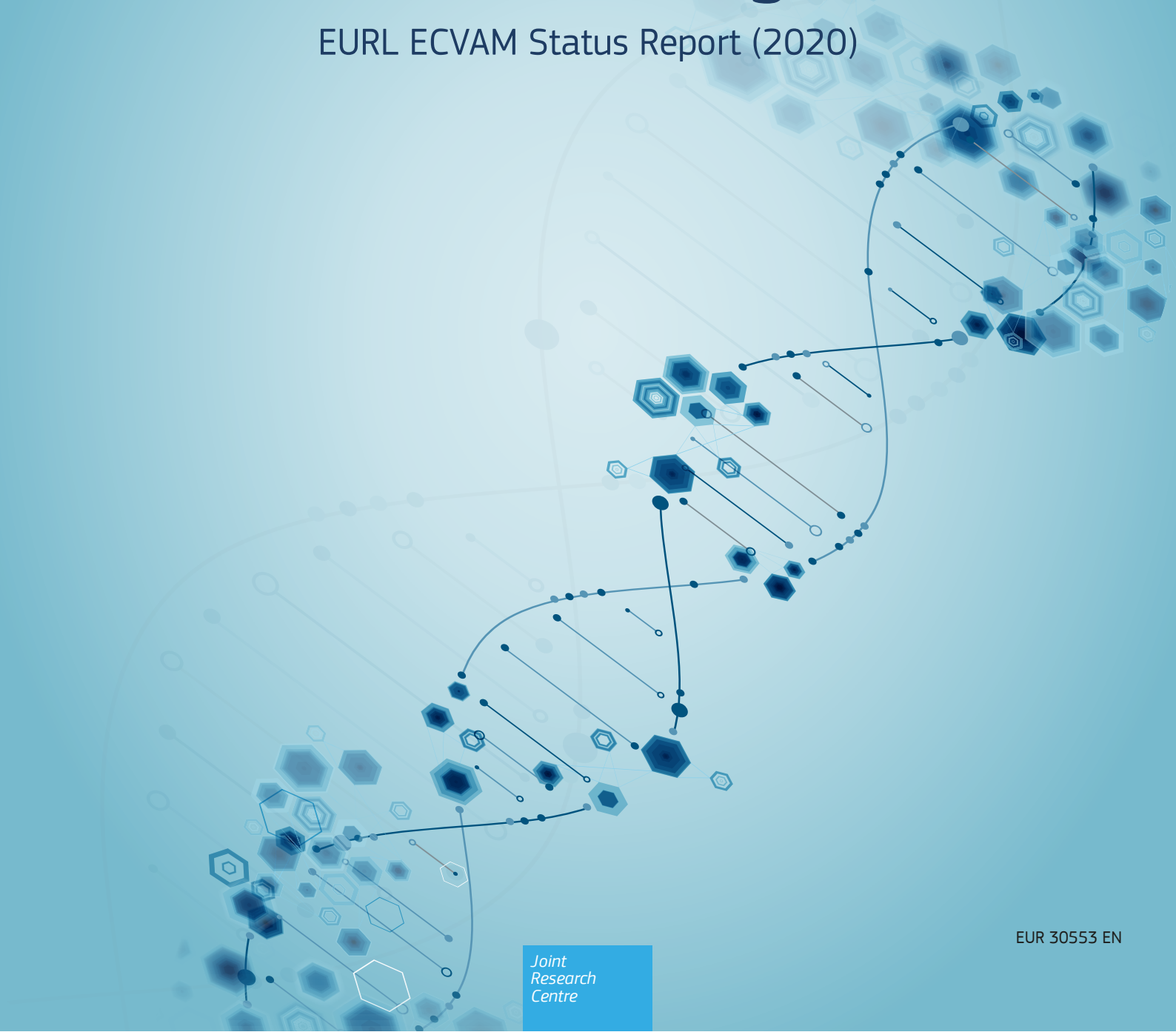
European Commission

JRC SCIENCE FOR POLICY REPORT

# Non-animal Methods in Science and Regulation

## EURL ECVAM Status Report (2020)



EUR 30553 EN

Joint Research Centre

Manuscript completed in December 2020.

**Contact information**

European Commission, Joint Research Centre (JRC), Chemical Safety and Alternative Methods Unit (F3)
Via E. Fermi 2749, I-21027 Ispra (VA), Italy
Email: JRC-F3-ENQUIRIES@ec.europa.eu

**EU Science Hub**

https://ec.europa.eu/jrc

JRC123531

EUR 30553 EN

| | | | |
|---|---|---|---|
| PDF | ISBN 978-92-76-28395-9 | ISSN 1831-9424 | doi:10.2760/719755 |
| Print | ISBN 978-92-76-28396-6 | ISSN 1018-5593 | doi:10.2760/163367 |

Luxembourg: Publications Office of the European Union, 2021



Chief editors: Zuang, V., & Dura, A.
Chapter editors: Worth, A., Corvi, R., Munn, S., Deceuninck, P., & Holloway, M.

How to cite this report: Zuang, V., Dura, A., *et al., Non-animal Methods in Science and Regulation - EURL ECVAM Status Report 2020,* EUR 30553 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-28395-9, doi:10.2760/719755, JRC123531.

# Non-animal Methods in Science and Regulation

## EURL ECVAM Status Report (2020)

# Table of contents

# List of authors

## (in alphabetical order)

David Asturiol Bofill
João Barroso
Sofia Batista Leite
Elisabet Berggren
Stephanie Bopp
Donatella Carpi
Silvia Casati
Sandra Coecke
Raffaella Corvi
Pierre Deceuninck
Adelaide Dura
Antonio Franco
Laura Gribaldo
Marlies Halder
Marcelle Holloway
Aude Kienzler
Ingrid Langezaal
Federica Madia
Anne Milcamps
Sharon Munn
Alicia Paini
Monica Piergiovanni
Francesca Pistollato
Anna Price
Pilar Prieto Peraita
Clemens Wittwehr
Maurice Whelan
Andrew Worth
Valérie Zuang

# Executive summary

The roles and responsibilities of the JRC's European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) are defined in EU Directive 2010/63/EU on the protection of animals used for scientific purposes (EU, 2010). The overarching and ultimate goal of the directive is a full replacement of procedures on live animals for scientific and educational purposes as soon as scientifically possible. The Directive cross-cuts and impacts on several other pieces of EU legislation and policies as it requests that Member States ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, is used instead of an animal procedure.

This EURL ECVAM 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.

In 2020, the development of non-animal methods and approaches continued predominantly under the EU Framework Programme for Research and Innovation, Horizon 2020, and in collaborative partnerships. These projects focused on the development of case studies on grouping and read-across[1], on the optimal combination of methods in Integrated Approaches to Testing and Assessment (IATA) for systemic toxicity testing, as well as on methodologies to improve the identification of endocrine disrupting chemicals. They foster the integration of non-animal methods, approaches and models in the risk assessment of chemicals and products to support regulatory decision making. Considering that there are over 100,000 chemicals on the market and that for 90% of them, little or no information on their hazardous properties exist, innovative and more efficient ways of safety testing and chemical risk assessment, not dependent on animal testing, are needed. This obligation is also expressed in the recently published EU Chemicals Strategy for Sustainability, aiming at a toxic-free environment under the European Green Deal (EC, 2020a).

For the safety and potency testing of vaccines, several non-animal methods developed and standardised within the project "Vaccine batch to Vaccine

1 Read-across involves the use of relevant information from analogous substance(s) (the 'source' information) to predict properties for the 'target' substance(s) under consideration (ECHA, 2017).

batch comparison by consistency testing" (VA2VAC)[2] have been transferred to partner laboratories to assess their transferability and reproducibility. EURL ECVAM is coordinating an activity within the project aimed at developing guidance for moving from the traditional *in vivo* test-based quality control strategy to a consistency test-based strategy relying on non-animal based assays. Such efforts are important since batch potency testing currently requires a high number of animals.

EURL ECVAM has started a project to explore how to better use the available mechanistic information from existing animal studies across different endpoint hazard assessments. The aim is to avoid redundancy in *in vivo* testing and to integrate novel non-animal methods in effective testing and assessment strategies.

Characterising, validating and standardising new non-animal methods and approaches are important steps towards their regulatory use and international adoption. Various thyroid methods, targeting different modes of action of thyroid disruption, are currently under validation by EURL ECVAM and its network of validation laboratories EU-NETVAL. Chemicals that disrupt thyroid homeostasis have the potential to be endocrine disruptors and thus associated with several adverse health effects. Promising methods or strategies are also evaluated and validated in the area of skin and respiratory sensitisation, genotoxicity, fish toxicity and for the quality control of vaccines. Qualification processes and standardisation needs for new emerging technologies such as organ-on-chip devices are being discussed too.

EURL ECVAM leads and contributes to activities to translate scientifically valid non-animal methods and approaches into international standards, such as e.g., OECD test guidelines and guidance documents for the safety testing of chemicals or VICH guidelines for the safety testing of vaccines for veterinary use. In addition, it leads activities at the United Nations to define criteria based on non-animal methods for the Globally Harmonized System (GHS) for classification, so that chemicals can be classified and labelled according to their hazardous properties and transported and handled safely across the globe, without resorting to animal data.

Statistics from 2017 on uses of animals in research and testing in the EU indicate that 68% of 9.58 million animals are used for basic, applied and translational research, while regulatory testing represents 23%. The same trend is likely to be confirmed in the upcoming EC report on the statistics on the use of animals for scientific purposes in the Member States of the European Union in 2018. It is thus important to invest efforts to promote the uptake of non-animal methods and models in the research area too.

In 2020, EURL ECVAM completed the reviews on non-animal models in seven disease areas. EURL ECVAM also started a study to assess the impact of EU-funded research projects in the biomedical area (based on animal models or not). Moreover, considering the emergency of the COVID-19 pandemic in 2020, EURL ECVAM launched an exploratory project to gather and disseminate knowledge on the pathogenesis of COVID-19 using Adverse Outcome Pathways (AOP) and cross-disciplinary collaboration.

EURL ECVAM undertook several education and training activities aiming at increasing the awareness of the Three Rs (Replacement, Reduction and Refinement of animal use in science) and alternative methods at the levels of secondary school, university and early professional training. These include the introduction of the Three Rs and related teaching material into the curriculum of secondary schools and universities. EURL ECVAM also developed, in collaboration with partners, a massive open online course (MOOC) entitled “The Three Rs and animal use in science”. The MOOC was tailored for life sciences teachers in secondary schools to provide them with materials, support and tutorials to integrate the Three Rs in their own classrooms.

Raising the awareness of students and teachers on 21st century technologies and the European values related to animal welfare and animal protection in science, through specific education and training programmes, is a crucial endeavour towards the ultimate aim of abandoning animal testing.

Likewise, a collective investment of all stakeholders is needed too. EURL ECVAM therefore continues to work with all its established networks, such as its network of regulators (PARERE), its stakeholder forum (ESTAF), its network of validation laboratories (EU-NETVAL), the European Partnership on Alternatives to Animal Approaches (EPAA) and European and international bodies such as CEN, EDQM, OECD, WHO, ICH, VICH, and the UN.

2 The consistency approach for batch release testing of established vaccines consists in using physicochemical, immunochemical, cell-based and/or multiparametric tests, instead of animal tests, to ensure that each vaccine batch produced is consistent with a batch already proven to be safe and efficacious.

# Introduction

EU policies and legislation call for innovative and more efficient ways of safety testing and chemical risk assessment that do not depend on animal testing.

Advanced technologies such as computational models, *in vitro* methods and organ-on-chip devices are being developed, evaluated and integrated to translate mechanistic understanding of toxicity into safety testing strategies. The ultimate goal is to achieve better protection of human health and the environment while supporting EU innovation and industrial competitiveness, without the use of animals. The development and use of non-animal models and methods are also essential for advancing basic, applied and translational research.

Education also plays an essential role in enabling a shift to non-animal methods through the introduction of the Three Rs (Replacement, Reduction and Refinement of animal use in science) into secondary school curricula and programmes of higher education.

The annual EURL ECVAM status report describes research, development and validation activities, as well as initiatives that promote the regulatory use and international adoption of non-animal methods and approaches and their dissemination in the regulatory and research arenas.

# 1. Introduction

Every year, EURL ECVAM prepares a report to inform stakeholders and interested parties about on-going activities in the field of alternative (non-animal) approaches in science and regulation.

This report provides updates on activities since the last report published in March 2020. It describes development and validation activities together with initiatives that promote the regulatory application and international adoption and use of non-animal methods and approaches and their dissemination.

It also informs about EURL ECVAM's activities to promote the uptake and use of non-animal models in the areas of basic, applied and translational research, as well as EURL ECVAM's engagement in education and training activities on the Three Rs (Replacement, Reduction and Refinement of animal use in science) at the levels of secondary school, university and early professional training. It describes primarily, but not exclusively, the activities that EURL ECVAM has undertaken or has been involved in together with a wide range of collaborators.

The mandate of EURL ECVAM is described in Directive 2010/63/EU (EU, 2010) on the protection of animals used for scientific purposes (Article 48 and Annex VII). This mandate includes (a) coordinating and promoting the development and use of alternatives to procedures including in the areas of basic and applied research and regulatory testing; (b) coordinating and participating in the validation of alternative approaches at Union level; (c) acting as a focal point for the exchange of information on the development of alternative approaches; (d) setting up, maintaining and managing public databases and information systems on alternative approaches and their state of development and; (e) promoting dialogue between legislators, regulators, and all relevant stakeholders, in particular, industry,

biomedical scientists, consumer organisations and animal-welfare groups, with a view to the development, validation, regulatory acceptance, international recognition, and application of alternative approaches.

In addition to Directive 2010/63/EU, EURL ECVAM supports a broad range of European policies on chemicals and products ranging from industrial chemicals, plant protection and biocidal products, medicinal products for human and veterinary uses to cosmetic products. Moreover, EURL ECVAM supports the work in the field of biologicals and on cross-cutting topics such as endocrine disruptors and chemical mixtures.

All these policies have an aim in common, namely to protect human and environmental health without, or minimally, resorting to animal testing. Instead, they promote the use of advanced tools, methods and models, and data analysis capacities. In fact, non-animal methods are not just “alternatives” to animal tests, but innovative key enabling technologies.

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

# Development

Considerable scientific and technical progress is being made on the development of non-animal methods via a range of EU-funded projects and collaborative partnerships. EURL ECVAM plays a supporting role in such activities by contributing its expertise, providing advice on method characterisation and standardisation, and by sharing the results of relevant in-house research activities.

The overall aim is to identify promising methods and facilitate their translation into safety assessment practice, which remains a considerable barrier to acceptance and application.

# 2. Development

## 2.1 Systemic toxicity

### *2.1.1 EU-ToxRisk*

EU-ToxRisk is a European collaborative project funded by the EU Framework Programme for Research and Innovation, Horizon 2020, to advance mechanism-based toxicity testing and risk assessment of chemicals. EURL ECVAM through the JRC has a formal collaboration agreement in place with the consortium to support its science programme with a view to eventual translation and dissemination of research results to advance safety assessment practice.

**READ MORE**

- EU-ToxRisk: www.eu-toxrisk.eu
- IATA case studies project: www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm#Project

Four read-across case studies developed by the consortium were submitted to the OECD for review in its 5th Meeting of the IATA case studies project. All case study reports have been endorsed and published.

EURL ECVAM is now collaborating in a second round of case studies, providing chemicals to the partners carrying out experimental work, and actively participating in the development of an *ab initio* safety assessment workflow designed to avoid animal testing.

### *2.1.2 APCRA*

Accelerating the Pace of Chemical Risk Assessment (APCRA) is a government-to-government initiative whose aim is to promote collaboration and dialogue on the scientific and regulatory needs for the application and acceptance of new approach methodologies (NAMs) in regulatory decision making. It was initiated in

2016 and involves participants from the US, Canada, South Korea, Japan, Singapore, Australia, the OECD and the EU.

The consortium hosted a public webinar in March 2020, designed to share outcomes with the public and stakeholders. In addition, a closed workshop took place in August 2020 to discuss the integration of NAMs in the risk assessment process. Progress is made by conducting case studies, which trigger focused discussions to increase the understanding of regulatory needs and potential solutions.

EURL ECVAM is involved in a "prospective" case study aimed at assessing chemicals with limited toxicological data, using both NAM data and classical toxicological studies. The idea is to compare a (conservative) point of departure derived from an *in vitro* assay battery with those already used in risk assessment. This case study also builds upon the findings and learnings from the APCRA retrospective case study, reported in "Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization" (Paul Friedman *et al.*, 2020).

**READ MORE**

- APCRA: cfpub.epa.gov/si/si_public_record_Report.cfm?Lab=NCCT&dirEntryId=345649
- Presentations of the APCRA public webinar: ftp://newftp.epa.gov/COMPTOX/TEMP/APCRA2020_presentations

### *2.1.3 Mechanistic analysis of repeated dose toxicity studies*

Following a call for tender[3] in January 2020, EURL ECVAM kicked off a study in October with the Vrije Universiteit Amsterdam to collect and analyse relevant mechanistic information contributing to effects observed on target organs in animal studies with repeated exposure to chemicals (Figure 2.1). The study builds on previous work conducted by EURL ECVAM (Prieto *et al.*, 2019), recently awarded by the scientific journal ALTEX, to investigate specific mechanisms at the cellular level that are associated with acute oral systemic toxicity.

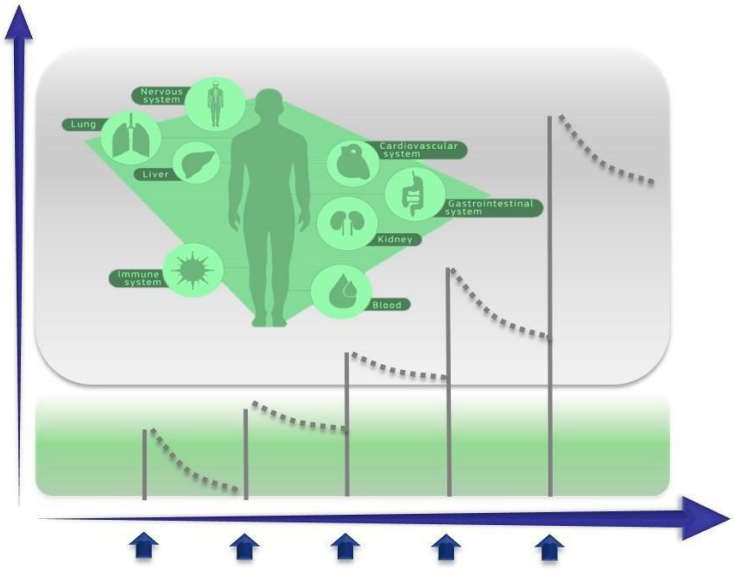



**Figure 2.1:** Mapping mechanistic knowledge on target organ toxicological effects caused by repeat exposure to chemicals.

The aim of this new study is to better understand the nature of repeated dose toxicities by describing the molecular mechanisms or modes of action according to key characteristics of repeated dose systemic toxicity (i.e., key chemical properties by which agents contribute to systemic target organ toxic effects), including their relevance to human health. To help the process, a well-defined set of data rich chemicals will be used to demonstrate the relevance and completeness of the key

3 https://europa.eu/!ux33un

characteristics described. Among the systems or organs relevant for the repeated dose toxicity studies that will be considered are liver, kidney, cardiovascular system, lung, nervous system and gastrointestinal tract. Depending on the analysis and data available, other tissues may be added.

**READ MORE**

Pilar Prieto wins the ALTEX Award 2020: www.altex.org/index.php/altex/announcement/view/255

This study is complementary to EURL ECVAM activities aimed at developing a strategy for evaluating hazard by combining information across different systemic toxicity endpoints (see Section 2.1.4).

### *2.1.4 Making better use of toxicity data by extrapolating across endpoints*

EURL ECVAM has started a project to explore how to develop and evaluate scientifically robust and innovative approaches for the safety assessment of chemicals across multiple regulatory sectors. The aim is to minimise reliance on *in vivo* testing, to avoid redundancy and facilitate the integration of novel non-animal methods in the regulatory setting, with the ultimate goal of designing sustainable testing strategies.

A number of examples were recently published (Madia *et al.*, 2020a) to illustrate and trigger further discussions within the scientific and regulatory communities on how useful information for predicting toxicity can be extrapolated from one toxicity endpoint to another as a mechanistically informed read across approach (Figure 2.2). The work has also highlighted that it is essential to formulate defined questions addressing specific regulatory needs and to define specific scenarios where new integrated approaches can be applied.

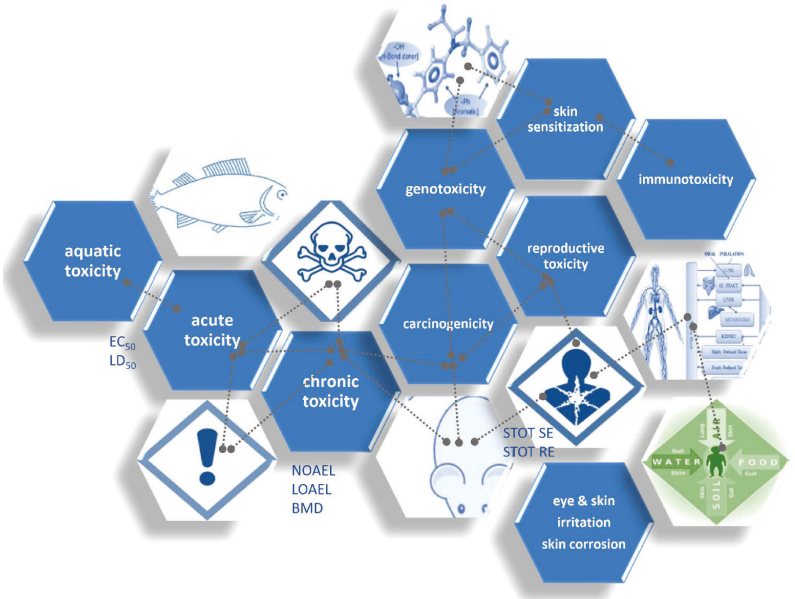



**Figure 2.2:** Extrapolation of information based on mechanistic knowledge across multiple sources and endpoints can help a sustainable and more human-relevant prediction of toxicity. This avoids redundancy of testing and reduces the use of animals (©Madia *et al.*, 2020a, under Creative Common License CC BY 4.0).

Moreover, a number of practical steps need to be made to further evolve such approaches: inclusion of toxicokinetic information; integration of quantitative data into AOP networks; integration of human physiology and pathophysiology in the description of toxicity pathways; using exposure considerations to inform biological relevance; analysis of uncertainties. A project aiming at evaluating the feasibility of a mechanistically informed read across is being finalised within the carcinogenicity testing scenario (Madia *et al.*, manuscript in preparation).

## 2.2 Expanded genotoxicity and carcinogenicity database

In July 2020, EURL ECVAM released an extension of its genotoxicity and carcinogenicity consolidated database. This is in line with EURL ECVAM activities to avoid and reduce animal use in genotoxicity testing (Corvi *et al.*, 2013) and published recommendations on chemicals that would be appropriate to evaluate the sensitivity and specificity of new or modified mammalian cell genotoxicity tests (Kirkland *et al.*, 2016; Kirkland *et al.*, 2007).

The database now includes genotoxicity and carcinogenicity data from various tests for 211 new chemicals with negative results in the Ames test (Box 2.1). This builds on the previously published database of 726 Ames-positive substances.

**READ MORE**

- EURL ECVAM genotoxicity and carcinogenicity consolidated database of Ames negative chemicals: europa.eu/!fU84ym
- EURL ECVAM genotoxicity and carcinogenicity consolidated database of Ames positive chemicals: europa.eu/!RX73pD

### Box 2.1 Expanded genotoxicity and carcinogenicity database

Damage to DNA caused by chemicals is concerning since it can contribute to the initiation and development of many serious health effects including birth defects and cancer.

Thus, the assessment of genotoxicity and carcinogenicity is an essential requirement for the safety assessment of chemical substances. This is typically done in a step-wise approach whereby a battery of *in vitro* tests in both bacterial (Ames test) and mammalian cells are employed to detect genotoxicity effects. In certain cases, *in vitro* testing is then followed by *in vivo* studies in rodents.

In addition, due to its relative simplicity and low cost, the Ames test is usually employed as an initial screen to determine the mutagenic potential of new substances in the early stages of product development.

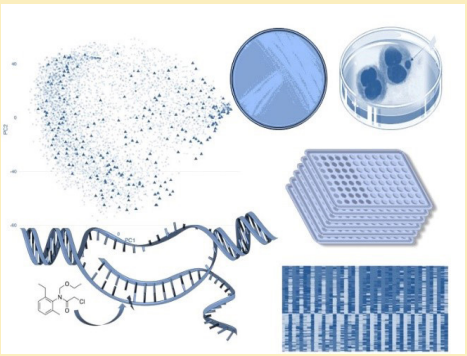
EURL ECVAM scientists collaborated with a group of international experts to compile this unique dataset from several sources. The database is a highly valuable resource for evaluation of genotoxicity studies and the development and validation of alternative methods to animal testing.

According to JRC scientist and study coordinator Federica Madia, “Our genotoxicity database contains rigorously curated information which we’re confident will advance the identification of genotoxicants and our understanding of them”.

The new dataset adds to the EURL ECVAM genotoxicity and carcinogenicity consolidated database of Ames-positive chemicals previously published in the EURL ECVAM Collection of the JRC Data Catalogue.

The database has become a key resource for academia, industry and the regulatory community. It contributes for example to the development and validation of new *in vitro* and computational methods and the design of testing and assessment strategies used in a variety of sectors including food, cosmetics, pesticides and industrial chemicals. Presented at the 17th meeting of the OECD QSAR Toolbox Management Group, the database is proposed to be incorporated in the next QSAR Toolbox update.

A detailed description of the new Ames-negative dataset together with suggestions for further analysis are provided in the related scientific paper 'EURL ECVAM Genotoxicity and Carcinogenicity Database of Substances Eliciting Negative Results in the Ames Test: Construction of the Database' (Madia *et al.*, 2020b).



## 2.3 Endocrine Disruptors

EURION is a cluster of eight EU-funded projects (2019-2024) developing methodology to improve the identification of endocrine disrupting chemicals. It is funded by the European Commission's Horizon 2020 Research and Innovation Programme. Areas of focus are thyroid disruption (ATHENA, ERGO, SCREENED), metabolic disruption (OBERON, GOLIATH and EDCMET), endocrine disruptor induced developmental neurotoxicity (ENDPoinTs) and female reproductive toxicity (FREIA).

Thirteen cross-cluster working groups have been set up to facilitate pooling knowledge and sharing best practices on cross-cutting issues such as validation, chemicals selection, collecting tissue samples to make best use of *in vivo* animal studies, data collection and dissemination, reporting and interpretation of 'omics data, and development of adverse outcome pathways.

A cross-cluster International Advisory Panel (IAP) chaired by EURL ECVAM has also been established with the goal of providing a bridge to other European and international initiatives and regulatory bodies dealing with endocrine disruptors. It also serves to provide guidance and feedback on the regulatory relevance of test methods.

Prior to the annual meeting held in Paris in February 2020, EURL ECVAM conducted a validation workshop for around 70 participants working across all the projects. The focus was on establishing an assay in-house using the OECD guidance on Good *In Vitro* Methods Practice (GIVIMP; OECD, 2018) with emphasis on good documentation of the method description to facilitate transfer of a given method to another laboratory.

**READ MORE**

- EURION: eurion-cluster.eu/
- AOP-Wiki: aopwiki.org/

Training on the principles and best practices of Adverse Outcome Pathway (AOP) development has also taken place in 2020 and an AOP workshop to share progress is planned for January 2021 to facilitate collaborative AOP development and entering of project data into the AOP-Wiki.

## 2.4 Quality control of vaccines

VAC2VAC is a collaborative five-year research project (2016-2021) funded under the Innovative Medicines Initiative, a joint undertaking of the EU Horizon 2020 Research and Innovation Programme and the European Federation of Pharmaceutical Industries and Associations (EFPIA). It started in 2016 as a five-year project and following a one-year, no-cost extension will now end in 2022. The project brings together 22 public and private partners including the JRC represented by EURL ECVAM.

VAC2VAC

The overall objective is to demonstrate proof-of-concept of the consistency approach for batch release testing of established vaccines. For this purpose, non-animal methods are being developed, standardised and validated for the quality control of vaccines for human use (e.g., diphtheria, tetanus, acellular pertussis, tick-borne encephalitis) and veterinary use (e.g., tetanus, rabies, clostridial, leptospiral, infectious bronchitis).

Recent achievements and future work were discussed during the 4th Annual Meeting in March 2020. Several method developers started to transfer their assays to partner laboratories (e.g., monocyte activation test, ELISA for tetanus, diphtheria and tick-borne encephalitis, clostridial vaccines). Several small-scale collaborative studies to assess assay transferability and reproducibility are planned to take place during 2021.

EURL ECVAM is coordinating an activity aimed at developing guidance on how to move from the classical *in vivo* test-based quality control strategy to a consistency test-based strategy relying on non-animal based assays. Initial discussions with international experts (e.g., VAC2VAC, EDQM, WHO, national control authorities) took place in 2020 and will be continued in 2021.

**READ MORE**

- VAC2VAC: www.vac2vac.eu
- VAC2VAC newsletter: www.vac2vac.eu/news-events/news/vac2vac-newsletter-voli-ii-november-2020
- Innovative Medicine Initiative (IMI): www.imi.europa.eu

## 2.5 Biokinetics

### *2.5.1 In vitro biokinetic data*

Although not typically a regulatory requirement, information on the biokinetics of a substance, characterised by physiological processes of absorption, distribution, metabolism and excretion (ADME), can be valuable for informing risk assessment.

To this end, a study was conducted and published in the JRC data catalogue to make available *in vitro* data on blood-plasma protein binding (relevant to absorption) and liver hepatocyte clearance (relevant to metabolism) for a set of 77 (primarily) industrial chemicals. The study also explored the utility of these data for biokinetic profiling and quantitative *in vitro* to *in vivo* extrapolation (qIVIVE). The data will also be exploited in a Next Generation Chemical Risk Assessment case study being developed within the context of the APCRA project (see Section 2.1.2).

### *2.5.2 Physiologically based kinetic models*

Within the context of the EPAA, EURL ECVAM is a "champion" (i.e., responsible to coordinate the project team and keep the communication active with the EPAA steering committee) of two projects related to physiologically based kinetic (PBK) models (see also Box 4.3).

EURL ECVAM was also involved in an international effort led by HESI to propose a harmonised PBK model reporting template. This activity was carried out to support good modelling practices and to facilitate the regulatory acceptance of the growing number of available PBK models (Tan *et al.*, 2020).

EURL ECVAM further explored the use of PBK models in the context of interpreting human biomonitoring data (see Box 2.2).

### *2.5.3 Advancing the kinetically derived maximum dose concept*

The kinetically derived maximum dose (KMD) is an important concept relevant to the dose selection in repeated dose toxicity studies. It is defined as the internal concentration of a chemical (area under the curve) that marks a departure from linear kinetics. Non-linear kinetics can arise from the saturation of ADME processes (like metabolism, Figure 2.3), resulting in chemical concentrations in the body that are disproportionally high or low relative to the change in external dose.

A public symposium on the "Kinetically Derived Maximum Dose Concept to Refine Risk Assessment" was held in September 2020. The symposium was organised by NICEATM, the U.S. Environmental Protection Agency, and the Health and Environmental Sciences Institute.

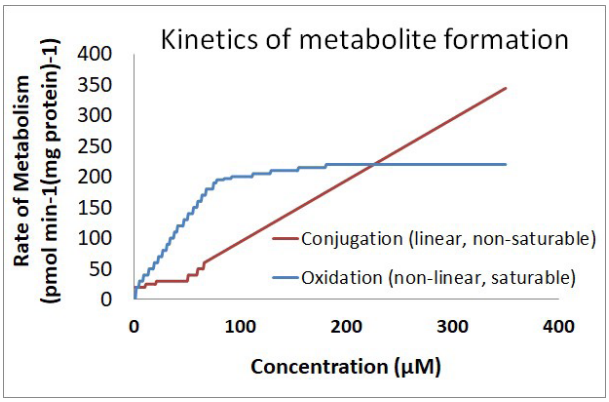



**Figure 2.3:** Non-linear and linear kinetics of metabolism.

The public forum was followed by a scientific workshop focussing on four topics: inflection point (appropriate use of kinetic data to determine dose non-linearity); exposure (likelihood of human exposure levels close to a KMD value); dose-setting and 3Rs[4] (determination of a KMD from human blood or tissue concentration data); and pharmacodynamics / weight of evidence (application of *in silico* models to predict systemic dose and key ADME parameters to inform a KMD in a WoE approach to select the top dose for an *in vivo* toxicity study). EURL ECVAM was involved in the scientific organisation of the workshop and presented during the pharmacodynamics / weight of evidence session. One of the outcomes of the scientific workshop was that recent advances in the development of *in vitro* and *in silico* methods for kinetics and toxicodynamics will be crucial in establishing a "new approach methodologies toolbox" for informed KMD setting in regulatory toxicology.

**READ MORE**

Symposium Webinar: Opportunities and Challenges in Using the Kinetically Derived Maximum Dose Concept to Refine Risk Assessment: ntp.niehs.nih.gov/go/kmd-2020

### *2.5.4 Species differences in metabolism*

EURL ECVAM has contributed to a project led by Wageningen University and Research (the Netherlands), to analyse the variability in liver metabolism (clearance) between rats and humans, using literature-derived *in vitro* clearance data for rat and human primary hepatocytes (Louisse *et al.*, 2020). A large variability in reported Cmax value was identified (1- to 8-fold for different compounds), which partly relates to differences in study designs but also to variability in the methodological components, such as materials and reagents used in *in vitro* culture like foetal bovine serum (FBS). This work highlights the need to harmonise clearance methods. Ongoing work is investigating the implications of using such clearance data in the development of PBK models for interspecies variability.

## 2.6 From *in silico* medicine to *in silico* toxicology

In July 2020, EURL ECVAM hosted an online workshop aimed at understanding how the acceptance of mathematical models in the pharmaceutical domain differs from the chemical domain, and what lessons can be learned in terms of translating successful applications from one domain to the other. As a follow-up to the workshop, a case study is being developed with the University of Catania, Italy, to explore the translatability of the Universal Immune System Simulator (UISS) from the pharmaceuticals to the environmental chemicals domain. The UISS is being developed in the EU-funded STRITUVAD project, which is applying computer simulations to test the efficacy of new anti-tuberculosis therapies (*in silico* trials). This will also be a contribution to the CIAO project (see Section 5.3).

**READ MORE**

Developing *in silico* trials to fight tuberculosis: www.strituvad.eu/

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

## Box 2.2 Use of PBK modelling to interpret human biomonitoring data of mixtures

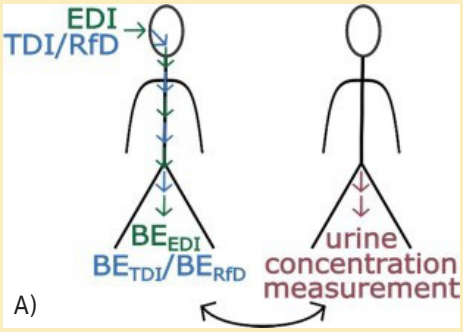
Human biomonitoring (HBM) data can provide insight into co-exposure patterns resulting from exposure to multiple chemicals from various sources and over time. Therefore, such data are particularly valuable for assessing potential risks from combined exposure to mixtures (see also Section 4.8.2).

One way of interpreting HBM data is to establish safe levels in blood or urine, called Biomonitoring Equivalents (BE) or HBM values. These can be derived from established external reference values, such as tolerable daily intake (TDI) values, by using physiologically based kinetic (PBK) models (see also Section 2.5.2). The process of establishing BE values using chemical-specific models can be time consuming and cumbersome.

For this reason, the derivation of BE values for several chemicals was investigated by using generic PBK models (Pletz *et al.*, 2020).

Two generic models were selected, the IndusChemFate tool and the High-Throughput Toxicokinetics package, for two different classes of chemicals, phenols and phthalates. HBM data from two cohorts, Danish children and Norwegian mothers and children, were used to evaluate the quality of the predictions and to illustrate the overall approach in the context of mixture risk assessment.

The study showed that use of PBK models enables a better understanding and interpretation of HBM data. However, establishing safety threshold levels of metabolites in urine is a challenging task, compared with the analysis of parent compounds in blood.




$$\frac{dC_{liver}}{dt} = \frac{\left[C_{blood} - \left(C_{liver} / R_{liver}\right)\right]}{V_{liver}} \cdot Q_{liver}$$

B)

A) Schematic Representation taken from Pletz *et al.*, (2020) showing Tolerable Daily Intake (TDI), Reference Dose (RfD) and Estimated Daily Intake (EDI) values as input doses in a forward dosimetry approach to obtain Biomonitoring Equivalents $BE_{TDI}$, $BE_{RfD}$ and $BE_{EDI}$ values for risk assessment. The derived BE values can subsequently be compared to actual measured urinary concentrations. B) Example of a PBK model ordinary differential equation (of the liver) governing the chemicals fate in the body (C=concentration, t=time, V=volume, Q=blood flow rate, R=partition coefficient).


## 2.7 Modelling AOPs and AOP networks

The digital transformation is expected to have a considerable impact on toxicology. The last decade has seen an enormous growth in e-resources capturing data related to the toxicological effects of chemicals and supporting assessments of hazardous properties based on chemistry alone (Madden *et al.*, 2020). However, it remains a challenge to identify and exploit these resources in an optimal way to support decision making.

To this end, EURL ECVAM together with the Liverpool John Moores University and RIVM organised a workshop in October 2019 at the Lorentz Center (Leiden, NL) entitled 'e-Resources to Revolutionise Toxicology: Linking Data to Decisions'. This workshop aimed to map out how and where digital resources could contribute to the digital revolution in toxicology, with specific objectives to: a) identify how digital resources could enhance the AOP paradigm; and b) develop four quantitative

AOPs by utilising currently available digital resources, providing better insight into data requirements including accessibility. The three quantitative AOPs, related to skin sensitisation, neurotoxicity and carcinogenicity, were considered as case studies to facilitate the evaluation of e-tools and e-infrastructure for developing quantitative AOPs. A paper is being drafted to capture key learnings and set out future perspectives.

In related work, the emerging concept of quantitative AOP (qAOP) was analysed and further developed on the basis of an extensive literature review of published qAOP models (Spinu *et al.*, 2020).

## 2.8 *In silico* toxicology protocol

A global consortium on *in silico* toxicology protocols, led by Leadscope Inc. US and partly funded by the NIEHS, is developing *in silico* protocols for major toxicological endpoints, similar to test guidelines routinely used in the application of *in vitro* or *in vivo* methods. The aim is to improve the efficiency, quality, and acceptance of assessments based on *in silico* methods. A genetic toxicology and a skin sensitisation *in silico* protocol have been completed (Hasselgren *et al.*, 2019; Johnson *et al.*, 2020), while protocols on acute toxicity, neurotoxicity, endocrine activity, and environmental toxicity are under development.

The work on the carcinogenicity protocol started in 2019, with the aim of assembling the available information on different aspects of carcinogenicity associated with the ten key characteristics of carcinogens, as defined by Smith *et al.*, (2016). *Ad hoc* sub-teams, including EURL ECVAM, have been established to work on these aspects. A position paper summarising the current status of *in silico* methods useful for carcinogenicity hazard assessment together with a description and discussion of identified gaps, has been prepared (Tice *et al.*, submitted).

## 2.9 Efficient design of testing strategies

Hazard classification forms the basis of generic risk assessment and subsequent risk management of chemicals in the EU. While chemical classification based on standard checklists of information triggers risk management measures, the link is not one-to-one. Toxicity testing may end up being performed with no impact on the safe use of chemicals. Based on the premise that the hazard class of a chemical reflects its level of concern, EURL ECVAM carried out a study to explore how an equivalent level of concern could be derived through the use of more efficient testing strategies.

The testing strategy could be optimised according to different criteria, minimising the total number of tests conducted, or minimising the use of animals. This work has been published as a thought starter to trigger discussions on how classification and labelling of chemicals might be carried out more efficiently (Da Silva *et al.*, 2020).

Mixture risk assessment (MRA) strategies for the evaluation of developmental neurotoxicity (DNT) should be implemented, since infants and children are co-exposed to more than one chemical at a time. In a recent study (Pistollato *et al.*, 2020b; see Box 2.3), EURL ECVAM described a possible approach to tackle MRA: DNT chemicals were clustered in mixtures on the basis of their mode of action

(MoA), and DNT assays were anchored to common key events (CKEs) identified in DNT-specific adverse outcome pathways.

Analyses of chemical mixtures effects were carried out in human induced pluripotent stem cell (hiPSC)-derived neuronal and glial cultures, which is considered a suitable *in vitro* model to enable mechanistic understanding of chemically-induced adverse effects, avoiding species extrapolation. Data showed that chemicals working through similar MoA, at non-cytotoxic or very low toxic concentrations, induce DNT effects in mixtures, as shown by increased number of neurons, impairment of neurite outgrowth and synaptogenesis. Synaptogenesis was the most sensitive endpoint as confirmed by mathematical modelling.

## Box 2.3 New approaches for assessing the neurotoxicity of mixtures

A EURL ECVAM study shows that the effects of chemical mixtures on the developing brain can be assessed using human cells *in vitro* combined with mathematical modelling (Pistollato *et al.*, 2020b).

EURL ECVAM scientists found that chemicals at non-toxic concentrations combined in mixtures can cause adverse effects in developing human brain cells. The chemical mixtures altered significantly several cellular processes which are known to be indicators of developmental neurotoxicity (DNT), particularly those linked to cognitive deficits in children.

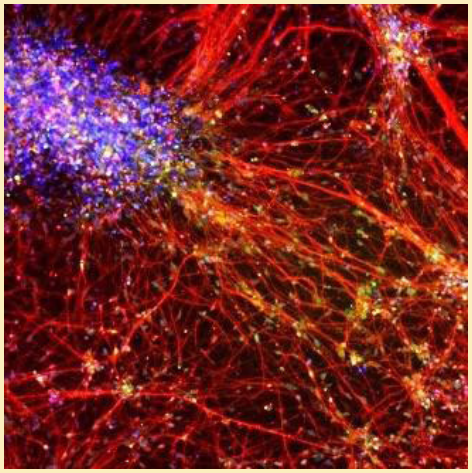
The EURL ECVAM team exposed human developing brain cells to single chemicals and their mixtures at concentrations found in human samples, mimicking real life exposure.

"We have exposed the cells to single chemicals and chemical mixtures because it is well documented that so called mixture effects can be greater than the effects triggered by the most potent single chemical in a mixture, due to their additive or, in some cases, even synergistic effects" explains Francesca Pistollato, EURL ECVAM scientist and lead author of the article.

This is potentially a matter of concern because different environmental chemicals have been found together in human biofluids such as breast milk and cord blood. Moreover, the developing brain is highly sensitive to the effects of these chemicals. "We are all exposed to more than one chemical at a time but more vulnerable individuals are pregnant women, infants and children, since these life stages are particularly sensitive to the effects of chemicals" continues Francesca.

After exposing the cells to the mixtures, the scientists evaluated whether key neurodevelopmental processes critical for learning and memory formation were impaired. Mathematical modelling confirmed that the combined effects of chemicals in a mixture can generally be predicted without testing the mixture itself, provided that the individual chemicals have been tested at relevant concentrations. Notably, these effects found in cell-based assays reproduce to a certain extent some autism-like cellular changes that are also observed in the brains of autistic children.

Overall, this study illustrates how mechanistic knowledge of DNT effects, captured as adverse outcome pathways, can guide the design and integration of *in vitro* methods and mathematical modelling to predict possible adverse effects that may result from exposure to chemical mixtures. It therefore represents a step forward towards more reliable DNT testing for protecting public health.



# Validation

At the core of EURL ECVAM activities is the validation of test methods and approaches. Validation is at the interface between test method development/optimisation and regulatory acceptance/international recognition and ensures a science-based and rigorous evaluation of test methods and approaches by establishing their performance and fitness for a given purpose, i.e., their scientific validity.

The EURL ECVAM validation process encompasses four key steps, including the assessment of test method submissions, the planning and conduct of validation studies, the coordination of independent scientific peer reviews and the development of EURL ECVAM Recommendations on the validity status of test methods. These steps involve close interaction with key stakeholders and international partners.

The status of each test method can be monitored using the EURL ECVAM Tracking System for Alternative methods towards Regulatory acceptance (TSAR).

# 3. Validation

## 3.1 Test method submissions

The following sections summarise the progress of selected test method submissions that EURL ECVAM has recently received. A comprehensive list of test method submissions and their status can be found in TSAR (https://tsar.jrc.ec.europa.eu/).

### *3.1.1 ALIsens*

Air–liquid interface (ALI) *in vitro* lung cell models can be used to investigate physiological and pathophysiological responses of the respiratory tract, molecular events, modes of action and interaction of different cell types. Being apically exposed to air, these models potentially mimic more closely the *in vivo* situation of cells in the respiratory tract than standard cell culture methods. EURL ECVAM received a test presubmission on an *in vitro* method (named ALIsens) for the prediction of respiratory irritation and sensitisation of inhalable substances. The ALIsens method uses a test system consisting of a 3D lung tissue model cultured at the air-liquid interface. It addresses two steps in the respiratory sensitisation process by measuring the differential induction of cytokines at the epithelial barrier and the activation of immature dendritic cells (DCs) using different biological endpoints (i.e., cell surface markers, cytokines and gene expression).

EURL ECVAM consulted its Preliminary Assessment of Regulatory Relevance (PARERE) network on the regulatory relevance of the test method and shared with its members its preliminary assessment. Overall, there was a general view that the submitted test method is promising and might be of potential use in a regulatory context, also for defining classification criteria under the EU CLP Regulation

and the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). However, the method needs to be further developed and additional testing is required to evaluate whether it can distinguish between irritants and sensitisers. Between-laboratory reproducibility is also crucial to assess the reliability of the test method, particularly as a step towards the development of a test guideline for widespread application. Finally, there was general agreement that the method could eventually be used to investigate molecular pathways and specific reactions related to different parts of the respiratory tract in basic and translational research. EURL ECVAM invited the submitter to address the issues raised and to resubmit an updated presubmission.

**READ MORE**

- ALIsens method: tsar.jrc.ec.europa.eu/test-method/tm2019-01

### *3.1.2 Genotoxicity testing*

In an effort to improve the predictive capacity of the *in vitro* genotoxicity test battery, Cosmetics Europe has led an initiative to develop and validate *in vitro* human skin-based genotoxicity assays for topically exposed substances, such as cosmetics ingredients. An international validation study was carried out specifically for the reconstructed skin (RS) comet assay and micronucleus test (RSMN). Between March and June 2020, Cosmetics Europe submitted these two methods to EURL ECVAM for consideration to undergo an ESAC peer-review.

The RS Comet Assay is an adaptation of the known alkaline comet assay to address the potential of test items to cause genotoxicity in the form of DNA strand breaks, which can lead to clastogenic effects, and DNA lesions leading to gene mutations (Pfuhler *et al.*, 2020a). The method uses specifically the Phenion® Full-Thickness Skin Model, which is composed of primary and p53 competent keratinocytes and fibroblasts of human origin. The RSMN Assay applies the known micronucleus test in a three-dimensional reconstructed human skin model and addresses the potential of test items to cause genotoxicity in the form of chromosomal damage (clastogenicity and aneugenicity; Pfuhler *et al.*, in press). The method uses the EpiDerm™ Skin Model, composed of normal human epidermal keratinocytes derived from neo-natal foreskin tissue.

EURL ECVAM invited the submitter to put forward a full submission for the two test methods, taking into consideration the information provided, the implications of the tests to predict the genotoxic potential of primarily dermally exposed substances in combination and/or in support of the standard *in vitro* test battery and their potential regulatory relevance.

**READ MORE**

- RS Comet Assay: tsar.jrc.ec.europa.eu/test-method/tm2020-01
- 3D Reconstructed Human Skin Micronucleus assay: tsar.jrc.ec.europa.eu/test-method/tm2020-04

A proposal for the development of new OECD Test Guidelines for both assays has been included in the OECD work plan in 2019. In addition, recent regulatory guidance documents within the pharmaceuticals and cosmetics sectors (i.e., SCCS, ICH) and the International Workshops on Genotoxicity Testing (IWGT) working group on "Use of 3D Tissues in Genotoxicity Testing" have expressed favourable opinions with regard to the appropriateness of these test methods (ICH, 2011; Pfuhler *et al.*, 2020b; SCCS, 2018).

### *3.1.3 SEN-SIS*

Subsequent to the EURL ECVAM communication to ImmunoSearch in July 2019 that there were still some shortcomings in the SENS-IS re-submission that prevented the method from entering peer review, in October 2020, EURL ECVAM received additional information from the test submitter to address the comments made. Following a completeness check of the information provided, EURL ECVAM concluded that the

**READ MORE**

SENS-IS method: tsar.jrc.ec.europa.eu/test-method/tm2011-11

SENS-IS submission is sufficiently complete to enter independent peer-review by ESAC. This decision was communicated to the test submitter in December 2020 with a request to re-arrange some of the information and data provided in order to facilitate the ESAC assessment of the submission. The information on the SENS-IS, re-arranged according to EURL ECVAM suggestions was received in December 2020.

## 3.2 Validation studies

Currently, EURL ECVAM is coordinating a validation study on thyroid methods. Information is also provided in this section on the progress made by the EDQM Biological Standardisation Programme.

### *3.2.1 Thyroid validation study*

Thyroid hormones regulate metabolic homeostasis and impairment of homeostasis has been associated with several adverse health effects. Chemicals that disrupt thyroid function have the potential to be endocrine disruptors (EDs). With a view to increasing the available battery of non-animal methods for identifying EDs, EURL ECVAM is conducting a multi-laboratory study aimed at validating a number of *in vitro* methods (OECD, 2017) for different modes of action on the thyroid system (see an example in Box 3.1).

Fifteen laboratories from the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL, see Box 3.2), are participating in this two-part validation study. Further to the thyroid expert meetings in 2019 and 2020, one laboratory has been formally added to evaluate the important block of methods that integrate multiple modes of action (thyroid methods 8b and 8c).

Part 1, currently ongoing, aims to characterise the methods through the development of standard operating procedures (SOPs) and the assessment of transferability and within-laboratory reproducibility. Known positive/negative control and reference chemicals are being used. Different challenges were encountered depending on the available methods, i.e., available peer-reviewed papers are old and the developer is no longer available; test systems were not commercially available or standardised; reference items did not show the expected outcome; methods were not user-friendly and needed to be adapted and; reproducibility of the methods was not sufficient. Part 2 will aim to assess the overall relevance of selected test methods, using a set of 30 reference chemicals, selected on the basis of known modes of action and available *in vivo* study data.

The OECD GIVMP guidance document on the development and proper conduct of *in vitro* methods (OECD, 2018) provides a sound basis for conducting the thyroid validation study according to the latest study quality guidelines. The OECD GIVIMP guidance document provided practical guidance to test method developers and EU-NETVAL test facilities participating in the validation study on aspects such as, test system characterisation, method performance, choice of reference and control items, and SOP design.

A challenge for the success of this validation study is the appropriate selection of a relevant set of chemicals, which should cover the various modes of action, to be tested in the validation study. In support of the chemical selection for Part 2, an expert meeting was organised in November 2019. Prior to this meeting, experts were surveyed to propose potential validation set chemicals with known

activity in at least one of the methods/mechanisms of action. This resulted in an initial list of 87 possible test chemicals. During the meeting, 51 chemicals were short-listed, including negative control chemicals, which were considered to best cover the methods and their modes of action. The shortlist was further reduced to 30 chemicals based on information provided by chemoinformatics and Artificial Intelligence (AI) tools, i.e., machine learning and text analytics.

Part 2 testing was initiated at the end of 2020 for a human liver microsome based diodinase 1 (DIO 1) inhibition method by one of the participating EU-NETVAL test facilities (BASF, Germany). Diodinases are one of the important regulators of systemic and local thyroid hormone balance by activation of T4 to T3 and degradation of thyroid hormones via deiodination. DIO1, one of the three isoforms, serves as one main source for circulating T3 via deiodination of T4 in liver, kidney and thyroid and has a role in rescuing thyroid hormone bound iodide from biliary excretion. Jointly with the original developer (Charité Berlin and BfR, Germany), a non-radioactive approach to determine substance induced DIO1 inhibition based on iodide release activity (Renko *et al.*, 2012) of human liver microsomes was established. Standardisation efforts regarding batch specific enzyme activity further improved the performance of the human liver microsome based DIO inhibition *in vitro* method.

The thyroid validation study will also serve as a case study to implement the recently updated OECD Harmonised Template (OHT) 201 to report mechanistic data generated in the thyroid validation study. EURL ECVAM and selected EU-NETVAL test facilities provided critical input in the update of the OHT 201 template (see also Section 4.2.9). The description of the methods under consideration can be found in TSAR.

**READ MORE**

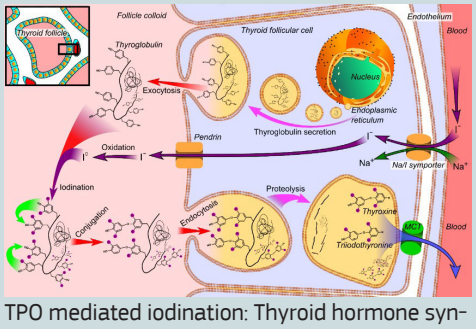
- Thyroid methods: tsar.jrc.ec.europa.eu/search-test-methods-a?search_combined_anonymous=thyroid+method
- Thyroid method 8b: tsar.jrc.ec.europa.eu/test-method/tm2019-18
- Thyroid method 8c: tsar.jrc.ec.europa.eu/test-method/tm2019-19

## Box 3.1 Thyroid validation study - example of one of the methods under validation: tyrosine iodination using liquid chromatography

Within the EU-NETVAL network of European validation laboratories currently various *in vitro* methods are developed and validated targeting different modes of action (MoA) of thyroid disruption (OECD, 2014). One of the targeted MoAs is inhibition of thyroperoxidase (TPO), an enzyme critical in thyroid hormone synthesis. Although various methods for investigating inhibition of TPO mediated iodination are described in literature, most methods are not completely animal-free and are not, or partially, validated, and as Valsuch, there is a need for alternative methods.

In the current work, an *in vitro* method to assess chemicals for potential inhibition of TPO iodination using FTC-238-hrTPO cell homogenates containing TPO was developed and implemented to be ready for validation. The model substrate L-tyrosine was incubated with potassium iodide, FTC-238-hrTPO cell homogenate and peroxide. TPO enzymatically converts L-tyrosine into the metabolite mono-iodotyrosine (MIT). MIT formation was quantified using Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) as a direct measurement of TPO activity. As such, this *in vitro* TPO method is a promising tool for medium-throughput screening of chemicals on inhibition of TPO, hereby contributing to the setup of a robust toolbox of relevant *in vitro* methods to predict thyroid disruption in the framework of the ECHA/EFSA Guidance on identification of endocrine disruption and other global regulatory needs.

Read more on the method in TSAR: https://tsar.jrc.ec.europa.eu/test-method/tm2019-06




TPO mediated iodination: Thyroid hormone synthesis, with thyroid peroxidase performing the oxidation step seen at center-left (©Häggström, 2014 under Creative Common Licence CC0 1.0).

**Box 3.2 European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL)**

EURL ECVAM set up a consortium that currently counts 35 European laboratories (mostly GLP approved), called EU-NETVAL in 2013. Fifteen EU-NETVAL test facilities are experimentally involved in the development and validation of new cell and tissue-based mechanistic methods to target different modes of action (MoA) of thyroid disruption (see Section 3.2.1).

EU-NETVAL also participated in the AR-CALUX validation study that led to OECD test guideline 458 on androgen receptor transactivation assays (see Section 4.2.4).

Together with the EU-NETVAL test facilities, the thyroid validation study will be a pilot case study to actively start to implement the recently updated OECD Harmonised Templates (OHT) 201 (see Section 4.2.9) to report thyroid validation study data obtained with the evaluated thyroid methods.

EURL ECVAM and selected EU-NETVAL test facilities (BASF, Aptuit, Charles River, Wageningen University & Research and Sciensano) have given critical input in the update of the OHT 201 template and indicated during the review process, the need for GIVIMP (OECD, 2018) in *in vitro* method development and validation.

OHT 201 has been implemented in IUCLID, a software used by industry to fulfil reporting obligations under more and more legislative programmes (e.g., REACH). *In vitro* and *in silico* mechanistic study data can be reported for regulatory use which is an important step towards the acceptance of *in vitro* mechanistic thyroid results.

EURL ECVAM and three EU-NETVAL test facilities (BASF, ARPEA, ABICH) were in 2020 selected to become members of the new "EFSA Technical group on Notification of Studies Database" to test the usability of this new EFSA database. Together with other stakeholders, the EU-NETVAL test facilities review proposed requirements, data model and workflows. EFSA sought the participation of EU-NETVAL facilities since they are expert test facilities in performing studies within the scope of the General Food Law (GFL) Regulation.

Two new EU-NETVAL facilities joined EU-NETVAL in 2020: the Norwegian Institute for Water Research, Norsk Institutt for Vannforskning from the EFTA country Norway and, ERT, Centre of Experimental Medicine (CEM), Slovak Academy of Sciences from the Slovak Republic.

The new members reinforce the network with their experimental experience, but also with their GIVIMP compliant in-house validation experience and their experience in regulatory multi-laboratory validation trials.

### *3.2.2 Vaccine quality control – EDQM Biological Standardisation Programme*

The Biological Standardisation Programme (BSP) of the European Directorate for the Quality of Medicines & HealthCare (EDQM, Council of Europe) is a joint initiative of the Council of Europe and the EU, partly funded by the European Commission. It focuses on the establishment of reference preparation and validation of analytical methods for the quality control of biologicals, including Three Rs methods.

**READ MORE**

▸▸ EDQM Biological Standardisation Programme: www.edqm.eu/en/Biological-Standardisation-Programme-mission-60.html

Recently, two projects validating a serological method for potency testing of whole-cell pertussis vaccines and *in vitro* methods for the testing of *Clostridium septicum* vaccines (see also Section 4.6), have been finalised. Other projects reported in 2019 (Zuang *et al.*, 2020) are ongoing.

## 3.3 EURL ECVAM Scientific Advisory Committee (ESAC) peer reviews

The EURL ECVAM Scientific Advisory Committee (ESAC) advises EURL ECVAM on scientific issues. ESAC's main role is to conduct independent peer reviews of non-animal methods/approaches at the request of EURL ECVAM, assessing their scientific validity for a given purpose. ESAC peer reviews are formally initiated with a "EURL ECVAM Request for ESAC Advice", which provides the necessary background for the review and establishes its objectives, timelines and the questions to be addressed. ESAC peer reviews are normally prepared by specialised ESAC Working Groups (WGs) and the ESAC's advice to EURL ECVAM is formally provided as a "WG Report" and an "ESAC Opinion" at the end of the review.

The ESAC consists of a minimum of nine core members plus *ad hoc* members. The core members are appointed as individual experts based on their personal capacity and scientific excellence. The current mandate of the core membership of the ESAC will end on 15 April 2021. The *ad hoc* members are selected to serve in ESAC WGs based on their expertise on the methods or approaches and/or the scientific questions under review.

The last plenary meeting of the ESAC was held on 2-3 December 2019. During this meeting, the ESAC endorsed its final Opinion on the Bioelution test method (EURL ECVAM Scientific Advisory Committee, 2020) and agreed to peer review the Genomic allergen rapid detection (GARD™) assay for skin sensitisation testing (see Section 3.3.1).

Due to the situation with the COVID-19 pandemic, an ESAC plenary meeting could not be organised in June 2020 and was postponed to March 2021. In November, there was a virtual meeting of the ESAC WG on the GARD (see Section 3.3.1 below).

**READ MORE**

EURL ECVAM Scientific Advisory Committee (ESAC): europa.eu/!UU87JH

### *3.3.1 GARD*

After receiving a revised submission of the GARD potency method in August 2019, EURL ECVAM assessed the GARD skin and GARD potency submissions and deemed that both methods could enter the ESAC peer review process. An ESAC WG on the GARD was established early in 2020 and met virtually for the first time in June 2020. Subsequent meetings took place in September, October, November and December to advance the review and draft a WG Report. EURL ECVAM has facilitated interactions between the WG and the test submitter SenzaGen to clarify aspects of the submissions and to provide additional information requested by the ESAC WG to conduct a proper assessment of the GARD prediction model. The WG aims to finalise its peer review report with a view to discussing and possibly endorsing the ESAC Opinion in March 2021.

**READ MORE**

GARD method: tsar.jrc.ec.europa.eu/test-method/tm2011-09

## 3.4 EURL ECVAM Recommendations

EURL ECVAM has issued a Recommendation urging end-users and other stakeholders to recognise the scientific validity of non-animal-derived antibodies and to stop using animals for antibody development and production if not scientifically justified (Barroso *et al.*, 2020, see also Box 3.3).

The Recommendation is based on the opinion of EURL ECVAM's Scientific Advisory Committee (ESAC) and states that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic

and therapeutic applications, if not justified on purely scientific grounds. It also challenges misconceptions existing in the scientific community about non-animal-derived antibodies and highlights the scientific and economic benefits of their use.

Every year in the EU, close to 1 million animals are used for antibody generation and production despite the availability of technologies that do not necessitate the use of animals. Not only is this number high but the procedures employed often cause severe suffering. The Recommendation proposes concrete actions for key actors including end-users, commercial providers, authorities, research funding bodies and journal editors.

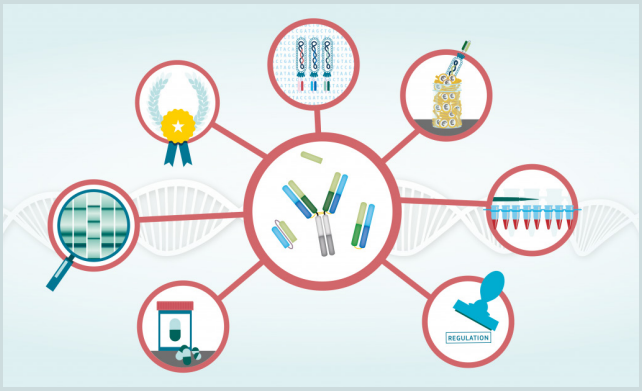
### Box 3.3 EURL ECVAM Recommendation on non-animal-derived antibodies

EURL ECVAM recommends that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic applications. In the EU, the provisions of Directive 2010/63/EU should be respected and EU countries should no longer authorise the development and production of antibodies through animal immunisation, where robust, legitimate scientific justification is lacking.

The EURL ECVAM Recommendation is published as a JRC Science for Policy Report and is based on an independent scientific peer review conducted by EURL ECVAM's Scientific Advisory Committee (ESAC). The ESAC Opinion on the "Scientific validity of replacements for animal-derived antibodies" and the accompanying ESAC Working Group report are annexed to the Recommendation report.

During the development of the Recommendation, EURL ECVAM consulted with other Commission services and relevant EU regulatory agencies, EURL ECVAM's advisory body for Preliminary Assessment of Regulatory Relevance (PARERE), the EURL ECVAM Stakeholder Forum (ESTAF) and with partner organisations of the International Collaboration on Alternative Test Methods (ICATM).

EURL ECVAM has published responses to frequently asked questions (FAQs) on the Recommendation to clarify several important issues and facilitate proper interpretation and communication of the recommendations made. The FAQS are available at the following link: https://ec.europa.eu/jrc/en/eurl/ecvam/faqs/non-animal-derived-antibodies.



## 3.5 Meeting of the Preliminary Assessment of Regulatory Relevance (PARERE) network

The ninth PARERE meeting took place at the JRC, Italy on 21 October 2019. It included a round-table on activities within the PARERE network, updates from EURL ECVAM, other Commission services and EU agencies. EURL ECVAM also provided updates on the AOP framework analysis study carried out by EURL ECVAM for which PARERE members had been surveyed (see Section 4.3.1 and Section 5.7.4 of Zuang *et al.*, (2020)). In addition, a session on "Non-animal approaches under EU Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP regulation) and the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)" had been organised.

Regarding the latter, EURL ECVAM provided an overview of the activities of the informal working group on non-animal testing methods established at the UN GHS in 2017. The task of the working group is to revisit the current GHS text to enable classification based on non-animal methods and approaches (see Section 4.7.1). GHS is implemented in the EU through the CLP regulation. More information on PARERE and on the outcome of the PARERE meeting can be found in Box 3.4 and at the link in the "Read more" section, respectively.

**Box 3.4 Preliminary Assessment of Regulatory Relevance (PARERE) network**

The Preliminary Assessment of Regulatory Relevance (PARERE) network was established by EURL ECVAM further to a provision of Directive 2010/63/EU that 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.

This trans-sectorial network is composed of regulators nominated by 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 Commission services.

In order to expedite the process of regulatory acceptance of alternative methods, it was considered that regulators operating within all sectors of relevance to alternative methods should be 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 has some additional tasks which are described on the EURL ECVAM website: https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/advisory-bodies/parere.

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 and case studies being developed within research projects funded by the EU Framework Programme for Research and Innovation.

On 22 November 2019, both PARERE and ESTAF networks were invited to the ICATM workshop on "The Future of Alternative Methods for Regulatory Testing and their Contribution to Public Health", held at the JRC, Ispra, Italy (see Section 3.7).

In 2020, the PARERE meeting was held online on 25 November. The purpose of the meeting was to provide updates on activities related to alternative methods

and approaches being undertaken in EU Member States and by Commission services and EU agencies; to seek input on a possible validation framework for non-animal methods in the area of respiratory sensitisation; and to have an exploratory discussion on organ-on-a-chip technologies and their potential for translation into the regulatory arena.

**READ MORE**

▸▸ Summary record of PARERE meeting 2019: europa.eu/!pf73wd

## 3.6 TSAR

TSAR is a web-based tracking system, which provides the latest information on the status of alternative methods which have been proposed for validation with a view to their use for a regulatory purpose. It contains methods under evaluation by EURL ECVAM and by members of the International Cooperation on Alternative Testing Methods (ICATM, see Box 3.5).

TSAR provides an overview of alternative (non-animal) methods that have been proposed for regulatory safety or efficacy testing of chemicals or biological agents such as vaccines. It tracks the progress, in a transparent manner, from proposal of an alternative method for validation through its final adoption by inclusion into the regulatory framework (e.g., EU legislation, Organisation for Economic Collaboration and Development (OECD) Test Guidelines or Guidance documents, European Pharmacopoeia, International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human/veterinarian use (ICH), International Organization for Standardization (ISO), etc.).

TSAR provides information on the validation status of a method and indicates the stages it has reached in terms of acceptance as a recognised test method for use in various sectors together with a method summary description. Where available, TSAR also includes relevant documents associated with a method linked to the different steps of the entire process: submission, validation, peer-review, recommendations and regulatory acceptance including international standards.

In 2020, EURL ECVAM improved TSAR to increase knowledge sharing on the regulatory acceptance of alternative methods with its main stakeholders. With the enhancement of the query mechanism and the provision of additional information, users can now benefit from a better overview of the evaluation process.

**READ MORE**

▸▸ TSAR: tsar.jrc.ec.europa.eu

## 3.7 International Cooperation on Alternative Test Methods (ICATM)

EURL ECVAM hosted a workshop on "The Future of Alternative Methods for Regulatory Testing and their Contribution to Public Health" on 22 October 2019. It was held in conjunction with meetings of ECVAM's regulatory advisory network (PARERE; see Section 3.5) and its stakeholder forum (ESTAF).

The aim of the workshop was to celebrate the 10$^{th}$ anniversary of ICATM, to raise ICATM's visibility and to discuss the future outlook of alternatives in the different jurisdictions of the ICATM partners. Invited participants from validation bodies, European agencies, national regulatory authorities and stakeholder organisations reflected on past achievements and looked to the future.

During the discussion session, the participants were asked to discuss three questions:
1) what do you see as ingredients for success;

2) what are the challenges encountered; and
3) what would be an added value for ICATM.

The three major ingredients for success for alternative methods in future regulatory testing according to the participants at the ICATM workshop were collaboration, funding and harmonisation. Some proposals on how to achieve these ingredients for success were made. Regarding the challenges, most participants voted for a lack of confidence of regulators, a real-life complexity of organisms and exposure, and a lack of cross-disciplinarity. Here again, participants made several suggestions on how to tackle these challenges.

**Figure 3.1:** Participants of the workshop on "The Future of Alternative Methods for Regulatory Testing and their Contribution to Public Health", 22 October 2019, JRC Ispra, Italy.

Several proposals were also made on how ICATM could contribute to reinforce the ingredients for success and meet the challenges in a future of alternative methods for regulatory testing. With regard to the ingredients for success, the participants suggested e.g., that ICATM could facilitate communication between different interest groups; support case study developments within their own networks to gain confidence through the application of new approaches and share case studies undertaken within the respective Member Countries; discuss common goals and roadmaps; facilitate data sharing and data curation among Member Countries; share information on reference chemicals; share information on data requirements; support the harmonisation of data requirements; as well as share experiences on how to more efficiently use funds.

### Box 3.5 International Cooperation on Alternative Test Methods

On 27 April 2009, representatives from Health Canada, the European Commission, the National Institute of Health Sciences in Japan and the National Institute of Environmental Health Sciences in the United States signed the memorandum of cooperation establishing the International Cooperation on Alternative Test Methods (ICATM).

In 2011, the National Institute of Environmental Health Sciences in South Korea formally joined the cooperation. Since then, other governmental institutions from Brazil, Singapore, China and Taiwan have been participating in ICATM initiatives on an *ad hoc* basis.

With regard to the challenges that had been discussed, the participants suggested to share education and training initiatives and resources on new methodologies; continue to exchange information on new methodologies; continue to organise dedicated ICATM workshops with additional experts and share experiences for better ways of using funds to stimulate cross-disciplinarity.

More information on ICATM and on the outcome of the ICATM workshop can be found in Box 3.5 and in the read more section, respectively.

In 2020, the ICATM meeting was hosted by JaCVAM at the National Institute of Health Sciences in Japan and took place virtually on 9 October. ICATM partners updated on their activities and discussions revolved around the concept of standards, follow-up activities to the EURL ECVAM recommendation on non-animal derived antibodies, the development of OECD Detailed Review Papers, integrated approaches to testing and assessment, and human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations.

**READ MORE**

- Summary record of ICATM workshop: europa.eu/!TP87Gt

## 3.8 Qualification of organ-on-chip models

Among new non-animal *in vitro* technologies, organ-on-chip (OoC) devices have gained considerable interest within the scientific community. The way this technology can recreate body physiology promises to revolutionise science, including biomedical research, drug development, chemical safety and personalised medicine. Nevertheless, a comprehensive qualification process is necessary to demonstrate its relevance and encourage implementation by end users and regulators.

In this regard, EURL ECVAM has been actively collaborating with the European Organ-on-Chip Society (EUROoCS), which originated in part from the H2020 project ORCHID. A regulatory advisory board of the society, chaired by EURL ECVAM aims at supporting and facilitating the regulatory acceptance of OoC. In addition, EURL ECVAM is investigating the role of standards to support OoC qualification, as well as identifying potential entry points for acceptance and regulatory application of OoC.

**READ MORE**

- EUROoCS: euroocs.eu/
- ORCHID: h2020-orchid.eu/

### *3.8.1 EURL ECVAM survey on complex in vitro models*

In 2018, EURL ECVAM conducted a survey to collect scientific opinions on issues influencing end-user confidence in complex *in vitro* models, including OoC. A total of 645 replies to the 14-question survey were received from 36 different countries. Results were analysed and presented in different fora and are now compiled in a JRC technical report (Batista Leite *et al.*, 2021).

Three main results are worth mentioning:
1) there is high interest of stakeholders in the subject, independently of their background (academia, industry or regulatory body);
2) the majority of the respondents believe that some type of assessment is necessary, not only for regulatory purposes, but also in research;
3) the assessment process is not yet established and agreed by the community.

The complete data can be found in the JRC data catalogue. EURL ECVAM is continuing to collaborate with the community to explore ways of enhancing end-users confidence in these models.

**READ MORE**

- JRC Data Catalogue - Survey on complex *in vitro* models: europa.eu/!XU47nb

### *3.8.2 Contribution to standardisation*

The need for standardisation is recognised by major stakeholders in the OoC community as a fundamental step to advance the field, both in a regulated and a non-regulated framework. A EURL ECVAM proposal on OoC has been selected as a topic for the "Putting Science Into Standard (PSIS) 2021 workshop".

This initiative, co-organised by the JRC and the CEN/CENELEC, two European Standardisation organisations that develop standards in a wide range of fields, aims to identify standardisation needs in areas of emerging science and technologies. The goal of the PSIS workshop on OoC led by EURL ECVAM and involving experts in the area is to identify priority areas and establish a European roadmap for standardisation of OoC.

**READ MORE**

» Putting Science into Standards (PSIS) 2021 workshop "Organ on Chip: Towards Standardization": www.cen.eu/news/events/Pages/EV-2021-08.aspx

4

# Regulatory applications

One of EURL ECVAM's main aims is to support the uptake of new approach methodologies for safety assessment into actual regulatory use through its engagement with the activities of numerous EU and international bodies, such as the OECD, WHO, UN and ICH.

Uptake is achieved through the transformation of methods into regulatory test guidelines, or inclusion in guidance documents as well as through the advancement of hazard assessment methodologies, in general.

Focus at OECD level is currently on integrated approaches to testing and assessment (IATA) which accommodate the combination of information from many different sources provided by a variety of available tools.

# 4. Regulatory applications

## 4.1 Activities in the OECD Working Party on Hazard Assessment

The JRC (through EURL ECVAM) participates as a Commission representative in the OECD Working Party on Hazard Assessment (WPHA). The objectives of the WPHA are to facilitate and support the work of the OECD on the hazard assessment of chemicals, with special emphasis on the harmonisation of hazard assessment methodologies and integrated approaches to testing and assessment (IATA), and improving access to information on chemicals. During 2020, the WPHA adopted two documents drafted under the leadership of EURL ECVAM.

One document provides an overview of concepts and available guidance related to Integrated Approaches to Testing and Assessment (OECD, 2020a). The aims, characteristics and key concepts of IATA are explained, including an overview of IATA components (information sources). Basic definitions are provided and compared, identifying some inconsistencies in the way terminology is used. A mapping exercise identified 153 guidance documents that are systematically described in a supporting file. Emphasis was given to documents developed by the OECD, other international organisations and member country agencies. Recommendations to fill identified gaps in existing guidance were also made. The document was presented during the SOT/FDA symposium on Integrated Approaches to Testing and Assessment: The Future of Regulatory Toxicology Assessment (May 2020).

The other document provides guidance on the characterisation, validation and reporting of physiologically based (PBK) models for regulatory applications (OECD, 2021). This builds on existing guidance to address the need to build confidence in PBK models that are developed by relying on *in vitro* and *in silico* data, rather than

**READ MORE**

- OECD Working Party on Hazard Assessment: www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm
- SOT/FDA colloquia: www.toxicology.org/events/shm/fda/fda.asp
- ASCCT annual meeting 2020: www.ascctox.org/annualmeeting

*in vivo* data. Such models cannot be validated in the conventional way, i.e., by comparing predictions with existing *in vivo* data. The guidance outlines an assessment framework for PBK models, with emphasis on the major uncertainties underlying model inputs and outputs. A template for documenting PBK models, and a checklist for evaluating their quality, are also provided as supporting tools. To help increase dissemination and uptake, the PBK model guidance was presented during the SOT/FDA symposium on route to route extrapolation in the 21st century (February 2020), to the Scientific Committee on Consumer Safety (March 2020), at the OECD WNT meeting (April 2020) and at the 9th annual meeting of the American Society for Cellular and Computational Toxicology (ASCCT; October 2020).

## 4.2 Activities in the OECD Test Guidelines Programme

### *4.2.1 Outcome of the 32nd meeting of the Working Group of National Coordinators of the OECD Test Guidelines Programme*

The OECD Test Guidelines Programme (TGP) is managed by the Working Group of National Coordinators of the OECD Test Guidelines Programme (WNT) under the Joint Meeting (renamed as Chemicals and Biotechnology Committee (CBC) in 2020). The JRC acts as a National Coordinator for the OECD TGP, representing the European Commission and EU, and as such is a member of the WNT.

The 32nd meeting of the WNT was held virtually on 21 to 24 April 2020. The following updated Test Guidelines (TG) were approved:

- Updated TG 437 on the Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage.
- Updated TG 491 on the Short Time Exposure *In Vitro* Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage.
- Updated TG 488 on Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays.
- Updated TG 458 on Stably Transfected Human Androgen Receptor Transcriptional Activation Assays for Detection of Androgenic Agonist and Antagonist Activity of Chemicals.

In addition, the following Guidance Documents (GD) and Detailed Review Papers (DRP) were approved:

- New Guidance Document on aquatic toxicity testing of nanomaterials.
- New Guidance Document for the testing and interpretation of data on dissolution rate and dispersion stability of nanomaterials for effects and exposure assessment.
- Detailed Review Paper on the Pig-a assay for genotoxicity testing.

Minor corrections were also made to TG 405, TG 439, TG 442C and TG 471.

Eleven new projects were adopted on the OECD Workplan.

Additional discussion points included adjustments to the Standard Project Submission Form (SPSF), the status of FRAND declarations to be submitted to OECD for methods subject to licensing agreements, confidential business information in candidate OECD TGs, outcome and follow-up from the joint teleconference between the National Coordinators and the Good Laboratory Practices Working Group (WG

GLP) on challenges related to new generation test methods, expansion of Mutual Acceptance of Data (MAD) to computational methods, way forward with the CYP induction TG and GD on hepatic induction and clearance methods, status of the guideline on defined approaches for skin sensitisation, status of the revision of the dermal absorption Guidance Note 156, dose selection in chronic, reproductive and developmental toxicity studies, and the use of concentration-response information from *in vitro* assays.

More information can be found on the OECD website of the TGP.

**READ MORE**

- OECD Test Guidelines Programme: www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm

The following sub-sections mainly focus on TGs and GDs for which the EC (through JRC/EURL ECVAM) has the lead or co-lead. However, some selected projects, with relevance to the alternatives field, led by other Member Countries are also briefly described. Besides those, EURL ECVAM participated in numerous OECD expert groups and validation management groups and commented on several other draft TGs and GDs led by other OECD Member Countries.

### *4.2.2 Activities related to toxicity testing in fish*

There are three projects aiming at the overall reduction of the number of fish used in acute or chronic fish studies.

Austria and the International Council on Animal Protection in OECD Programmes (ICAPO) are co-leading a project, which aims to develop an IATA for fish acute toxicity testing. A recent paper authored by contributors to the OECD project summarises the limitations and uncertainties of acute fish tests and how they could be reduced using alternative methods (Paparella *et al.*, 2021). How information derived from alternative methods could be combined is currently explored in the Cefic Long Range Research Initiative (LRI) project ECO51, which aims to develop a systematic weight of evidence approach integrating fish embryo testing (OECD TG 236; OECD, 2013) to predict potential acute fish toxicity. Both projects closely collaborate.

**READ MORE**

- Cefic LRI project ECO51: cefic-lri.org/request-for-proposals/lri-eco51-integrating-the-fish-embryo-test-into-the-weight-of-evidence-to-inform-acute-fish-toxicity/
- Public consultation on drafting a new test guideline for a fish cell line acute toxicity test - the RTgill-W1 cell line assay: search.oecd.org/env/ehs/testing/section-2-effects-biotic-systems.htm

Another alternative method, the RTgill-W1 (rainbow trout gill cell line) cytotoxicity assay, is in the process of becoming an OECD TG. Switzerland and Norway are responsible for drafting the new test guideline and the first public consultation was launched in autumn 2020.

ICAPO and the EC (through JRC/EURL ECVAM), who are co-leading an OECD project on the use and analysis of control fish in fish toxicity studies, received a lot of support and interest from experts representing regulators, industry and academia. Data gathering and analysis is progressing well. From December 2020 onwards, the project will be co-led by ICAPO and USA.

### *4.2.3 Transgenic fish and amphibian models to identify endocrine disruptors*

Three projects focusing on assays for screening the endocrine disrupting potential of chemicals using genetically modified fish embryos are ongoing. The EASZY assay, under the lead of France, is based on the use of the transgenic cyp19a1b-GFP zebrafish embryos for the detection of endocrine active substances acting through the estrogen pathway, more precisely estrogen receptor agonists (Brion *et al.*, 2012). After the first commenting round, the EASZY protocol and validation report were amended. The second commenting round on the revised TG was launched at the end of 2020, and it is expected that the final draft documents are circulated for approval by the WNT in spring 2021.

The Rapid Androgen Disruption Adverse outcome Reporter (RADAR) assay aims at screening potential androgen and steroidogenesis disrupting chemicals using fluorescent markers in medaka fry (Spiggin-GFP Medaka) immediately after hatch. It is co-led by France and UK. A ring test was carried out in 2019, and the related draft validation report and draft test guideline have been submitted in 2020.

The Rapid Estrogen ACTivity *In Vivo* (REACTIV) assay aims at detecting estrogen axis active chemicals in transgenic Choriogenin-GFP Medaka embryos. The chemicals potentially detected are estrogen receptor agonists and antagonists, chemicals altering the activity and/or expression of aromatase, and chemicals disrupting the steroidogenic enzyme 5α-reductase. A detailed proposal for the interlaboratory validation of the REACTIV has been submitted.

**READ MORE**

Revised Draft new Test Guideline for the Detection of Endocrine Active Substance, acting through estrogen 1 receptors, using transgenic cyp19a1b-GFP Zebrafish embrYos: search.oecd.org/env/ehs/testing/section-2-effects-biotic-systems.htm

### *4.2.4 Androgen Receptor Transactivation Assays (ARTA)*

The EURL ECVAM coordinated validation study of the AR-CALUX® method was finalised in 2019. This method is an androgen receptor transactivation assay (ARTA) where the cell line is transfected with the cDNA of a human androgen receptor and a luc-reporter gene. The assay provides for an increase or decrease in luminescence when the cell line is presented with chemicals that have either androgenic or anti-androgenic potential, respectively. The method was experimentally evaluated by three laboratories of EURL ECVAM's network of specialised laboratories, EU-NETVAL (see Box 4.1) and the test method developer BioDetectionSystems (NL). A peer-review was conducted by ESAC, which concluded that the reproducibility and relevance was very good. EURL ECVAM took the lead in updating OECD test guideline TG 458 (which included one ARTA) with two additional mechanistically similar methods (see Box 4.1). The TG 458 now contains the AR-EcoScreen™ (validated by Japan), the 22Rv1/MMTV GR- KO TA (validated by Korea) and the AR-CALUX® (validated by EURL ECVAM). This TG was adopted by OECD in 2020 (see also 4.2.1; OECD, 2020b).

**READ MORE**

AR-CALUX: tsar.jrc.ec.europa.eu/test-method/tm2010-07

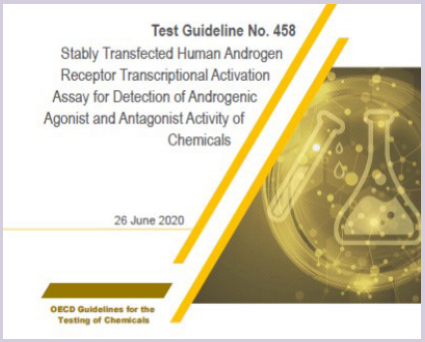
The validation and statistical report, the SOP and data analysis forms, the ESAC opinion and the link to TG 458 are all publicly available through EURL ECVAM's TSAR.

### *4.2.5 Defined Approaches for skin sensitisation*

The EC (through JRC/EURL ECVAM), the United States and Canada are leading a project for the development of an OECD Guideline for Defined Approaches (DAs) for skin sensitisation that will have the potential to replace the *in vivo* skin sensitisation methods to a large extent. The project is supported by an Expert Group composed of regulators, DA developers, QSAR experts, statisticians, and skin sensitisation experts. A defined approach to testing and assessment consists of a fixed data interpretation procedure (DIP) applied to data generated with a defined set of information sources (*in chemico*, *in vitro*, *in silico*) to derive a result that can either be used on its own, or together with other information sources to satisfy a regulatory need. Results generated with DAs will adhere to the agreement on Mutual Acceptance of Data (MAD), designed to reduce duplicative testing, to reduce the number of animals used in chemical safety testing and facilitate the sharing of data among OECD member countries. The DAs currently considered in the first version of the Guideline will provide data for hazard identification (skin sensitiser / non skin sensitiser) and potency categorisation (severe vs. moderate skin sensitiser). In the future, the Guideline is anticipated to be updated to include DAs that can be used for risk assessment. An extensive amount of work was conducted in 2020 to finalise the curation of the murine and human *in vivo* reference data

**Box 4.1** **Endocrine disruptors: updated OECD TG 458, now including three mechanistically similar *in vitro* methods**

Societal concerns about endocrine disruptors stem from the fact that they can adversely affect the health of humans and animals by altering the functioning of the endocrine (hormonal) system. Minimising our exposure to endocrine disruptors is important to reduce the risk that they contribute to causing endocrine-mediated diseases, such as some cancers. Standardised and internationally accepted methods to measure such exposure are of utmost importance. Government agencies, industry, and contract research organisations need this type of mechanistic information to identify potential endocrine disruptors.




EURL ECVAM led an OECD project to update test guideline TG 458 that includes the AR-CALUX® method along with two other similar methods. All three methods use genetically engineered cell lines that respond to either activation or blocking of the androgen receptor.

As described in related OECD guidance, information on androgen activity derived from *in vitro* methods is typically combined with other information sources, for example within an integrated approach to testing and assessment (IATA), to arrive at a conclusion about the endocrine properties of a chemical.

Updating of TG 458 is an important step towards achieving the Commission's goal of making more endocrine-specific methods available while at the same time reducing reliance on animal testing (EC, 2018).

**Short overview of the 3 mechanistically similar methods included in TG458**

| | AR-EcoScreen™ | AR-CALUX® | 22Rv1/MMTV/GR-KO TA |
|---|---|---|---|
| Parental cell line | Chinese hamster ovarian cancer | Human osteosarcoma | Human prostate carcinoma epithelial |
| Androgen receptor | Human cDNA | Human cDNA | endogenous |
| Reporter gene | Firefly-luc<br>Renilla-luc | Firefly-luc | Firefly-luc |

classifications; analyse the performance of the DAs against the curated reference data; characterise the applicability domain of the DAs and define the level of confidence in DA predictions. An updated draft guideline and supporting document addressing the work conducted in 2020 was provided to the expert group and WNT members for review and commenting in December 2020, with the aim of endorsement by the WNT in April 2021.

### *4.2.6 Developmental neurotoxicity in vitro assays*

Current *in vivo* developmental neurotoxicity (DNT) testing following OECD TG 426 is not efficient and is rarely conducted. Therefore, the use of an *in vitro* battery of assays based mainly on human stem cell-derived neuronal models and the application of the AOP and IATA concepts have been proposed for permitting more efficient and predictive regulatory DNT testing. These approches deliver mechanistic knowledge and facilitate better understanding of induced DNT effects, leading to the development of more reliable prediction models regarding human adverse

outcomes. Indeed, decades of *in vitro* work using rodent and human neuronal and glial cellular models has delivered a range of reliable *in vitro* assays and data that permit quantitative evaluation (via concentration-response relationships) of the impact of a compound on various stages of brain development.

Towards this goal, the preparation of a guidance document on the application and interpretation of *in vitro* DNT assays for testing and assessment is ongoing. This project is led by EFSA/OECD, US EPA and the Danish EPA, with the participation of EURL ECVAM and interested OECD member countries. In order to provide an adequate scientific background and facilitate the preparation of this GD, EFSA launched a procurement for the conduct of experimental work to accelerate the development and use of *in vitro* test methods based on cost and time efficient testing of chemicals for their potential to disrupt the development of the nervous system.

The procurement was awarded to the Leibniz Research Institute for Environmental Medicine (IUF, Düsseldorf) and University of Konstanz (UKN, Konstanz) and includes a collaboration with the US Environmental Protection Agency (EPA), the US National Toxicology Program (NTP) as well as additional testing sponsored by the Danish EPA. The IUF and UKN experimental work has been finalised while the US EPA and Danish EPA projects are still running.

To support the development of an AOP network for DNT adverse outcomes induced by exposure to selected pesticides, a systematic review was applied to *in vivo*, epidemiological and *in vitro* studies. The identified body of evidence was evaluated taking into consideration various potential sources of uncertainty and appraised for Risk of Bias. EFSA also applied a Bayesian Network approach to assess the Key Event Relationships.

Currently, the EFSA DNT working group is developing AOP network-based IATA case studies for hazard identification and characterisation of DNT for a selected pesticide where the outcome of the *in vitro* studies will be used as a part of the available evidence.

A guidance document, which should be finalised before the end of 2021, will provide information on a suitable *in vitro* DNT battery of assays and the interpretation and integration of *in vitro* data into IATA for hazard identification and characterisation.

### *4.2.7 IATA for non-genotoxic carcinogens*

Non-genotoxic carcinogens contribute to an increased cancer risk through a variety of mechanisms that are not yet directly included in international regulatory approaches. With the objective of addressing this gap, in 2016, the OECD established an expert group to develop an IATA for non-genotoxic carcinogens.

Through that work, a definition of non-genotoxic carcinogens in a regulatory context was first agreed (Jacobs *et al.*, 2016). Various cancer models were developed by using the AOP concept, and overarching mechanisms and modes of action were identified. After further refining and structuring with respect to the common hallmarks of cancer and knowing that non-genotoxic carcinogens act through a wide variety of specific mechanisms, an overarching IATA for non-genotoxic carcinogens was developed. The structure of the agreed IATA, will now be used as a transparent basis to distil, evaluate and organise relevant assays. In turn, this will facilitate future test guideline development for addressing the key events in cancer hazard assessment, for which currently there are no suitable test methods.

The process undertaken to derive the IATA scheme for non-genotoxic carcinogens are based upon commonalities between different human cancer models as reported in Jacobs *et al.*, (2020). The manuscript also describes the methodology that is currently used to evaluate and prioritize appropriate (mainly *in vitro*) assays to address the respective key events.

### *4.2.8 Bioelution test method*

In November 2019, the EC (through JRC/EURL ECVAM) submitted to OECD a project proposal aimed at drafting a new Test Guideline on the Bioelution test method for the determination of the relative *in vitro* bioaccessibility of metals and metalloids, inorganic metal compounds and metal(metalloids)-containing materials using a simulated gastric fluid composed of hydrochloric acid (HCl 0.032 M) at pH 1.5. The Bioelution test method was evaluated by EURL ECVAM and peer reviewed by ESAC. All relevant documents regarding these processes are available in TSAR.

The OECD project proposal was reviewed and discussed by the WNT. A number of concerns were raised by some member countries regarding the regulatory application and some technical/scientific aspects of the method. The issues were extensively discussed and it was agreed to separate the discussion on the regulatory application of the method, which will take place at CARACAL (Competent Authorities for REACH and CLP) level within the EU, from the discussion on the technical/scientific aspects of the method, which will take place at OECD level. In May 2020, the WNT accepted the Commission's proposal and in July the OECD established an expert group on metal release. A first draft of the TG produced by JRC/EURL ECVAM in collaboration with Eurometaux was discussed by the expert group during the kick-off meeting in September 2020. The aim is to circulate a revised version of the TG by early 2021 for formal commenting by the WNT.

DG Environment established a CARACAL sub-group on the use of relative *in vitro* bioaccessibility data in March 2020 with the aim to provide advice and exchange views on legislative and policy issues in relation to the potential use of the relative *in vitro* bioaccessibility of a hazardous metal in metal compounds or alloys, i.e., for the refinement of their classification under CLP. The group had the first meeting on 25 September 2020 and it is expected to be active until June 2021.

**READ MORE**

▸▸ Bioleution: tsar.jrc.ec.europa.eu/test-method/tm2016-02

### *4.2.9 OECD Harmonised Template 201*

EURL ECVAM has always supported and promoted ways to increase regulatory acceptance of mechanistic information. For regulators being able to trust and acknowledge such information, it is important that the (mostly) NAM-derived data are captured and reported in an internationally agreed and useful template. Data exchange among data producers and among regulatory authorities is only possible when the underlying reporting format is the same for all parties. This becomes even more important with the recently published EU Chemicals Strategy for Sustainability, which requires that tools and practices be established to ensure that relevant data is easily and readily accessible for safety assessments and is suitable for regulatory purposes.

The OECD Harmonised Template for reporting intermediate effects (OHT 201) is exactly such a tool and EURL ECVAM was the driving force behind it from the beginning. OHT 201 had been available since April 2016, and progress in reporting standards for mechanistic data as well as the necessity to better reflect AOP knowledge suggested that an update of the template was needed. EURL ECVAM

therefore led and finalised an OHT 201 revision activity - in collaboration with OECD, ECHA and reviewers from OECD working groups and EU-NETVAL. The revised template was officially adopted at OECD level on 6 November 2020.

OHT 201 allows the reporting of mechanistic information from *in vitro / in chemico* testing, *in silico* testing, or *ex vivo* testing, obtained with either OECD test guideline methods or non-guideline methods. It also allows the reporting of other classes of methods providing mechanistic information, including *in vivo* testing or read across.

OHT 201 is not limited to any specific technology. However, to make OHT 201 immediately useful for *in vitro* method users, the reporting fields are geared towards *in vitro* methods, while retaining the option to report the outcome of *in silico* models such as (Q)SARS or *in vivo* methods that provide mechanistic information. The revised OHT 201 includes specific fields related to the test system (usually cell lines), detection method, test material preparation / concentration selection, control and reference items, experimental conditions, data analysis, parameters to report and the option to upload attachments.

Another important new feature is the establishment of a link with the ontologies used in the AOP-Wiki, where key events are tagged with ontology terms to describe them as biological processes using an object and action vocabulary. Such terms relevant for skin sensitisation and endocrine disruption from existing test guidelines facilitate the selection of the process, object and action that best describe the biological effect. Matching these ontology terms across AOP-Wiki and OHT 201 will facilitate the linkage between an AOP Key Event and the test methods relevant for its detection.

Implemented in the IUCLID[5] software, the revised OHT 201 provides a multi-purpose tool to gather mechanistic information that can be used in the context of both regulatory and non-regulatory decision-making.

**READ MORE**

▸▸ OECD Harmonised Template 201: Intermediate effects: www.oecd.org/ehs/templates/harmonised-templates-intermediate-effects.htm

## 4.3 Extended Advisory Group on Molecular Screening and Toxicogenomics

The Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST) serves to explore, discuss, and facilitate application of new technologies and approaches in chemical risk assessment. The JRC acts as the EU co-chair of EAGMST, together with the USA, represented by the US EPA. The group was originally established in 2005 to focus on toxicogenomics, but the scope of its work was later expanded to molecular (high throughput *in vitro*) screening in 2009, and to managing the AOP programme in 2012. Currently the AOP programme of EAGMST has four different sub-groups working on: AOP development methodology and practice; scientific peer review; AOP knowledge-base; and education, training and outreach. Other ongoing projects include dealing with best practices for reporting 'omics data intended for regulatory risk assessment purposes and a guidance document on the characterisation, validation and reporting of physiologically based kinetic (PBK) models for regulatory applications (jointly with the WPHA; see Section 4.1). EAGMST also cooperates closely with the OECD Test Guidelines Programme (see Section 4.2).

5 IUCLID plays a central role in the IT environments of all organisations that manage scientific data on chemicals in a regulatory context, for example under the OECD HPV, EU Biocides and EU REACH programmes.

### *4.3.1 AOP framework analysis study*

The AOP Framework is one of the cornerstones of an international effort to modernise chemicals regulation. By dissecting highly complex toxicological processes into a series of more manageable individual Key Events, AOPs are key to a better understanding of toxicity, which should ultimately lead to more mechanism-focused approaches in chemicals testing and increased regulatory acceptance of NAM test results.

In 2018, EURL ECVAM commissioned a study to analyse how and to what extent the AOP Framework actually leads to better regulatory decisions. The study delivered its final report in 2020 which will be made publically available in 2021.

The study aimed to gain insights into stakeholders' perceptions of i) the main challenges facing chemicals regulation, ii) alternative approaches to conducting toxicological studies, and iii) the role and added-value of the AOP Framework. The focus was on key stakeholders who are directly involved in decision-making in regulatory or industry contexts, i.e., regulatory toxicologists, risk assessors and risk managers. Numerous interviews and interactions in face-to-face meetings and in focus groups at conferences, teleconferences, an online survey, and follow-up questioning were the main methods applied in the study, in addition to in-depth literature review and evidence gathering.

The study revealed that the stakeholders are deeply divided on the best methods for obtaining data and evidence fit for the purpose of informing decisions on particular substances, and on policy in general (see Box 4.2). This lack of consensus leads to fundamental disagreements regarding what counts as good evidence for decision making in the chemicals domain. Added to this, there is significant mistrust between sectors and stakeholders, which affects the way that data and studies are considered.

**Box 4.2 Main outcome of the AOP framework study**

The AOP Framework Study found that the main challenges of current chemicals regulation are:

- the science directly informing policy and regulatory decision-making often lags behind current science;
- there is a lack of consensus on the use and value of different methods and approaches in toxicological sciences, exacerbated by difficulty of access to large quantities of dispersed and non-standard data;
- there is mistrust among stakeholders in different sectors;
- there is not a shared understanding of how data is constituted as evidence for regulatory decisions, or for current and future policy regarding chemicals;
- in view of the likely increasingly contentious nature of the use and safety of chemicals and other potential stressors, transparency of the decision-making process in regulation and policy, for all stakeholders, becomes an ever greater challenge.

Lack of trust and lack of consensus regarding the criteria for sound decision-making undermine the chemicals domain, and make it less able to keep up with scientific developments.

The study makes a series of recommendations for nurturing a healthy trust culture in chemicals regulation. Conceptually, these recommendations centre on ensuring

a common framework through 1) shared understandings of the reasoning in decision-making processes, 2) facilitating the comparison between established and new approach methods, 3) bridging between these approaches, 4) showing ways to convert scientific data into policy evidence, and 5) proactive engagement with relevant stakeholders (see Figure 4.1).

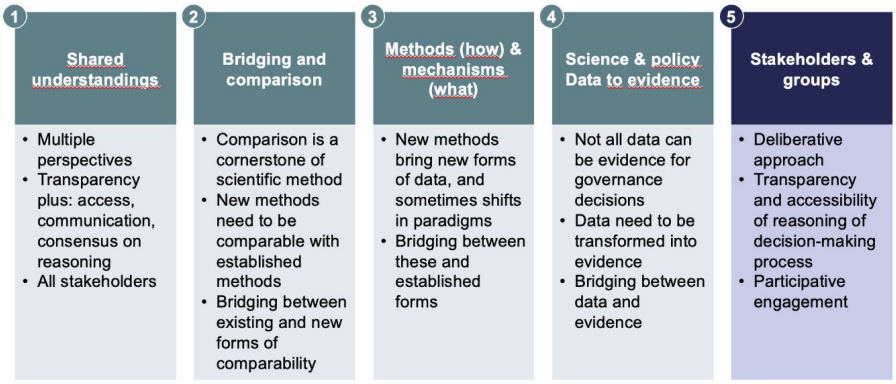



**Figure 4.1:** Five recommendations for a healthy trust culture in chemicals regulation and policy.

Practically, these recommendations are to be enacted in a knowledge management approach leading to increased understanding of the commonalities of applied methods and ultimately mutual trust (see Figure 4.2).

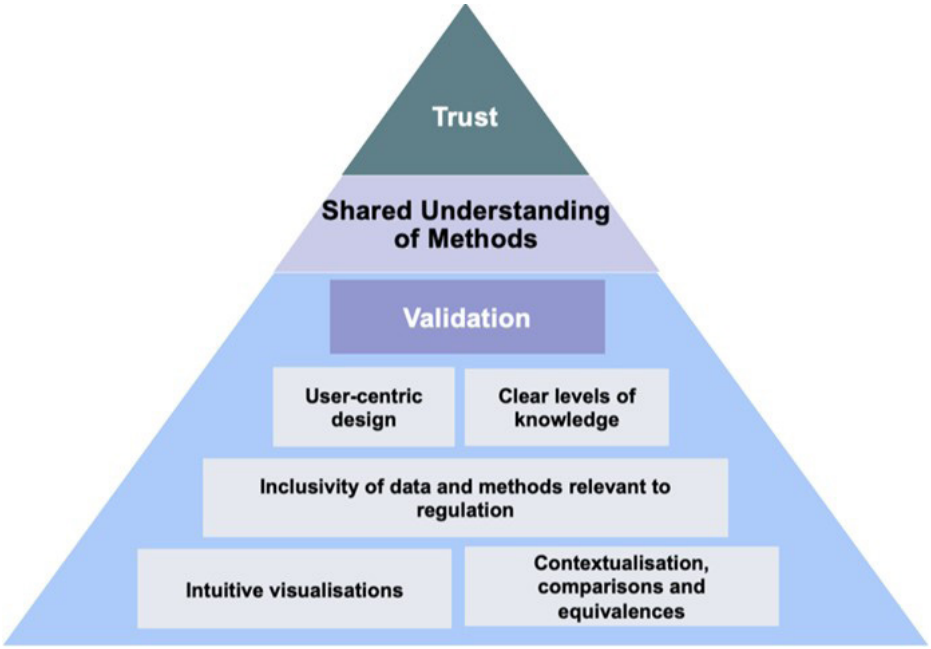



**Figure 4.2:** Trust building knowledge management pyramid in the chemical safety domain.

The findings and recommendations of the study were shared with the OECD, the governing body of the AOP framework. The full study report will become public very soon, and in the coming months and years, EURL ECVAM, together with its international partners, will follow up on these recommendations with the aim to further improve regulatory decision-making.

### *4.3.2 Transcriptomics and metabolomics reporting framework*

To promote the use of omics technology in the regulatory context, an expert group has been established to draft guidance for consistent reporting of 'omics data from various sources. In particular, EURL ECVAM is involved in the drafting of the transcriptomics and metabolomics reporting frameworks (TFR and MRF, respectively). In the last months, the modular structure of the two guidance documents was harmonised. They now share the first section, the experimental module, defining how an 'omics experiment should be illustrated in sufficient detail to allow another researcher to replicate it. Then, both reporting formats include two additional sections: i) the processing and analysis of 'omics data; modules related to the various omics platforms (RNA-Seq and microarray in the TRF and mass spectrometry and nuclear magnetic resonance in the MRF), descriptions of sample processing procedures, and methods used to collect and transform raw data; ii) the downstream analysis reporting modules; which serve to gather resources and steps necessary to reproduce a computational analysis of the processed data.

Recently, the expert group has proposed to add a further module, focused on possible applications of the techniques, i.e., point of departure calculation and read-across. Some modules, including the one on the experiment characterisation, which was led by EURL ECVAM, have been finalised. While the remaining modules are under finalisation, the focus is now on the conduct of case studies to test the clarity and completeness of the guidance, with a particular emphasis on data analysis.

### *4.3.3 AOP Knowledge Base*

The Adverse Outcome Pathway Knowledge Base (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 browse through the KB to find the AOPs or AOP elements they need.

EURL ECVAM co-chairs the OECD working group dealing with the maintenance and further development of the AOP-KB, which consists of several modules and interacts with a series of third party tools (see Figure 4.3):

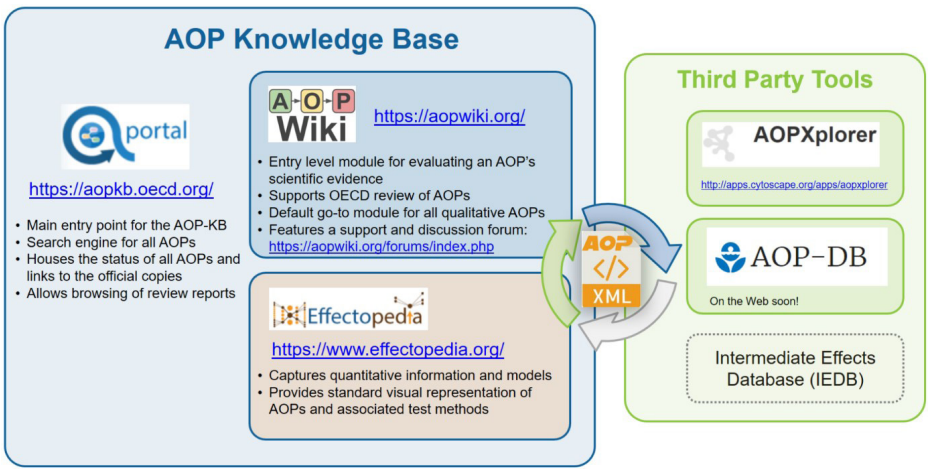



**Figure 4.3:** Modules and third party tools of the AOP Knowledge Base.

The most prominent and most relevant module is the AOP-Wiki, which was first released in 2014, and of which an updated version was published in 2016.

In 2020, EURL ECVAM commissioned a series of further enhancements to the Wiki:

A completely overhauled user interface improves the user experience and gives an overview of all Wiki functionalities at one glance (see Figure 4.4).

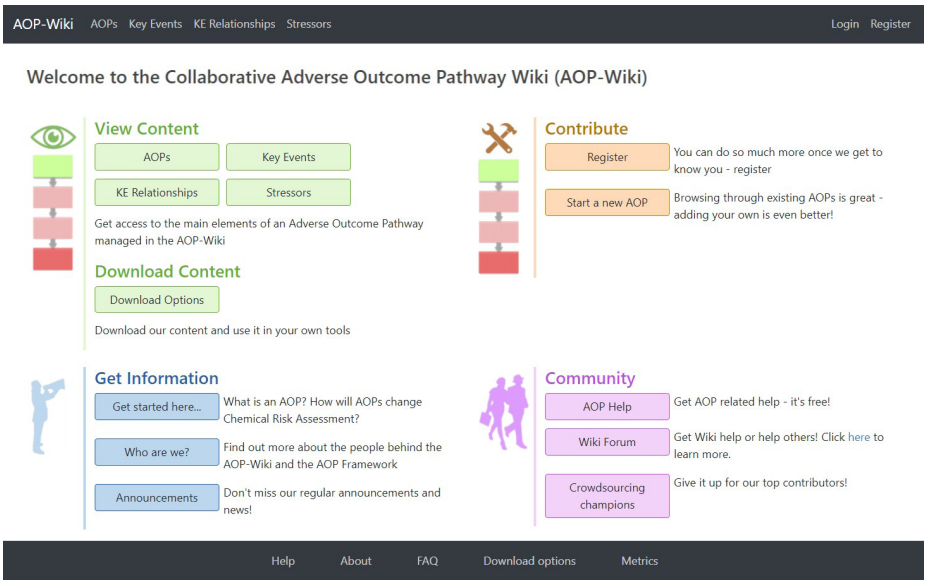



**Figure 4.4:** Overview of Wiki functionalities on the AOP-Wiki homepage. ©aopwiki.org.

It is now also possible to access the Wiki with a mobile device for content browsing (content editing must happen on the desktop version).

In addition to several back-office technical improvements, a new functionality was introduced to foster re-use of existing content, which is a key concept in the AOP Framework:

- Authors who want to create a new Key Event are now warned if a similar Key Event already exists in the Wiki. Authors can then either select one of the suggested already existing Key Events, or override the warning and proceed with the creation of their own new Key Event.
- Authors who want to re-use and update an already existing Key Event or Key Event Relationship can now contact the author of the original description to start a discussion about the changes they suggest.

An AOP Forum was introduced to make it easier for the people managing the AOP Framework to engage in exchange of information and opinions about the Wiki or scientific questions.

In the coming years, EURL ECVAM will continue to collaborate with the AOP Framework stakeholders to add further functionality to the AOP-Wiki.

## 4.4 Waiving of laboratory animal batch safety testing of vaccines for veterinary use (VICH)

**READ MORE**

- VICH laboratory animal batch safety testing (GL59): vichsec.org/en/guidelines/biologicals/bio-safety/target-animal-batch-safety.html
- VICH target animal batch safety testing (GL50R and GL55): vichsec.org/en/guidelines/biologicals/bio-safety/target-animal-batch-safety.html

The draft VICH GL59 on Harmonisation of Criteria for Waiving of Laboratory Animal Batch Safety Testing of Vaccines for Veterinary Use underwent a 6-months public consultation, which ended in spring 2020. As topic leaders, EURL ECVAM and the Paul-Ehrlich-Institute (Germany) addressed the comments in collaboration with the VICH experts. The VICH Scientific Committee approved GL59 at its meeting in November 2020. VICH countries have now one year for its implementation. In line with two VICH guidelines addressing the target animal batch safety test for inactivated (VICH GL50(R)) and live vaccines (VICH GL55), manufacturers can apply for waivers after demonstration of safe and consistent production.

## 4.5 NC3Rs/WHO review of animal use requirements in WHO biologics guidelines

**READ MORE**

- Review of animal use requirements in WHO biologics guidelines: nc3rs.org.uk/review-animal-use-requirements-who-biologics-guidelines

The WHO has tasked the NC3Rs to review WHO recommendations and guidelines for biologics and identify animal test requirements and currently recommended 3Rs approaches. Furthermore, the project aims to explore opportunities of better implementation of the 3Rs, identification of potential barriers to the uptake of the 3Rs in WHO countries, e.g., at the level of manufacturers, regulators or control laboratories.

The project started in 2020 and will end in 2022. EURL ECVAM is part of the international expert working group overseeing the project.

## 4.6 EPAA promotion of the regulatory acceptance of alternative methods

The European Partnership for Alternative Approaches to Animal Testing (EPAA) is a public-private collaboration between the European Commission, European trade associations and companies from seven business sectors. JRC, represented by EURL ECVAM, is one of the Commission services that are members of the EPAA, together with DG GROW, DG ENV, DG SANTE and DG RTD.

The partners have a shared vision to accelerate the development, validation and acceptance of alternative approaches to animal testing. The overall aim is the replacement, reduction and refinement (Three Rs) of animal use for meeting regulatory requirements through better and more predictive science. The wide range of partners from industry and regulators working together on this common goal with experts from academia and animal welfare groups constitutes an effective forum for dialogue and collaboration.

**READ MORE**

- EPAA achievement brochure: europa.eu/!xr34qt
- EPAA Annual report 2020: europa.eu/!xM47RH
- EPAA Action Programme 2021-2025: europa.eu/!Qj66nX
- EPAA Annual Conference 2020 '15 years of EPAA contribution to the 3Rs': europa.eu/!mK99Xj

In recent years, EPAA focused mainly on the promotion of regulatory issues and user acceptance. In this context, EPAA runs a number of projects that are briefly described in Box 4.3. EURL ECVAM supports this EPAA project platform by co-chairing a number a projects, as well as the platform itself. An overview of the EPAA activities in 2020 is given in the EPAA Annual Report. This year the EPAA has adopted a new action programme for 2021-2025 and has renewed the Mirror Group, its consultative body. In 2020, the EPAA also celebrated its 15$^{th}$ anniversary, from its launch in 2005, on the initiative of two European Commissioners (G. Verheugen and J. Potocnik) and a Member of the European Parliament (D. Roth-Behrendt). Therefore, the 2020 annual Conference aimed to give insight on 15

years of contribution to the 3Rs, to report on EPAA achievements and milestones, announce the EPAA 3Rs Science prize-winner, and to discuss current and future challenges on the way towards human-based testing models.

**Box 4.3 Overview and status of EPAA projects to which EURL ECVAM contributes**

### Acute toxicity

A data mining exercise carried out as part of the EPAA acute toxicity project evaluates since 2015 whether clinical signs (evident toxicity) are predictive of mortality at higher dose levels in acute oral toxicity studies and are an appropriate alternative to death as an endpoint. During 2020, access to a larger dataset, covering different chemical classes has been possible thanks to new relevant data provided by EPAA member companies.

### Clostridial vaccines

A collaborative study carried out in the framework of EDQM BSP (BSP130) resulted in three Vero cell based assays to replace the three *in vivo* tests required for in-process testing of *Clostridium septicum* vaccines. The outcome of phases 1-2 of the study have been published (Daas *et al.*, 2020), while the final report of the study is in preparation and a dissemination workshop is planned for March 2021 (https://www.edqm.eu/en/events/save-date-workshop-quality-control-veterinary-vaccines).

### Rabies vaccines

This project aims at replacing the current *in vivo* potency test for the release of human rabies vaccines (NIH, mice intracranial challenge test) with an *in vitro* antigen (G glycoprotein) quantification assay using an ELISA technology. A collaborative study to validate the transferability and robustness of the ELISA is coordinated by EDQM BSP (BSP 148). Phase II of the study is ongoing and is expected to be completed in 2021.

### Skin sensitisation user forum

Based on the recommendations of the EPAA/LRI/IFRA workshop held in Helsinki in 2019 (Basketter *et al.*, 2020), a User's Forum has been established as a mechanism to build confidence in the use of NAMs and avoid silo-ed thinking. The User's Forum is being evaluated by the Skin Sensitisation group in 2020-2021. The format of the Users' Forum is currently being trialled via virtual presentation and discussion of skin sensitisation safety decision-making case studies from across different industry sectors.

### Monoclonal antibodies

This is the most recent EPAA project, which aims to reduce the use of animal studies in the non-clinical safety studies during the drug development programmes of monoclonal antibodies for humans. EPAA member companies have submitted data for a substantial number of antibodies, which are currently being analysed and will lead to establishing criteria for decision-making.

### Harmonisation of 3Rs in biologicals

This project aims at facilitating harmonisation and international convergence of 3Rs in regulatory testing requirements for biological products. It has so far focused on requirements for batch testing of vaccines and on pyrogenicity testing.

### Prediction of carcinogenic potential of agrochemicals

This project aims to (1) enhance the prediction of carcinogenic potential of agrochemicals in humans using mechanistic information together with 90-days repeated dose toxicity data to reduce or replace the need for the 2-year carcinogenicity studies and (2) establish a virtual waver approach for the 2-y carcinogenicity animal assay. The results of the initial study are published in Luijten *et al.*, (2020) and Heusinkveld *et al.*, (2020).

### New ideas for systemic toxicity

A list of recommendations is under discussion on a research strategy as how to bring innovative approaches for repeated-dose systemic toxicity forward, which was formulated during a workshop held in October 2019 (Mahony *et al.*, 2020).

### Physiologically based kinetic (PBK) models

There are two projects related to PBK models. One project, led by the Health Safety Executive (UK), focuses on the quantification of *in vitro* to *in vivo* extrapolation using PBK modelling, while the second project, led by Liverpool John Moores University (UK), is developing an infrastructure to provide access to PBK models and their underlying data.

## 4.7 Transport of dangerous goods and classification of chemicals

Chemicals are classified and labelled with respect to their hazardous properties to ensure safe transport, handling of chemicals by workers and consumers and to protect the environment. For this aim, two United Nations Economic and Social Council (ECOSOC) Sub-Committees were established at UN level, one on Transport of Dangerous Goods (TDG) and one on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). The classification criteria are harmonised between the TDG and GHS as far as the same hazard classes and categories are defined in both systems.

The UN Model Regulations for TDG are directly transposed into EU law and implemented at Member State level to guarantee safe transport and facilitate trade worldwide. The GHS is implemented in the EU through the Regulation on Classification, Labelling and Packaging of substances and mixtures (CLP) with impact on a vast number of downstream legislation leading to risk management of chemicals within the EU.

Under the TDG, minor revisions to Chapter 2.8 on class 8 - corrosive substances were discussed. The chapter was updated in 2019 to include non-animal methods in the 21st revision of the TDG for guaranteeing safe transport of corrosive materials without animal testing. Under the GHS, an important activity towards replacement of animal testing is the work carried out by the Informal Working Group (IWG) on the use of Non-Animal Testing Methods for classification of health hazards, chaired by the Netherlands and the United Kingdom (see Section 4.7.1). In addition, EURL ECVAM on behalf of the EC took the initiative in 2020, to suggest clarification of the GHS criteria for germ cell mutagenicity further described in Section 4.7.2.

### *4.7.1 Use of non-animal test methods for classification of health hazard*

EURL ECVAM continued to lead the revision of GHS Chapter 3.3 on serious eye damage / eye irritation, to include criteria for classification based on non-animal methods/approaches.

The IWG was able to resolve an issue that was still pending from Chapter 3.2 and that also applies to Chapter 3.3, namely, the current ambiguity on whether the appropriate classification is corrosive or inconclusive where a substance/mixture has extreme pH and low acid/alkaline reserve. The IWG was able to reach an agreement on the final text to include in Chapter 3.3 and agreed to make conforming changes in Chapter 3.2.

Another issue that required consideration by the IWG is the absence of a full replacement alternative to animal data due to the lack of *in vitro* / *ex vivo* OECD Test Guideline methods directly identifying Category 2 eye irritants. The currently available *in vitro* / *ex vivo* OECD Test Guidelines for serious eye damage / eye irritation often result in an outcome that is inconclusive according to the Test Guidelines' criteria.

To solve this issue, the IWG agreed to introduce the possibility to use other validated *in vitro* / *ex vivo* non-guideline methods in Chapter 3.3, some of which may be useful to classify in Category 2. Moreover, the concept of Defined Approaches (DAs) was introduced into Chapter 3.3 because it was recognised that single *in vitro* methods would not be able to fully replace the *in vivo* method. The OECD

has agreed to publish DAs in OECD Guidelines falling under Mutual Acceptance of Data. Therefore, it was considered that DAs could be given the same weight and included in the same tier as *in vitro* / *ex vivo* methods. This is the first time the concept of DAs is introduced in GHS and it will have a major impact on follow up chapters covering more complex endpoints.

Due to the many issues that needed to be discussed and agreed, the IWG was not able to complete the revision of Chapter 3.3 during this biennium. However, it is expected that it will be possible to finalise it in time for adoption by the Sub-Committee early in the next biennium. The IWG will thereafter start to discuss the GHS chapter on respiratory and skin sensitisation.

### *4.7.2 Clarification of the classification criteria for germ cell mutagenicity*

On behalf of the EC, the JRC's EURL ECVAM has submitted a proposal to the GHS subcommittee to revise the chapter on germ cell mutagenicity (United Nations, 2019). The classification criteria described in the chapter would benefit from certain clarifications as they have caused considerable problems when implementing the current GHS, since the chapter was first published in 2003. It is thus timely to revise the chapter and to include newly available OECD Test Guidelines embracing scientific progress and when possible the replacement of animal testing.

In particular, the requirement for "demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells" has caused difficulties to enable classification in category 1B. In fact, data proving the substance's molecular interaction with germ cell DNA are so far very rarely available. Therefore, this wording is prone to different interpretations.

Based on current scientific evidence and, as stated in the EFSA Scientific Opinion on genotoxicity testing strategy (EFSA, 2011), systemic exposure to a substance should usually result in it reaching the germ cells if there is systemic distribution. A positive *in vivo* genotoxicant in somatic tissues would be assumed to be a germ cell mutagen, unless it has been demonstrated that the gonadal-blood barrier prevents the substance reaching the germ cells. There is a strong concern that substances can be classified in a lower hazard Category under the GHS, based on lack of sufficient evidence that the substance interacts with germ cells genetic material. This has considerable impact in terms of risk management measures coming into force through legal instruments based on the GHS classification, for example within the EU under REACH or the Biocides Regulation.

Indirect information could demonstrate the ability of a substance or its metabolite(s) to interact with germ cell genetic material and should be sufficient to justify classification in category 1B if substances elicit positive results in *in vivo* somatic cell mutagenicity tests in mammals. This could involve a weight of evidence-based assessment, considering all available data, including toxicokinetic data from currently accepted *in vivo* studies or supporting evidence from other available studies that the substance or its metabolite(s) reaches the germ cells. This approach would then enable classification in category 1B without necessarily requiring additional resourceful *in vivo* germ cell mutagenicity study(ies).

At the GHS sub-committee meeting in December 2020, it was agreed to establish an informal GHS working group led by the JRC (through EURL ECVAM), to discuss the possible revisions in the next biennium with the aim to achieve a coherent and clear text.

## 4.8 EU chemicals strategy for sustainability

Almost 20 years after the first major overarching approach to chemicals management in Europe (the 2001 white paper paving the way to REACH), the Commission has outlined a new long-term vision for the EU's chemical policy. The chemicals strategy for sustainability (EC, 2020a) steps up the Commission's commitment towards a toxic free environment. The JRC contributed to the formulation of the strategy and led some of the underpinning activities supporting the formulation and implementation of the strategy, namely, the fitness check on endocrine disruptors (EC, 2020b) and the progress report on the assessment and management of combined exposures to multiple chemicals (chemical mixtures) and associated risks (EC, 2020c), which were published together with the strategy. The IPCHEM platform was mentioned in the strategy as a key component of the evolving knowledge base supporting chemical policy (see Section 4.8.3).

**READ MORE**

▸▸ Chemicals Strategy for Sustainability Towards a Toxic-Free Environment: ec.europa.eu/environment/strategy/chemicals-strategy_en

### *4.8.1 Endocrine disruptors fitness check*

Following its communication on a comprehensive strategy for endocrine disruptors (EC, 2018), the Commission appointed the JRC (through EURL ECVAM) to lead a fitness check. The fitness check evaluated whether relevant EU laws deliver on their objectives to protect people and the environment by minimising exposure to endocrine disruptors. It also evaluated whether the relevant laws work in a coherent way. In close consultation with several other Commission services, EURL ECVAM analysed about 30 regulations and directives relevant to the identification and management of endocrine disruptors. Stakeholder groups (companies and business associations, regulatory authorities, civil society organisations and academia), citizens and small and medium-sized enterprises were also consulted.

Results were published in October 2020 as part of the chemicals strategy for sustainability (EC, 2020b). They were also presented at the second EU Forum on endocrine disruptors in December 2020.

The fitness check found no cases of inconsistent identification across legislation. A horizontal approach to identification and assessment is broadly supported by stakeholders but still needs to be defined. The rapid and consistent identification of endocrine disruptors across legislation requires:

- updating information requirements (ongoing for pesticides, biocides and REACH substances);
- establishing horizontal identification criteria, building on the experience with pesticides and biocides;
- streamlining regulatory assessment processes between Agencies and Commission services;
- accelerating the development and regulatory uptake of methods for identifying endocrine disruptors focusing on new approaches that minimise the use of animals (e.g., *in vitro* and *in silico* approaches).

Some stakeholders find that differences in risk management approaches for identified endocrine disruptors are not justified. Principles guiding risk management of these substances should be consolidated across legislation and better communicated.

**READ MORE**

▸▸ Second Annual Forum on Endocrine Disruptors: europa.eu/!jY43vu

Regarding the effectiveness of legislation in protecting against the adverse effects of endocrine disruptors by minimising exposure, no firm conclusions could be drawn due to lack of sufficient evidence. To improve future evaluations, research should focus on better health and ecosystem indicators (e.g., biomonitoring).

*In loving memory of our friend and colleague Alfonso Lostia.*

*In the recent years, the JRC and its EURL ECVAM has been supporting many activities related to endocrine disruptors (ED). In some of these activities, the contribution of our dear friend and colleague, Alfonso Lostia, was pivotal. Sadly, in the summer of 2020 Alfonso lost his life in a tragic accident.*

*Alfonso joined the JRC in September 2012 and started by contributing to the Horizon 2020 project cluster dedicated to Safety Evaluation Ultimately Replacing Animal Testing (SEURAT-1). In particular, he conceived and coordinated an ambitious case study introducing the use of mechanistic knowledge to predict the potential hepatotoxicity of chemicals using in vitro methods.*

*When in 2014 the JRC was asked to support the impact assessment on policy options for criteria for the identification of EDs, under the respective plant protection and biocidal products regulations, Alfonso enthusiastically jumped at the opportunity. He played a key role in the design of a unique rapid-screening methodology that was applied to 600 substances, at the heart of which was a highly innovative spreadsheet to capture, integrate and visualise complex data streams underpinning the screening process. Later this novel tool was adopted by EFSA for use in actual regulatory assessments. He also played an important role as part of the team from JRC, EFSA and ECHA that developed the guidance now in use by EU agencies and Member State authorities for the identification of endocrine disruptors.*

*Furthermore, outside of the ED activities, he developed new approaches to build chemical categories based on both biological and structural similarity to improve and strengthen read-across used in chemical risk assessment. He was also involved in establishing a framework to characterise in vitro methods for human hepatic metabolic clearance in collaboration with international experts in the field.*

*In 2018, Alfonso joined the pesticides unit of EFSA where unsurprisingly he made an immediate and significant impact both professionally and personally.*

*Alfonso was an accomplished scientist with a terrific analytical mind and skills to match, full of passion, enthusiasm and dedication for his work. We will remember him for his sense of humour, his radiant smile and for not only being an exceptional colleague, but a great friend too.*

*Alfonso, you will be sadly missed.*

### *4.8.2 Combined exposure to multiple chemicals*

Based on the activities in recent years to improve the assessment and management of combined exposures to multiple chemicals, the Commission published the "Progress report on the assessment and management of combined exposures to multiple chemicals (chemical mixtures) and associated risks" (EC, 2020c as part of the chemical strategy for sustainability (EC, 2020a). It summarises progress made in the development of methodologies, guidance, case studies and achievements in filling some of the knowledge gaps.

EURL ECVAM has contributed to progress in the area by reviewing regulatory requirements (Kienzler *et al.*, 2014; Kienzler *et al.*, 2016), scientific methodologies (Bopp *et al.*, 2015), case studies (Bopp *et al.*, 2016), and investigating how scientific advancements can help addressing remaining challenges in the regulatory assessment and management of mixtures (Bopp *et al.*, 2018; Bopp *et al.*, 2019).

The progress report shows achievements so far, but identifies a need to reinforce provisions to take account of the combination effects more consistently across relevant legislation, and highlights in particular remaining difficulties in addressing unintentional mixtures.

One of the knowledge gaps highlighted in the Commission Communication on Chemical Mixtures of 2012 (EC, 2012) was the lack of knowledge on the relevance of chemical interactions in mixtures. The risk of combined exposures is often predicted in component-based approaches using the additivity concept. If chemicals in a mixture interact, e.g., through metabolic interactions, this can lead to synergistic effects, which might be underpredicted using additivity-based approaches. EURL ECVAM funded a Systematic Literature Review to investigate the frequency and magnitude with which such synergistic effects occur. The study found that only few claims in the literature of synergistic or antagonistic effects exceeded the boundaries of acceptable between-study variability. The results confirm the utility of default application of the dose (concentration) addition concept for predictive assessments of simultaneous exposures to multiple chemicals. However, application of dose addition must be complemented by an awareness of the synergistic potential of specific classes of chemicals (Martin *et al.*, 2020).

Moreover, in a recently published article, EURL ECVAM has shown that the possible effects of chemical mixtures on the developing brain can be assessed using human cells *in vitro* combined with mathematical modelling (Pistollato *et al.*, 2020b; see Box 2.3).

### *4.8.3 IPCHEM - Information Platform for Chemical Monitoring*

Monitoring chemical occurrence in humans, the environment, and other media, can support chemical policy in various ways. Chemical monitoring can improve the risk assessment of (groups of) substances. It can provide evidence to evaluate the effect of regulatory interventions. It helps researchers to investigate links between exposure and adverse effects in humans and ecosystems. The chemical strategy for sustainability (EC, 2020a) recognises such potential. In its effort to strengthen the scientific evidence base, it strives to make chemical data "*easily findable, interoperable, secure, shared and reused by default*".

IPCHEM, the Information Platform for Chemical Monitoring, is the European Commission's reference access point for searching, accessing, and retrieving chemical occurrence data collected and managed in Europe (Figure 4.5). As scientific and technical lead of the platform, the JRC has integrated more than 150 datasets (representing a total of >450 million data points) in IPCHEM. Many more are in the pipeline. While efforts for data integration continue, the aim now is to illustrate how data providers and users can benefit from data in IPCHEM.

The JRC organised a virtual workshop "EU chemicals, environment and health policies: How can IPCHEM contribute?" in September 2020. Participants were experts from the areas of human biomonitoring, epidemiology, human and environmental health, environmental impact assessment, chemical mixtures, statistical analysis and modelling and representatives from the Commission's Directorate Generals (JRC, ENV, SANTE, GROW, EMPL, RTD) and EU Agencies (ECHA, EEA, EFSA, OSHA). Participants shared ideas for possible uses of monitoring data in combination with additional data sets from related scientific areas, such as disease registries, epidemiology, and stress ecology. The discussion led to the definition of policy questions, targeting various stages of the policy cycle (e.g., policy options analysis,

policy implementation, policy evaluation) and possible use cases to address them. A description of the use cases and of how these can benefit from data provided via IPCHEM was published in the IPCHEM 2020 report (Bopp *et al.*, 2020). Since 2017, IPCHEM is a contributor to the OECD eChemPortal. In August 2020, IPCHEM data were updated to reflect the increase of the number of substances for which data are available.

**READ MORE**

- IPCHEM: ipchem.jrc.ec.europa.eu/
- OECD eChemPortal: www.echemportal.org/

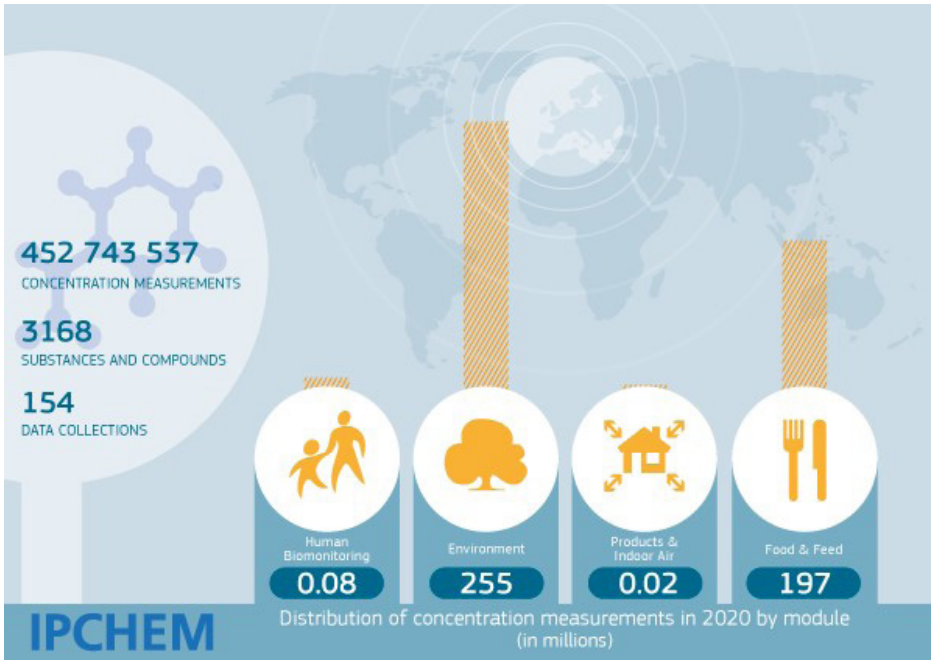



**Figure 4.5:** Overview of IPCHEM, the European Commission's Information Platform for Chemical Monitoring.

5

# Alternatives in basic and applied research

Approximately 70% of animals used for scientific purposes are for basic, translational and applied research. Thus expediting the uptake and use of non-animal models, especially in areas where animal testing is high (nervous and mental disorder, oncology), is essential to reduce numbers and advance science.

In 2020, EURL ECVAM led several activities in order to identify the availability of alternatives in basic, translational and applied research, completing the review of available and emerging non-animal models in seven disease areas.

Furthermore, EURL ECVAM started a study to assess the impact of EU-funded research projects in the biomedical domain (based on animal models or not) to see how their work contributed to scientific innovation and benefited society.

With the aim to stimulate and support more knowledge sharing on new alternatives within and between biomedical research communities, EURL ECVAM also launched an exploratory project to model the pathogenesis of COVID-19 using Adverse Outcome Pathways.

# 5. Alternatives in basic and applied research

## 5.1 Review of advanced non-animal models in biomedical research

EURL ECVAM has carried out a series of studies to review available and emerging non-animal models being used for research in seven disease areas:

1. respiratory tract diseases
2. breast cancer
3. immune-oncology
4. immunogenicity of advanced medicinal products
5. neurodegenerative disorders
6. cardiovascular disease
7. autoimmunity.

These particular areas have been selected based on disease incidence and prevalence, the reliance of related research on animal models (see Figure 5.1), and the amount of animal procedures conducted.

The aim was to identify and describe specific research contexts where animal models have been put aside in favour of novel non-animal techniques that use, for example, *in vitro* methods based on human cells and engineered tissues or *in silico* approaches employing computer modelling and simulation. By understanding and sharing information on successful alternative models in biomedical research, EURL ECVAM expects that the transition towards non-animal approaches will be facilitated and potentially accelerated.

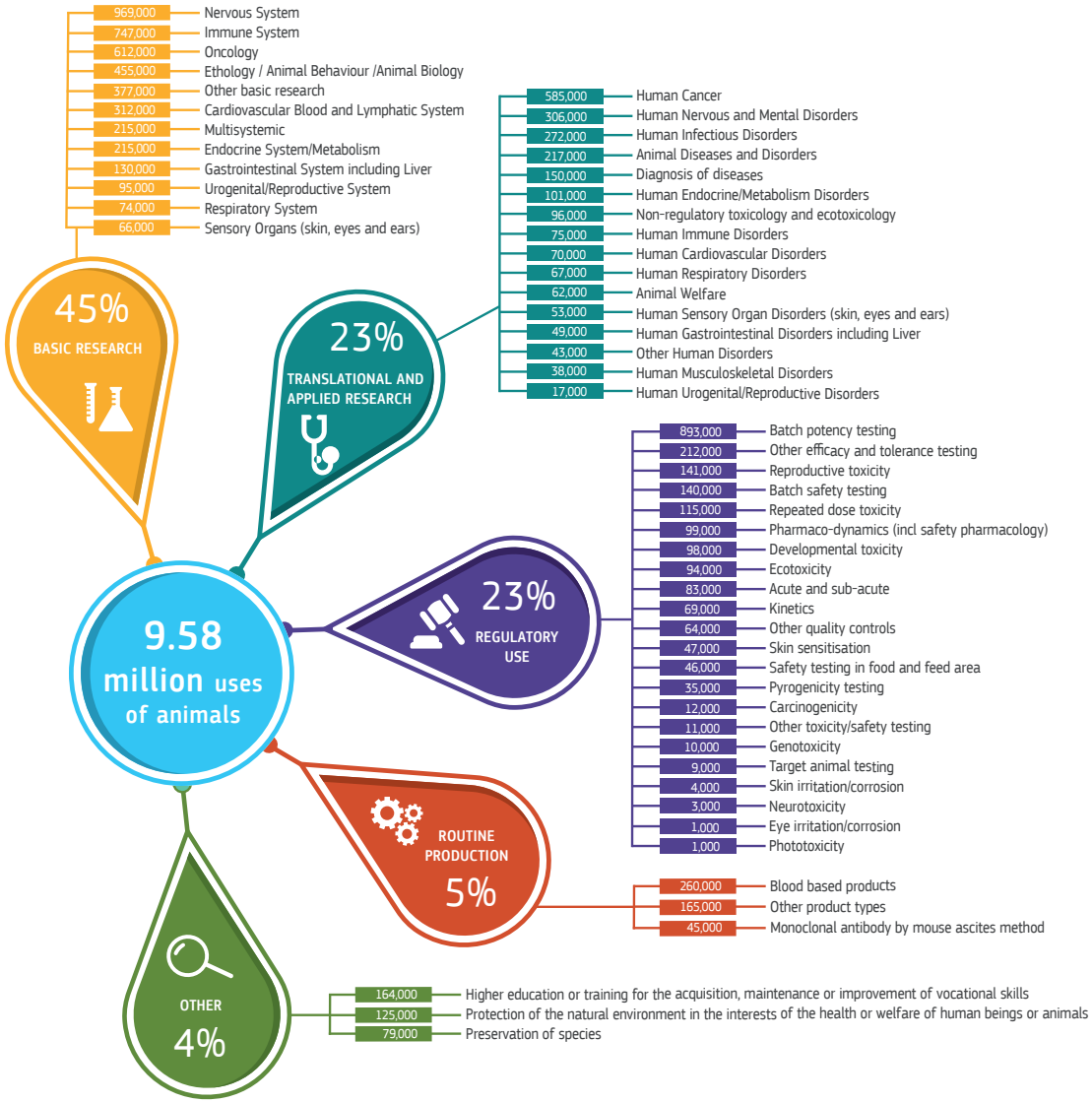



**Figure 5.1:** All uses of animals for research and testing in the European Union in 2017.

Moreover, since complex human-relevant non-animal methods offer the promise of recapitulating human physiology more effectively than many animal models, shifting to new animal-free methodologies and research strategies can in fact enhance the understanding of human-specific biology and disease.

In 2020, the two first studies on respiratory tract diseases and breast cancer were published (see Box 5.1), while the others will follow in 2021.

### *5.1.1 Respiratory tract diseases*

Currently there is still a lack of effective new therapies for serious respiratory conditions, such as asthma, chronic obstructive pulmonary disease (COPD) and lung cancer, which are among the most common of all diseases and causes of death globally.

Research strategies for the development of safe and efficacious therapies for respiratory diseases are therefore beginning to exploit non-animal methods that recapitulate the mechanistic basis of human disease rather than continuing to use animal models which poorly reflect the human clinical situation.

In the EURL ECVAM study, over 21,000 abstracts from the scientific literature were screened and from these, 284 publications were selected that described the most representative and innovative models of respiratory diseases. These models produced a unique knowledge base freely available in an easy-to-use spreadsheet format from the EURL ECVAM Collection in the JRC Data Catalogue.

The collection of models shows that simple models such as cell cultures are still prominent; however, research is shifting towards more sophisticated bioengineering approaches e.g., 3D human tissue cultures, spheroids, organoids, and microfluidic / 'lung-on-a-chip' systems that more accurately mimic human respiratory diseases.

**READ MORE**

JRC Data Catalogue - EURL ECVAM review of non-animal models in biomedical research - respiratory tract diseases: europa.eu/!Tn39yB

A technical report describes the review methodology and presents the main findings of the study (Hynes *et al.*, 2020) while an executive summary has been produced for a wider audience (Gribaldo & Whelan, 2020).

### *5.1.2 Breast cancer*

In the area of breast cancer, the most common cancer among women in the European Union and worldwide, approximately 30% of all patients have recurrent disease despite advances in early detection and therapies. Preclinical research currently relies on animal models, mostly rodents. However, animal models mimic limited aspects of human breast cancer. For this reason, research is gradually moving towards developing advanced non-animal systems, to offer better treatment with increased efficacy and low toxicity.

In this study about 120,000 scientific papers were screened for relevant human-based models and from those 935 models were identified as being the most representative and promising (see Box 5.1). The analysis of these models shows that immortalised cell lines represent the most used approach for breast cancer research. These cell lines are mostly commercially available or already qualified lines. The use of a scaffolding system emerged as the main technique employed to generate 3D models, followed by organoids and spheroids, known as mammospheres. This collection of models is freely available for download from the JRC Data Catalogue in an easy-to-use spreadsheet format.

**READ MORE**

JRC Data Catalogue - EURL ECVAM review of non-animal models in biomedical research - breast cancer: europa.eu/!bM83pv

This knowledge base is complemented by a technical report, which provides an in-depth meta-analysis of the approaches being used (Folgiero *et al.*, 2020) and a separately published executive summary intended for the general reader (Gribaldo *et al.*, 2020).

## 5.2 Gauging the output and impact of biomedical research

The prevalence of noncommunicable diseases, such as Alzheimer's disease, breast cancer and prostate cancer, has been increasing in Western countries. In the last two decades, the European Union has supported research activities focused on these biomedical research areas in order to elucidate the onset and evolution of these pathologies, identify associated risk factors, and ultimately design new preventive and therapeutic strategies (Pistollato *et al.*, 2020a).

EURL ECVAM, in collaboration with DG RTD, has initiated an activity aimed at retrospectively monitoring the outputs and impact of EU-funded biomedical research. As a first step, a survey addressed to former participants of EU-funded research projects (under FP5, FP6, FP7, and H2020) in the fields of Alzheimer disease and

## Box 5.1 Human-based methods for better breast cancer research

In October 2020, EURL ECVAM delivered a freely available knowledge base of over 900 non-animal models for breast cancer research.

Breast cancer is now estimated to be the most frequently occurring cancer, accounting for 13.3% of all new cancer diagnoses during 2020 in EU-27 countries. One woman in eleven is at risk of developing breast cancer.

While important scientific breakthroughs have been made in the field of early detection and understanding of the molecular bases of breast cancer biology, further progress is needed to offer women more effective treatments with fewer side effects.

Why non-animal models? "The problem we're facing is that current breast cancer research is too reliant on animal models, mostly using rodents. But rodents provide a poor model for human diseases. We need therapies based on the patient and the clinical and molecular characteristics of the tumour", explains JRC scientist Laura Gribaldo.

A key feature of breast cancer is the high level of heterogeneity observed both between tumours and even within the same tumour. This has driven the development of three-dimensional tissues, often called 'mammospheres',

that reflect the different cell types present and, crucially, the interactions between them.

There are already many advanced non-animal methods being used fruitfully for breast cancer research. However, knowledge about them is scattered across the scientific literature, limiting their diffusion and impact within the scientific community.

The study involved an extensive review of scientific literature published from January 2014 to March 2019 (Folgiero *et al.*, 2020) which identified 935 papers describing relevant non-animal models for breast cancer.

These models are based mainly on cells and tissues cultured in the laboratory (*in vitro*), computer modelling and simulation (*in silico*), or explanted cells and tissues taken from patients (*ex vivo*).

other dementias, breast cancer, and prostate cancer has been conducted to gain insights related to: (i) How EU-funded projects have contributed to innovation and major scientific breakthroughs; (ii) how scientific results have been translated into socioeconomic impacts of benefit to the society; (iii) what ingredients determined the success of research projects; and (iv) what scientific methods and research approaches underpinned the advances made. The main results of the survey have been published in a factual summary report, and show that 93% of the 202 participants think either that their research had an impact beyond their project, or that an impact may be seen in the future. Effective collaboration with project partners, multidisciplinarity, the design of the research strategy, and the international dimension of their project were considered as major drivers of research success (see also Box 5.2).

As a follow-up to the survey, in-depth interviews with a number of survey respondents are currently being conducted by EURL ECVAM in order to gain a more exhaustive understanding on aspects concerning the translatability of research, social impact, and lay public engagement. A synopsis report summarising these results will be published in spring 2021.

**Box 5.2 Gauging the output and impact of biomedical research**

EURL ECVAM has published initial results of a survey it conducted to gather the views of EU-funded researchers on how their work has contributed to scientific innovation and benefitted society.

The survey targeted researchers working on breast cancer, prostate cancer, Alzheimer's disease and other dementias who have participated in EU-funded projects over the past 20 years under framework programs FP5, FP6, FP7 or Horizon 2020. The JRC report provides a brief factual summary of the 202 responses received.

Respondents to the survey mostly work in academia on basic, translational and clinical research. A large majority of respondents indicated that the development of a new research methodology or approach was a major outcome of their activities.

Almost all participants (93%) stated that their research had an impact beyond their project, or that impact may be seen in the future. In particular, 46% of respondents claimed that their scientific results had a positive contribution to diagnostic or prognostic tools, treatment and prevention actions and the design of clinical trials.

Respondents consider the following as the major drivers of research success:

- effective collaboration with project partners
- interdisciplinarity
- the design of research strategies
- the international dimension of EU projects.

The report will provide valuable input into several activities EURL ECVAM is currently pursuing in collaboration with the Commission's Directorate-General for Research and Innovation. These include the development of indicators to assess the output and impact of EU-funded research in several important disease areas, and a recent initiative to bridge across method in the biosciences to promote better crossdisciplinarity in biomedical research.

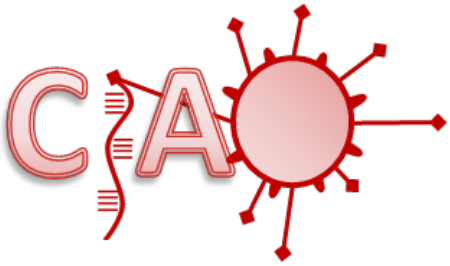
EURL ECVAM is currently working in collaboration with a DG ESTAT contractor on the definition of sets of indicators suitable to retrospectively assess EU-funded research contribution to scientific innovation and social impact.

## 5.3 CIAO - modelling the pathogenesis of COVID-19 using AOPs

Immediately after the COVID-19 outbreak, it became clear that a plethora of data were being made available on a daily basis concerning the population impacts of COVID-19 around the world. These have formed the basis for multiple approaches to modelling the path of the disease, which in turn have been used to support critical policy and healthcare decisions.

What was (and still is) missing however, is the linking of this information on population effects with an understanding of the pathogenesis of the disease. In particular, there is an urgent need to develop insights into why some population groups are more vulnerable than others, as a result of biological, epidemiological and socioeconomic factors. The nature of the vulnerability will be crucial in determining appropriate prevention and mitigation measures.

EURL ECVAM therefore decided to instigate an activity that would help to make sense of the disparate information sources on COVID-19 pathogenesis by exploiting a well-established knowledge sharing paradigm, the Adverse Outcome Pathway (AOP) Framework. The exploratory research project was therefore named "Modelling the pathogenesis of COVID-19 using the AOP Framework" – CIAO. The AOP Framework had originated in the toxicology domain, but as a generic knowledge management paradigm, it could easily be adapted to the COVID-19 disease domain. While in toxicology a chemical is the stressor triggering the pathway, with COVID-19 it is the SARS-CoV-2 virus.




The CIAO project consolidates knowledge from different disciplines and specialists in a way that is understandable and actionable by relevant stakeholders (e.g., medical professionals, public health officials and policy makers, managers in the healthcare sector, etc.). Moreover, this mechanistic knowledge is used to develop an understanding of the biological modulating factors that determine different clinical outcomes and will be exploited to develop mathematical models that simulate the pathogenesis and health impact of COVID-19.

Ideally, AOPs are created in a crowdsourcing effort, i.e., volunteers from many different research areas work together, and the total is much more than the sum of its parts due to the value added by crossdisciplinarity. In a series of introductory webinars, more than 100 scientists were informed about the project. In a COVID-19 AOP Design Workshop held on 1 to 2 October 2020, about 40 international participants from numerous prestigious organisations, universities, NGOs and authorities assembled and identified a first batch of candidate AOPs and AOP Key Events to be further developed in four working groups organised along the Key Events' position in the AOPs (see Figure 5.2). A fifth working group dealt with the modulating factors influencing the course of the disease (sex, age, diet ,etc.).

The working groups met online numerous times before the second COVID-19 AOP Design Workshop (27-28 January 2021). First results will be published in 2021.

**READ MORE**

- CIAO project: www.ciao-covid.net/

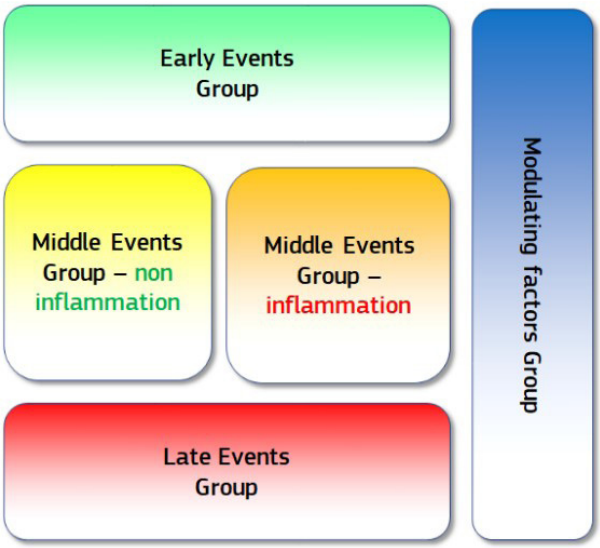



**Figure 5.2:** COVID-19 related Key Events' position in the AOPs.

6

# Education and training

Education and training are fundamental to boosting the uptake of alternative methods and the application of the Three Rs in science. In 2016, EURL ECVAM conducted a study to gain an overview of existing Three Rs knowledge, identifying the sources and how they are used and shared (Holley *et al.*, 2016). A main conclusion was that, although Three Rs courses and resources are abundant, there is a need to further complement and amplify current education and training opportunities, especially at secondary school level but also at the level of higher education.

Therefore, EURL ECVAM has been engaged in several education and training activities aiming at increasing the awareness of the Three Rs and alternative methods and approaches at the levels of secondary school, university and early professional training.

EURL ECVAM continues to build and expand on the experiences so far and to strengthen efforts to support Three Rs teaching. This is achieved by adding to its catalogue of resources and stepping up outreach towards educators, education experts and decision makers who play a key role in incorporating the Three Rs into education programmes and curricula. Furthermore, EURL ECVAM collaborates with university networks to develop a strategy for implementing enhanced teaching of the Three Rs and alternatives.

Finally, the third edition of the popular JRC Summer School, aimed at post-graduate students and early-career scientists, will take place on 17 to 21 May 2021.

# 6. Education and training

## 6.1 Introducing the Three Rs into secondary schools, universities and continuing education programmes

EURL ECVAM, with the support from selected experts, investigated how to facilitate the development and uptake of Three Rs content and resources into secondary schools, universities and continuing education programmes (see section 6.3.2 of Zuang *et al.*, (2020)). The aim is to make new generations aware of the ethical basis to the European Union's Three Rs policy, the scientific and technological progress underpinning new approach methods, and the opportunities for European innovation and competitiveness.

A first step was to investigate how to incorporate the Three Rs and related teaching material into the curriculum of schools and universities. Finally, a strategy was devised on how to reach out to decision-makers and influencers within education systems who contribute to educational policy-making at European, Member State or local level.

EURL ECVAM have produced a report to stimulate and facilitate the uptake of new Three Rs educational content (Holloway *et al.*, 2021). The report describes how to incorporate Three Rs teaching into the curriculum of schools and universities and aims to reach decision-makers and influencers within education systems who contribute to educational policy-making at European, Member State or local level. The content includes an introduction to the principles of the Three Rs; the relevant EU legal framework promoting the Three Rs; ethical considerations of animal use in science; as well as how innovative non-animal science is opening up new career and job opportunities for young people.

The current state of play of teaching the Three Rs in education is described and recommendations are made on how to introduce new learning scenarios and resources to make Three Rs education more attractive and more consistent for education programmes at secondary school, university and professional levels.

**READ MORE**

JRC Data Catalogue - Three Rs educatin and training learning scenarios: europa.eu/!pK93yf

In this context, EURL ECVAM is building up a pool of learning scenarios[6] and teaching resources to both inspire and facilitate teachers and professors to include Three Rs in education (see Section 6.2).

## 6.2 Educational resources

EURL ECVAM has produced a set of learning scenarios and teaching resources on various related subjects. These resources are freely available to educators and students alike to facilitate inclusion of this key STEM (Science, Technology, Engineering and Maths) topic in the classroom.

Providing teaching resources forms part of a two-track approach to securing a place for the Three Rs on the curriculum. Whilst adaptations to the curriculum are under discussion at education ministry-level, the Three Rs can already become a feature of the classroom through introducing the subject directly to teachers and providing them with the means to teach it. In this way, through a so-called 'bottom-up' approach, EURL ECVAM complements the challenge of including the Three Rs on the curriculum through traditional channels (see Section 6.1).

The resources can be found on the EU Science Hub website:

1. six learning scenarios (see Box 6.1) which can be downloaded and used by educators to support delivering a lesson;
2. a selection of university-level learning scenarios developed by university professors to be used as examples to follow when building a module or course for higher education purposes;
3. infographics, with links to more dynamic content and background information;
4. a slide set (in Microsoft PowerPoint) and an accompanying storyboard that can be edited and re-purposed containing background information on four themes:
    - Theme 1 – introduction and the scientific and social context
    - Theme 2 – the European Union and animals used for scientific purposes
    - Theme 3 – more about The Three Rs – Replace, Reduce, Refine
    - Theme 4 – innovations enabling replacement.

**READ MORE**

EU Science Hub - education & training: europa.eu/!FV94DM

6 Learning scenarios are stand-alone descriptions of educational activities to be carried out in a classroom connected to a specific topic, including objectives, pedagogical methodologies, duration, target age, etc.

## Box 6.1 Learning scenarios for secondary schools

In the context of the European Parliament Pilot Project on promoting alternatives to animal testing (see Section 6.3.2 of Zuang *et al.,* (2019)), and in collaboration with external partners (Ecorys Ltd., Syrcle and European Schoolnet) EURL ECVAM has developed some teaching resources on the Three Rs for secondary schools.

Twelve pilot teachers were selected from six countries: Belgium, Italy, Malta, Portugal, Spain and Turkey to co-created six learning scenarios, which are 'standard operating procedures' containing objectives, pedagogical methodologies, duration, target age, resources and several other elements to help deliver effective lessons. They cover the following subjects:

- Animal welfare: animals in society, animals in science
- Sustainable science: the Three Rs, human-based science
- Critical thinking: debate acknowledging facts, emotion and science literacy.

From October 2019 to January 2020, 23 teachers and 568 students tested these learning scenarios throughout Europe in order to ascertain their effectiveness. This was verified with the use of pre- and post-course questionnaires to both teachers and students.

According to the questionnaires, all teachers involved in the validation phase agreed that the learning scenarios provided an opportunity for their professional development. They also thought that students were more motivated to learn the curriculum content after the implementation of the learning scenarios.

From the students' perspective, after the introduction of the learning scenarios, they felt more comfortable in finding resources that do not use live animals to learn about body systems or other biology topics. Overall students perceived the Three Rs subject in a favourable way as evidenced by the large number of positive comments.

A final version of the six learning scenarios is available in English on the Scientix platform: http://www.scientix.eu/pilots/pilot-3rs.

## 6.3 The Three Rs MOOC

A massive open online course (MOOC) entitled "The Three Rs and animal use in science" was developed by EURL ECVAM in collaboration with Ecorys Ltd., SYRCLE and European Schoolnet.

The MOOC was tailored for life sciences teachers in secondary schools to provide them with materials, support and tutorials to integrate the Three Rs in their own classrooms.

The ultimate goal is to inspire students to develop their critical thinking and science literacy skills by exploring topics such as ethics and research methods, how the European Union is protecting the welfare of animals used in science and how to improve the welfare of animals in general.

From 13 January to 19 February 2020, the MOOC was run and moderated with interactive and live sessions, and included a final assignment consisting of developing an own learning scenario as well as reviewing the learning scenarios suggested by three other participants. The course was completed by 264 participants and it is estimated that over 8,000 students were therefore reached only in 2020 (see Box 6.2). EURL ECVAM plans to re-run an updated version of the MOOC in 2021.

The MOOC, available in English, is currently hosted on the European Schoolnet Academy platform and is freely accessible to anyone who signs up. It is based on six learning scenarios developed, tested and validated by twelve pilot science teachers (see Section 6.2 and Box 6.1).

**READ MORE**

▸▸ MOOC on "The Three Rs and animal use in science": www.europeanschoolnetacademy.eu/courses/course-v1:3Rs+-AnimalsInScience+2020/about

### Box 6.2 "The Three Rs and animal use in science" MOOC

Six learning scenarios, co-created by 12 science teachers (see Section 6.2) were showcased in a massive open online course (MOOC) in order to train other teachers to use them, as well as create their own learning scenarios to introduce the Three Rs in their classes.

The course explains the Three Rs and the alternatives to animal testing and addresses various ways that teachers can introduce this topic in their classrooms. To help teachers integrate the Three Rs in their daily teaching, the online course also addresses topics relevant to 21st century skills in the context of animal testing, such as fake news/media literacy, science literacy and critical thinking.

The course is based on the six learning scenarios mentioned in Box 6.1 and divided into four modules:

- Module 1: animal welfare and science
- Module 2: human-based science
- Module 3: critical thinking
- Module 4: design your own Three Rs learning scenario.

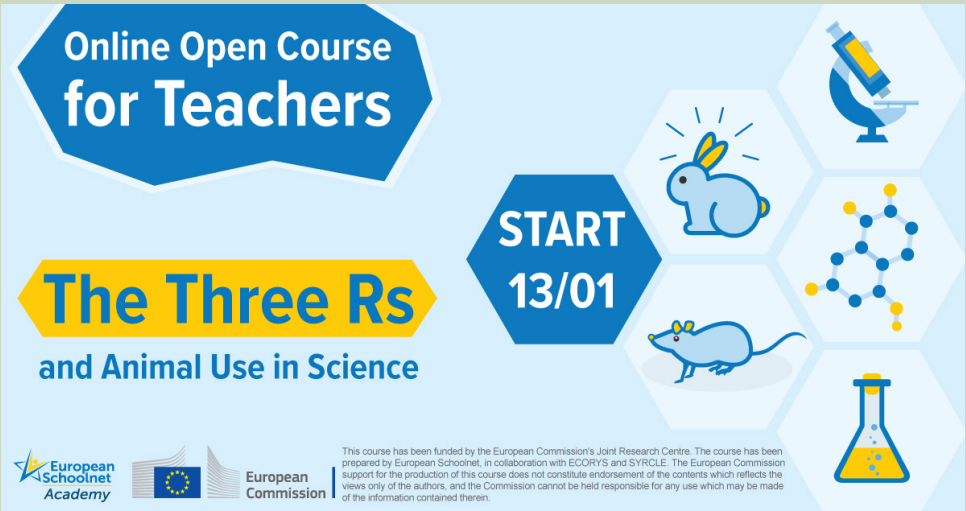
The MOOC was live, run between 13 January and 19 February 2020, and included interactive and live sessions, webinars, a forum and a wiki space. Upon completion of the four modules and the final assignment, consisting of developing a learning scenario and reviewing those of three other participants, the participants received a course certificate and a digital badge. This course was formally recognised as a valid continuous professional development for Portuguese teachers.

Overall, the course had 1,215 registrants, 692 active participants and 264 completions, reaching over 8,000 students. The engagement rate was 57%, which is very high compared to the average of 5-13% reported in literature for MOOCs.

The active participants were from 54 countries worldwide. From the post-course survey, it emerged that the majority of participants were researchers or upper secondary school teachers and with 10 years or more experience in education. A total of 98% of the

course participants rated the course as "Very good" or "Good".

Although the "The Three Rs and animal use in science" MOOC is currently not being moderated by a coordinator, its content is freely available to anyone who signs up on the European Schoolnet Academy platform: https://www.europeanschoolnetacademy.eu/courses/course-v1:3Rs+AnimalsInScience+2020/about. An updated version of the MOOC will be re-run in 2021.




## 6.4 E-learning modules

Six open access and interactive e-learning modules to provide training on aspects of animal use in science and the Three Rs will be available in Q1 2021. The modules have been created by the Commission's Directorate General for Environment, responsible for legislation protecting animals used for scientific purposes, in close collaboration with EURL ECVAM.

The module content elaborates on priority training areas established in the EU Education and Training Framework (DG ENV, 2014) under Directive 2010/63/EU on the protection of animals used for scientific purposes and aims to facilitate a harmonised approach in the EU to laboratory animal science education and training.

The modules cover the following specific subjects: project design, project evaluation, the application of the severity assessment framework for procedures on animals and best practice on searching for and retrieval of non-animal methods and good practice for alternative method development in accordance with Good *In Vitro* Method Practices (GIVIMP, OECD 2018) which are high international standards. The modules are designed to ensure competence of scientists in the conduct and approval of animal procedures, and that the Three Rs are applied fully with a focus on Replacement.

The modules are aimed at the entire animal user community, including laboratory animal practitioners, technicians, project designers and evaluators, researchers, but also alternative method developers, validation bodies, regulators, young scientists at university and early-career levels and any other stakeholder with an interest in the field who wants to learn more.

Although designed to be standalone, the courses, which take around one and a half hours to complete, also fit well with other related training courses and programmes. They will allow students to gain a thorough knowledge of the subjects addressed, and be able to test their understanding through quizzes and other activities. Further modules are planned to complete the suite.

**Box 6.3 Learning scenarios for secondary schools**

1. Project evaluation
2. Design of procedures and projects part 1
3. Design of procedures and projects part 2
4. Implementation of the severity assessment framework within projects using live animals
5. Searching for, and identification of, alternative (non-animal) methods and approaches
6. Developing reliable and relevant *in vitro* methods and approaches for scientific purposes.

When available the modules can be accessed here: https://ec.europa.eu/environment/chemicals/lab_animals/index_en.htm

7

# Conclusions

In 2020, EURL ECVAM continued to advance the field of non-animal methods and approaches through their development, validation and international promotion for regulatory uses and through the evaluation, dissemination and promotion of human-relevant non-animal models for use in basic, applied and translational research.

# 7. Conclusions

In the regulatory field, this was achieved by working on a variety of projects and activities ranging from research and development projects, evaluation of submitted test methods, validation of promising methods, issuing of EURL ECVAM recommendations and promotion of the regulatory use and international adoption of non-animal methods, in particular within defined approaches and integrated approaches to testing and assessment.

EURL ECVAM intensified its engagement with legislators and regulators to find common solutions to current gaps in chemicals hazard and risk assessments. For instance, in 2020, EURL ECVAM's work contributed to the European Green Deal, one of the EC's priorities for 2019-2024. In particular, EURL ECVAM led the EC fitness check on endocrine disruptors and contributed to the progress report on the assessment and management of combined exposures to multiple chemicals (chemical mixtures) and associated risks, both published together with the EU Chemicals Strategy for Sustainability.

The European Green Deal includes a zero-pollution ambition with strategies to protect citizens' health from environmental degradation and pollution, addressing air and water quality, hazardous chemicals, industrial emissions, pesticides and endocrine disrupters.

The EU Chemicals Strategy for Sustainability is part of the EU's zero pollution ambition and is one of the first deliverable of the European Green Deal. The strategy can only be successful and deliver on its promise if non-animal methods and approaches are used for chemicals hazard and risk assessments. The aim should be to move away from a check-list approach based on *in vivo* test guidelines that does not efficiently meet legislative mandates which require increased numbers

of chemical assessments without a parallel increase in the use of animals. In the coming years, EURL ECVAM will also contribute, within the European Green Deal, to the new "Farm to Fork Strategy" on sustainable food along the whole value chain, as well as to Europe's beating cancer plan within the European Commission's priority "Promoting our European way of life".

The higher number of animals used for research (basic, applied and translational research) when compared to regulatory use already seen in the EC report on the 2015 to 2017 statistics on the use of animals for scientific purposes (EC, 2020d) seems to continue in the upcoming new EU figures of 2018 (EC forthcoming publication). It is thus also important to invest efforts and activities to reduce the high number of animal use in research.

In 2020, activities included comprehensive reviews on non-animal models and methods for investigating seven disease areas. Two of these reviews, i.e., on respiratory tract diseases and breast cancer, were published in 2020. The study outcomes will be useful for several actors involved in the implementation of Directive 2010/63 such as the National Committees, research groups submitting new project proposals, Competent Authorities who are responsible for project evaluation and dissemination of information, animal welfare bodies advising research groups on project proposals and the National Contact Points responsible for the implementation of the Directive in the respective Member States. EURL ECVAM also started to monitor the outputs and impact of EU-funded biomedical research and to explore the pathogenesis of COVID-19 using Adverse Outcome Pathways.

Education and training activities teaching the new generation on the Three Rs and non-animal methods and approaches, were also prominent in 2020. These included among others the development of freely available teaching resources and a strategy to implement enhanced teaching of the Three Rs and alternatives at secondary school and university levels.

Finally and importantly, as evidenced by the many activities described in this report, the transition to non-animal approaches in science and regulation depends heavily on collaboration and dedication from committed stakeholders – together we are stronger!

# References


Barroso, J., Halder, M., & Whelan, M. (2020). *EURL ECVAM Recommendation on Non-Animal-Derived Antibodies*. EUR 30185 EN, Publications Office of the European Union, Luxembourg, JRC120199. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC120199/jrc120199pdf.pdf

Basketter, D., Beken, S., Bender, H., Bridges, J., Casati, S., Corvaro, M., Cuvellier, S., Hubesch, B., Irizar, A., Jacobs, M.N., Kern, P., Lamplmair, F., Manou, I., Muller, B.P., Roggeband, R., & Rossi, L.H. (2020). Building confidence in skin sensitisation potency assessment using new approach methodologies: report of the 3rd EPAA Partners Forum, Brussels, 28th October 2019. *Regulatory Toxicology and Pharmacology*, 117. doi:10.1016/j.yrtph.2020.104767

Batista Leite, S., Cipriano, M., Carpi, D., Coecke, S., Holloway, M., Corvi, R., Worth, A., Barroso, J., & Whelan, M. (2021). *Establishing the scientific validity of complex in vitro models.: Results of a EURL ECVAM survey*. Publications Office of the European Union, Luxembourg, Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC122394/civm_survey_report_pdf_online.pdf

Bopp, S., Berggren, E., Kienzler, A., van der Linden, S., & Worth, A. (2015). *Scientific methodologies for the assessment of combined effects of chemicals – a survey and literature review*. EUR 27471 EN, Publications Office of the European Union, Luxembourg, JRC97522 Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC97522/jrc_tech_rep_sci%20meth%20for%20mix_final.pdf

Bopp, S., Barouki, R., Brack, W., Dalla Costa, S., Dorne, J.C.M., Drakvik, P.E., Faust, M., Karjalainen, T.K., Kephalopoulos, S., van Klaveren, J., Kolossa-Gehring, M., Kortenkamp, A., Lebret, E., Lettieri, T., Norager, S., Ruegg, J., Tarazona, J.V., Trier, X., van de Water, B., van Gils, J., & Bergman, A. (2018). Current EU research activities on combined exposure to multiple chemicals. *Environment International*, 120, 544-562. doi:10.1016/j.envint.2018.07.037

Bopp, S., Franco, A., Cusinato, A., Kephalopoulos, S., & Ceridono, M. (2020). *Information Platform for Chemical Monitoring IPCHEM 2020 – Update on the state of play of IPCHEM*. European Commission, Ispra, JRC123154. Available at: https://ipchem.jrc.ec.europa.eu/documents/JRC123154_ipchem_2020.pdf

Bopp, S.K., Kienzler, A., Linden, V.D., Lamon, L., Paini, A., Parissis, N., Richarz, A.-N., & Worth, A. (2016). *Review of case studies on the human and environmental risk assessment of chemical mixtures future needs*. EUR 27968 EN, Publications Office of the European Union, Luxembourg, JRC102111 Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC102111/jrc102111_jrc_tech-rep_mix%20case%20studies_2016_vf.pdf

Bopp, S.K., Kienzler, A., Richarz, A.N., van der Linden, S.C., Paini, A., Parissis, N., & Worth, A.P. (2019). Regulatory assessment and risk management of chemical mixtures: challenges and ways forward. *Critical Reviews in Toxicology*, 49(2), 174-189. doi: 10.1080/10408444.2019.1579169

Brion, F., Le Page, Y., Piccini, B., Cardoso, O., Tong, S.K., Chung, B.C., & Kah, O. (2012). Screening estrogenic activities of chemicals or mixtures *in vivo* using transgenic (cyp19a1b-GFP) zebrafish embryos. *PLoS ONE*, 7(5), e36069. doi:10.1371/journal.pone.0036069

Corvi, R., Madia, F., Worth, A., & Whelan, M. (2013). *EURL ECVAM strategy to avoid and reduce animal use in genotoxicity testing*. EUR 26375 EN, Publications Office of the European Union, Luxemburg, JRC86616. Available at: https://publications.jrc.ec.europa.eu/repository/handle/JRC86616

Da Silva, E., Baun, A., Berggren, E., & Worth, A. (2020). Optimising testing strategies for classification of human health and environmental hazards - A proof-of-concept study. *Toxicology Letters*, 335, 64-70. doi:10.1016/j.toxlet.2020.10.008

Daas, A., Behr-Gross, M.E., Bruckner, L., & Redhead, K. (2020). Collaborative study for the validation of cell line assays for in-process toxicity and antigenicity testing of *Clostridium septicum* vaccine antigens - Part 1. *Pharmeur Bio Sci Notes*, 2020, 53-124.

DG ENV. (2014). Directive 2010/63/EU - Education and Training Framework. Available at: https://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_E-T.pdf

EC. (2012). Communication from the Commission to the Council "The combination effects from chemicals – Chemical Mixtures". COM/2012/0252 final. Available at: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A52012DC0252

EC. (2018). Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions - Towards a comprehensive European Union framework on endocrine disruptors. COM(2018) 734 final. Available at: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A52018DC0734

EC. (2020a). 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. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=COM%3A2020%3A667%3AFIN

EC. (2020b). Commission Staff Working Document - Fitness Check on endocrine disruptors accompanying the document 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. Available at: https://ec.europa.eu/environment/pdf/chemicals/2020/10/SWD_on_Endocrines_disruptors.pdf

EC. (2020c). Commission Staff Working Document - Progress report on the assessment and management of combined exposures to multiple chemicals (chemical mixtures) and associated risks accompanying the document 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. Available at: https://ec.europa.eu/environment/pdf/chemicals/2020/10/SWD_mixtures.pdf

EC (2020d). Report from the Commission to the European Parliament and the Council - 2019 report on the statistics on the use of animals for scientific purposes in the Member States of the European Union in 2015-2017. COM(2020) 16 final. Available at: https://ec.europa.eu/transparency/regdoc/rep/1/2020/EN/COM-2020-16-F1-EN-MAIN-PART-1.PDF

ECHA. (2017). Read-Across Assessment Framework (RAAF). ECHA-17-R-01-EN Available at: https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

EFSA. (2011). Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA Journal*, 9(9), 2379. doi:10.2903/j.efsa.2011.2379

EU. (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, 2008/0211 (COD). Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063

EURL ECVAM Scientific Advisory Committee. (2020). *ESAC Opinion on the Scientific Validity of the Bioelution Test Method*. EUR 30281 EN, Publications Office of the European Union, Luxembourg, JRC121143. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC121143/esac_opinion_2019-03_bioelution_%28online%29.pdf

Folgiero, V., Romania, P., Rossi, F., Caforio, M., Nic, M., Dibusz, K., Novotny, T., Busquet, F., Straccia, M., & Gribaldo, L. (2020). *Advanced Non-animal Models in Biomedical Research: Breast cancer*. EUR 30334/1 EN, Publications Office of the European Union, Luxembourg, JRC122309. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC122309/final_report_online.pdf

Gribaldo, L., Dura, A., Straccia, M., & Whelan, M. (2020). *Advanced Non-animal Models in Biomedical Research: Breast Cancer: Executive Summary*. EUR 30334/1 EN, Publications Office of the European Union, Luxembourg, RC122309. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC122309/exe_sum_online.pdf

Gribaldo, L., & Whelan, M. (2020). *Advanced Non-animal Models in Biomedical Research: Respiratory Tract Diseases: Executive Summary*. EUR 30334 EN, Publications Office of the European Union, Luxembourg, RC118161. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC118161/kjnb30334enn.pdf

Häggström, M. (2014). Medical gallery of Mikael Häggström 2014. *Wiki Journal of Medicine*, 1(2). doi:10.15347/WJM/2014.008

Hasselgren, C., Ahlberg, E., Akahori, Y., Amberg, A., Anger, L.T., Atienzar, F., Auerbach, S., Beilke, L., Bellion, P., Benigni, R., Bercu, J., Booth, E.D., Bower, D., Brigo, A., Cammerer, Z., Cronin, M.T.D., Crooks, I., Cross, K.P., Custer, L., Dobo, K., Doktorova, T., Faulkner, D., Ford, K.A., Fortin, M.C., Frericks, M., Gad-McDonald, S.E., Gellatly, N., Gerets, H., Gervais, V., Glowienke, S., Van Gompel, J., Harvey, J.S., Hillegass, J., Honma, M., Hsieh, J.H., Hsu, C.W., Barton-Maclaren, T.S., Johnson, C., Jolly, R., Jones, D., Kemper, R., Kenyon, M.O., Kruhlak, N.L., Kulkarni, S.A., Kummerer, K., Leavitt, P., Masten, S., Miller, S., Moudgal, C., Muster, W., Paulino, A., Lo Piparo, E., Powley, M., Quigley, D.P., Reddy, M.V., Richarz, A.N., Schilter, B., Snyder, R.D., Stavitskaya, L., Stidl, R., Szabo, D.T., Teasdale, A., Tice, R.R., Trejo-Martin, A., Vuorinen, A., Wall, B.A., Watts, P., White, A.T., Wichard, J., Witt, K.L., Woolley, A., Woolley, D., Zwickl, C., & Myatt, G.J. (2019). Genetic toxicology *in silico* protocol. *Regulatory Toxicology and Pharmacology*, 107, 104403. doi:10.1016/j.yrtph.2019.104403

Heusinkveld, H., Braakhuis, H., Gommans, R., Botham, P., Corvaro, M., van der Laan, J.W., Lewis, D., Madia, F., Manou, I., Schorsch, F., Wolterink, G., Woutersen, R., Corvi, R., Mehta, J., & Luijten, M. (2020). Towards a mechanism-based approach for the prediction of nongenotoxic carcinogenic potential of agrochemicals. *Critical Reviews in Toxicology*. doi:10.1080/10408444.2020.1841732

Holley, T., Bowe, G., Campia, I., Belz, S., Berggren, E., Janusch-Roi, A., & Wittwehr, C.W.M. (2016). *Accelerating progress in the Replacement, Reduction and Refinement of animal testing through better knowledge sharing*. EUR 28234 EN, Publications Office of the European Union, Luxemburg, JRC103906. Available at: https://publications.jrc.ec.europa.eu/repository/handle/JRC103906

Holloway, M., Berggren, E., Dura, A., Gribaldo, L., & Whelan, M. (2021). *Introducing the Three Rs into secondary schools, universities and continuing education programmes*. Publications Office of the European Union, Luxembourg, JRC123343.

Hynes, J., Marshall, L., Adcock, I., Novotny, T., Nic, M., Dibusz, K., & Gribaldo, L. (2020). *Advanced Non-animal Models in Biomedical Research: Respiratory Tract Diseases*. EUR 30334 EN, Publications Office of the European Union, Luxembourg, JRC118161. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC118161/final_report_online.pdf

ICH. (2011). Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use - S2(R). Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S2_R1/Step4/S2R1_Step4.pdf

Jacobs, M.N., Colacci, A., Louekari, K., Luijten, M., Hakkert, B.C., Paparella, M., & 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., & 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

Johnson, C., Ahlberg, E., Anger, L.T., Beilke, L., Benigni, R., Bercu, J., Bobst, S., Bower, D., Brigo, A., Campbell, S., Cronin, M.T.D., Crooks, I., Cross, K.P., Doktorova, T., Exner, T., Faulkner, D., Fearon, I.M., Fehr, M., Gad, S.C., Gervais, V., Giddings, A., Glowienke, S., Hardy, B., Hasselgren, C., Hillegass, J., Jolly, R., Krupp, E., Lomnitski, L., Magby, J., Mestres, J., Milchak, L., Miller, S., Muster, W., Neilson, L., Parakhia, R., Parenty, A., Parris, P., Paulino, A., Paulino, A.T., Roberts, D.W., Schlecker, H., Stidl, R., Suarez-Rodrigez, D., Szabo, D.T., Tice, R.R., Urbisch, D., Vuorinen, A., Wall, B., Weiler, T., White, A.T., Whritenour, J., Wichard, J., Woolley, D., Zwickl, C., & Myatt, G.J. (2020). Skin sensitization *in silico* protocol. *Regulatory Toxicology and Pharmacology*, 116, 104688. doi:10.1016/j.yrtph.2020.104688

Kienzler, A., Berggren, E., Bessems, J., Bopp, S., Linden, S.V.D., & Worth, A. (2014). *Assessment of Mixtures - Review of Regulatory Requirements and Guidance*. EUR 26675 EN, Publications Office of the European Union, Luxemburg, JRC90601. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC90601/lb1a26675enn.pdf

Kienzler, A., Bopp, S.K., van der Linden, S., Berggren, E., & Worth, A. (2016). Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives. *Regulatory Toxicology and Pharmacology*, 80, 321-334. doi:10.1016/j.yrtph.2016.05.020

Kirkland, D., Pfuhler, S., Tweats, D., Aardema, M., Corvi, R., Darroudi, F., Elhajouji, A., Glatt, H., Hastwell, P., Hayashi, M., Kasper, P., Kirchner, S., Lynch, A., Marzin, D., Maurici, D., Meunier, J.R., Muller, L., Nohynek, G., Parry, J., Parry, E., Thybaud, V., Tice, R., van Benthem, J., Vanparys, P., & White, P. (2007). How to reduce false positive results when undertaking *in vitro* genotoxicity testing and thus avoid unnecessary follow-up animal tests: Report of an ECVAM Workshop. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 628(1), 31-55. doi:10.1016/j.mrgentox.2006.11.008

Kirkland, D., Kasper, P., Martus, H.J.r., Müller, L., van Benthem, J., Madia, F., & Corvi, R. (2016). Updated recommended lists of genotoxic and non-genotoxic chemicals for assessment of the performance of new or improved genotoxicity tests. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 795, 7-30. doi:10.1016/j.mrgentox.2015.10.006

Louisse, J., Alewijn, M., Peijnenburg, A., Cnubben, N.H.P., Heringa, M.B., Coecke, S., & Punt, A. (2020). Towards harmonization of test methods for *in vitro* hepatic clearance studies. *Toxicology in Vitro*, 63, 104722. doi:10.1016/j.tiv.2019.104722

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., & 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

Madden, J.C., Enoch, S.J., Paini, A., & Cronin, M.T.D. (2020). A Review of *In Silico* Tools as Alternatives to Animal Testing: Principles, Resources and Applications. *Alternatives to Laboratory Animals*, 48(4), 146-172. doi:10.1177/0261192920965977

Madia, F., Corvi, R., Worth, A., Mathys, I., & Prieto, P. (2020a). Making better use of toxicity studies for human health by extrapolating across endpoints. *ALTEX*, 37(4), 519-531. doi:10.14573/altex.2005061

Madia, F., Kirkland, D., Morita, T., White, P., Asturiol, D., & Corvi, R. (2020b). EURL ECVAM Genotoxicity and Carcinogenicity Database of Substances Eliciting Negative Results in the Ames Test: Construction of the Database. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 854. doi:10.1016/j.mrgentox.2020.503199

Madia, F., Pillo, G., Worth, A., Corvi, R., & Prieto, P. (manuscript in preparation). Integration of data across toxicity endpoints to explore new ways for safety assessment of chemicals: the example of carcinogenicity testing.

Mahony, C., Ashton, R.S., Birk, B., Boobis, A.R., Cull, T., Daston, G.P., Ewart, L., Knudsen, T.B., Manou, I., Maurer-Stroh, S., Margiotta-Casaluci, L., Muller, B.P., Nordlund, P., Roberts, R.A., Steger-Hartmann, T., Vandenbossche, E., Viant, M.R., Vinken, M., Whelan, M., Zvonimir, Z., & Cronin, M.T.D. (2020). New ideas for non-animal approaches to predict repeated-dose systemic toxicity: Report from an EPAA Blue Sky Workshop. *Regulatory Toxicology and Pharmacology*, 114. doi:10.1016/j.yrtph.2020.104668

Martin, O., Scholze, M., Ermler, S., McPhie, J., Bopp, S.K., Kienzler, A., Parissis, N., & Kortenkamp, A. (2020). Ten years of research on synergisms and antagonisms in chemical mixtures: A systematic review and quantitative reappraisal of mixture studies. *Environment International*, 146, 106206. doi:10.1016/j.envint.2020.106206

OECD. (2013). *Test No. 236: Fish Embryo Acute Toxicity (FET) Test*. OECD Publishing, Paris. doi:10.1787/9789264203709-en

OECD. (2017). *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. 9789264274716. doi:10.1787/9789264274716-en

OECD. (2018). *Guidance Document on Good In Vitro Method Practices (GIVIMP)*. OECD Series on Testing and Assessment, No. 286. OECD Publishing, Paris. doi:10.1787/9789264304796-en

OECD. (2020a). *Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA)*. OECD Series on Testing and Assessment, No. 329. OECD Publishing, Paris. ENV/JM/MONO(2020)25. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2020)25&docLanguage=en

OECD. (2020b). *Test No. 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals. O*ECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.doi:10.1787/9789264264366-en.

OECD (2021). *Guidance Document on the Characterisation, Validation and Reporting of PBK Models for Regulatory Purposes*. Series on Testing and Assessment No. 330. OECD Publishing, Paris. ENV/CBC/MONO(2021)1.

Paparella, M., Scholz, S., Belanger, S., Braunbeck, T., Bicherel, P., Connors, K., Fassbender, C., Halder, M., Lillicrap, A., Liska, R., Schirmer, K., Stoddart, G., Thomas, P., & Walter-Rohde, S. (2021). Limitations and uncertainties of acute fish toxicity assessments can be reduced using alternative methods. *ALTEX*, 38(1). doi:10.14573/altex.2006051

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., & 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., Pirow, R., Downs, T.R., Haase, A., Hewitt, N., Luch, A., Merkel, M., Petrick, C., Said, A., Schafer-Korting, M., & Reisinger, K. (2020a). 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*, geaa009. doi:10.1093/mutage/geaa009

Pfuhler, S., van Benthem, J., Curren, R., Doak, S.H., Dusinska, M., Hayashi, M., Heflich, R.H., Kidd, D., Kirkland, D., Luan, Y., Ouedraogo, G., Reisinger, K., Sofuni, T., van Acker, F., Yang, Y., & Corvi, R. (2020b). Use of *in vitro* 3D tissue models in genotoxicity

testing: Strategic fit, validation status and way forward. Report of the working group from the 7th International Workshop on Genotoxicity Testing (IWGT). *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 850-851, 503135. doi:10.1016/j.mrgentox.2020.503135

Pfuhler, S., Downs, T.R., Hewitt, N.J., Hoffmann, S., Mun, G.C., Ouedraogo, G., Roy, S., Curren Rodger, D., & Aardema, M.J. (in press). 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*.

Pistollato, F., Bernasconi, C., McCarthy, J., Campia, I., Desaintes, C., Wittwehr, C., Deceuninck, P., & Whelan, M. (2020a). Alzheimer's Disease, and Breast and Prostate Cancer Research: Translational Failures and the Importance to Monitor Outputs and Impact of Funded Research. *Animals (Basel)*, 10(7). doi:10.3390/ani10071194

Pistollato, F., de Gyves, E.M., Carpi, D., Bopp, S.K., Nunes, C., Worth, A., & Bal-Price, A. (2020b). Assessment of developmental neurotoxicity induced by chemical mixtures using an adverse outcome pathway concept. *Environmental Health*, 19(1). doi:10.1186/s12940-020-00578-x

Pletz, J., Blakeman, S., Paini, A., Parissis, N., Worth, A., Andersson, A.M., Frederiksen, H., Sakhi, A.K., Thomsen, C., & Bopp, S.K. (2020). Physiologically based kinetic (PBK) modelling and human biomonitoring data for mixture risk assessment. *Environment International*, 143, 105978. doi:10.1016/j.envint.2020.105978

Prieto, P., Graepel, R., Gerloff, K., Lamon, L., Sachana, M., Pistollato, F., Gribaldo, L., Bal-Price, A., & Worth, A. (2019). Investigating cell type specific mechanisms contributing to acute oral toxicity. *ALTEX*, 36(1), 39-64. doi:10.14573/altex.1805181

Renko, K., Hoefig, C.S., Hiller, F., Schomburg, L., & Kohrle, J. (2012). Identification of Iopanoic Acid as Substrate of Type 1 Deiodinase by a Novel Nonradioactive Iodide-Release Assay. *Endocrinology*, 153(5), 2506-2513. doi:10.1210/en.2011-1863

SCCS. (2018). SCCS's Notes of Guidance (NoG) for the Testing of Cosmetic Ingredients and their Safety Evaluation, 10th Revision (SCCS/1602/18). Available at: https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_224.pdf

Smith, M.T., Guyton, K.Z., Gibbons, C.F., Fritz, J.M., Portier, C.J., Rusyn, I., DeMarini, D.M., Caldwell, J.C., Kavlock, R.J., Lambert, P.F., Hecht, S.S., Bucher, J.R., Stewart, B.W., Baan, R.A., Cogliano, V.J., & Straif, K. (2016). Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. *Environmental Health Perspectives*, 124(6), 713-721. doi:10.1289/ehp.1509912

Spinu, N., Cronin, M.T.D., Enoch, S.J., Madden, J.C., & Worth, A.P. (2020). Quantitative adverse outcome pathway (qAOP) models for toxicity prediction. *Archives of Toxicology*, 94(5), 1497-1510. doi:10.1007/s00204-020-02774-7

Tan, Y.M., Chan, M., Chukwudebe, A., Domoradzki, J., Fisher, J., Hack, C.E., Hinderliter, P., Hirasawa, K., Leonard, J., Lumen, A., Paini, A., Qian, H., Ruiz, P., Wambaugh, J., Zhang, F., & Embry, M. (2020). PBPK model reporting template for chemical risk assessment applications. *Regulatory Toxicology and Pharmacology*, 115, 104691. doi:10.1016/j.yrtph.2020.104691

Tice, R.R., Bassan, A., Amberg, A., T., A.L., Beal, M.A., Bellion, P., Benigni, R., Birmingham, J., Brigo, A., Bringezu, F., Ceriani, L., Crooks, I., Cross, K., Elespuru, R., Faulkner, D.M., Fortin, M.C., Fowler, P., Frericks, M., Gerets, H.H.J., Jahnke, G.D., Jones, D.R., Kruhlak, N.L., Lo Piparo, E., Lopez-Belmonte, J., Luniwal, A., Luu, A., Madia, F., Manganelli, S., Manickam, B., Mestres, J., Mihalchik-Burhans, A.L., Neilson, L., Pandiri, A., Pavan, M., Rider, C.V., Rooney, J.P., Trejo-Martin, A., Watanabe-Sailor, K.H., White, A.T., Woolley, D., & Myatt, G.J. (submitted). *In Silico* Approaches In Carcinogenicity Hazard Assessment: Current Status and Future Needs. *Regulatory Toxicology and Pharmacology*.

United Nations. (2019). Globally Harmonized System of Classification and Labelling of Chemicals (GHS, Rev.8): Published: May 2019.

Zuang, V., Dura, A., Asturiol Bofill, D., Barroso, J., Batista Leite, S., Berggren, E., Bernasconi, C., Bopp, S., Bowe, G., Campia, I., Carpi, D., Casati, S., Coecke, S., Cole, T., Corvi, R., Deceuninck, P., Fortaner Torrent, S., Franco, A., Gribaldo, L., Grignard, E., Halder, M., Holloway, M., Kienzler, A., Macko, P., Madia, F., Milcamps, A., Morath, S., Munn, S., Paini, A., Palosaari, T., Pistollato, F., Price, A., Prieto Peraita, P., Sund, J., Wittwehr, C., Worth, A., & Whelan, M. (2020). *EURL ECVAM status report on the development, validation and regulatory acceptance of alternative methods and approaches (2019)*. EUR 30100 EN, Publications Office of the European Union, Luxembourg, JRC119292. Available at: https://publications.jrc.ec.europa.eu/repository/handle/JRC119292

# List of abbreviations and definitions

| | |
|---|---|
| ADME | Absorption, distribution, metabolism and excretion |
| AI | Artificial intelligence |
| ALI | Air-liquid interface |
| AOP | Adverse Outcome Pathway |
| AOP-KB | Adverse Outcome Pathway-Knowledge Base |
| APCRA | Accelerating the Pace of Chemical Risk Assessment |
| AR | Androgen receptor |
| ARTA | Androgen receptor transactivation assay |
| ASCCT | American Society for Cellular and Computational Toxicology |
| BCOP | Bovine Corneal Opacity and Permeability |
| BE | Biomonitoring equivalents |
| BfR | German Federal Institute for Risk Assessment |
| BSP | Biological standardisation programme |
| CALUX | Chemically activated luciferase gene expression |
| CARACAL | Competent Authorities for REACH and CLP |
| CBC | Chemicals and biotechnology committee |
| CEM | Centre of experimental medicine |
| CEN | European Committee for Standardization |
| CENELEC | European Committee for Electrotechnical Standardization |
| CKE | Common key event |
| CLP | Classification, Labelling and Packaging |
| COPD | Chronic obstructive pulmonary disease |
| CYP | Human cytochrome P450 |

| | |
|---|---|
| DA | Defined approach |
| DC | Dendritic cell |
| DG EMPL | Directorate-General for Employment, Social Affairs and Inclusion (EC) |
| DG ENV | Directorate-General for Environment (EC) |
| DG GROW | Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs (EC) |
| DG RTD | Directorate-General for Research and Innovation (EC) |
| DG SANTE | Directorate-General for Health and Food Safety (EC) |
| DIP | Data interpretation procedure |
| DNA | Deoxyribonucleic Acid |
| DNT | Developmental neurotoxicity |
| DRP | Detailed review paper |
| EAGMST | Extended Advisory Group for Molecular Screening and Toxicogenomics |
| ECHA | European Chemicals Agency |
| ECOSOC | Economic and Social Council |
| ED | Endocrine disruptor |
| EDI | Estimated daily intake |
| EDQM | European Directorate for the Quality of Medicines & HealthCare (Council of Europe) |
| EEA | European Environment Agency |
| EFPIA | European Federation of Pharmaceutical Industries and Associations |
| EFSA | European Food Safety Authority |
| EFTA | European Free Trade Association |
| ELISA | Enzyme-linked immunosorbent assay |
| EMA | European Medicines Agency |
| EPAA | European Partnership for Alternatives to Animal Testing |
| ESAC | ECVAM Scientific Advisory Committee |
| ESTAF | ECVAM Stakeholder Forum |
| EU | European Union |
| EU-NETVAL | European Union Network of Laboratories for the Validation of Alternative Methods |
| EURL ECVAM | European Union Reference Laboratory for Alternatives to Animal Testing |
| FBS | Foetal bovine serum |
| FDA | Food and Drug Administration (USA) |
| FRAND | Fair, reasonable and non-discriminatory |
| GARD | Genomic allergen rapid detection test |
| GD | Guidance Document |
| GFL | General food law |
| GHS | Globally Harmonised System of Classification and Labelling of chemicals |
| GIVIMP | Good *In Vitro* Method Practices |
| GLP | Good Laboratory Practice |
| HBM | Human biomonitoring |
| HESI | Health and Environmental Sciences Institute (US) |
| HPV | High production volume |
| IATA | Integrated Approaches to Testing and Assessment |
| ICAPO | International Council on Animal Protection in OECD Programmes |
| ICATM | International Cooperation on Alternative Test Methods |

| | |
|---|---|
| ICH | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| IFRA | International Fragrance Association |
| IMI | Innovative Medicines Initiative |
| IPCHEM | Information platform for chemical monitoring |
| iPSC | induced pluripotent stem cell |
| ISO | International Organization for Standardization |
| IUCLID | Inrenational Uniform Chemical Information Database |
| IVIVE | *In vitro* to *in vivo* extrapolation |
| IWG | Informal working group |
| IWGT | International Workshops on Genotoxicity Testing |
| JaCVAM | Japanese Center for the Validation of Alternative Methods |
| JRC | Joint Research Centre (EC) |
| KB | Knowledge base |
| KMD | Kinetically derived maximum dose |
| LRI | Cefic's Long-Range Research Initiative |
| MAD | Mutual acceptance of data |
| MIT | Mono-iodotyrosine |
| MoA | Mode of action |
| MOOC | Massive open online course |
| MRA | Mixture risk assessment |
| MRF | Metabolomics reporting framework |
| NAM | New approach method |
| NC3Rs | National Centre for the Replacement, Refinement & Reduction of Animals in Research (UK) |
| NGO | Non-governmental organisation |
| NICEATM | National Toxicology Programme Interagency Center for the Evaluation of Alternative Toxicological Methods (US) |
| NIEHS | National Institute of Environmental Health Sciences (US) |
| NL | the Netherlands |
| NTP | National Toxicology Programme (US) |
| OECD | Organisation for Economic Co-operation and Development |
| OHT | OECD Harmonised Template |
| OoC | Organ-on-chip |
| ORCHID | Organ-on-chip in Development |
| OSHA | European Agency for Safety and Health at Work |
| PARERE | Preliminary Assessment of Regulatory Relevance network |
| PBK | Physiologically based kinetic (also PBPK, PBBK, PBTK) |
| PSIS | Putting Science Into Standard |
| QSAR | Quantitative Structure Activity Relationship |
| RADAR | Rapid androgen disruption adverse outcome reporter |
| REACH | European Regulation (EC) no 1907/2006 Registration, Evaluation, Authorisation and Restriction of Chemicals |
| REACTIV | Rapid estrogen activity *in vivo* |
| RfD | Reference dose |
| RIVM | National Institute for Public Health and the Environment (the Netherlands) |
| RNA | Ribonucleic acid |

| | |
|---|---|
| RS | Reconstructed skin |
| RSMN | Reconstructed skin micronucleus |
| SCSS | Scientific Committee on Consumer Safety (EU) |
| SOP | Standard Operating Procedure |
| SOT | Society of toxicology |
| SPSF | Standard project submission form |
| STE | Short time exposure |
| STEM | Science, Technology, Engineering and Maths |
| TDG | Transport of Dangerous Goods (United Nations) |
| TDI | Tolerable daily intake |
| TFR | Transcriptomics reporting framework |
| TG | Test Guideline (OECD) |
| TGP | Test Guidelines Programme (OECD) |
| TPO | Thyroperoxidase |
| TSAR | EURL ECVAM Tracking System on Alternative Methods towards Regulatory acceptance |
| UISS | Universal immune system simulator |
| UK | United Kingdom |
| UN | United Nations |
| UPLC-MS/MS | Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry |
| US(A) | United States (of America) |
| VAC2VAC | "Vaccine batch to vaccine batch comparison by consistency testing" |
| VICH | International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products |
| WG | Working group |
| WHO | World Health Organization |
| WNT | Working Group of the National Coordinators of the Test Guidelines Programme (OECD) |
| WoE | Weight of evidence |
| WPHA | Working Party on Hazard Assessment (OECD) |

# Figures and boxes

## GETTING IN TOUCH WITH THE EU

### IN PERSON

All over the European Union there are hundreds of Europe Direct information centres. You can find the address of the centre nearest you at: ***https://europa.eu/european-union/contact_en***.

### ON THE PHONE OR BY EMAIL

Europe Direct is a service that answers your questions about the European Union. You can contact this service:

- by freephone: **00 800 6 7 8 9 10 11** (certain operators may charge for these calls),
- at the following standard number: **+32 22999696,** or
- by electronic mail via: ***https://europa.eu/european-union/contact_en***.

## FINDING INFORMATION ABOUT THE EU

### ONLINE

Information about the European Union in all the official languages of the EU is available on the Europa website at: ***https://europa.eu/european-union/index_en***.

### EU PUBLICATIONS

You can download or order free and priced EU publications from EU Bookshop at: ***https://publications.europa.eu/en/publications.*** Multiple copies of free publications may be obtained by contacting Europe Direct or your local information centre (see ***https://europa.eu/european-union/contact_en***).

### EU LAW AND RELATED DOCUMENTS

For access to legal information from the EU, including all EU law since 1952 in all the official language versions, go to EUR-Lex at: ***http://eur-lex.europa.eu***.

### OPEN DATA FROM THE EU

The EU Open Data Portal ***(http://data.europa.eu/euodp/en)*** provides access to datasets from the EU. Data can be downloaded and reused for free, for both commercial and non-commercial purposes.

KJ-NA-30553-EN-N

## The European Commission's science and knowledge service

Joint Research Centre

### JRC Mission

As the science and knowledge service of the European Commission, the Joint Research Centre's mission is to support EU policies with independent evidence throughout the whole policy cycle.

### EU Science Hub

ec.europa.eu/jrc

@EU_ScienceHub

EU Science Hub - Joint Research Centre

EU Science, Research and Innovation

EU Science Hub

Publications Office of the European Union

doi:10.2760/719755
ISBN 978-92-76-28395-9