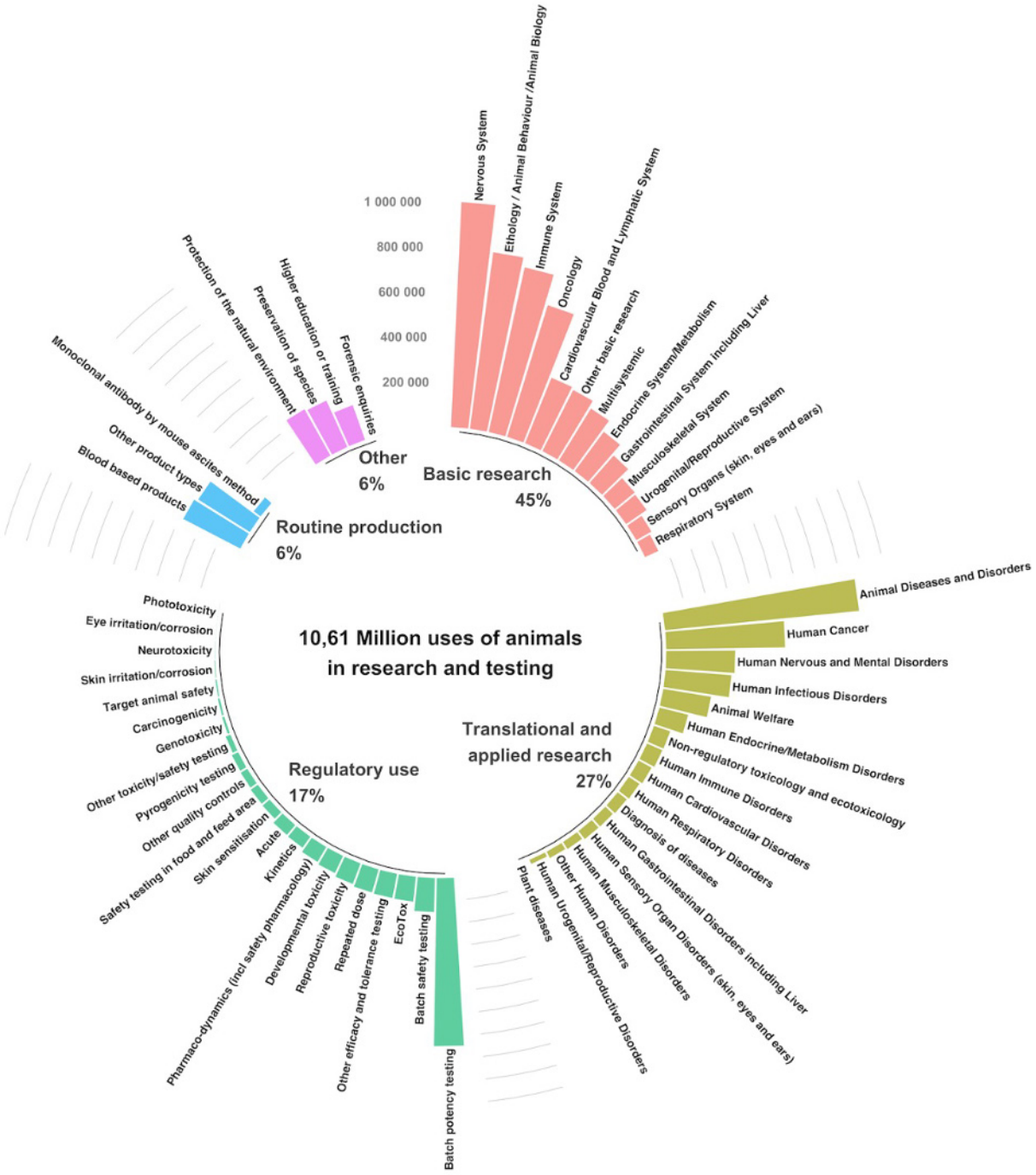

Figure 11: Statistics on the use of animals for scientific purposes for EU and Norway, including re-uses, in 2019.

5.1 Biomedical research

In 2022, EURL ECVAM achieved an important milestone with the publication of the remaining three reviews of advanced non-animal models in biomedical research. Looking forward, EURL ECVAM initiated projects to build on this comprehensive knowledge base with the aim to consolidate, disseminate and share the knowledge gathered with these reviews.

5.1.1 Review of advanced non-animal models in biomedical research

Three more reviews, on immunogenicity testing for advanced therapy medicinal products (ATMPs), cardiovascular disease and autoimmune disease, were published in 2022, thus closing the series of studies to review available and emerging non-animal models being used for research in seven disease areas[27] (see section 5.1 in Zuang *et al.*, 2021).

ATMPs are innovative therapies expected to reshape our approach towards several pathologies. Immunogenicity testing is an important step in the development of these therapies, since it aims to predict an adverse immune response of a patient prior to receiving a particular treatment. Results from immunogenicity testing carried out using animal models might be misleading because of fundamental biological differences between the immune system of different species. Therefore, EURL ECVAM carried out a review on the state-of-the-art of advanced non-animal models in use for immunogenicity testing and selected 88 models[28] that are described in Canals *et al.*, 2022. The majority of these models are *in vitro* techniques with a limited amount of *in silico* methods, and most of them focused on the investigation of cell therapy products.

This study highlighted that there is certainly room for improvement in several areas. These include: achieving a more reliable and scalable supply of immune cells, commonly based on voluntary donors; increasing the number of samples that can be tested in an immunogenicity experiment; and implementing more advanced measurement technologies such as 'omics to generate more information from a test. More innovative approaches could also be pursued through the incorporation of new technologies for high-throughput and high-content analysis. In addition, *in silico* models of the immune system should definitely be better exploited.

On World Heart Day that occurred on 29 September 2022, EURL ECVAM released another freely available knowledge base[29] describing 449 non-animal models used for cardiovascular disease research (Celi *et al.*, 2022). The most predominant models are based on computer simulation, underpinning *in silico* methods used to reproduce complex systems, including tissue/organ electrophysiology and tissue remodelling. These innovative models recapitulate well human physiology and functionality with the perspective of advancing research in the fight against cardiovascular diseases. As technology and computational power advance, *in silico* methods are gaining more interest and application in a clinical context, and are paving the way for personalised medicine.

27 *https://joint-research-centre.ec.europa.eu/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/biomedical-research_en*

28 *https://data.jrc.ec.europa.eu/dataset/c1c15110-2338-422e-a228-d3f8e19262c3*

29 *https://data.jrc.ec.europa.eu/dataset/20947a04-86ef-473f-8907-c658e4050c24*

Simple *in vitro* models (i.e. 2D) are very commonly used for effectively understanding and diagnosing disease and developing new therapies. However, rapid advances in the growing field of cardiovascular tissue engineering are making complex 3D constructs more readily available. OoC devices are emerging to meet the need for human-relevant models able to represent the more complex electrophysiological, biomechanical and pathological aspects of the human heart and vascular system.

Finally, since the prevalence of autoimmune disorders is increasing worldwide, and we still lack effective options to stop and/or reverse the progression of the majority of these diseases, EURL ECVAM performed a systematic review of non-animal models employed in this field. We identified 183 advanced models[30], the majority being based on the use of cells, particularly human primary cells. These models are described in Otero *et al.*, 2022. Specific models are preferentially exploited for certain types of conditions, such as in the case of *ex vivo* models like biopsies for skin diseases, and stem cells in the case of type 1 diabetes. EURL ECVAM is of the opinion that human-based alternative methods would represent more efficient options to elucidate disease mechanisms and discover potential drug targets.

5.1.2 European Parliament pilot project on the development of AI/ML approaches for biomedical models review updates

Given that the collection of models mentioned in the studies described under *section 5.1.1* covered the period until 2019, the effort should continue to identify relevant non-animal models developed since then.

On request of the European Parliament, EURL ECVAM launched a pilot study to develop an automated approach, based on Artificial Intelligence (AI) / Machine Learning (ML) approaches that collects and structures the NAMs in use for biomedical research. Using AI to mine the vast body of published literature will enable the creation and maintenance of up-to-date, fit-for-purpose knowledge sources collating NAMs applied to biomedical research, allowing the extension of already collected models both in time and in scope with the addition of new diseases categories.

By understanding and sharing information on successful NAMs in biomedical sciences, it is expected that the transition of the scientific community towards human biology-based methodologies will be encouraged, facilitated and potentially accelerated. In fact, the use of human biology-based models and methods is vital to improve the relevance of biomedical research, to enhance the likelihood that results will translate to patients and to accelerate the transfer of research results into clinical and public health practices. It is also expected that such dynamic databases would ensure long term sustainability to all other stakeholders such as Member States and competent authorities responsible for project evaluation or research project funding organisations.

5.1.3 CIAO – modelling the pathogenesis of COVID-19 using AOPs

The AOP framework provides a publicly accessible means for organising and reviewing biological knowledge across multiple levels when examining

30 *https://data.jrc.ec.europa.eu/dataset/700397b2-edd7-4ed6-86f7-fc1b164ed432*

adverse effects triggered by a stressor. While AOPs are widely acknowledged in chemical safety assessment and regulatory toxicology, they have the potential to be of great value also for biomedical research. The COVID-19 pandemic was a unique opportunity to introduce the biomedical community to the AOP framework.

As a hands-on initiative into that direction, EURL ECVAM has been facilitating an interdisciplinary crowdsourcing project, called CIAO (Modelling the pathogenesis of COVID-19 using AOPs)[31]. CIAO is based on the assumption that AOPs can provide an integrative means for organising the abundant, fast evolving and dispersed knowledge on COVID-19 pathogenesis (see *Figure 12*).

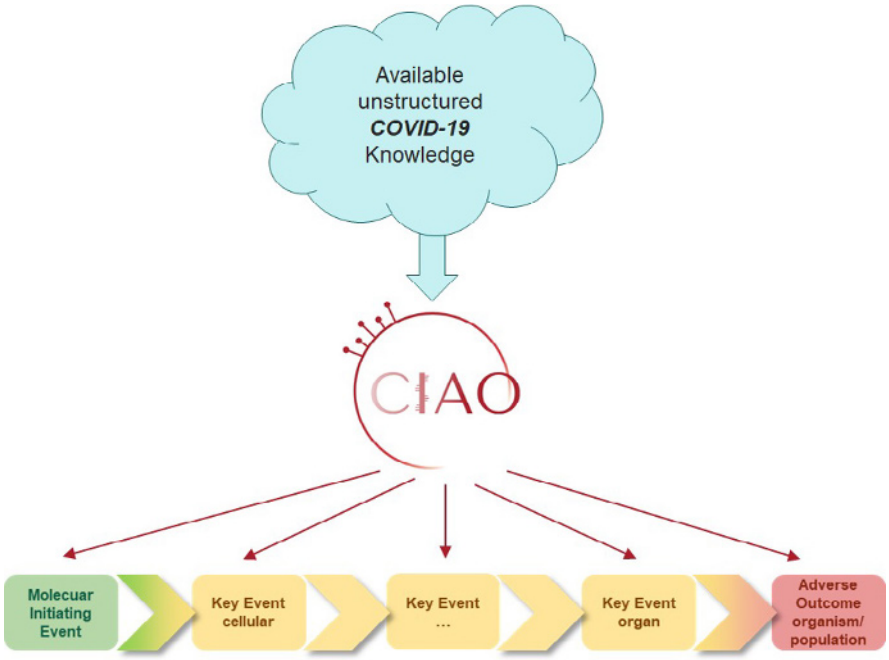

Figure 12: CIAO workflow exploiting the AOP framework.

A mechanistic understanding of the disease permits the combination of data from *in vitro* models for virus characterisation with data from animal and human studies depicting the inflammatory response and various clinical outcomes. This AOP-based description of how the SARS-CoV-2 virus infects the body also helps to capture how various factors modulate the clinical outcomes, increasing our understanding of why some populations are more vulnerable than others. The modular aspect of AOPs also allows the development of a COVID-19-related AOP network where central biological key events, the interrelation between the outcomes and knowledge gaps become more evident.

As in the years before, in 2022 more than 70 scientists around the world were (and are still) participating in the CIAO project and develop AOPs (and publish peer-reviewed papers, see below), which model the COVID-19 disease process (Clerbaux *et al.*, 2022a; Clerbaux *et al.*, 2022b; Clerbaux *et al.*, 2022c; Clerbaux *et al.*, 2022d; Shahbaz *et al.*, 2022; Pistollato *et al.*, 2022).

31 *https://ciao-covid.net*

EURL ECVAM steers the project infrastructure (crowd management, knowledge collection, publication facilitation), and this task was successfully completed in 2022. EURL ECVAM will continue to support the ongoing CIAO work on structuring COVID-19 knowledge, and will also participate in identifying follow-up activities and funding opportunities.

A promising perspective for EURL ECVAM in 2023 and beyond will be to extrapolate the CIAO approach and related processes to other areas of science where the AOP framework can foster interdisciplinary systematic organisation of the knowledge (see *Figure 13*).

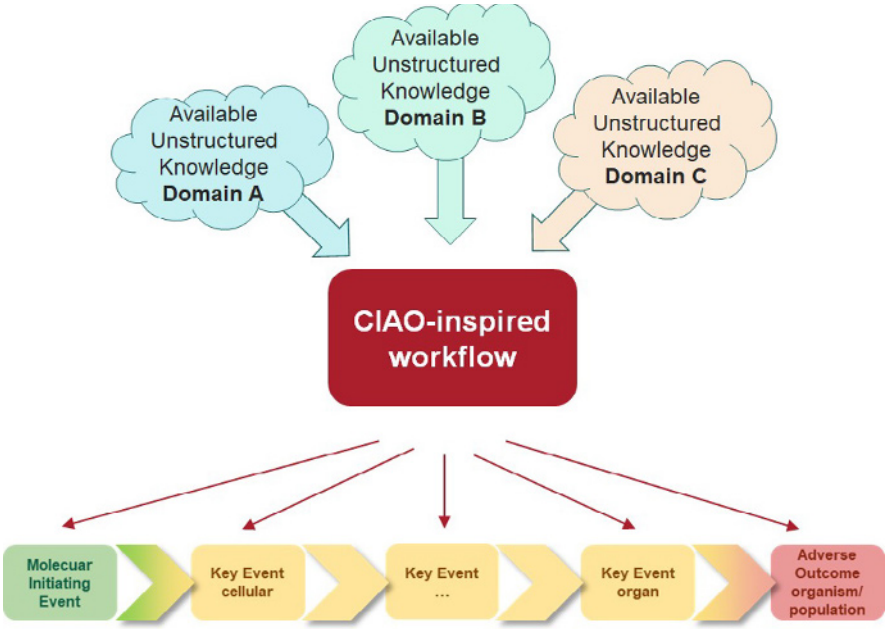

Figure 13: CIAO-inspired workflow exploiting the AOP framework.

Relevant candidate areas in public health are numerous, and 2023 will show which of them are the most promising.

In 2022, additional AOPs were developed and published in addition to the achievements of 2020 and 2021 (see *Table 3*).

Table 3: COVID-19 related AOPs developed within CIAO (OECD project 1.96)[32].

ID	Title	MIE	AO
Inflammatory and pulmonary outcomes			
468	*Binding of SARS-CoV-2 to ACE2 leads to acute respiratory distress (via cell death)*	Binding to ACE2	Hyperinflammation
430	*Binding of SARS-CoV-2 to ACE2 leads to viral infection proliferation*	Binding to ACE2	Viral infection, proliferated
392	*Decreased fibrinolysis and activated bradykinin system leading to hyperinflammation*	Fibrinolysis, decreased	Hyperinflammation

32 *https://www.oecd.org/chemicalsafety/testing/projects-adverse-outcome-pathways.htm*

ID	Title	MIE	AO
379	*Binding to ACE2 leading to thrombosis and disseminated intravascular coagulation*	Binding to ACE2	Thrombosis and Disseminated Intravascular Coagulation
412	*Endothelial cell dysfunction leading to thromboinflammation*	Endothelial cell dysfunction	Thromboinflammation
320	*Binding of SARS-CoV-2 to ACE2 receptor leading to acute respiratory distress associated mortality*	Binding to ACE2	Mortality
319	*Binding to ACE2 leading to lung fibrosis*	ACE2 dysregulation	Lung fibrosis
Neurological outcomes			
394	*SARS-CoV-2 infection of olfactory epithelium leading to impaired olfactory function (short-term anosmia)*	Binding to ACE2	Olfactory function, impaired (short-term anosmia)
374	*Binding of Sars-CoV-2 spike protein to ACE 2 receptors expressed on brain cells (neuronal and non-neuronal) leads to neuroinflammation resulting in encephalitis*	Binding to ACE2	Encephalitis
395	*Binding of Sars-CoV-2 spike protein to ACE 2 receptors expressed on pericytes leads to disseminated intravascular coagulation resulting in cerebrovascular disease (stroke)*	Binding to ACE2	Cerebrovascular disease (stroke)
Intestinal outcomes			
428	*Binding of S-protein to ACE2 in enterocytes induces ACE2 dysregulation leading to gut dysbiosis*	Binding to ACE2	Gut microbiota, alteration
422	*Binding of SARS-CoV-2 to ACE2 in enterocytes leads to intestinal barrier disruption*	Binding to ACE2	Intestinal barrier, disruption

5.1.4 Gauging the output and impact of biomedical research

EURL ECVAM continued its work initiated with the publication of a survey (2020) designed to retrospectively monitor the outputs and societal impact of EU-funded biomedical research conducted over the last 20 years, in the fields of Alzheimer's disease, breast cancer and prostate cancer. The survey was addressed to researchers who participated in projects funded under the EU framework programmes FP5, FP6, FP7 and H2020.

Following a round table organised with Directorate Generals for Research and Innovation (RTD) and for Health and Food Safety (SANTE), EURL ECVAM gathered the main findings of this analysis and proposed a set of priority actions that could be considered to help improve the translation of scientific

innovation of biomedical research into societal impact. The design of the research methodology can play an important role in determining translational impact of biomedical research, especially in the three disease fields under investigation (i.e., Alzheimer's disease, breast cancer and prostate cancer), as it can influence the way in which research problems are formulated and tackled. In particular, our analysis showed that research focused on human subjects and the generation and sharing of human relevant, large clinical data sets may have great potential to generate societal impact. In addition, cross-disciplinarity and better communication should be fostered to bridge the gap between scientific research and clinical practice. To this end, proactive collaboration among different EC DGs, Agencies and Member States, complementing different expertise and interests is important to match qualitative and quantitative information stemming from the impact building cycle, accelerate the development and the uptake of human-relevant methods, and incentivise progress particularly in the field of biomedical research.

To complete this work with a quantitative approach, EURL ECVAM also concluded an external study that started in 2020, designed to build indicators for capturing the societal impact of EU-funded projects in the same disease areas.

5.1.5 Methods and protocols in life sciences: building a roadmap to transparency and clarity in peer reviewed publications

On 28 to 29 June 2022, EURL ECVAM hosted a workshop entitled "Scientific Methods and Protocols: Roadmap to increase clarity in Life Sciences peer reviewed publications" at the JRC.

Researchers' access to detailed protocols especially in peer-reviewed publications is still limited. This lack of information affects the reproducibility and reliability of science and can be a constraint for scientific progress (EC, 2022); (EC, 2020). Open data has recently received a great deal of attention, but in reality, the usability of data is only ensured when the methods for generating it are clearly described as well.

The workshop brought together people working towards or concerned by the need to increase clarity in protocol descriptions. It has been agreed that scientific methods and protocols need to be detailed, clear, complete, transferable, reproducible, reliable, dynamic and open. However, progressing towards a more transparent, trustable and open reporting of methods requires significant investment from the different agents in the publication system, from researchers to publishers, the institutions in which they work and the funders that support them. A concerted community effort by all of these different agents is needed. The main actions identified include:

1. Increase awareness of the problem
2. Provide guidance on good methods and protocols reporting
3. Having better means and tools to publish and share protocols
4. Reward and support the time spent on good reporting
5. Invest on education.

The group has been working towards defining recommendations to support different key agents in the creation of a culture that embraces good method reporting. The final recommendations will be published during 2023 but the

ongoing work has already been presented at the December 2022 ASCCT/ESTIV webinar[33] and in an OECD WNT webinar (see *section 4.1.7*).

It is believed that this initiative can increase the quality and impact of scientific research but also the acceptance of new methods in the regulatory field. This is a way to promote non-guideline methods in support of the use of academic data for regulatory purposes, to establish more confidence in methods and give scientists the tools and methods to generate quality data for regulatory assessment

5.2 Education and training

5.2.1 Visit of students of the Karolinska Institute

The second-year international Masterclass of the Karolinska Institute from Stockholm, Sweden, made a study visit to EURL ECVAM on 19 May 2022 (*Figure 14*). All students made a two-minute pitch presenting their master thesis, and the colleagues from EURL ECVAM presented some of their activities and the progress of non-animal approaches both in biomedical research and in regulatory toxicology. The students also visited EURL ECVAM's *in vitro* facility including four main experimental setups used for NAMs, the High Throughput Screening robotic platforms, the High Content Imaging microscope, the Micro-Electrode Array platform, and an Organ-on-Chip (OoC) device.

Figure 14: Visit of second-year international Masterclass of the Karolinska Institute from Stockholm, Sweden.

33 *https://www.ascctox.org/webinar/102*, minute 23.30.

5.2.2 New educational resources

EURL ECVAM has developed open access teaching resources to support Three Rs education at primary, secondary school and university level. At school level, the aim is to support teachers in promoting the Three Rs and specifically replacement approaches, as an important science, technology, engineering and mathematics (STEM) topic and an inspiration to young people in their career choices. Through these resources, teachers can also alert their students to the value of good animal welfare and advance their critical thinking skills through discussion and debate of the many facets of animal use in science.

EURL ECVAM provides supporting resources for teachers who wish to bring the Three Rs into the classroom. The resources have been co-developed with teachers themselves, facilitated through European Schoolnet and their scientific platform, Scientix. The available resources which can be accessed from the dedicated Scientix page[34] are:

- Learning scenarios for primary school, secondary school (age 6-19 years);
- Eight podcasts of interviews with scientists talking about their work with replacement approaches;
- New resources including slides and games;
- Four career profiles of Three Rs professionals to support teachers giving career advice.

To complete the project, a workshop was organised in January 2023 with representatives from European education ministries to showcase our results and to highlight the importance of bringing the Three Rs into the classroom. The hope is that the Three Rs principle is further, and more permanently integrated into the syllabus.

At university level, a collection of learning scenarios from the expert's network has been reviewed and published in the JRC data catalogue[35]. This tool will be the basis for the implementation of a teaching programme on the EU academy platform.

5.2.3 Virtual reality and "Learn and Tell"

EURL ECVAM is developing an open access virtual reality teaching resource to support Three Rs education for students aged 14-19. The project is combined with the JRC Learn and Tell initiative, which allows valuable collaboration with students who will ultimately be the audience for this resource.

The goal is to provide a virtual reality experience which educates on important technologies used in science that do not require animals. The featured technologies used in the EURL ECVAM lab are the following:

- Cell imaging
- Microscopy
- High throughput screening
- *In chemico* screening (without cells)
- Organ-on-Chip
- Cell culturing
- Microelectrode array

34 *http://www.scientix.eu/projects/steam-partnerships/3rs#resources*

35 *https://data.jrc.ec.europa.eu/dataset/5803050b-bdc4-4032-bbda-f794a0fc58c0*

EURL ECVAM will collaborate with a small group of science students and their teachers from the European School gaining valuable input in a 'co-creation' initiative for this innovative tool. Along the way, the students will learn about and be inspired by state-of–the-art alternative technologies, various other lab activities and the equipment used. They will learn about the Three Rs and how EURL ECVAM is supporting the implementation of the legislation protecting lab animals and promoting the Three Rs principle. The hardware chosen should allow the widest accessibility to schools in terms of cost and ease of use, although the EURL ECVAM lab will also be available to explore in a web-based version.

As well as contributing to the development of the virtual reality experience, the students will be asked to sum up their knowledge, reinforce the learning and to 'tell' others what they have learned. For example, they could choose to do a virtual lab visit for other students and teachers through the headsets and large screen, which would inform, disseminate and encourage wider use of the system.

5.2.4 Participation in the FELASA congress and animal caretaker manual

In June 2022, EURL ECVAM organised and participated in three sessions at the 2022 congress of FELASA – Federation of Laboratory Animal Science Associations.

EURL ECVAM also helped to run the EC booth together with DG Environment, responsible for Directive 2010/63/EU on the protection of animals used for scientific purposes.

It was an excellent opportunity to present EURL ECVAM's work on Three Rs education and training (see *section 5.2.2*) and exchange thoughts and ideas with a community which bases its work on the use of animals and animal welfare, i.e. the refinement/reduction community. EURL ECVAM's booth material, talks and interactions were well received at this event.

EURL ECVAM's session "*Breaking the Deadlock by Drawing Innovation from Multidisciplinary Approaches*", aimed to address interdisciplinary approaches in the biosciences, namely the combination of all types of models in biomedical research to answer specific questions. The session was moderated by JRC colleagues together with a philosopher of science and was divided into two parts: i) a series of talks about activities that use different scientific models and cross-disciplinary approaches; ii) working together with participants to discuss points that can be common to different disciplines, i.e., how models are considered and chosen, why certain models are chosen and justified and whether cross-disciplinarity is an important factor when justifying the choice of models.

Exchange in this setting allowed understanding of the reality of why animals are used and replacement methods are often not used or not considered, and helped identify ways to tackle the hurdles and bottlenecks. Another take-home message from these sessions was that not all decisions are strictly scientific, and socio-economic aspects also play a role when deciding what models to use.

This was the second of a series of three workshops on cross-disciplinarity in biosciences. The experience with the different communities helped to understand the different possible dynamics and explore different strategies to share knowledge on non-animal methods as part of the life sciences models.

6. Conclusions

In 2022, EURL ECVAM continued to invest efforts at various levels to increase the acceptance and use of NAMs. This ranged from offering expertise and advice in EU-funded research projects, supporting an increased uptake of NAMs into regulatory frameworks to investing in targeted projects aimed at addressing the high number of animals used in basic and applied research.

Progress has been made on several fronts, however the promotion of NAMs needs to remain high on the political agenda with a collective investment and honest interest in progressing NAMs by all relevant actors in order to really achieve a break-through. Importantly, the acceptance and use of NAMs also require careful monitoring and appraisal by the Competent Authorities in EU Member States.

In 2021, the EP adopted a resolution[36] including concrete plans and actions to accelerate a transition to innovation without the use of animals in research, regulatory testing and education. The key message of that resolution is to draw up an EU-wide action plan to drive active phase-out of animal use in research and testing. After the first European Citizens' Initiative (ECI) "Stop Vivisection"[37] in 2015, a second ECI[38] collected again more than one million signatures in 2022. It calls on the EC to protect and strengthen the cosmetics animal testing ban by initiating a legislative change to achieve consumer, worker, and environmental protection for all cosmetics ingredients without testing on animals for any purpose at any time. It furthermore calls for a transformation of EU chemicals regulation to ensure human health and the environment are protected by adequately managing the risk posed by chemicals, without the addition of new animal testing requirements. Finally, it asks for a modernisation of science in the EU by committing to a legislative proposal plotting a roadmap to phase-out all animal testing in the EU before the end of the current legislative term.

The three major ambitions of the EC Chemicals Strategy for Sustainability, namely ensuring the highest levels of protection of human health and the environment, supporting innovation to reduce dependency on animal testing, and improving the quality and efficiency of chemical hazard and risk assessments can only be met if European legislation is ready to embrace modern safety science.

For example, for systemic health effects, the current CLP criteria are largely based on effects observed in animals with the information requirements under REACH tailored to meet the CLP criteria. This may guarantee legal certainty but impedes a smooth introduction of NAMs in regulation, since the latter do not provide the same information as animal (primarily rodent) studies. In fact, trying to fit new technologies into the mould of traditional older animal models hampers progress.

There are also different opinions over the value of information of animal tests versus NAMs, and this is exasperated by the apparent lack of trust between regulatory authorities and industry.

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36 *https://oeil.secure.europarl.europa.eu/oeil/popups/ficheprocedure.do?reference=2021/2784(RSP)&l=en*

37 *http://www.stopvivisection.eu/*

38 *https://europa.eu/citizens-initiative/initiatives/details/2021/000006_en*

In the framework of the EPAA, a unique public-private partnership promoting NAMs, EURL ECVAM collaborates on a Next Generation Risk Assessment (NGRA) framework. NGRA has been defined as an exposure-led, hypothesis-driven risk assessment approach that integrates NAMs to ensure safety without the use of animal testing (Thomas *et al.*, 2019; Baltazar *et al.*, 2020; Dent *et al.*, 2021). The overarching premise of NGRA is that if *in vitro* bioactivity is not seen in a suitable panel of NAMs at human-relevant concentrations, then the risk of adverse effects is low (Carmichael *et al.*, 2022; Paul Friedman *et al.*, 2020). In addition, EURL ECVAM is working on a vision for a future chemicals system in which a generic risk assessment is based on the combined use of exposure information and toxicological information from NAMs. The aims of this future option would be to achieve higher safety by having a basic level of knowledge about a higher number of substances while requiring less information for each substance and minimise animal testing with the eventual aim of complete replacement.

With the advancement of new technologies and models in bioscience developed by academia and industry, dialogue and knowledge sharing should span beyond the regulatory testing arena.

In the field of basic and applied research, which consumes most of the animals used for scientific purposes, EURL ECVAM is focusing on some specific activities where it can have impact. For example, EURL ECVAM promotes knowledge sharing on methods through better access to detailed protocols, in particular in peer-reviewed publications, and through publication of reviews and catalogues on advanced non-animal models in several biomedical research areas.

EURL ECVAM also promotes cross-disciplinarity as illustrated by the example of the CIAO project where the AOP framework is used for an interdisciplinary systematic organisation of the current knowledge on the various biological pathways to COVID-19 pathogenesis.

EURL ECVAM furthermore increased and complemented its education and training activities on the Three Rs and non-animal methods, as these remain foundations for changes in societal behaviours and beliefs.

A smart and optimal combination of test methods based on the use of human cells and tissue cultures (from monolayer cell (co)cultures, to organotypic three-dimensional (3D) cell models, OoC systems, 3D- and 4D-bioprinting), multiple highthroughput 'omics technologies, and computational analytical methods (e.g., IVIVE, PBK, and pharmacodynamics) promise to reduce and substantially replace animal use in both biomedical research and regulatory toxicology.

References

Bal-Price, A., Pistollato, F., Sachana, M., Bopp, S. K., Munn, S., and Worth, A. (2018). Strategies to improve the regulatory assessment of developmental neurotoxicity (DNT) using in vitro methods. *Toxicology and Applied Pharmacology, 354*, 7-18. doi:10.1016/j.taap.2018.02.008

Ball, N., Bars, R., Botham, P. A., Cuciureanu, A., Cronin, M. T. D., Doe, J. E., Dudzina, T., Gant, T. W., Leist, M., and van Ravenzwaay, B. (2022). A framework for chemical safety assessment incorporating new approach methodologies within REACH. *Arch Toxicol, 96*(3), 743-766. doi:10.1007/s00204-021-03215-9

Baltazar, M. T., Cable, S., Carmichael, P. L., Cubberley, R., Cull, T., Delagrange, M., Dent, M. P., Hatherell, S., Houghton, J., Kukic, P., Li, H., Lee, M. Y., Malcomber, S., Middleton, A. M., Moxon, T. E., Nathanail, A. V., Nicol, B., Pendlington, R., Reynolds, G., Reynolds, J., White, A., and Westmoreland, C. (2020). A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products. *Toxicol Sci, 176*(1), 236-252. doi:10.1093/toxsci/kfaa048

Bartnicka, J., Langezaal, I. and Coecke, S. (2021). *Towards animal-free in vitro methods in the Thyroid Validation Study,* EUR 30818 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-41164-2, doi:10.2760/544332, JRC125904. Retrieved from https://publications.jrc.ec.europa.eu/repository/handle/JRC125904

Canals, J. M., Romania, P., Belio-Mairal P., Nic, M., Dibusz, K., Novotny, T., Busquet, F., Rossi, F., Straccia, M., Daskalopoulos, E. P., and Gribaldo, L. (2022). *Advanced Non-animal Models in Biomedical Research – Immunogenicity testing for advanced therapy medicinal products,* EUR 30334/4 EN, Publications Office of the European Union, Luxembourg, 2022, ISBN 978-92-76-49091-3, doi:10.2760/7190, JRC126997. Retrieved from https://publications.jrc.ec.europa.eu/repository/handle/JRC126997

Carmichael, P. L., Baltazar, M. T., Cable, S., Cochrane, S., Dent, M., Li, H., Middleton, A., Muller, I., Reynolds, G., Westmoreland, C., and White, A. (2022). Ready for regulatory use: NAMs and NGRA for chemical safety assurance. *ALTEX, 39*(3), 359-366. doi:10.14573/altex.2204281

Carusi, A., Wittwehr, C., and Whelan, M. (2021). *Addressing evidence needs in chemicals policy and regulation,* EUR 30941 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-76-45889-0, doi:10.2760/9130, JRC126724. Retrieved from https://publications.jrc.ec.europa.eu/repository/handle/JRC126724

Celi, S., Cioffi, M., Capellini, K., Fanni, B. M., Gasparotti, E., Vignali, E., Positano, V., Haxhiademi, D., Costa, E., Landini, L., Daskalopoulos, E. P., Piergiovanni, M. and Gribaldo, L. (2022). *Advanced non animal models in biomedical research – Cardiovascular diseases,* Publications Office of the European Union, Luxembourg, 2022, doi:10.2760/94608, JRC130702. Retrieved from https://publications.jrc.ec.europa.eu/repository/handle/JRC130702

Chang, X., Tan, Y. M., Allen, D. G., Bell, S., Brown, P. C., Browning, L., Ceger, P., Gearhart, J., Hakkinen, P. J., Kabadi, S. V., Kleinstreuer, N. C., Lumen, A., Matheson, J., Paini, A., Pangburn, H. A., Petersen, E. J., Reinke, E. N., Ribeiro, A. J. S., Sipes, N., Sweeney, L. M., Wambaugh, J. F., Wange, R., Wetmore, B. A., and Mumtaz, M. (2022). IVIVE: Facilitating the Use of In Vitro Toxicity Data in Risk Assessment and Decision Making. *Toxics, 10*(5). doi:10.3390/toxics10050232

Clerbaux, L. A., Albertini, M. C., Amigo, N., Beronius, A., Bezemer, G. F. G., Coecke, S., Daskalopoulos, E. P., Del Giudice, G., Greco, D., Grenga, L., Mantovani, A., Munoz, A., Omeragic, E., Parissis, N., Petrillo, M., Saarimaki, L. A., Soares, H., Sullivan, K., and Landesmann, B. (2022a). Factors Modulating COVID-19: A Mechanistic Understanding Based on the Adverse Outcome Pathway Framework. *J Clin Med, 11*(15). doi:10.3390/jcm11154464

Clerbaux, L. A., Amigo, N., Amorim, M. J., Bal-Price, A., Batista Leite, S., Beronius, A., Bezemer, G. F. G., Bostroem, A. C., Carusi, A., Coecke, S., Concha, R., Daskalopoulos, E. P., De Bernardi, F., Edrosa, E., Edwards, S. W., Filipovska, J., Garcia-Reyero, N., Gavins, F. N. E., Halappanavar, S., Hargreaves, A. J., Hogberg, H. T., Huynh, M. T., Jacobson, D., Josephs-Spaulding, J., Kim, Y. J., Kong, H. J., Krebs, C. E., Lam, A., Landesmann, B., Layton, A., Lee, Y. O., Macmillan, D. S., Mantovani, A.,

Margiotta-Casaluci, L., Martens, M., Masereeuw, R., Mayasich, S. A., Mei, L. M., Mortensen, H., Munoz Pineiro, A., Nymark, P., Ohayon, E., Ojasi, J., Paini, A., Parissis, N., Parvatam, S., Pistollato, F., Sachana, M., Sorli, J. B., Sullivan, K. M., Sund, J., Tanabe, S., Tsaioun, K., Vinken, M., Viviani, L., Waspe, J., Willett, C., and Wittwehr, C. (2022b). COVID-19 through Adverse Outcome Pathways: Building networks to better understand the disease - 3rd CIAO AOP Design Workshop. *ALTEX, 39*(2), 322-335. doi:*10.14573/altex.2112161*

Clerbaux, L. A., Fillipovska, J., Munoz, A., Petrillo, M., Coecke, S., Amorim, M. J., and Grenga, L. (2022c). Mechanisms Leading to Gut Dysbiosis in COVID-19: Current Evidence and Uncertainties Based on Adverse Outcome Pathways. *J Clin Med, 11*(18). doi:*10.3390/jcm11185400*

Clerbaux, L. A., Mayasich, S. A., Munoz, A., Soares, H., Petrillo, M., Albertini, M. C., Lanthier, N., Grenga, L., and Amorim, M. J. (2022d). Gut as an Alternative Entry Route for SARS-CoV-2: Current Evidence and Uncertainties of Productive Enteric Infection in COVID-19. *J Clin Med, 11*(19). doi:*10.3390/jcm11195691*

Dent, M. P., Vaillancourt, E., Thomas, R. S., Carmichael, P. L., Ouedraogo, G., Kojima, H., Barroso, J., Ansell, J., Barton-Maclaren, T. S., Bennekou, S. H., Boekelheide, K., Ezendam, J., Field, J., Fitzpatrick, S., Hatao, M., Kreiling, R., Lorencini, M., Mahony, C., Montemayor, B., Mazaro-Costa, R., Oliveira, J., Rogiers, V., Smegal, D., Taalman, R., Tokura, Y., Verma, R., Willett, C., and Yang, C. (2021). Paving the way for application of next generation risk assessment to safety decision-making for cosmetic ingredients. *Regul Toxicol Pharmacol, 125*, 105026. doi:*10.1016/j.yrtph.2021.105026*

EC (2019). Communication from the Commission - The European Green Deal, COM(2019) 640 final. Retrieved from *https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52019DC0640*

EC (2020). Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and The Committee of the Regions - Chemicals Strategy for Sustainability Towards a Toxic-Free Environment. Retrieved from *https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=COM%3A2020%3A667%3AFIN*

EC (2022). *Assessing the reproducibility of research results in EU Framework Programmes for Research: final report,* Publications Office of the European Union, Luxembourg. Retrieved from *https://data.europa.eu/doi/10.2777/186782*

Hilton, G. M., Adcock, C., Akerman, G., Baldassari, J., Battalora, M., Casey, W., Clippinger, A. J., Cope, R., Goetz, A., Hayes, A. W., Papineni, S., Peffer, R. C., Ramsingh, D., Williamson Riffle, B., Sanches da Rocha, M., Ryan, N., Scollon, E., Visconti, N., Wolf, D. C., Yan, Z., and Lowit, A. (2022). Rethinking chronic toxicity and carcinogenicity assessment for agrochemicals project (ReCAAP): A reporting framework to support a weight of evidence safety assessment without long-term rodent bioassays. *Regul Toxicol Pharmacol, 131*, 105160. doi:*10.1016/j.yrtph.2022.105160*

Holzer, A. K., Karreman, C., Suciu, I., Furmanowsky, L. S., Wohlfarth, H., Loser, D., Dirks, W. G., Pardo Gonzalez, E., and Leist, M. (2022a). Generation of Human Nociceptor-Enriched Sensory Neurons for the Study of Pain-Related Dysfunctions. *Stem Cells Transl Med, 11*(7), 727-741. doi:*10.1093/stcltm/szac031*

Holzer, A. K., Suciu, I., Karreman, C., Goj, T., and Leist, M. (2022b). Specific Attenuation of Purinergic Signaling during Bortezomib-Induced Peripheral Neuropathy In Vitro. *Int J Mol Sci, 23*(7). doi:*10.3390/ijms23073734*

Jacobs, M. N., Colacci, A., Louekari, K., Luijten, M., Hakkert, B. C., Paparella, M., and Vasseur, P. (2016). International regulatory needs for development ofan IATA

for non-genotoxic carcinogenic chemical substances. *ALTEX, 33*(4), 359-392. doi:*10.14573/altex.1601201*

Jacobs, M. N., Colacci, A., Corvi, R., Vaccari, M., Aguila, M. C., Corvaro, M., Delrue, N., Desaulniers, D., Ertych, N., Jacobs, A., Luijten, M., Madia, F., Nishikawa, A., Ogawa, K., Ohmori, K., Paparella, M., Sharma, A. K., and Vasseur, P. (2020). Chemical carcinogen safety testing: OECD expert group international consensus on the development of an integrated approach for the testing and assessment of chemical non-genotoxic carcinogens. *Archives of Toxicology, 94*(8), 2899-2923. doi:*10.1007/s00204-020-02784-5*

Khalidi, H., Onasanwo, A., Islam, B., Jo, H., Fisher, C., Aidley, R., Gardner, I., and Bois, F. Y. (2022). SimRFlow: An R-based workflow for automated high-throughput PBPK simulation with the Simcyp((R)) simulator. *Frontiers in Pharmacology, 13*, 929200. doi:*10.3389/fphar.2022.929200*

Luijten, M., Corvi, R., Mehta, J., Corvaro, M., Delrue, N., Felter, S., Haas, B., Hewitt, N. J., Hilton, G., Holmes, T., Jacobs, M. N., Jacobs, A., Lamplmair, F., Lewis, D., Madia, F., Manou, I., Melching-Kollmuss, S., Schorsch, F., Schutte, K., Sewell, F., Strupp, C., van der Laan, J. W., Wolf, D. C., Wolterink, G., Woutersen, R., Zvonar, Z., Heusinkveld, H., and Braakhuis, H. (2020). A comprehensive view on mechanistic approaches for cancer risk assessment of non-genotoxic agrochemicals. *Regulatory Toxicology and Pharmacology, 118*, 104789. doi:*10.1016/j.yrtph.2020.104789*

OECD (2014). *New Scoping Document on in vitro and ex vivo Assays for the Identification of Modulators of Thyroid Hormone Signalling*, OECD Series on Testing and Assessment, No. 207, OECD Publishing, Paris. doi:*10.1787/9789264274716-en*

OECD (2018). *Guidance Document on the Determination of In Vitro Intrinsic Clearance Using Cryopreserved Hepatocytes (RT-HEP) or Liver S9 Sub-Cellular Fractions (RT-S9) From Rainbow Trout and Extrapolation to In Vivo Intrinsic Clearance*, Series on Testing and Assessment, No. 280, OECD Publishing, Paris. Retrieved from *http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2018)12&doclanguage=en*

OECD (2019). *Saving Costs in Chemicals Management: How the OECD Ensures Benefits to Society*, OECD Publishing, Paris. doi:*10.1787/9789264311718-en*

OECD (2021). *Guidance document on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes*, OECD Series on Testing and Assessment, No. 331, Environment, Health and Safety, Environment Directorate, OECD. Retrieved from *https://www.oecd.org/chemicalsafety/risk-assessment/guidance-document-on-the-characterisation-validation-and-reporting-of-physiologically-based-kinetic-models-for-regulatory-purposes.pdf*

Otero, M. J., Canals, J., Belio-Mairal, P., Nic, M., Dibusz, K., Novotny, T., Busquet, F., Rossi, F., Gastaldello, A., Gribaldo, L., Straccia, M., Dura, A., and Whelan, M. (2022). *Advanced Non-animal Models in Biomedical Research: Autoimmune Diseases,* Publications Office of the European Union, Luxembourg, 2022, doi:*10.2760/617688*, JRC131505. Retrieved from *https://publications.jrc.ec.europa.eu/repository/handle/JRC131505*

Pallocca, G., Mone, M. J., Kamp, H., Luijten, M., Van de Water, B., and Leist, M. (2022). Next-generation risk assessment of chemicals - Rolling out a human-centric testing strategy to drive 3R implementation: The RISK-HUNT3R project perspective. *ALTEX, 39*(3), 419-426. doi:*10.14573/altex.2204051*

Pappalardo, F., Russo, G., Corsini, E., Paini, A., and Worth, A. (2022). Translatability and transferability of in silico models: Context of use switching to predict the effects of environmental chemicals on the immune system. *Comput Struct Biotechnol J, 20*, 1764-1777. doi:*10.1016/j.csbj.2022.03.024*

Paul Friedman, K., Gagne, M., Loo, L. H., Karamertzanis, P., Netzeva, T., Sobanski, T., Franzosa, J. A., Richard, A. M., Lougee, R. R., Gissi, A., Lee, J. J., Angrish, M., Dorne, J. L., Foster, S., Raffaele, K., Bahadori, T., Gwinn, M. R., Lambert, J., Whelan, M., Rasenberg, M., Barton-Maclaren, T., and Thomas, R. S. (2020). Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization. *Toxicological Sciences, 173*(1), 202-225. doi:*10.1093/toxsci/kfz201*

Pfuhler, S., Downs, T. R., Hewitt, N. J., Hoffmann, S., Mun, G. C., Ouedraogo, G., Roy, S., Curren, R. D., and Aardema, M. J. (2021a). Validation of the 3D reconstructed human skin micronucleus (RSMN) assay: an animal-free alternative for following-up positive results from standard in vitro genotoxicity assays. *Mutagenesis, 36*(1), 1-17. doi:*10.1093/mutage/geaa035*

Pfuhler, S., Pirow, R., Downs, T. R., Haase, A., Hewitt, N., Luch, A., Merkel, M., Petrick, C., Said, A., Schafer-Korting, M., and Reisinger, K. (2021b). Validation of the 3D reconstructed human skin Comet assay, an animal-free alternative for following-up positive results from standard in vitro genotoxicity assays. *Mutagenesis, 36*(1), 19–35. doi:*10.1093/mutage/geaa009*

Piergiovanni, M., Cangar, O., Leite, S. B., Mian, L., Jenet, A., Corvi, R., Whelan, M., Taucer, F., and Ganesh, A. (2021). Putting Science into Standards workshop on standards for organ-on-chip. *Stem Cell Reports, 16*(9), 2076-2077. doi:*10.1016/j.stemcr.2021.07.010*

Pistollato, F., Petrillo, M., Clerbaux, L. A., Leoni, G., Ponti, J., Bogni, A., Brogna, C., Cristoni, S., Sanges, R., Mendoza-de Gyves, E., Fabbri, M., Querci, M., Soares, H., Munoz, A., Whelan, M., and Van de Eede, G. (2022). Effects of spike protein and toxin-like peptides found in COVID-19 patients on human 3D neuronal/glial model undergoing differentiation: Possible implications for SARS-CoV-2 impact on brain development. *Reprod Toxicol, 111*, 34-48. doi:*10.1016/j.reprotox.2022.04.011*

Reynolds, J., MacKay, C., Gilmour, N., Miguel-Vilumbrales, D. M., and Mawell, G. (2019). Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment. *Computational Toxicology, 9*, 36-49. doi:*10.1016/j.comtox.2018.10.004*

Reynolds, J., Gilmour, N., Baltazar, M. T., Reynolds, G., Windebank, S., and Maxwell, G. (2022). Decision making in next generation risk assessment for skin allergy: Using historical clinical experience to benchmark risk. *Regul Toxicol Pharmacol, 134*, 105219. doi:*10.1016/j.yrtph.2022.105219*

Russo, G., Crispino, E., Corsini, E., Iulini, M., Paini, A., Worth, A., and Pappalardo, F. (2022). Computational modelling and simulation for immunotoxicity prediction induced by skin sensitisers. *Comput Struct Biotechnol J, 20*, 6172-6181. doi:*10.1016/j.csbj.2022.10.032*

Shahbaz, M. A., De Bernardi, F., Alatalo, A., Sachana, M., Clerbaux, L. A., Munoz, A., Parvatam, S., Landesmann, B., Kanninen, K. M., and Coecke, S. (2022). Mechanistic Understanding of the Olfactory Neuroepithelium Involvement Leading to Short-Term Anosmia in COVID-19 Using the Adverse Outcome Pathway Framework. *Cells, 11*(19). doi:*10.3390/cells11193027*

Socianu, S., Bopp, S. K., Govarts, E., Gilles, L., Buekers, J., Kolossa-Gehring, M., Backhaus, T., and Franco, A. (2022). Chemical Mixtures in the EU Population: Composition and Potential Risks. *Int J Environ Res Public Health, 19*(10). doi:*10.3390/ijerph19106121*

Spînu, N. (2021). *Modelling of quantitative Adverse Outcome Pathways.* (Doctoral thesis). Liverpool John Moores University, Retrieved from *https://researchonline.ljmu.ac.uk/id/eprint/15012/*

Thomas, R. S., Bahadori, T., Buckley, T. J., Cowden, J., Deisenroth, C., Dionisio, K. L., Frithsen, J. B., Grulke, C. M., Gwinn, M. R., Harrill, J. A., Higuchi, M., Houck, K. A., Hughes, M. F., Hunter, E. S., Isaacs, K. K., Judson, R. S., Knudsen, T. B., Lambert, J. C., Linnenbrink, M., Martin, T. M., Newton, S. R., Padilla, S., Patlewicz, G., Paul-Friedman, K., Phillips, K. A., Richard, A. M., Sams, R., Shafer, T. J., Setzer, R. W., Shah, I., Simmons, J. E., Simmons, S. O., Singh, A., Sobus, J. R., Strynar, M., Swank, A., Tornero-Valez, R., Ulrich, E. M., Villeneuve, D. L., Wambaugh, J. F., Wetmore, B. A., and Williams, A. J. (2019). The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. *Toxicol Sci, 169*(2), 317-332. doi:*10.1093/toxsci/kfz058*

Thompson, C. V., Firman, J. W., Goldsmith, M. R., Grulke, C. M., Tan, Y.-M., Paini, A., Penson, P. E., Sayre, R. R., Webb, S., and Madden, J. C. (2021). A Systematic Review of Published Physiologically based Kinetic Models and an Assessment of their Chemical Space Coverage. *Alternatives to Laboratory Animals, 49*(5). doi:*10.1177/02611929211060264*

van der Stel, W., Yang, H., Vrijenhoek, N. G., Schimming, J. P., Callegaro, G., Carta, G., Darici, S., Delp, J., Forsby, A., White, A., le Devedec, S., Leist, M., Jennings, P., Beltman, J. B., van de Water, B., and Danen, E. H. J. (2022). Mapping the cellular response to electron transport chain inhibitors reveals selective signaling networks triggered by mitochondrial perturbation. *Arch Toxicol, 96*(1), 259-285. doi:*10.1007/s00204-021-03160-7*

van der Zalm, A. J., Barroso, J., Browne, P., Casey, W., Gordon, J., Henry, T. R., Kleinstreuer, N. C., Lowit, A. B., Perron, M., and Clippinger, A. J. (2022). A framework for establishing scientific confidence in new approach methodologies. *Arch Toxicol, 96*(11), 2865-2879. doi:*10.1007/s00204-022-03365-4*

Westmoreland, C., Bender, H. J., Doe, J. E., Jacobs, M. N., Kass, G. E. N., Madia, F., Mahony, C., Manou, I., Maxwell, G., Prieto, P., Roggeband, R., Sobanski, T., Schutte, K., Worth, A. P., Zvonar, Z., and Cronin, M. T. D. (2022). Use of New Approach Methodologies (NAMs) in regulatory decisions for chemical safety: Report from an EPAA Deep Dive Workshop. *Regul Toxicol Pharmacol, 135*, 105261. doi:*10.1016/j.yrtph.2022.105261*

Zuang, V., Dura, A., Asturiol Bofill, D., Batista Leite, S., Berggren, E., Bopp, S., Carpi, D., Casati, S., Coecke, S., Corvi, R., Deceuninck, P., Franco, A., Gribaldo, L., Halder, M.E., Holloway, M., Kienzler, A., Langezaal, I., Madia, F., Milcamps, A., Munn, S., Paini, A., Piergiovanni, M., Pistollato, F., Price, A., Prieto Peraita, M.D.P., Viegas Barroso, J.F., Wittwehr, C., Worth, A. and Whelan, M. (2021). *Non-animal Methods in Science and Regulation*, EUR 30553 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-28396-6, doi:*10.2760/719755*, JRC123531. Retrieved from *https://publications.jrc.ec.europa.eu/repository/handle/JRC123531*

Abbreviations and definitions

2D	Two-dimensional
3D	Three-dimensional
3Rs	Replacement, Reduction, Refinement
ACE2	Angiotensin-converting enzyme 2
ADME	Absorption, distribution, metabolism and excretion
ADRA	Amino acid Derivative Reactivity Assay
AG EDTA	Advisory Group on Testing and Assessment of Endocrine Disruptors
AI	Artificial intelligence
ANSES	French National Agency for Food, Environmental and Occupational Health and Safety
AOP	Adverse Outcome Pathway
AOP-KB	Adverse Outcome Pathway Knowledge Base
ARM	Application Reporting Module
ASPIS	Animal-free Safety assessment of chemicals: Project cluster for Implementation of novel Strategies (H2020)
ASTM	American Society for Testing and Materials
ATMPs	Advanced Therapy Medicinal Products
cAMP	cyclic adenosine monophosphate
CARACAL	Competent Authorities for REACH and CLP
CEN	European Committee for Standardization
CENELEC	European Committee for Electrotechnical Standardization
CG	Chemical Grouping
CIAO	Modelling the Pathogenesis of COVID-19 using the Adverse Outcome Pathway Framework
CLP	Classification, Labelling and Packaging
COVID-19	Coronavirus disease
DA	Defined approach
DG ENV	Directorate-General for Environment (EC)
DG RTD	Directorate-General for Research and Innovation (EC)
DG SANTE	Directorate-General for Health and Food Safety (EC)
DIO 1-SK	Deiodinase 1 Sandell-Kolthoff reaction
DNA	Deoxyribonucleic Acid
DNT	Developmental neurotoxicity
DNT-IVB	Developmental neurotoxicity in vitro testing battery
DoI	Declaration of Interest
DPRA	Direct peptide reactivity assay
EAGMST	Extended Advisory Group for Molecular Screening and Toxicogenomics
EASIS	Endocrine Active Substances Information System
EATS	Estrogen, Androgen, Thyroid and Steroidogenesis
EC	European Commission (EU)
EEA	European Environment Agency (EU)

ECCC	Environment and Climate Change Canada
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency (EU)
ED	Endocrine disruptor
EDCs	Endocrine-disrupting chemicals
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EMPODAT	Environmental Monitoring of POllutants DATabase
EPA	Environmental Protection Agency
EPAA	European Partnership for Alternatives to Animal Testing
ERDC	US Army Engineer Research and Development Center
ESAC	EURL ECVAM Scientific Advisory Committee
EU	European Union
EURION	European Cluster to Improve Identification of Endocrine Disruptors
EUROoCS	European Organ-on-Chip Society
EU-NETVAL	European Union Network of Laboratories for the Validation of Alternative Methods
EU-ToxRisk	An Integrated European 'Flagship' Programme Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st century
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FAIR	Findability, accessibility, interoperability, and reusability (of data)
FDA	US Food and Drug Administration
FELASA	Federation of European Laboratory Animal Science Associations
FHAIVE	Finnish Hub for Development and Validation of Integrated Approaches
FP	Framework programme
GD	Guidance Document
GHS	Globally Harmonised System of Classification and Labelling of chemicals
GIVIMP	Good *In Vitro* Method Practices
GL	Guideline
GTTC	Genetic Toxicology Technical Committee (HESI)
GTX	Genotoxic
H2020	Horizon 2020
hASC-HUVEC	Human adipose stromal cell – human umbilical vein endothelial cell
HB	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative
HESI	Health and Environmental Sciences Institute (US)
hiPSC	human induced pluripotent stem cells
IATA	Integrated Approaches to Testing and Assessment

ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICT	Information and communication technologies
IDAEA CSIC	Institute of Environmental Assessment and Water Research
IPCHEM	Information platform for chemical monitoring
IPCS	International Programme on Chemical Safety
IQ Consortium	International Consortium for Innovation and Quality in Pharmaceutical Development
IUCLID	International Uniform Chemical Information Database
IUF	Institut für Umweltmedizinische Forschung, Leibniz
IVIVE	*In vitro* to *in vivo* extrapolation
JRC	Joint Research Centre (EC)
KCs	Key characteristics
KE	Key event (AOP)
KER	Key event relationship (AOP)
LC/MS	Liquid chromatography / mass spectrometry
MAD	Mutual acceptance of data
MCT8	Monocarboxylate transporter 8
MD	Method developers
ML	Machine Learning
MoA	Mode of action
MPS	Microphysiological systems
NAMs	New approach methodologies
NATM	Non-Animal test methods
NEN	Nederlands Normalisatie Instituut
NGRA	Next generation risk assessment
NGTX	Non-genotoxic
NICEATM	National Toxicology Programme Interagency Center for the Evaluation of Alternative Toxicological Methods (US)
NIH	National Institutes of Health
NIS	Sodium iodide symporter
NLP	Natural language processing
NTP	National Toxicology Programme (US)
NWA-ORC	National Science Agenda Research on Routes by Consortia
NWO	Nederlandse Organisatie voor Wetenschappelijk Onderzoek
OECD	Organisation for Economic Co-operation and Development
OHTs	OECD Harmonised Templates
ONTOX	Ontology-driven and artificial intelligence-based repeated dose toxicity testing of chemicals for next generation risk assessment (ASPIS cluster)
OoC	Organ-on-chip
PARC	European partnership for the assessment of risks from chemicals
PARERE	Preliminary Assessment of Regulatory Relevance network

PATROLS	Physiologically Anchored Tools for Realistic nanOmateriaL hazard aSsessment
PBD	Physiologically based dynamic
PBK	Physiologically based kinetic (also PBPK, PBBK, PBTK)
PBT	Persistent, Bio-accumulative and Toxic
PMT	Persistent and Mobile
PETA	People for the Ethical Treatment of Animals
PFAS	Perfluoroalkyl chemicals
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PrecisionTOX	Toward Precision Toxicology: New Approach Methodologies for Chemical Safety (ASPIS cluster)
qAOP	quantitative AOP
QIVIVE	Quantitative *in Vitro* to *in Vivo* Extrapolation
QSAR	Quantitative Structure Activity Relationship
RAB	Regulatory Advisory Board
RADAR	Rapid Androgen Disruption Activity Reporter Assay
REACH	European Regulation (EC) No. 1907/2006 Registration, Evaluation, Authorisation and Restriction of Chemicals
RISE	Research Institutes of Sweden
RISK-HUNT3R	RISK assessment of chemicals integrating HUman centric Next generation Testing strategies promoting the 3Rs (ASPIS cluster)
ROS	Reactive oxygen species
RS	Reconstructed skin
RSMN	Reconstructed skin micronucleus
RTI	Research Triangle Institute
SAAOP	Society for the Advancement of AOPs
SOPs	Standard Operating Procedures
SRIA	Strategic Research and Innovation Agenda
STEM	Science, technology, engineering, and mathematics
STOT	Specific target organ toxicity
T3-FITC	Triiodothyroxine antibody conjugated to fluoroscein isothiocyanate
TBG	Thyroxine-binding globulin
TDM-EG	Thyroid Disruption Methods Expert Group
TG	Test Guideline (OECD)
TGP	Test guidelines programme
THs	Thyroid hormones
TPO	Thyroid peroxidase
TRα	Human thyroid hormone receptor alpha
TRβ	Human thyroid hormone receptor beta
TSAR	EURL ECVAM Tracking System on Alternative Methods towards Regulatory acceptance

TSH	Thyrotrophin-stimulating hormone
TTR	Thyroxine-binding prealbumin
UISS	Universal immune system simulator
UK	United Kingdom
UN	United Nations
US	United States (of America)
VHP4Safety	Virtual Human Platform for safety assessment
vPvB	very Persistent and very Bio-accumulative
vPvM	very Persistent and very Mobile
VU	Vrije Universiteit
WG	Working Group
WHO	World Health Organization
WNT	Working Party of the National Coordinators of the Test Guidelines Programme (OECD)
WPHA	Working Party on Hazard Assessment (OECD)
WPP	Working Party on Pesticides (OECD)

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