



JRC SCIENCE FOR POLICY REPORT

# EURL ECVAM Status Report

on the Development, Validation and Regulatory Acceptance  
of Alternative Methods and Approaches (2019)

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2019

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# **Abstract and List of Authors**

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The principle of the Three Rs, Replacement, Reduction and Refinement of animal use in basic, applied and translational research as well as for regulatory testing, is anchored in EU legislation.

Innovative technologies such as computational models, *in vitro* methods and organ-on-chip devices are being developed, evaluated and integrated into safety assessment strategies for chemicals and products to ensure a high level of protection of human health and the environment.

Important activities are also ongoing to promote the development and use of alternative methods and approaches in basic and applied research as well to incorporate the Three Rs into educational programmes.

The annual EURL ECVAM Status Report provides an extensive overview of research and development projects, test method validation studies and peer reviews, initiatives to promote the regulatory acceptance and international adoption of alternative methods, and initiatives that share knowledge and information across communities and sectors.

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# Executive Summary

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The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) is an integral part of the European Commission's Joint Research Centre (JRC). This annual EURL ECVAM status report provides updates on the progress being made in the development, validation and regulatory acceptance of alternative-to-animal methods and approaches and their dissemination.

Research initiatives funded by the EU Framework Programme for Research and Innovation, Horizon 2020, are fostering mechanistic-based toxicity testing to support risk assessment of chemicals with a primary focus on repeated dose, developmental and reproductive toxicity. Progress made includes the development of 'read-across' approaches that exploit (chemical) structural features and *in vitro* bioactivity data to identify similar chemicals to fill data gaps and avoid unnecessary animal testing. Attention is also being given to '*ab initio*' strategies with the aim of carrying out a safety assessment using only *in vitro* and *in silico* methods without resorting to read-across. Other EU-funded research projects have been dealing with new testing methods and approaches to identify endocrine disrupting chemicals and to assess chemical mixtures. In the "Vaccine batch to vaccine batch comparison by consistency testing (VAC2VAC)" project, funded under the Innovative Medicines Initiative 2, considerable progress has been made in making standard, scientifically valid non-animal based methods available for the safety and potency testing of vaccines for human and veterinary use.

The broader relationship between chemical exposure and public and environmental health is being explored at EURL ECVAM, with particular attention being given to the area of carcinogenicity. Current emphasis is on investigating how carcinogenic properties of chemicals could be better assessed taking account of the nature of the actual cancer burden in our society and making better use of human relevant data and assessment tools.

The potential impact of Digital Transformation and Artificial Intelligence on decision making in the chemicals arena is also being evaluated.

For environmental effects, R&D projects related to fish toxicity and bioaccumulation continue to be funded through the Cefic Long-Range Research Initiative. Other projects focus on effluents and the development of the ecological Threshold of Toxicological Concern (eco-TTC) concept for environmental risk assessment.

Validation activities on *in vitro* methods have progressed in the areas of endocrine disruption (thyroid), skin sensitisation (transcriptomics-based *in vitro* methods) and

genotoxicity (micronucleus and comet assay on 3D skin models). EURL ECVAM's Scientific Advisory Committee (ESAC) carried out peer reviews on an androgen receptor transactivation assay (being included in updated OECD Test Guideline 458) and a bioelution test method. The latter measures the relative bioaccessibility of metals in inorganic metal compounds and metal-containing materials when in contact with simulated biological media. With an aim to replace the use of animals for the production of antibodies used for scientific purposes, ESAC also reviewed the scientific validity of non-animal-derived antibodies and non-antibody affinity reagents used for research, diagnostics, and regulatory testing applications.

EURL ECVAM continues to collaborate closely with EU agencies (ECHA, EFSA, EMA), its network of regulators (PARERE), its stakeholder forum (ESTAF), its network of laboratories for validation of alternative methods (EU-NETVAL), the European Partnership on Alternatives to Animal Approaches (EPAA) and international harmonisation bodies (OECD, WHO, ICH, VICH, EDQM, UN) on a large number of projects. The aim is to promote the wider international acceptance and application of non-animal approaches (*in vitro* tests, computational models, and integrated assessment strategies) in regulatory testing of chemicals and products.

According to EU statistics on the use of animals in scientific procedures, most animals are still being used in basic, applied and translational research. With a view to encouraging the development and use of alternatives in these areas too, major reviews on non-animal models and methods currently applied for investigating respiratory tract diseases, neurodegenerative disorders, cardiovascular diseases, breast cancer, immunogenicity testing for advanced therapy medicinal products, autoimmune diseases and immune oncology models, were undertaken in 2019 and will be published in 2020. Other activities include the monitoring of the level of uptake of alternative approaches in various contexts and the attempt to bridge existing gaps between disciplines in life sciences.

Finally yet importantly, a complete paradigm shift away from the use of animals for scientific purposes will only be possible by properly educating the next generation. With the support of the European Parliament therefore, in 2019 major effort was invested by EURL ECVAM in the development of guidance for decision-makers in educational organisations on ways to introduce the Three Rs in curricula, together with a range of teaching resources targeting secondary school and university students and early career scientists. The guidance and teaching material will be made freely available in early 2020.

# 1

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## Introduction

# 1.

## Introduction

This is the sixth EURL ECVAM Status Report on the development, validation and regulatory acceptance of alternative methods and approaches. The status reports follow up on the ECVAM progress reports published from 2008 to 2013 in the framework of Regulation No 1223/2009 on cosmetic products which introduced a definitive ban on cosmetics tested on animals and marketed in the EU and the need to monitor the status of alternative approaches.

The primary purpose of the EURL ECVAM status reports is to inform its stakeholders and all interested parties on the progress made in the development, validation and regulatory acceptance and use of alternative approaches in general, and more specifically on EURL ECVAM's activities in the field. The 2019 report provides updates since the last EURL ECVAM status report published early February 2019 and covering the year 2018.

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

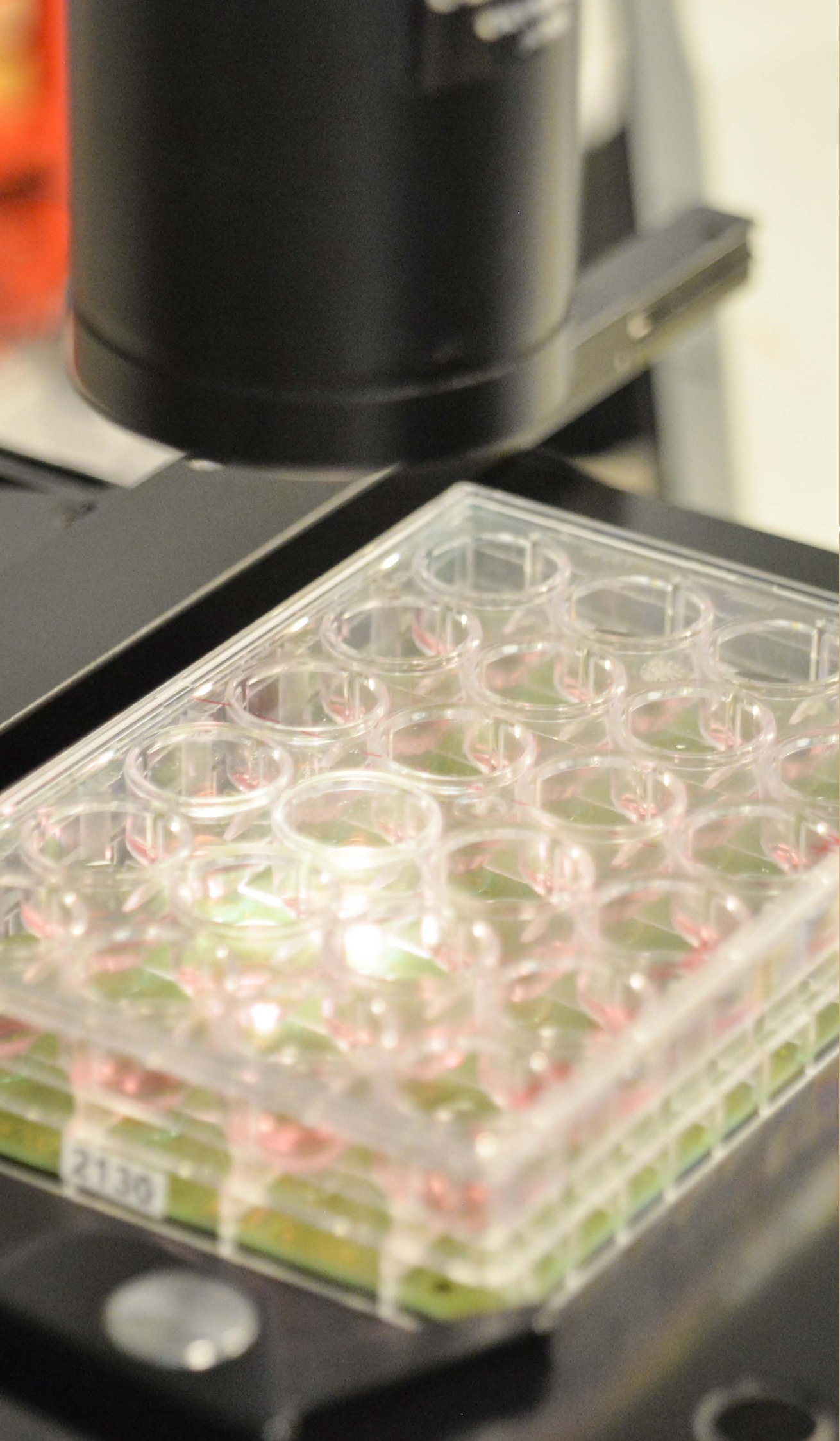
of alternative approaches; (d) setting up, maintaining and managing public databases and information systems on alternative approaches and their state of development and; (e) promoting dialogue between legislators, regulators, and all relevant stakeholders, in particular, industry, biomedical scientists, 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.

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

The 2019 EURL ECVAM status report informs about research and development activities, test method submissions, validation and peer reviews, activities which promote the regulatory use of alternatives and their dissemination for use in basic and applied research as well as for regulatory testing, and international collaborations.





# 2

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## **Research and Development Activities on Alternative Methods and Approaches**

# 2.

## Research and Development Activities on Alternative Methods and Approaches

### 2.1 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 in various ways and to support the eventual validation, translation and dissemination of research results to advance safety assessment practice.



A prominent activity during the first three years of the EU-ToxRisk project has been the development of a novel approach to 'read-across' toxicological properties between similar chemicals to fill data gaps and avoid unnecessary animal testing. Mechanistic data from *in vitro* and computational methods are combined with information on chemical structure to characterise the degree of similarity between chemicals, thereby reducing scientific uncertainty and increasing confidence in read-across proposals.

A set of read-across case studies developed by the project consortium were presented to regulators from national authorities and EU agencies at a workshop on "New Approach Method (NAM)-supported read-across: from case studies to regulatory guidance in safety assessment" held in Espoo, Finland, on 21 to 22 May 2019. Some case studies have also been submitted to the Integrated Approaches to Testing and Assessment (IATA) case study project at the OECD (see [Section 5.5](#)) for formal review by international regulatory experts. The intention is to exploit the experience gained and the feedback received to produce a guidance document on how best to use NAMs (ECHA, 2016) to support read-across to fill data gaps and satisfy regulatory information requirements.

A second round of case studies was initiated during the summer of 2019 and these are now entering the execution phase. A number of these case studies will deal with an '*ab initio*' scenario where the challenge is to carry out a safety assessment using only *in vitro* and *in silico* methods and without being able to resort to read-across. In April 2019, EURL ECVAM joined with EU-ToxRisk and Cosmetics Europe to organise a workshop at the JRC to help elaborate the conceptual basis to *ab initio* safety assessment approaches. The resulting report was shared within the project consortium and participating organisations. Going forward, EURL ECVAM will continue to procure and distribute



case-study chemicals to all participating laboratories and will actively contribute expertise and experimental data to selected case studies that are taking an *ab initio* approach.

Furthermore, EURL ECVAM has been supporting the consortium on reporting the protocols of the test methods used. After an initial guidance, 15 methods have already been submitted and included in the EURL ECVAM Database on Alternative Methods to Animal Experimentation (DB-ALM; see [Section 6.1.1](#)). As a follow up, EU-ToxRisk has extended that exercise and proposed a protocol template – ToxTemp, recently published in ALTEX (Krebs *et al.*, 2019).

#### READ MORE

- ▶▶ EU-ToxRisk: [www.eu-toxrisk.eu](http://www.eu-toxrisk.eu)
- ▶▶ Horizon 2020: [europa.eu/!Fm38rr](http://europa.eu/!Fm38rr)

## 2.2 European Research Projects on Chemical Mixtures

Several EU funded Horizon 2020 research projects that are relevant in the context of chemical mixtures, have just been finalised or are still ongoing (Bopp *et al.*, 2018). The two projects EDC-MixRisk and EuroMix ended in May 2019 and a common final event called "The chemical cocktail challenge" was organised in Brussels, Belgium, on 26 March 2019.

EDC-MixRisk studied the effects of prenatal exposure to mixtures of suspected endocrine disruptive chemicals (EDCs) on the development and health in children. The work emphasises potential effects of EDC mixtures during foetal development and provides new tools and approaches for mixture risk assessment. An overview of results is presented in the EDC-MixRisk Policy Brief (Bergman *et al.*, 2019).



**EDC-MixRisk**  
safe chemicals for future generations

EuroMix aimed at establishing novel testing and assessment strategies for chemical mixtures, to develop appropriate mixture risk assessment methodology and to implement this in a well-accessible interoperational data and model platform. Several

stakeholder groups were trained and tested the EuroMix platform in case studies, and for performing dietary exposure assessments including large numbers of chemicals. More results of these projects can be found via their webpage.



HBM4EU, the Initiative for coordinating and advancing human biomonitoring in Europe to provide evidence for chemical policy making, has started the second half of their project. Several videos, factsheets, reports and protocols have already been developed showing the progress made so far and new policy briefs are under development. Regular updates can also be received via the HBM4EU newsletter. One of the work packages in HBM4EU is dedicated to chemical mixtures. Case studies evaluating existing data in view of combined exposures are conducted, and new joint surveys with a view on exposure to multiple pesticides in hotspot and control areas, will be performed.



science and policy  
for a healthy future

EURL ECVAM looked further into current challenges and future perspectives for risk assessment and risk management of chemical mixtures (Bopp *et al.*, 2019). Not only scientific challenges in the hazard, exposure, and risk assessment of mixtures remain to be addressed, but another major challenge is to develop appropriate risk management measures. If a particular mixture raises a concern, restrictions may need to be placed on chemicals that individually do not pose a risk.

#### READ MORE

- ▶▶ EDC-MixRisk: [edcmixrisk.ki.se](http://edcmixrisk.ki.se)
- ▶▶ EuroMix: [www.euromixproject.eu](http://www.euromixproject.eu)
- ▶▶ HBM4EU: [www.hbm4eu.eu](http://www.hbm4eu.eu)
- ▶▶ "The chemical cocktail challenge" workshop: [www.euromixproject.eu/the-chemical-cocktail-challenge](http://www.euromixproject.eu/the-chemical-cocktail-challenge)

## 2.3 European Research Projects on Endocrine Disruptors

### 2.3.1 EURION

In January 2019, the eight projects which were funded under the European Commission (EC) Directorate-General Research and Innovation (RTD) call "New testing and screening methods to identify endocrine disrupting chemicals" started (see section 2.3 in Zuang *et al.*, 2018).

These projects, which will last five years, are focusing on less studied areas of endocrine disruption; namely thyroid disruption (three projects), metabolic disorders

(three projects), developmental neurotoxicity (one project), and female reproduction (one project).



The projects are grouped in a cluster called EURION (European Cluster to Improve Identification of Endocrine

**Table 2.1:** List of projects included in the European Cluster to Improve Identification of Endocrine Disruptors (EURION).

Acronym, title	Focus	Website
<b>ATHENA</b> Assays for the identification of thyroid hormone axis-disrupting chemicals: elaborating novel assessment strategies	Thyroid hormone disruption	<a href="http://bit.ly/2KrTkuo">http://bit.ly/2KrTkuo</a>
<b>ERGO</b> Breaking down the wall between human health and environmental testing of endocrine disruptors: endocrine guideline optimisation	Thyroid hormone disruption	<a href="https://ergo-project.eu">https://ergo-project.eu</a>
<b>SCREENED</b> A multistage model of thyroid gland function for screening endocrine-disrupting chemicals in a biologically sex-specific manner	<i>In vitro</i> assays, thyroid disruption	<a href="https://www.screened-project.eu">https://www.screened-project.eu</a>
<b>EDCMET</b> Metabolic effects of endocrine disrupting chemicals: novel testing methods and adverse outcome pathways	Test systems for metabolic disorders	<a href="https://www.uef.fi/en/web/edcmet">https://www.uef.fi/en/web/edcmet</a>
<b>GOLIATH</b> Beating Goliath: Generation of novel, integrated and internationally harmonised approaches for testing metabolism disrupting compounds	Testing metabolic disruption	<a href="https://goliath.wp.hum.uu.nl">https://goliath.wp.hum.uu.nl</a>
<b>OBERON</b> An integrative strategy of testing systems for identification of EDs related to metabolic disorders	Test systems for metabolic disorders	<a href="https://oberon-4eu.com">https://oberon-4eu.com</a>
<b>ENDpoiNTs</b> Novel testing strategies for endocrine disruptors in the context of developmental neurotoxicity	Testing strategy for developmental neurotoxicity	<a href="https://endpoints.eu">https://endpoints.eu</a>
<b>FREIA</b> Female reproductive toxicity of EDCs: a human evidence-based screening and identification approach	Female reproductive toxicity	<a href="http://www.freiaproject.eu/">www.freiaproject.eu/</a>

Disruptors) in order to optimise synergies between them. The details of these projects can be found on the dedicated websites (as listed in [Table 2.1](#)), or at a link to EURION (<http://eurion-cluster.eu>).

The outcome of the projects should contribute to international activities on endocrine disruptors at OECD level and, in order to facilitate regulatory uptake, the inclusion of a validation step in the proposals was considered essential. The JRC/EURL ECVAM will provide support to the projects on the translation of research results into regulatory application, providing specific support on aspects such as validation and Adverse Outcome Pathway (AOP) development.

**READ MORE**

▶ EURION: [eurion-cluster.eu](http://eurion-cluster.eu)

### 2.3.2 Thyroid Disruptor Testing in the Mammalian System

At the end of 2017, EC Directorate-General for Environment launched a call for tender for the development of a study protocol for thyroid disruptor testing in the mammalian system to improve the identification of substances disturbing the thyroid system, by either enhancing already existing OECD test guidelines and/or developing a new test guideline.

This 18-month project was finalised in June 2019. It focused on the neurodevelopmental effects of thyroid disrupting chemicals in mammalian organisms, looking at the potential addition of endpoints to the Extended One-Generation Reproductive Toxicity Study (OECD TG 443; OECD, 2018e). The endpoints considered during the feasibility study of this project were: heterotopias (cellular malformations with clusters of neurons in atypical places, resulting from defective neuronal migration during key stages of brain development), hormone measurements and cortical gene expression.

The results of this study showed a heterogeneous pattern of effects amongst the parameters measured across the substances tested. Some of the substances tested induced a pattern of effects only partly in agreement with their known mode of action. The results of this study will be published on the website of Directorate-General for Environment, and will be used to inform the H2020 EURION project ATHENA (see [Table 2.1](#)).

### 2.3.3 Thyroid Disruptor Testing in Non-mammalian Vertebrates

In June 2018, Directorate-General for Environment also launched a call for tender for the development of a study protocol for regulatory testing to identify endocrine disrupting substances in non-mammalian vertebrates or invertebrates, *i.e.*, regulatory relevant endpoints for environmental hazard assessment, by either enhancing already existing test guidelines and/or developing a new test guideline.

The selected project will last 24 months, and has two objectives:

- (a) Merge two already existing test guidelines in fish, TG 229 (fish short-term reproduction assay, on adults) and TG 234 (fish sexual development test, from eggs to juveniles), in order to obtain a test guideline that would cover all relevant life stages of the fish for endocrine disruptors identification. These test guidelines include endpoints aiming at identifying estrogenic and androgenic effects in fish, but no thyroid-related endpoints.
- (b) Evaluate the feasibility to add thyroid-related endpoints such as eye development, swim bladder inflation, and thyroid histopathology in this merged protocol.

EURL ECVAM is part of the Steering Committee of this project.

**READ MORE**

▶ Call for tender 105-238732: [etendering.ted.europa.eu/cft/cft-display.html?cftId=3657](https://etendering.ted.europa.eu/cft/cft-display.html?cftId=3657)

## 2.4 Relationship between Health and Exposure to Environmental Chemicals

Exposure to chemicals constitutes one of several risk factors in developing disease or impacting the ecosystem. In order to make chemical risk assessment more relevant and efficient, it is important to look into the contribution of chemicals to the processes of developing diseases in comparison to other risk factors. This includes looking into prevalent diseases, the established links between exposures and health effects, and the consideration of combined exposures to multiple chemicals.

The activity started exploring the area of carcinogenicity. Following the recommendations of the ECVAM-ESTIV workshop on carcinogenicity (Corvi *et al.*, 2017), EURL ECVAM recently performed an analysis of the relevance and possible gaps of the current available carcinogenicity testing paradigm in the context of

chemical environmental changes and disease burden challenges (see [Box 2.1](#); Madia *et al.*, 2019).

In fact, rising rates of cancer incidence and prevalence identified by the World Health Organisation (WHO) are of serious concern. Furthermore, due to the increasing global trend of chemical production including novel compounds, chemical exposure patterns are foreseen to change, posing increasing demands on chemical safety assessment, and creating potential protection gaps.

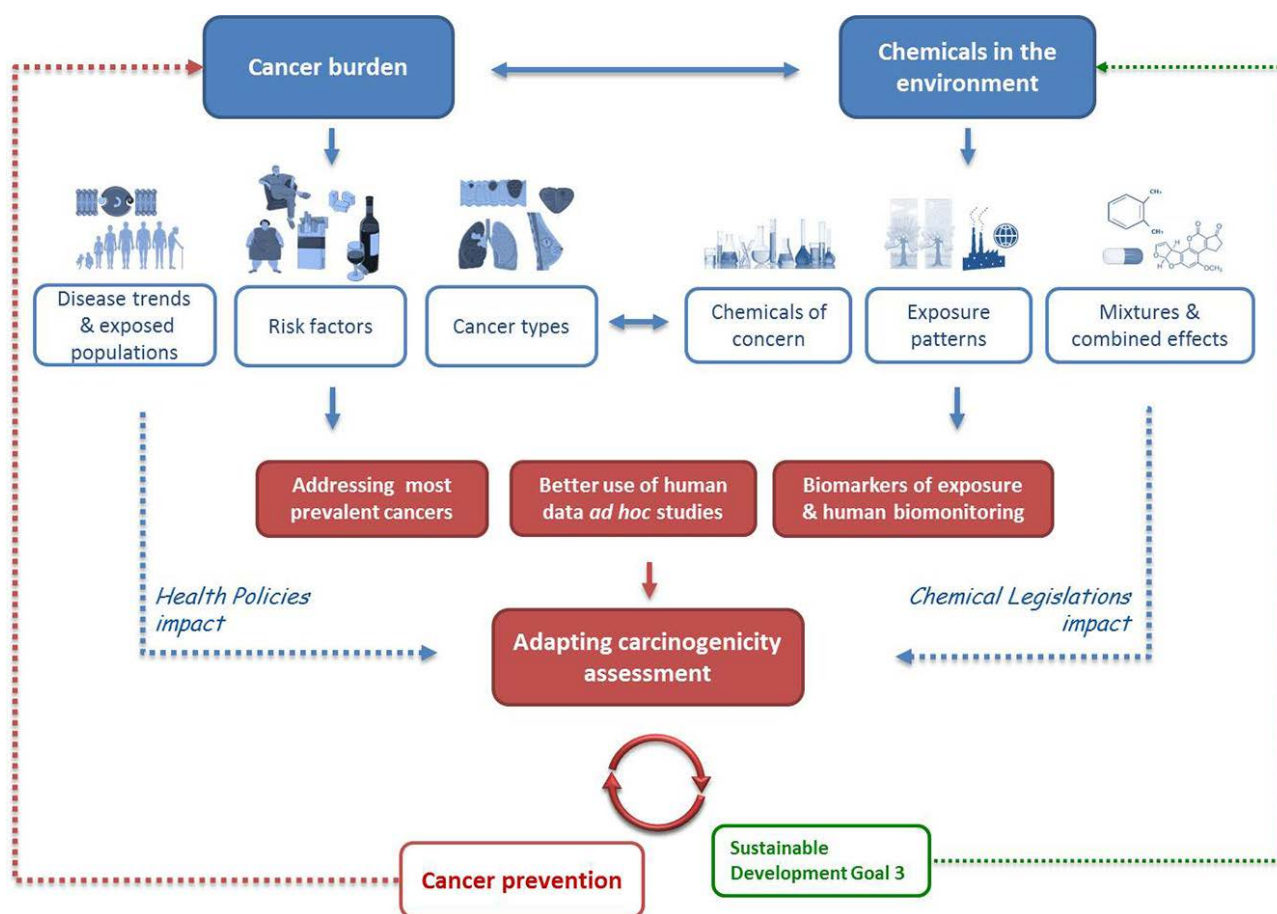
EURL ECVAM is of the opinion that the safety assessment of carcinogenicity needs to evolve to keep pace with changes in the chemical environment and cancer epidemiology ([Figure 2.1](#)). A number of tools are available or under development to more accurately assess the carcinogenicity endpoint. However, it is recommended that future strategies for assessing carcinogenicity take also into account the prevalence of certain cancers, the contribution of different risk factors to the disease, the study of relationships

between chemical exposure and risk factors, the disease aetiology and links with other disorders. In addition, changes in chemical exposure patterns and exposed populations are also critical considerations.

The outcome of this study provided put the basis for a number of internal exploratory activities related to the better understanding of chemical contribution to diseases, including cancer and/or other non-communicable diseases. Opportunities lie in the analysis of available health indicators and the definition of novel indicators, but also in comparative analysis between approaches for human and environment risk assessment and sensitivity analysis of risk factors.

**READ MORE**

▶▶ ECHA’s guest corner - New study shows how to better assess chemicals and help prevent cancer: [chemicallife.echa.europa.eu/fr/guest-corner/-/asset\\_publisher/vcrOSpi91ebF/blog/new-study-shows-how-to-better-assess-chemicals-and-help-preventing-cancer](https://chemicallife.echa.europa.eu/fr/guest-corner/-/asset_publisher/vcrOSpi91ebF/blog/new-study-shows-how-to-better-assess-chemicals-and-help-preventing-cancer)



**Figure 2.1:** Recommended options to evolve the safety assessment of carcinogenicity (from Madia *et al.*, 2019, *Environment International*, 128, under CC BY 4.0).

## Box 2.1

**Better assessment of chemicals to reduce the public health burden of cancer**

A new study by EURL ECVAM scientists (Madia *et al.*, 2019) advocates a holistic approach to the safety assessment of chemicals that keeps pace with a changing environment and reduces their contribution to cancer risk.

We are surrounded by chemicals – in our food, cosmetics, household products, medicines, and the air we breathe. Some of them are potentially carcinogenic, so managing our exposure to these chemicals can significantly reduce the incidence of cancer in Europe and worldwide. The study focuses on how we can better assess carcinogenic properties of chemicals by taking better account of actual cancer burden and making better use of human relevant data and assessment tools.

*The cancer burden in society*

Incidence and prevalence rates for many cancers are increasing. The figures speak for themselves: in 2018, more than 4 million new cancer cases were reported in Europe. Almost 2 million European citizens died because of cancer the same year. Is cancer an inevitable burden in our society? "We have of course unavoidable risk factors, such as family history. For 5 to 10 per cent of cancer cases, significant correlations with specific inherited genes have been identified. Simply getting older also increases cancer risk", explains EURL ECVAM scientist Federica Madia. Importantly however, preventable risk factors collectively contribute to the development of most cancers. These include infections, exposure to sunlight, poor diet, being overweight, excessive consumption of tobacco and alcohol, and exposure to carcinogens at work or through everyday living. "If we cannot completely avoid cancer, we can at least reduce its occurrence worldwide. Today, we can count on more sophisticated therapies than 20 years ago. However, we should also effectively manage relevant risk factors, including our exposure to chemicals", says Federica.

*Chemicals and cancer*

The European Union produces approximately 300 million tonnes of chemicals per year, of which 12-15% are classified as carcinogenic, mutagenic or toxic to reproduction (CMRs). It is difficult to establish clear links between chemical exposure and the burden of cancer, and estimates are variable and uncertain. Studies put the contribution of chemicals to various types of cancer somewhere between 1 and 19%. Moreover, approximately 5% of childhood cancers have also been estimated to result from environmental exposure to pollutants.

*A necessary legal framework*

The European Union already has extensive regulatory measures in place to tackle the problem. In 2007 for example, the EU started to implement a comprehensive cross-cutting legislation on chemicals called REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) which contributes significantly to the identification and management of health risks linked to chemicals. "We saw that all legislative measures put in place have successfully reduced cancer at work over the last 20 years. But today, we still have uncertainties regarding the potential risk posed by chemical exposure to the population through food, air, soil and water. It is difficult to assess the effects of mixtures, especially when there is a long latency of effects following exposure." EURL ECVAM scientists therefore call for new strategies and approaches for carcinogenicity assessment. They also call into





question the standard testing procedures still relying on animal studies. "At the moment, chemicals are usually tested on mice and rats. But the results they give are not always relevant to humans and often lack sufficient reproducibility." Carcinogenicity testing using animals has other issues too such as obvious ethical concerns, high costs and long study durations, all of which support a transition to more modern human-relevant approaches that exploit our understanding of the disease and our environment and which employ the latest non-animal methods available in a modern safety assessment toolbox.

#### *Recommendations for adapting carcinogenicity assessment*

According to the EURL ECVAM researchers, public health policy actions cannot be decoupled from environmental policy actions. The study recommends a holistic approach to better assess the carcinogenic properties of chemicals and manage their risk:

1. Prioritising the carcinogenicity assessment of chemicals for their potential to contribute specifically to the development of the four most prevalent cancers (breast, colorectal, lung and prostate cancers).
2. Make better use of information on cancer aetiology and evolution in humans. For example, increased cancer risk has been associated with several chronic disorders such as cardiovascular disease, diabetes, chronic kidney disease and pulmonary disease. Moreover, interactions between chemical exposure and genetic and lifestyle factors may also play a role in the manifestation of various cancer types.
3. Use of biomarkers of exposure and human biomonitoring. Biomarkers give evidence of association between exposure to specific chemicals and a carcinogenic effect, and can therefore be used to enhance epidemiological studies.

Source: EU Science Hub (<https://europa.eu/ImC37vn>)

## 2.5 Integration of Data across Toxicity Endpoints to Explore New Ways for Carcinogenicity Testing

EURL ECVAM has started to investigate ways for exploiting existing data to perform a comparative analysis of several toxicological effects measured in different species and using different models. This is an attempt to avoid redundant *in vivo* studies and minimise reliance on apical endpoint tests.

The overall aim is to explore possibilities to develop a strategy for evaluating hazard by combining information across different systemic toxicity endpoints, rather than considering them individually, and integrating them also with *in vitro/in silico* data. This integrated "comparative toxicology" approach can in principle be applied to any toxicity endpoint and is expected to result in a set of options for waiving redundant toxicity studies (mainly long-term ones, including carcinogenicity) with the final goal to design sustainable testing strategies.

A concept paper (Madia *et al.*, submitted) on the rationale behind this integrated approach and its potential application to different systemic toxicity endpoints has been submitted for publication. This integrated approach has been applied to

carcinogenicity assessment by designing and developing a regulatory tool to guide the extrapolation and integration of information necessary for the evaluation of carcinogenic hazard.

In this regard, the tool allows linking the information stored within current test guidelines and non-standard method protocols to the key characteristics of carcinogens (Smith *et al.*, 2016). It is expected to help the full exploitation of stored information and avoidance of redundancy of studies. The results from this study and description of the tool are the subject of a second manuscript which is in preparation (Madia *et al.*, in preparation-b).

## 2.6 Mechanistic Analysis of Repeated Dose Toxicity Studies

In line with the activities aimed to explore possibilities to develop a strategy for evaluating hazard by combining information across different systemic toxicity endpoints (described in Section 2.5), EURL ECVAM is launching a study focused on the relevance of repeated-dose toxicity studies. Specifically, EURL ECVAM is proposing to undertake an analysis (through a Study

Contract<sup>1</sup>) which aims to gather, organise and analyse mechanistic knowledge related to the toxicological effects on target organs observed in animal models after repeated exposure to a test chemical.

The outcome of this analysis will be the description of a set of characteristics of chemicals inducing repeated dose systemic toxicity, as well as to identify novel *in vivo* approaches with modifications and enhancements of standard *in vivo* studies to improve the identification of toxicity. It is expected that the results from the study will give input into the design of alternative approaches for systemic toxicity that are underpinned by a better understanding of the mechanistic basis of current *in vivo* (animal) tests and their human relevance.

## 2.7 Artificial Intelligence and Chemical Risk Assessment

A EURL ECVAM organised workshop, titled "AI4CRA – Artificial Intelligence for Chemical Risk Assessment", was held in Ispra, Italy, 4 to 5 April 2018. The participants were scientists from both the Artificial Intelligence (AI) and the Chemical Risk Assessment (CRA) fields from Aalto University (Finland), Lancaster University, Leeds University (UK), the European Food Safety Authority (EFSA) and EURL ECVAM. The participants explored how, and to what extent, applying AI methods could support both the quantitative (the large number of insufficiently assessed chemicals and, the unmanageable extent of existing information sources) and the qualitative (speed, transparency, reproducibility, reliability, objectivity, credibility) aspects of CRA.

As a follow-up of the AI4CRA workshop, in 2019, EURL ECVAM, together with Aalto University (Finland), and Leeds and Lancaster Universities (UK), published a paper in "Computational Toxicology" (Wittwehr *et al.*, 2020), which points out the possible CRA topics that can be supported or enhanced by AI Identifying and prioritising problems, enhancing the evidence base (*e.g.*, systematic review), knowledge discovery, optimising expert identification, enhancing expert collaboration, process simulation, and building cognitive models (see [Box 2.2](#)).

While these topics are interconnected, they are organised and discussed under two main themes:

scientific-technical evaluation process and social aspects of the decision making process.

The level of AI-readiness of the individual topics (illustrated by their penetration of the AI area in [Figure 2.2](#)) will also indicate the short-term wins that can potentially be made. The AI4CRA endeavour will be followed up in 2020 and beyond.

## 2.8 Orchid Project

The ORCHID project (Organ-on-chip in Development) is an EU-funded project coordinated by Leiden University Medical Center and the Dutch Organ-on-Chip consortium hDMT in the Netherlands. The main goal of ORCHID is to create a roadmap for organ-on-chip (OoC) technology and to build a network of all relevant stakeholders working in this fast moving field. ORCHID started in October 2017 and involves a total of seven leading European research institutions.



EURL ECVAM has been collaborating with ORCHID on exploring the impact of organ-on-chip technology in different arenas, especially in the chemicals toxicity field. EURL ECVAM has been part of several ORCHID meetings, namely the Strategy and the Vision workshops from which relevant publications have been prepared (Mastrangeli *et al.*, 2019a,b).

This consortium has been the shell of the European Organ-on-Chip Society (EUROoCS) created in 2018 and is an "independent, not-for-profit organisation established to encourage and develop Organ-on-Chip research, and to provide opportunities to share and advance knowledge and expertise in the field towards better health for all." The project has recently been selected as one of the better projects of the Future and Emerging Technologies (FET)-Open call and its final report has been published (see "read more").

<sup>1</sup> Deadline for Tender offer is expected by second quarter 2020.

## Box 2.2

## Artificial intelligence could lead to better decisions on chemicals

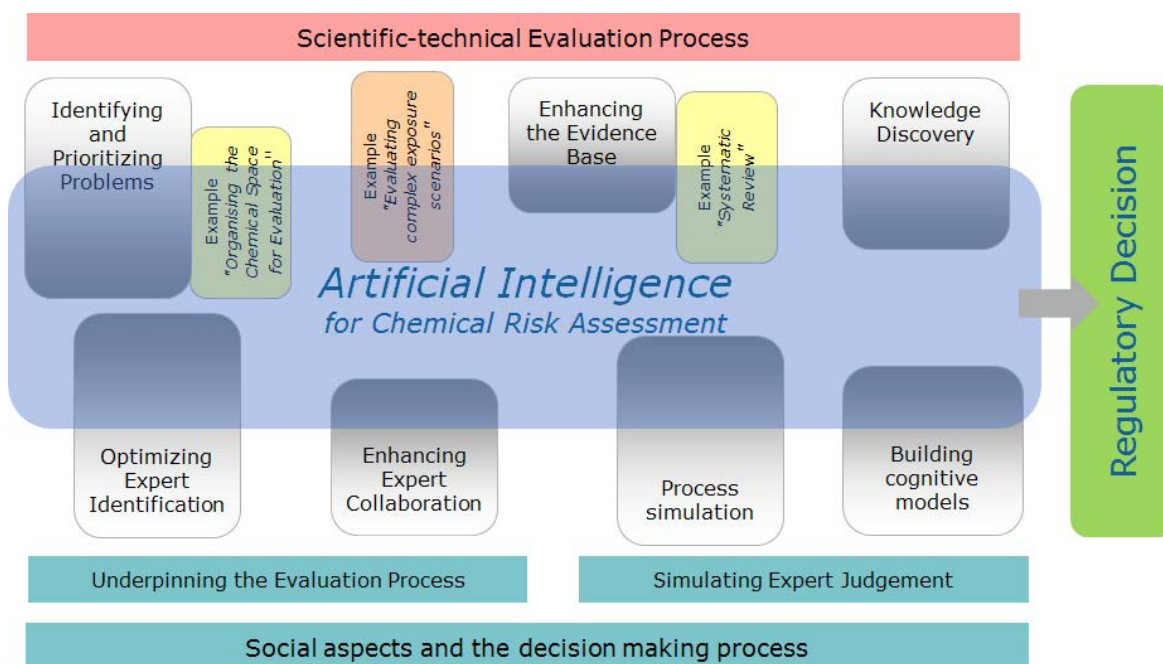
The huge potential of artificial intelligence to improve regulatory decision making on chemicals is discussed in a recent journal article, co-authored by EURL ECVAM researchers (Wittwehr *et al.*, 2020).

Artificial Intelligence (AI) promises not only to improve the scientific and technical aspects of the chemical evaluation process, but also the social dimension of regulatory decision making. As the basis for managing the risks of chemical exposure, the Chemical Risk Assessment (CRA) process can impact a substantial part of the economy, the health of hundreds of millions of people, and the condition of the environment. However, the number of properly assessed chemicals falls short of societal needs due to a lack of experts for evaluation, interference of third party interests, and the sheer volume of potentially relevant information on the chemicals from disparate sources.

To explore ways in which computational methods may help overcome this discrepancy between the number of chemical risk assessments required on the one hand and the current pace of the CRA process on the other, EURL ECVAM organised a workshop on Artificial Intelligence for Chemical Risk Assessment. The workshop identified a number of areas where AI could potentially increase the number and quality of regulatory risk management decisions based on CRA. Although interconnected, these areas were organised under two main themes: the scientific-technical process and social aspects of the decision making process.

Scientific and technical aspects include, for example, the use of “big data” for discovering biological knowledge and informing chemical safety assessments. The state-of-the-art in using big data in toxicology is reviewed extensively in a book (Neagu & Richarz, Eds., 2019) with significant contributions from EURL ECVAM scientists. Social aspects of decision making include, for example, identifying chemicals of concern, finding experts and facilitating collaboration. It is expected that further exploration of the topics covered in the workshop could eventually increase the efficiency and effectiveness of the CRA process. However, the only way to fully realise the applications of AI for CRA is to promote global and cross-disciplinary collaboration.

Source: EU Science Hub (<https://europa.eu/lwY69pT>)



**Figure 2.2:** Artificial Intelligence and Chemical Risk Assessment (from Wittwehr *et al.*, 2020, *Computational Toxicology*, 13, p. 3, under CC BY 4.0).



Although the ORCHID project has ended in October 2019, EURL ECVAM intends to continue to collaborate with specialists in the area of organ-on-chip, namely the students from the Marie Skłodowska-Curie funded Innovative Training Network (ITN) EUROoC or the European Organ-on-Chip Society (EUROoCS). Both initiatives resulted from ORCHID activities.

#### READ MORE

- ▶▶ ORCHID project summary: [h2020-orchid.eu/summary](https://h2020-orchid.eu/summary)
- ▶▶ EUROoCS: [euroocs.eu](http://euroocs.eu)
- ▶▶ EUROoC: [www.eurooc.eu](http://www.eurooc.eu)

## 2.9 Review of Non-animal Methods Used for Biomedical Research

According to the latest EU report on the statistics on the use of animals for scientific purposes that covers the statistics of the EU Member States from the years 2015 to 2017 (EC, 2020; see also [Section 6.2.3](#)), 68% of animal uses are in research, whereas regulatory use accounts for 23%.

The EURL ECVAM studies to review non-animal models and methods already in use for basic and applied research (see section 2.8 of Zuang *et al.*, 2018), were completed (see [Box 2.3](#)) and will become available in 2020 as a dataset of categorised models and two technical reports. The two areas covered by this project were respiratory tract diseases and neurodegenerative disorders. The criteria used for the areas selection were incidence/prevalence of human diseases, the high number of animals used and potential causal link between chemical exposure and the development of the disease.

The final goal is to disseminate and improve knowledge sharing on potentials and limitations of human based models at different levels: scientific communities, universities and secondary schools, National Committees for animal welfare and the public at large.

For the respiratory diseases area, the highest number of identified models were on lung cancer, based on 2D or 3D cultures, and applied to investigate the disease's mechanisms using protein and gene expression as biological endpoint.

For the neurodegenerative disorders, the highest number of models focus on Alzheimer (AD) and Parkinson's (PD) diseases, and are based on human/patient *ex-vivo* tissue or body fluids (brain biopsy,

cerebrospinal fluid (CSF), post-mortem). In the AD area, almost two thirds of all models/methods focused on generating greater insight into the disease mechanism. About a third was primarily aimed at improved diagnostics. A minority of models addressed disease therapy development or experimental model development. A similar trend is seen for PD, where two-thirds of all models/methods focused on generating greater insight into the disease mechanism while a third were primarily aimed at improved diagnostics.

A second call for tender was published in the areas of cardiovascular diseases, breast cancer, immunogenicity testing for advanced therapy medicinal products, autoimmune diseases and immune oncology models. The final results will be published in 2020.

## 2.10 The VAC2VAC Project

The VAC2VAC project - "Vaccine batch to vaccine batch comparison by consistency testing" brings together 22 public and private partners including the JRC represented by EURL ECVAM. This five years project (2016 – 2021) is funded under the Innovative Medicines Initiative 2 (IMI 2), a joint undertaking of the EU Horizon 2020 Research and Innovation Programme and the European Federation of Pharmaceutical Industries and Associations (EFPIA).

# 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 based methods are developed, standardised and validated for the safety and potency testing 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 at the 3<sup>rd</sup> Annual Meeting in March 2019 ([Figure 2.3](#)) and are summarised in the VAC2VAC newsletter (VAC2VAC, July 2019). After the publication of the recommendations for multi-centre validation studies (Halder *et al.*, 2018), the JRC-led workpackage on validation developed guidance for the VAC2VAC method developers on how to perform

### Box 2.3

#### Advanced non-animal models in biomedical research: neurodegenerative and respiratory tract diseases

Neurodegenerative and respiratory diseases are a leading cause of mortality and disability worldwide. Research on human diseases relies extensively on animal models, however, effective new therapies for such serious conditions are still lacking. One reason for this is that animal models often poorly represent human physiology and pathology.

EURL ECVAM has carried out an extensive review of advanced non-animal models being used for basic and applied research into neurodegenerative and respiratory tract diseases. The abstracts of scientific papers published between 2013 and 2018 were scanned for relevant human-based non-animal models of these diseases. From those papers researchers characterised and catalogued 568 and 284 models for neurodegenerative and respiratory tract disease respectively, to make them more accessible for human relevant studies that avoid the use of animals.

In 2020 this collection of models will be made publicly available from the JRC Data Catalogue, while two technical reports will provide an in-depth meta-analysis of the approaches being used and elucidate the main findings. In this meta-analysis the selected models are characterised according to:

- The disease feature investigated (*e.g.*, airway modelling, inflammation, protein aggregation)
- The type of non-animal model (*e.g.*, *in vitro*, *in silico*)
- The biological endpoint used to describe the health effect (*e.g.*, DNA damage, protein dysfunction)
- The application of the model (*e.g.*, drug testing, diagnosis of disease, disease mechanism)
- The throughput potential of the model for automated large scale experiments and studies

##### *A non-animal model for Alzheimer's Disease*

Alzheimer's Disease (AD) is an incurable and debilitating condition that result in progressive degeneration and death of nerve cells. Induced pluripotent stem cells offer a good model to study AD. Skin cells donated from patients affected by Alzheimer are reprogrammed into a pluripotent state capable to develop into any type of cell or tissue of the human body. Using differentiation factors, these pluripotent stem cells can generate one of the cell types of the brain called microglia. Microglia plays a key role in preserving the function of neural networks and responding to injury and disease. This model can be used to understand how microglia interact with other brain cells and influence the development of AD.



##### *A non-animal model for Pulmonary Fibrosis*



Idiopathic Pulmonary Fibrosis (IPF) is a respiratory disease in which scars are formed in the lung tissues, resulting in stiffness and difficulty in breathing. Microfluidic co-cultures also offer new opportunities for faithfully modelling human disease of pulmonary fibrosis. Lab-grown lung cells (epithelial cells, fibroblasts, and macrophages) are placed upon a chip and interconnected by channels to mimic the *in vivo* lung tissue – this is the lung-on-a-chip technology. A protein is then introduced in the system to cause contraction and stiffening of the engineered lung tissue. In this way, it is possible to mimic the scars of the lung tissue observed in people who suffer from the IPF. This system recapitulates the critical changes characteristic of pulmonary fibrosis.



**Figure 2.3:** VAC2VAC 3<sup>rd</sup> Annual Meeting (March 2019, RIVM, Bilthoven, NL).

assay validation in the context of the International Cooperation on Harmonisation of Technical Requirements for Registration of (Veterinary) Medicinal Products (ICH/VICH) guidelines, and is now discussing the validation of quality control strategies.

The planning of collaborative studies to assess the transferability and reproducibility of two methods for the quality control of tick-borne encephalitis vaccines is ongoing with public and private partners.

#### READ MORE

- ▶▶ VAC2VAC: [www.vac2vaceu](http://www.vac2vaceu)
- ▶▶ Innovative Medicine Initiative (IMI): [www.imi.europa.eu](http://www.imi.europa.eu)

### 2.11 EURL ECVAM Laboratory Studies

#### • High Throughput Transcriptomics

The EURL ECVAM laboratory started to explore the technical reliability and the biological relevance of *in vitro*-based transcriptomics test methods for their regulatory application.

Over the last decade, 'omics technologies have had a huge impact on the discovery of chemical mode of action, biomarkers, disease aetiology, nevertheless, the use of genome-wide analysis in the decision making process has been slow.

At EURL ECVAM, two High Throughput Transcriptomics studies were conducted so far. The first study, a pilot *in vitro* study, used the hepatic cell line HepaRG treated with 20 chemicals, at seven concentrations (see section 2.6 of Zuang *et al.*, 2018). The study was conducted in quantitative High Throughput Screening (qHTS) format and demonstrated the feasibility of the use of state of the art targeted sequencing technology in automated toxicology testing. Moreover, the dose-response modelling integrated with High Content Imaging data gave interesting insights into the mechanisms of action of toxic chemicals and demonstrated the biological relevance of this kind of approach in toxicological studies, crucial for translational applications.

A follow-up study has now been completed to investigate the dynamics of gene expression as a function

of both time and concentration of chemical exposure. With more than 40 million data points produced, the study was an excellent opportunity to optimise the data analysis approach efficiently and transparently. In fact the lack of a harmonised and reproducible data handling workflow is one of the bottlenecks in the application of transcriptomics in the decision making process.

Moreover, EURL ECVAM currently explores if these data could eventually be used for various purposes such as *e.g.* in a read-across approach or to calculate a Point of Departure or to formulate mode of action hypothesis. A particular interest lies in the dynamical response of transcripts across time and concentration, and it is investigated if these molecular changes could be informative about *e.g.* the severity of a toxic effect and/or its possible cumulative damage. Finally, the eventual possibility to include 'omics in a stepwise risk assessment workflow is also being considered.

#### • Hepatic Metabolic Clearance

Human hepatic metabolic clearance represents in many cases the main driving process of kinetics to determine the concentration-time profile of a chemical in a biological system and is an indispensable information source to support the chemical risk assessment. EURL ECVAM is currently running laboratory studies on hepatic metabolic clearance using hepatocytes which contain the full complement of metabolising enzymes maintained within the intact cell, and which, therefore, provide an adequate *in vitro* model for predicting *in vivo* metabolic clearance.

Essentially, the hepatocytes are exposed to the test chemical by incubation in culture medium. Replicate samples are prepared to enable the interaction to be terminated after appropriate intervals, *e.g.*, from time zero to two hours (*e.g.*, 0, 15, 30, 60, 90, 120 min). Determination of the supernatant chemical concentration with time allows calculation of any depletion rate occurring due to metabolism. Chemicals can then be ranked by their relative intrinsic clearance. In addition, the results contribute to weight of evidence assessments including read-across and the concept of data extrapolation between chemicals of similar structure (analogues) as a basis for estimating biological behaviour.

Three example results from six relevant analogues (Figure 2.4) show some differences in clearance rate.

#### • Developmental Neurotoxicity

EURL ECVAM has performed studies where the effects of mixtures of ten developmental neurotoxicity (DNT) chemicals have been evaluated using *in vitro* assays applied to induced pluripotent stem cell (hiPSC)-derived neuronal and glial cultures. These cultures were exposed to mixtures consisting of heterogeneous classes of chemicals at the concentrations that were found in human samples (*e.g.*, breast milk or children's blood), thus mimicking real life exposure.

The applied *in vitro* assays were anchored to common key events identified in AOPs where impairment of learning and memory in children was defined as the adverse outcome. The chemicals have been clustered on the basis of their mode of action into 'similar' and

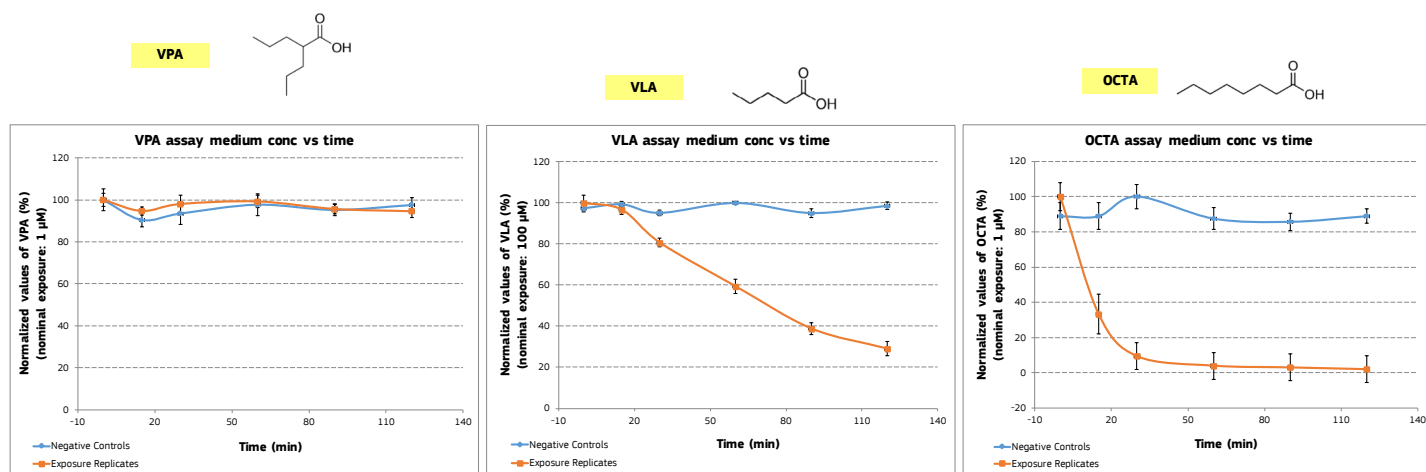


Figure 2.4: Differences in clearance rate values between valproic, valeric and octanoic acids.



'dissimilar'. Single chemicals working through similar mode of actions at non-cytotoxic concentrations, induce DNT effects in mixtures, to a certain extent reproducing autism-like cellular changes observed in the brain of autistic children (Pistollato *et al.*, submitted). The obtained results suggest that the use of human iPSC-derived mixed neuronal/glia cultures applied to a battery of assays anchored to common key events of an AOP network represents a valuable approach to identify mixtures of chemicals with potential to cause learning and memory impairment in children.

The same approach was applied to evaluate DNT effects induced by an exposure to persistent organic pollutants (POPs) mixtures. hiPSC-derived neural stem cells undergoing differentiation were exposed to POPs at concentrations similar or lower than those found in human blood. The obtained data suggest that POP mixtures at these concentrations altered brain derived neurotrophic factor (BDNF) levels, neurite outgrowth, synaptogenesis and neuronal cell number, indicating potential contribution to neurodevelopmental disorders. This study was performed in collaboration with the Norwegian Institute of Public Health (NIPH) in Oslo.

- **Complex Cell Models to Increase the Biological Relevance of *In Vitro* Assays**

EURL ECVAM is conducting pilot studies with complex cell models such as spheroids. In traditional two-dimensional (2D) monolayer cultures, cells are grown on top of a matrix, which results in unphysiological conditions. In contrast, spheroids and other three-dimensional (3D) cell models recapitulate better 3D architecture found in native tissues. As a consequence, the organisation and polarisation of the cells are better, hence their cellular differentiation, cell-cell communication and metabolic levels are considerably more enhanced than in 2D cell models. Because of these properties, complex cell models are widely used in drug discovery research. Moreover, complex cell models can be used in personalised medicine *e.g.*, by culturing tissue samples of patients and testing which drug would be most effective for treatment.

The better biological relevance is also advantageous in toxicity testing of chemicals, hence EURL ECVAM is exploring possibilities of utilising High Throughput Screening (HTS) to test chemical safety on complex cell models. So far, the efforts have been focused on producing spheroids from neuronal cells obtained from human induced pluripotent stem cells (hiPSC)

and hepatocytes (HepaRG cell line). Preliminary studies show that the electrical activity of hiPSC neuronal spheroids is considerably higher than in traditional 2D hiPSC neurons, which would place them as good candidate to test developmental neurotoxicity (DNT). Hepatocytes form efficiently spheroids and they can be readily grown in 384 well plates, enabling them to be used in HTS to test large chemical libraries efficiently.

- **Animal Free Test Systems**

It is often assumed that human cellular and tissue-based systems equate with animal free laboratory work. In reality though, animal-derived products are still being used and are important in the development of cell culturing techniques. There are three main categories of animal-derived components that are widely used in laboratory research and development activities: (1) serum, (2) antibodies, and (3) other biological extracts.

Foetal bovine serum, the liquid fraction of clotted blood from foetal calves, has been an integral component of cell culture for decades, however, its use has been discouraged in recent years due to its undefined nature. EURL ECVAM recommends to deliver *in vitro* methods that are animal-free and can be reliably used to support regulatory decision making. Many *in vitro* methods make still use of one or more animal-based reagents, such as Foetal Bovine Serum or antibodies. The use of serum-free, chemically-defined medium is recommended to avoid potential sources of uncertainty and for biosafety and ethical reasons (Gray *et al.*, 2016b; OECD, 2018b; Oredsson *et al.*, 2019; van der Valk *et al.*, 2018). Chemically-defined/controlled media are characterised by low qualitative and quantitative (batch-to-batch) variability, simplified isolation of synthetic products and metabolites and avoidance of use of animal source material (OECD, 2018b).

Conventional animal-derived antibodies are widely used in biomedical laboratory studies for *e.g.*, monitoring disease progression at the molecular level, localisation or quantification of cellular components, single protein isolation or studying protein-protein interactions. To overcome batch to batch variability and lack of target specificity, *in vitro* recombinant antibodies can be used (Gray *et al.*, 2016a; see [Section 4.6.1](#)).

There are other animal derived components such as Matrigel, trypsin, collagen that should be avoided to

be truly animal-free. The way forward for current and future high technology research is to strive towards fully defined systems with known composition. This will necessitate understanding of the *in vitro* methods and all its components (OECD, 2018b) and will include interdisciplinary efforts to ensure reproducible, credible, and trustworthy scientific data generation in the Three Rs research and development arena (Oredsson *et al.*, 2019).

## 2.12 Contributions to Cefic Long-range Research Initiative Projects

This chapter describes new and ongoing projects within the Cefic Long-range Research Initiative (LRI) related to fish toxicity, aquatic and terrestrial bioaccumulation, as well as to an open access PBPK modelling platform, of specific interest to EURL ECVAM.

### 2.12.1 Integrating the Fish Embryo Test in a Weight of Evidence Strategy for Acute Fish Toxicity Testing

The Cefic LRI launched a new project (ECO51) which aims at developing a systematic weight of evidence approach integrating fish embryo testing to predict potential acute fish toxicity, in particular addressing the regulatory purposes of hazard assessment and Classification & Labelling (see [Section 5.6.3](#)).

#### READ MORE

- ▶▶ LRI-ECO51: Integrating the Fish Embryo Test into the Weight of Evidence to inform Acute Fish Toxicity: [cefic-lri.org/request-for-proposals/lri-eco51-integrating-the-fish-embryo-test-into-the-weight-of-evidence-to-inform-acute-fish-toxicity](https://cefic-lri.org/request-for-proposals/lri-eco51-integrating-the-fish-embryo-test-into-the-weight-of-evidence-to-inform-acute-fish-toxicity)

### 2.12.2 Development of a Tiered Testing Strategy for Fish Bioaccumulation Testing Based on In Vitro Approaches

The aim of this Cefic LRI-funded project (ECO34) is to develop a tiered approach for the assessment of the bioaccumulation potential of chemicals. For this purpose, various *in vitro* approaches using fish cell lines from different tissues to estimate chemical uptake and biotransformation are combined with toxicokinetic and quantitative structure-activity relationship models. The principle of this work is further described in Stadnicka-Michalak *et al.* (2018). Recent results show that biotransformation does not occur only in liver, but also in intestine and gills cell lines, which

should be accounted for to more accurately estimate chemical accumulation and bioconcentration factors (BCF) (Stadnicka-Michalak *et al.*, 2019).

#### READ MORE

- ▶▶ LRI-ECO34: A tiered testing strategy for rapid estimation of bioaccumulation by a combined modelling – *in vitro* testing approach: [cefic-lri.org/projects/eco34-a-tiered-testing-strategy-for-rapid-estimation-of-bioaccumulation-by-a-combined-modelling-in-vitro-testing-approach](https://cefic-lri.org/projects/eco34-a-tiered-testing-strategy-for-rapid-estimation-of-bioaccumulation-by-a-combined-modelling-in-vitro-testing-approach)

### 2.12.3 Integrating Bioaccumulation Assessment Tools for Mammals

This Cefic LRI-funded project (ECO44) started in 2018 and aims at developing a toxicokinetic modelling framework for bioaccumulation assessment in mammals, combining *in vitro* and *in vivo* physiologically-based toxicokinetic (PBTK) data, field collected bioaccumulation data, and quantitative structure-activity relationship (QSARs) models.

In total, approximately 35,000 *in vivo* and *in vitro* toxicokinetics parameters have been collected for over 13,000 organic chemicals and a data quality scoring method has been developed to evaluate the general reliability of the *in vitro* and *in vivo* data. Existing QSAR models for predicting hepatic clearance in rodents and humans, whole body total elimination and biotransformation half-lives in humans and dietary absorption efficiency have been reviewed. A one-compartment and multi-compartment PBTK models for bioaccumulation assessment of air-breathing organisms is currently being developed and tested.

#### READ MORE

- ▶▶ LRI-ECO44 – A toxicokinetic mammalian modelling framework for B assessment: [cefic-lri.org/request-for-proposals/lri-eco44-a-toxicokinetic-mammalian-modelling-framework-for-b-assessment](https://cefic-lri.org/request-for-proposals/lri-eco44-a-toxicokinetic-mammalian-modelling-framework-for-b-assessment)

### 2.12.4 Improvement of In Vitro Approaches to Predict Fish Bioconcentration

The Cefic LRI project SNAPPFISH “Searching for refined *in vitro* Approaches to Predict bioconcentration in FISH” (ECO47) started in 2019. Experimental work focuses on increasing the reliability of the current methods, for example, OECD test guidelines 319A and 319B for determination of hepatic clearance using hepatocytes or fish S9 (OECD, 2018c,d; see also section 5.3.2 of [Zuang \*et al.\*, 2018](#)). The experimentally derived information is

used to refine *in vitro* to *in vivo* extrapolation (IVIVE) and prediction models for fish bioconcentration.

**READ MORE**

- ▶▶ LRI-ECO47 – SNAPFISH: Searching for refined *in vitro* Approaches to Predict bioconcentration in FISH: [cefic-lri.org/projects/eco47-snapfish-searching-for-refined-in-vitro-approaches-to-predict-bioconcentration-in-fish](https://cefic-lri.org/projects/eco47-snapfish-searching-for-refined-in-vitro-approaches-to-predict-bioconcentration-in-fish)

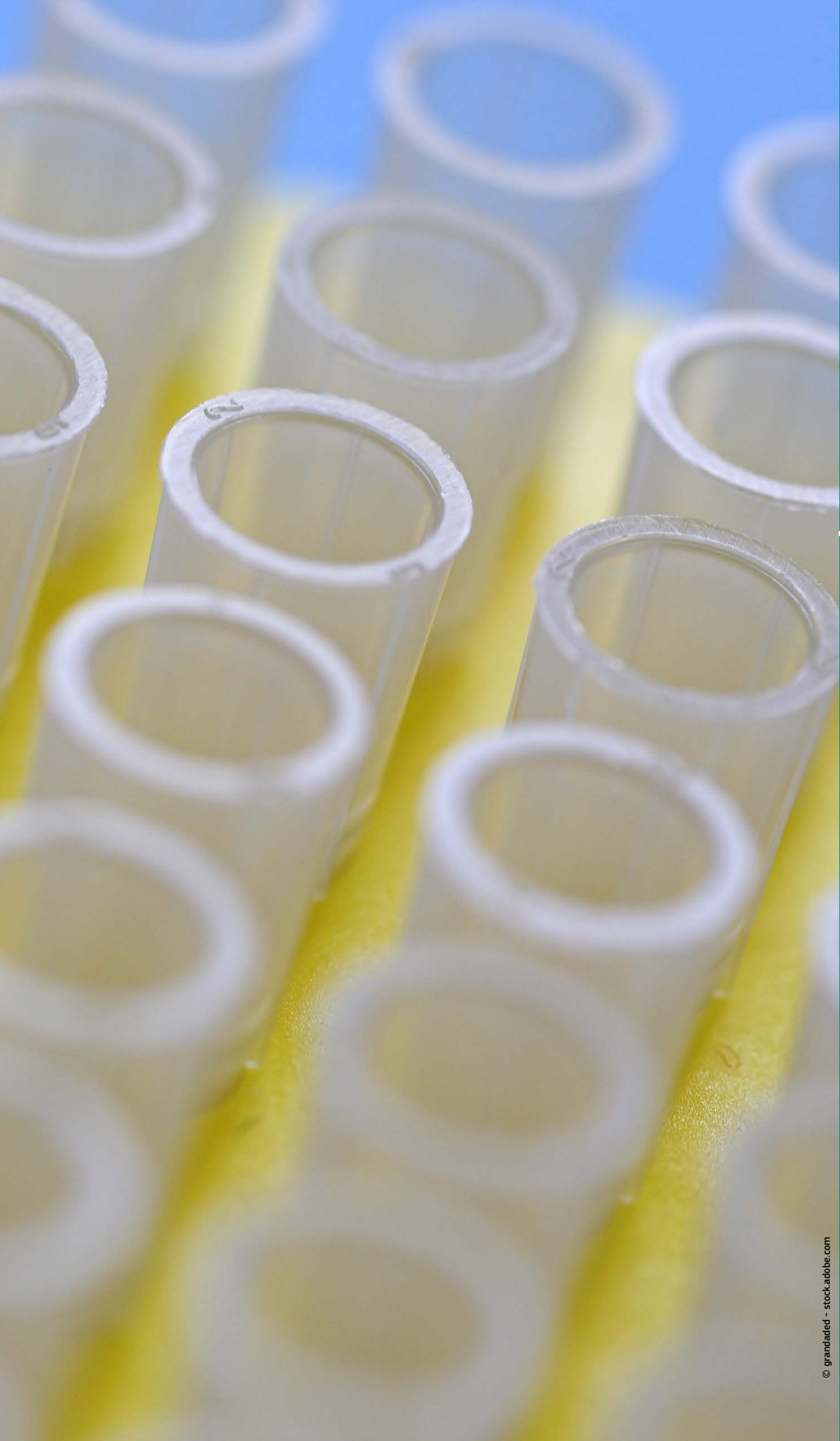
### 2.12.5 Open Access PBPK Modelling Platform

The objective of this Cefic LRI project (AIMT 7) is to further develop R-Vis, a prototype application for the analysis of structure and performance of physiologically based pharmacokinetic (PBPK), and other models, written in the free, open source syntax R or C++. The overall aim is to extend, improve and to provide more

features and make them more robust. The project was finalised at the end of 2019. The tool now features the possibility of performing a visual global sensitivity analysis for any species (human, rodents, farm animals) of PBK models, as well as parameter estimation using Markov Chain Monte Carlo simulation and Bayesian inference. The parameter estimation feature is used to perform “reverse dosimetry” to reconstruct human dose or exposure concentrations consistent with human biomonitoring data. The R-Vis will be available for download from the LRI tool box.

**READ MORE**

- ▶▶ AIMT7: RVis – Open Access PBPK Modelling Platform: [cefic-lri.org/projects/aimt7-rvis-open-access-pbpk-modelling-platform](https://cefic-lri.org/projects/aimt7-rvis-open-access-pbpk-modelling-platform)
- ▶▶ LRI tool box: [cefic-lri.org/lri-toolbox](https://cefic-lri.org/lri-toolbox)





# 3

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## Test Method Submissions

# 3.

## Test Method Submissions

A new pre-submission (ALLsens; see [Section 3.1](#)) and a revised full submission (Genomic Allergen Rapid Detection (GARD) potency, see [Section 3.4](#)) were received by EURL ECVAM since the last EURL ECVAM status report published in February 2019 (see section 3 in Zuang *et al.*, 2018). The assessment of this new pre-submission and of the GARD (skin and potency) full submission progressed in 2019.

The assessment of the full submission of the Bio-olution test method was concluded and the method entered peer review by the EURL ECVAM Scientific Advisory Committee (ESAC, see [Section 4.6.3](#)).

An assessment of the revised full submission of the Toxicogenomic analysis on 3D reconstituted epidermis for measuring skin sensitisation potential and potency (SENS-IS) assay received in 2018 was conducted and some shortcomings were still identified. The test submitter was invited to address those before the method can be considered for peer review by the ESAC (see [Section 3.3](#)).

The assessment of the GARD full submission was stopped in 2018 and the test submitter was informed of EURL ECVAM's decision not to pursue the assessment or an eventual peer review by the ESAC until several non-scientific issues were discussed and resolved at OECD level. During 2019, some of these issues, such as those related to Intellectual Property Rights (IPR), were resolved (see [Section 5.6.15](#)). Others, such as those related to GLP implementation and the use of

confidential business information to keep some elements secret (*i.e.*, the cloud-based GARD Data Analysis Application (GDAA) software) are still under discussion. EURL ECVAM has nevertheless, decided to commence evaluation of the GARD (see [Section 3.4](#)).

EURL ECVAM received a full submission of the RTgill-W1 (rainbow trout gill cell line) cytotoxicity assay in September 2018 (see section 3 in Zuang *et al.*, 2018), but in 2019 the test submitter decided to withdraw its submission and communicated to EURL ECVAM that the assay will be considered and evaluated in the context of an OECD test guideline (see [Section 5.6.6](#)).

The test submitters of the Toxtracker<sup>®</sup>, of the EDITOX and of the TR MARCoNI assays were invited in 2017 (Toxtracker<sup>®</sup> and EDITOX) and 2018 (TR MARCoNI) to provide a full submission (see section 3 in Zuang *et al.*, 2018) but no full submissions were received for these assays in 2019.

All updates on test method submission, assessment, peer review and regulatory acceptance can be regularly consulted on EURL ECVAM's Tracking System for Alternative methods towards Regulatory acceptance (TSAR) accessible at: <https://tsar.jrc.ec.europa.eu>.

### READ MORE

- ▶▶ TSAR: Tracking System for Alternative methods towards Regulatory acceptance: [tsar.jrc.ec.europa.eu](https://tsar.jrc.ec.europa.eu)
- ▶▶ EURL ECVAM test method submission: [eu/!ww88Mh](https://ec.europa.eu/!ww88Mh)

### 3.1 ALIsens

EURL ECVAM received a pre-submission on an *in vitro* model for the prediction of respiratory sensitisation of inhalable substances (ALIsens).

ALIsens is designed to directly replace the respiratory LLNA assay, which, in any case, is an adaptation of the skin LLNA and has not yet been validated for inhalation toxicology.

EURL ECVAM has evaluated the submission and prepared an assessment report on the basis of which it will decide on the best way forward for this type of test methods developed in the area of respiratory sensitisation.

#### READ MORE

▶ ALIsens: [tsar.jrc.ec.europa.eu/test-method/tm2019-01](https://tsar.jrc.ec.europa.eu/test-method/tm2019-01)

### 3.2 Bioelution

EURL ECVAM has finalised the evaluation of all information received in the Bioelution test method submission and considered to progress the method to peer-review by ESAC (see [Section 4.6.3](#)).

#### READ MORE

▶ Bioelution: [tsar.jrc.ec.europa.eu/test-method/tm2016-02](https://tsar.jrc.ec.europa.eu/test-method/tm2016-02)

### 3.3 SENS-IS

EURL ECVAM evaluated the SENS-IS re-submission of 2018 (see [section 3.3](#) of *Zuang et al., 2018*). Some of the concerns raised by EURL ECVAM on the original full submission received in 2016 were addressed (*e.g.*, inconsistencies in the data reported, gaps in the data submitted, clarifications to be made to the protocol etc.). Nevertheless, the EURL ECVAM evaluation of the re-submission indicated that there are still

shortcomings that need to be addressed by the test submitter before progression of the method into peer review. For example some of the experiments submitted in 2016 are no longer reported in the revised submission without providing a justification for it.

Regarding the questions on the terms and conditions of the patent of the SENS-IS test method and the impossibility to develop a similar method (see [section 3.3](#) in *Zuang et al., 2018*), the reader is referred to [Section 5.6.15](#).

#### READ MORE

▶ SENS-IS: [tsar.jrc.ec.europa.eu/test-method/tm2011-11](https://tsar.jrc.ec.europa.eu/test-method/tm2011-11)

### 3.4 GARD

As reported in [section 3.4](#) of *Zuang et al. (2018)*, the SenzaGen-led validation of the GARD test for sensitisation hazard assessment submitted to EURL ECVAM in January 2018 was not initiated knowing that additional work was conducted to generate information on the GARDpotency. In addition, EURL ECVAM decided to put on hold the evaluation of the GARD submission until clear indications on the outcome of the discussions taking place at the OECD would become available.

EURL ECVAM received the submission on the validation of the GARDpotency in July 2018. In December 2018 EURL ECVAM received a notification from the test submitter indicating that a number of inaccuracies were found in the GARDpotency submission requiring revision. A revised submission was received in August 2019 which is currently under evaluation.

#### READ MORE

▶ GARD: [tsar.jrc.ec.europa.eu/test-method/tm2011-09](https://tsar.jrc.ec.europa.eu/test-method/tm2011-09)



# 4

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## **Validation of Alternative Methods and Approaches**

# 4.

## Validation of Alternative Methods and Approaches

### 4.1 Endocrine Disruption - AR-CALUX Test Method

EURL ECVAM coordinated the validation of an androgen receptor transactivation method to determine endocrine activity from chemicals, the AR-CALUX method. This method was submitted by the Dutch company BioDetection Systems (BDS, The Netherlands). The validation was carried out by the test submitter BDS and three participating laboratories of EURL ECVAM's network of specialised laboratories, the European Union Network for the Validation of Alternative Methods (EU-NETVAL, see [Section 4.7](#)): RISE, Covance and Charles River Labs.

The experimental work of the AR-CALUX validation study was completed in November 2018, and the validation report finalised in April 2019, including review and approval by the validation management group. The ESAC peer review took place shortly after (see [Section 4.6.2](#)).

#### READ MORE

► AR-CALUX: [tsar.jrc.ec.europa.eu/test-method/tm2010-07](https://tsar.jrc.ec.europa.eu/test-method/tm2010-07)

### 4.2 Thyroid Hormone Disruption

EURL ECVAM is coordinating a large scale validation study of a set of mechanistically informative alternative methods to detect chemicals that disrupt normal

thyroid hormone function. The validation study will be performed with EU-NETVAL and the respective alternative method developers. A total of 17 methods have been identified as candidates taking into account the information reported in an OECD scoping document on *in vitro* and *ex vivo* methods for the identification of modulators of thyroid hormone signalling (OECD, 2017b), an OECD Detailed Review Paper (OECD, 2012), and feedback received at various OECD Expert Group meetings.

For part 1 of the validation study, efforts have been invested to assure the quality and long-term availability of the test systems (*e.g.*, cells and tissues), the selection of relevant reference and control items, the completeness of the 17 methods (*i.e.*, the description of their critical elements and decision details), and to ensure that the validation study is performed according to Good *In Vitro* Method Practices (GIVIMP; OECD, 2018b). Nineteen test systems are included in the thyroid validation study. EURL ECVAM provides all the test systems to the EU-NETVAL test facilities, which includes purchasing, shipments and quality control of the test systems and, if required, information and documentation on test system handling and GMO notification. To date, most shipments have been completed. Upon receipt of the test systems, EU-NETVAL facilities will create master and working cell banks (if applicable), prepare Standard Operating Procedures

(SOPs) for maintenance and handling of the test system(s) and generate historical datasets. In parallel, EURL ECVAM, EU-NETVAL, the method developers, and the test system suppliers are all contributing to safeguard that complete test system characterisation is performed and documented.

To date, 65 chemicals (mainly reference and control items) have been identified for 10 of the methods. EURL ECVAM procured, aliquoted, labelled and shipped the appropriate reference and control items, together with material safety data sheets and certificates of analysis, to the relevant EU-NETVAL facilities. For part 2 of the validation study, chemical selection took place with an expert meeting that was held in November 2019. A list with 171 potential chemicals was refined on the basis of specific selection criteria. For part 2, the final set of selected chemicals (test items) will be distributed to EU-NETVAL as coded chemicals.

The outline procedure(s) will be provided as word document(s) to the EU-NETVAL test facilities. These outline procedures contain a compilation of available information written in a stepwise fashion, which can be used to create SOPs. An initial assessment of (i) the completeness of the method and (ii) the presence of acceptance criteria revealed great disparities. Acceptance criteria (when available) were assessed for each method and will be completed (where necessary) in collaboration with the method developers and EU-NETVAL laboratories.

In April 2019, the OECD adopted a document "Guiding principles on good practices for the availability/distribution of protected elements in OECD TGs" (see [Section 5.6.15](#)). This document states that a declaration regarding accessibility to protected elements in the test method based on fair, reasonable and non-discriminatory conditions will need to be signed by method developers when submitting a new TG. To this end, elements protected by intellectual property rights (IPR) related to the 17 *in vitro* methods are being investigated and documented. The new OECD standard project submission form also includes information on the protected elements of a method. It has proved quite challenging to retrieve and document the information regarding protected elements for the thyroid validation methods. The process will continue in 2019/2020.

### 4.3 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.

As outlined in the EDQM Annual Report 2018 (EDQM, 2018), nine projects regard the development of new European Pharmacopoeia (Ph. Eur.) methods, and six of these are dedicated to applying the Three Rs principles to the field of quality control of biologicals, *e.g.*, a serological method for potency testing of whole-cell pertussis vaccines, the BINACLE assay for *in vitro* detection of toxicity in tetanus vaccines, and, in collaboration with the European Partnership on Alternative Approaches to Animal Testing (EPAA), BSP130 Phase III Validation of *in vitro* methods for the testing of *Clostridium septicum* vaccine (see [Section 5.10.4](#)) and BSP148 Validation of a rabies *in vitro* potency assay (see [Section 5.10.5](#)). Moreover, two projects are dedicated to quality control of erythropoietin and aim at removal of the current *in vivo* biological activity assay.

The establishment of a new reference standard for Pertussis toxin is needed for the recently validated Chinese Hamster Ovary (CHO) cell-clustering assay, which replaces the animal-based histamine sensitisation assay and is now included into the Ph. Eur. The BSP also aims at establishing a new non-endotoxin pyrogen reference standard for use in the monocyte activation test (Ph. Eur. 2.6.30), a replacement of the rabbit pyrogen test.

#### READ MORE

► EDQM Biological Standardisation Programme (BSP): [www.edqm.eu/en/work-programme-bsp](http://www.edqm.eu/en/work-programme-bsp)

### 4.4 Genotoxicity Testing - Micronucleus Test and Comet Assay in Reconstructed Skin Models

The validation work on the 3D skin comet and Reconstructed Skin Micronucleus (RSMN) assays, coordinated by Cosmetics Europe, has been finalised and the relative manuscripts are foreseen to be published in a dedicated issue of Mutagenesis on



3D skin genotoxicity assays. EURL ECVAM had been actively involved in the chemical selection for the validation studies. The International Workshop on Genotoxicity Testing (IWGT) workgroup on 3D tissue models that met in Tokyo in 2017, considered that the 3D skin comet and RSMN assays are now sufficiently validated to move towards peer review and the development of individual OECD test guidelines (Pfuhrer *et al.*, 2020).

A proposal of new OECD test guidelines on *in vitro* genotoxicity tests for dermal exposure using 3D skin models (*i.e.*, RSMN and 3D Skin Comet) led by Germany and France was included in the OECD Work Plan in April 2019.

Further discussion on the way forward to accelerate the implementation of these assays in regulatory decision making took place in a workshop organised by Cosmetics Europe in November 2018. The workshop aimed at engaging in discussions with toxicologists and risk assessors from industry and the public sector, including several members of the Scientific Committee on Consumer Safety (SCCS), to facilitate the regulatory use and acceptance of alternative methods. One of the sessions was chaired by EURL ECVAM, aimed at being truly "hands-on", with the participants being the real actors in addressing three case studies. The participants received data for different case studies in a coded manner and were tasked to come to a conclusion on whether the substance was considered hazardous or not. Interestingly, all sub-groups formed reached correct and same conclusions using data from conventional genotoxicity studies supplemented with mechanistic data and data from 3D reconstructed skin models. This can be considered promising in view of a safety assessment without animals.

#### 4.5 Genotoxicity Testing - Hen's Egg Test for Micronucleus Induction (HET-MN)

The hen's egg test for micronucleus induction (HET-MN) may represent a good supplementation to the genotoxicity assays in 3D skin models, because it uses the complex model of the incubated hen's egg, which mimics systemic exposure (Reisinger *et al.*, 2019). A validation study was carried out by a German consortium in three laboratories with testing of more than 30 chemicals and the manuscript is in preparation.

## 4.6 EURL ECVAM Scientific Advisory Committee Peer Reviews

### 4.6.1 Replacements for Animal-derived Antibodies

Antibodies are important tools used in research, therapeutics, and diagnostic and in many regulatory applications. The generation of monoclonal and polyclonal antibodies as well as other affinity reagents is still involving animals despite the availability of technologies for their generation and production that do not entail the use of animals.

For this reason, EURL ECVAM asked its Scientific Advisory Committee (ESAC) in April 2018 to review the scientific validity of non-animal-derived antibodies and non-antibody affinity reagents used for research, diagnostics, and regulatory applications, and an ESAC Working Group was established for that purpose. The ESAC Working Group met at the JRC in Ispra, Italy, on 8 to 9 November 2018 to advance the detailed review and draft a Working Group Report and an ESAC Opinion for discussion at the ESAC plenary meetings on 4 to 5 December 2018 and 3 to 5 June 2019 (see section 4.7.2.1 of Zuang *et al.*, 2018; [Figure 4.1](#)).

The review focused on non-animal-derived antibodies generated by phage-display technology since this is the most mature technology and already widely used. Based on the scientific literature, application examples and the experts' own extensive experience, the ESAC concluded:

- Non-animal-derived antibodies are mature reagents generated by a proven technology.
- Non-animal-derived antibodies offer significant scientific benefits. Their polypeptide sequences are established as part of the generation process, thus providing a unique identifier, and subsequently unlimited and sustainable supply. Use of non-animal-derived antibodies will therefore ultimately enhance experimental reproducibility.
- Non-animal-derived antibodies should be promoted as many scientific misconceptions still subsist regarding these reagents.

EURL ECVAM is currently preparing its Recommendation based on the outcome of the ESAC review and expects to publish it together with the ESAC Opinion and the Working Group Report in the beginning of 2020.



**Figure 4.1:** Core members of ESAC and of the ESAC Working Group on affinity reagents (JRC Ispra, Italy, 3-5 June 2019).

#### READ MORE

- ▶▶ EURL ECVAM Scientific Advisory Committee (ESAC): [europa.eu/lgp34jf](https://europa.eu/lgp34jf)
- ▶▶ ESAC in the Register of Commission Expert groups: [ec.europa.eu/transparency/regexpert/index.cfm?do=groupDetail.groupDetail&groupID=3602](https://ec.europa.eu/transparency/regexpert/index.cfm?do=groupDetail.groupDetail&groupID=3602)

#### 4.6.2 Endocrine Disruption - AR-CALUX Test Method

The peer-review by the ESAC working group of the AR-CALUX validation study took place in May 2019, concluding on a successful validation study (see Section 4.1), very good performance values of the method which were well in the range of publicly available similar methods such as the ER-CALUX method and the ARTA using EcoScreen cells, and a recommendation to have the method proposed as a test guideline at OECD.

The ESAC opinion was endorsed at the ESAC plenary meeting of June 2019.

#### 4.6.3 Bioelution Assay

In May 2019, EURL ECVAM mandated ESAC to review the available evidence and deliver an opinion on the scientific validity of the bioelution test method to assess the relative bioaccessibility of metals in

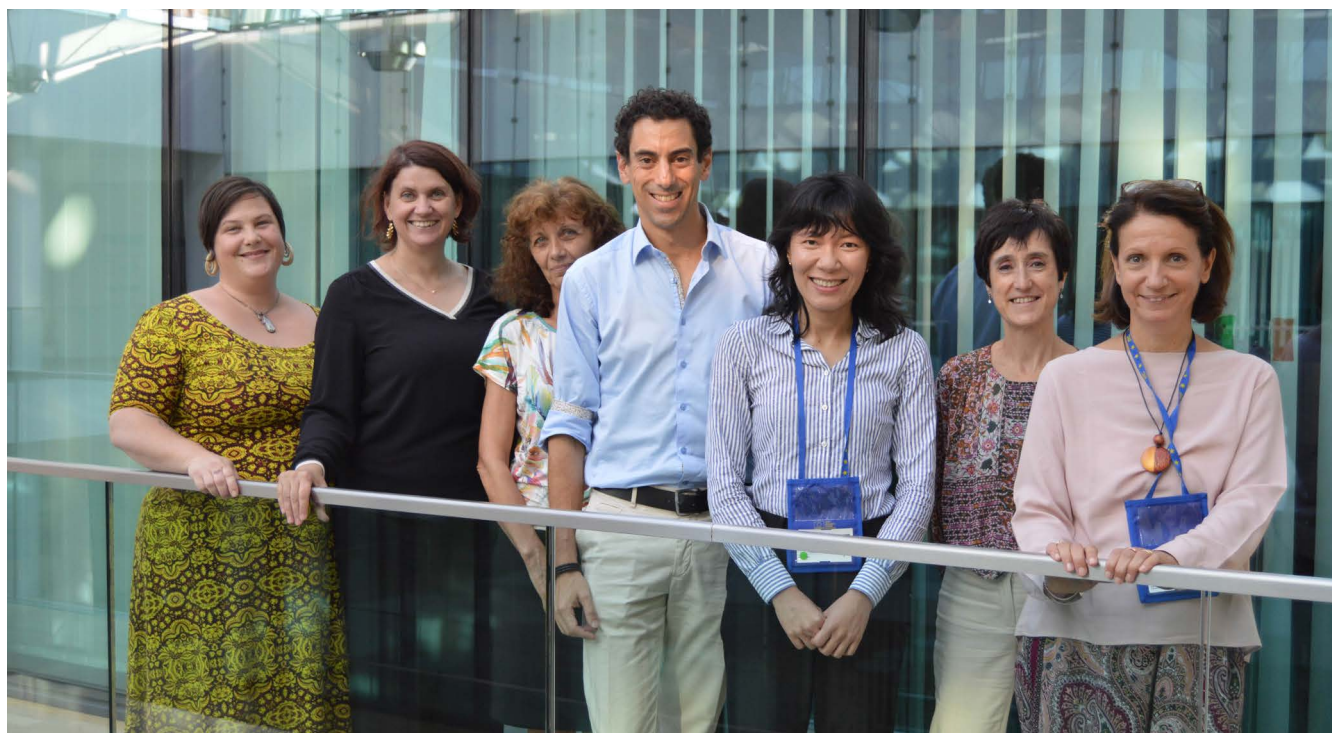
inorganic metal compounds and metal-containing materials using a simulated gastric fluid. Data from the bioelution test method are meant to support two proposed regulatory applications, namely grouping and read across and classification of alloys.

This bioelution test method generates:

- a) relative bioaccessibility data by comparing bioaccessibility results of a given metal released from different compounds of the same metal or from different materials containing this metal, and
- b) to calculate the relative bioaccessible concentration of a metal in an alloy by comparing the metal releases from the alloys to those from their pure metal ingredients.

The evaluation of how these *in vitro* relative bioaccessibility data will be used in a regulatory context is, nevertheless, not in the remit of the ESAC.

An ESAC Working Group was created to conduct the detailed review. It met at JRC in Ispra, Italy, on 2 to 3 May and on 24 to 25 September 2019 (see Figure 4.2) to advance the detailed review and draft a Working Group Report and an ESAC Opinion. The ESAC discussed and endorsed the opinion at the ESAC plenary meeting on 2 to 3 December 2019.



**Figure 4.2:** ESAC Working Group on the Bioelution assay.

#### 4.7 Meeting of the European Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL)

The EU Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) is a network of 37 highly qualified laboratories across Europe, coordinated by EURL ECVAM to support the *in vitro* method validation process. It represents a wide range of expertise and competences and includes laboratories experienced in advanced *in vitro* procedures, biological test systems and measurement techniques.

On 7 to 8 May 2019, EURL ECVAM hosted the 5<sup>th</sup> meeting of EU-NETVAL where international experts shared their knowledge on advancing *in vitro* methods to improve safety assessment and avoid animal testing (Figure 4.3).

Invited experts from the OECD, EFSA and ECHA gathered with EU-NETVAL members to share their experiences and knowledge on specific *in vitro* methods to detect androgen and thyroid hormone disrupting chemicals that are currently being validated using EU-NETVAL laboratories.

EU-NETVAL members discussed key considerations for the design and development of methods intended for regulatory use and reported on the significant

progress being made in using *in vitro* methods to assess chemicals for three potential health effects:

- skin sensitisation: allergic response following skin contact with a substance;
- genotoxicity: property of chemicals that can cause genetic alterations in cells, which may lead to cancer;
- developmental neurotoxicity: any adverse effect induced by chemicals that affect the developing nervous system.

EU-NETVAL test facilities and invited experts agreed on key issues and recommendations to be considered in aiding the translation of more sophisticated methods into the regulatory domain, with particular emphasis on handling more complex test systems (*e.g.*, 3-dimensional tissue models or human induced pluripotent stem cells), complex test items (*e.g.*, chemical mixtures, biomedical devices, nanomaterials) and complex measurement technologies (*e.g.*, automated imaging and 'omics). The importance of advancing and implementing Good *In Vitro* Method Practices (GIVIMP; OECD, 2018b)) was also emphasised.

Updates were provided on a joint initiative between Directorate-General for Environment and EURL ECVAM to promote alternative methods and support the implementation of Directive 2010/63 for the



protection of animals used for scientific purposes, through the development of a suite of education and training resources.

#### READ MORE

- ▶▶ EU Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL): [europa.eu/!jD93bV](http://europa.eu/!jD93bV)
- ▶▶ Summary record of EU-NETVAL meeting 2018: [europa.eu/!bb34Yf](http://europa.eu/!bb34Yf)

### 4.8 Meeting of the Preliminary Assessment of Regulatory Relevance (PARERE) network and the ECVAM Stakeholder Forum (ESTAF)

EURL ECVAM's Preliminary Assessment of Regulatory Relevance (PARERE) network met at the JRC, Ispra, Italy on 27 to 28 November 2018. The PARERE meeting was followed by a joint meeting of PARERE and the ECVAM Stakeholder Forum (ESTAF) on 28 (starting at lunch) to 29 November 2018. The PARERE network is composed of regulators from the EU Member States and relevant Commission services as well as EU agencies (EFSA, ECHA and EMA). The network provides advice to EURL ECVAM on the regulatory relevance and suitability of alternative approaches proposed for validation or peer review and facilitates information flow between EURL ECVAM and regulators regarding the development

and validation of methods and testing strategies. The network also supports the identification of areas that need specific attention whilst both PARERE and ESTAF networks contribute to EURL ECVAM strategies and recommendations as part of the validation process.

During the first part of the PARERE meeting, PARERE members and EURL ECVAM provided updates on relevant activities within the PARERE network in the respective Member States and within the EC, respectively. EURL ECVAM then informed on recent test method submissions, their assessment by EURL ECVAM and some related issues that had been identified.

EURL ECVAM also updated on relevant activities within different OECD groups such as the Working Party on Hazard Assessment (WPHA), the Working Group of National Coordinators of the OECD Test Guidelines Programme (WNT) and the Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST).

In the morning of 28 November, EURL ECVAM introduced the Adverse Outcome Pathway (AOP) framework and in particular why the AOP concept is useful, what AOPs actually are, and how AOPs are currently captured, managed and disseminated. This was followed by a discussion on how to move the AOP framework forward



**Figure 4.3:** Participants of the 5<sup>th</sup> meeting of EU-NETVAL members held on 7-8 May 2019 at the JRC Ispra, Italy.

with PARERE. One obvious role that was identified was PARERE's assessment of the regulatory relevance of AOPs, in particular during the early stages of AOP development, and PARERE's input to discussions on prioritisation. Another useful role that was highlighted was to pull the country representatives of the different OECD groups (EAGMST-WNT-WPHA) closer together to ensure better communication and more coordinated interactions and actions. The scientific review process of AOPs also needs support from experts as the OECD secretariat cannot sustain it on its own. In addition, information days and training courses could be organised (with support from JRC/EURL ECVAM).

More information on the outcome of the PARERE meeting of 2018 is available at the link in the "read more".

The joint meeting of PARERE and ESTAF started in the afternoon of 28 November (Figure 4.4). The ESTAF comprises organisations representing academia, industry and civil society (including animal welfare NGOs) with a stake in non-animal approaches. The forum provides input in a number of areas of EURL ECVAM's work including strategies to implement the Three Rs.

The first part of the joint meeting focused on validation of alternative methods and approaches whereas during the second part of the joint meeting, participants were asked to reflect on what multi-stakeholder initiatives they would propose that could lead to a

more purpose-driven development of new methods and the successful application of existing methods. The background to this question was the multitude of non-animal methods which are currently available or under development, many of which do not have a defined use for chemical safety assessment.

The discussion in breakout groups highlighted the following needs:

- better dialogue and coordination between regulators and test method developers. This could be achieved, for example, by means of advisory groups, which includes both scientists and regulators, providing adequate training courses, or involving regulators from an early stage of method development;
- ensuring a greater harmonisation of EU regulations across different sectors. Identifying the alternative approaches available could be a first step and this can be followed by a collective effort to evaluate their applicability under the existing regulations;
- translation of mechanistic knowledge to the regulatory domain, exploiting all information that is available, *e.g.*, read-across, toxicokinetics, threshold of toxicological concern (TTC), and human data. In addition, case studies represent a relevant tool to build confidence in alternative methods and to demonstrate their applicability within testing strategies;



**Figure 4.4:** Participants in the joint meeting of the Preliminary Assessment of Regulatory Relevance (PARERE) network and ECVAM Stakeholder Forum (ESTAF), 28 November 2018, JRC Ispra, Italy.

- call for funding and open access. The launch of open calls for input on a specific Three Rs or regulatory needs has proven very useful to move forward non-animal methods in a specific area. Results from such publicly funded research should be available as open access with availability of raw data.

The summary record of the joint meeting of PARERE and the ESTAF is publicly available (see “read more”).

In 2019, the PARERE meeting took place on 21 October. It included updates from EURL ECVAM and the PARERE network on relevant activities, updates on the AOP framework study carried out by JRC/EURL ECVAM for which PARERE members had been surveyed (see [Section 5.7.4](#)), as well as 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 Harmonised System of Classification and Labelling of Chemicals (GHS)”.

On 22 November, 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 7.1](#)).

Summary records of these meetings will become available on EURL ECVAM’s website early 2020.

#### READ MORE

- ▶ Preliminary Assessment of Regulatory Relevance (PARERE): [europa.eu/!gF94hp](https://europa.eu/!gF94hp)
- ▶ Summary record of PARERE meeting 2018: [europa.eu/!bQ77wU](https://europa.eu/!bQ77wU)
- ▶ ECVAM Stakeholder Forum (ESTAF): [europa.eu/!wU38uj](https://europa.eu/!wU38uj)
- ▶ Summary record of joint meeting of PARERE and the ESTAF 2018: [europa.eu/!DD86CV](https://europa.eu/!DD86CV)





# 5

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## **Promoting the Regulatory Acceptance and International Adoption of Alternative Methods and Approaches**

# 5.

## Promoting the Regulatory Acceptance and International Adoption of Alternative Methods and Approaches

### 5.1 High Level Conference EU Chemical Policy 2030

In the past years, the European Commission has been working on major evaluations of the EU chemicals policy. In collaboration with the Ministry for Environment and Food of Denmark, the Commission held a high-level conference in Brussels, Belgium, on 27 and 28 June 2019 to engage the different stakeholder groups in discussions on recent and possible future developments of EU chemicals policy. JRC, through EURL ECVAM, contributed to the organisation of the conference, which focussed on six main thematic areas for promoting green and sustainable chemistry through innovation, alternative technologies and processes and right skills:

- Chemicals and the circular economy: safe management of chemicals in products and waste and contribution to resource efficiency.
- Improving the regulatory framework for risk assessment and risk management of hazardous chemicals.
- Knowledge building, monitoring and early warning on emerging risks.
- Smarter communication, better protection and lower costs: meeting citizens' concerns.
- Completing the EU Single Market and ensuring a level playing field.
- The EU chemicals policy and global challenges: sustainability, innovation, competitiveness.

The outcome of the conference, available in the final report (DG Environment and Ministry of Environment and Food of Denmark, 2019), provided inputs for the Commission to define and prioritise future policy developments. The JRC, through EURL ECVAM, is contributing to a dedicated task force led by Directorate-General for Environment and Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs in charge of drafting a strategy with concrete actions to improve the design, implementation and enforcement of the EU chemical policy towards 2030. The strategy will be a crucial milestone for delivering the recently announced green deal including the zero pollution ambition and the shift towards climate neutrality and circular economy. Key to the success of the initiative is the ability to strengthen synergies and the coherence between chemical policy and other EU policy priorities including health, climate and biodiversity.

#### READ MORE

► EU Chemicals Policy 2030 Conference: [euchemicalspolicy2030.teamwork.fr/en/home](https://euchemicalspolicy2030.teamwork.fr/en/home)

### 5.2 Fitness Check on Endocrine Disruptors

In its Communication 'Towards a more comprehensive European Union framework on endocrine disruptors' (COM(2018) 734) published in November 2018, the Commission referred to the need to

organise a Fitness Check to assess whether relevant EU legislation on endocrine disruptors delivers its overall objective to protect human health and the environment by minimising exposure to these substances. The JRC is leading this activity, with the support of policy DGs through an Inter-service Steering Group. The main focus of this Fitness Check will be a systematic analysis of the coherence of relevant provisions on endocrine disruptors across the EU legal measures. The roadmap detailing the purpose and scope of the evaluation, as well as the methodology followed, has been published in June 2019. The publication of the Fitness Check is expected in Q2 2020.

#### READ MORE

- ▶▶ Harmful chemicals – endocrine disruptors, review of EU rules: [ec.europa.eu/info/law/better-regulation/initiatives/ares-2019-2470647\\_en](https://ec.europa.eu/info/law/better-regulation/initiatives/ares-2019-2470647_en)
- ▶▶ Fitness Check on the EU legislation on endocrine disruptors: [ec.europa.eu/info/policies/endocrine-disruptors\\_en](https://ec.europa.eu/info/policies/endocrine-disruptors_en)
- ▶▶ Citizens' consultation on endocrine disruptors: [ec.europa.eu/jrc/en/news/endocrine-disruptors-have-your-say-eu-legislation](https://ec.europa.eu/jrc/en/news/endocrine-disruptors-have-your-say-eu-legislation)

### 5.3 Extension of the EURL ECVAM Genotoxicity and Carcinogenicity Consolidated Database: Ames Negative Compounds

The bacterial reverse mutation test (Ames test) is the most commonly used genotoxicity test. Data on the Ames test are amongst the first ones to be required by regulatory agencies worldwide. Within the current standard *in vitro* genotoxicity test battery, the Ames test is considered capable of revealing DNA reactivity, and identifying substances that can produce gene mutations via different mechanisms. Therefore, evaluating the predictivity of the Ames test for *in vivo* genotoxicity and carcinogenicity, when considered alone or in association with mammalian cell assays for chromosome damage and/or gene mutations, is extremely important.

The consolidated EURL ECVAM Genotoxicity and Carcinogenicity Database of Ames positive test results represents a robust set of data (Kirkland *et al.*, 2014a,b); it serves as a reference for a number of regulatory activities in the area of genotoxicity testing. Consequently, EURL ECVAM has committed to expanding the database via inclusion of Ames negative test results.

The extension is expected to improve the evaluation of Ames test predictivity and evaluations of the genotoxic and carcinogenic potential of substances across product types and sectors.

Together with experts in the field of regulatory genetic toxicology, EURL ECVAM curated a collection of 211 Ames negative compounds, with a summary of complementary data available for other *in vitro* and *in vivo* genotoxicity test methods, plus all available carcinogenicity data. All collected information has been evaluated using defined criteria for reliability and data quality. A preliminary analysis of the data has been performed, which includes an evaluation of the database with respect to chemical space and initial groupings by structural features and alerts, plus an evaluation of correlations with carcinogenic properties of the chemicals.

The results will be presented in a manuscript (Madia *et al.*, in preparation-a).

### 5.4 European Food Safety Authority Scientific Committee and Panels

In 2011, a Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment was issued by the EFSA Scientific Committee (EFSA, 2011). However, in recent EFSA substance evaluations some limitations were identified in relation to the assessment of aneugenic substances, those that induce numerical chromosomal aberrations through the interactions with cellular targets other than DNA, such as proteins involved in the segregations of chromosomes during mitosis or meiosis. Considering these limitations, the EFSA Scientific Committee is now working on the development of a guidance in relation to what the most appropriate *in vivo* follow-up is for substances that are aneugenic *in vitro*, and how risk to human health should be assessed for a substance exhibiting aneugenicity. The related public consultation is foreseen to be launched in March 2020.

An EFSA Workshop on *in vitro* comparative metabolism studies in regulatory pesticide risk assessment, held in Parma, Italy, on 15 to 16 November 2018, discussed the use of *in vitro* methods to identify human metabolites (EFSA, 2019). These studies will also be used to support the assessment of human relevance of chemical-mediated thyroid disruption. Discussions focused on the minimum amount of information that should be provided to satisfy the data requirement

and should be included in the methods, the key elements to be considered for the interpretation of the studies outcome, and how metabolism data can be used in a weight of evidence (WoE) approach for endocrine mediated mechanisms of toxicity.

From the workshop it became clear that a guidance document is needed on how to conduct and interpret these studies for the identification of human metabolites. The workshop outcome, which recommended that some mechanistic relevant key events be measured as part of the WoE approach, can be used as the foundation for developing such guidance. The EU pesticides Regulation established that *in vitro* comparative metabolism studies shall be performed and that guidance is needed on this. At present there is no OECD test guideline for comparative metabolism studies available.

In order to provide an adequate scientific background and facilitate the preparation of an OECD guidance document (GD) on the application and interpretation of *in vitro* DNT assays (see Section 5.6.11), EFSA created a DNT Working Group which is intended to develop integrated approaches to testing and assessment (IATA) case studies for hazard identification and characterisation. The existing *in vitro* data and the outcome of the ongoing *in vitro* evaluation studies (see Annex 2) will be used as part of the available evidence. The developed IATA case studies will be used as a basis for the development of the GD on the use of alternative methods for DNT testing, including guidance on data interpretation.

#### READ MORE

- ▶ EFSA Workshop on "In vitro comparative metabolism studies in regulatory pesticide risk assessment": [www.efsa.europa.eu/en/events/event/181115-0](http://www.efsa.europa.eu/en/events/event/181115-0)
- ▶ EFSA Working Group on "Developing IATA case studies on Developmental Neurotoxicity": [www.efsa.europa.eu/en/pesticides/working-groups](http://www.efsa.europa.eu/en/pesticides/working-groups)

## 5.5 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), the elaboration of

OECD-wide agreed hazard assessments, and improving access to information on chemicals.

During the 3<sup>rd</sup> meeting of the WPHA, held on 17 to 19 June 2019, the JRC gave progress updates on two projects it is leading: an overview document on existing guidance for Integrated Approaches to Testing and Assessment (IATA); and a guidance document on the characterisation, validation and reporting of physiologically based (PBK) models for regulatory applications (Sachana, 2019). A separate guidance document on weight of evidence, drafted by Environment & Climate Change Canada and ECHA, including input from the JRC, was endorsed.

In addition, the JRC presented a thought starter on the Development of an Assessment Framework for Quantitative Structure-Activity Relationship (QSAR) Model Predictions, aimed at going beyond the 2007 OECD guidance on QSARs. Although the proposal was supported by the WPHA, and several Member Countries expressed a willingness to contribute, it will remain on hold until a Member Country has the resources to take the lead.

## 5.6 Activities in the OECD Test Guidelines Programme

### 5.6.1 Summary of the Outcome of the 31<sup>st</sup> Meeting of the Working Group of National Coordinators of the OECD Test Guidelines Programme

At the 31<sup>st</sup> meeting of the Working Group of National Coordinators of the OECD Test Guidelines Programme (WNT) held at the OECD headquarters in Paris on 9 to 12 April 2019, the following new and updated test guidelines and a new guidance document were approved (see also Annex 1 and Annex 2 for the adopted test guidelines based on *in vitro* methods):

- New TG 248 on the *Xenopus* Eleutheroembryo Thyroid Assay (see Section 5.6.5)
- Updated TG 203 on the Fish Acute Toxicity Test (see Section 5.6.4)
- Updated TG 442C on *in chemico* skin sensitisation assays addressing the AOP key event on covalent binding to proteins (inclusion of the Amino acid Derivative Reactivity Assay (ADRA) and the TG becomes a key event-based TG)
- New TG 494: Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification



and Labelling for Eye Irritation or Serious Eye Damage

- Updated TG 492 on Reconstructed human Cornea-like Epithelium (RhE) test methods for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage (inclusion of the MCCT\_HCE test method)
- Updated TG 439 on *in vitro* skin irritation: Reconstructed human Epidermis test method (inclusion of the epiCS® and Skin+® test methods)
- Updated TG 431 on *in vitro* skin corrosion: Reconstructed human Epidermis test method (inclusion of the LabCyte EPIMODEL-24 test method)
- Updated TG 432 on 3T3 NRU for Phototoxicity Testing
- New TG 495 on Reactive Oxygen Species for Photoreactivity
- New GD 298 on Guiding Principles on Good Licensing Practices for Protected Elements in OECD TGs (see Section 5.6.15).

In addition, a correction to TG 456 on the H295R Steroidogenesis Assay in relation to the list of proficiency substances was also approved. The proposal introduces some flexibility to use another well-studied substance if, for example, any substance listed is difficult to obtain. Several other *in vitro* TGs listing proficiency substances also contain a similar sentence.

The TG on a Macromolecular assay for eye irritation was not approved during the WNT meeting due to a few remaining issues, but it was approved later through written procedure.

Seventeen new projects were adopted on the OECD Workplan. More information can be found on the OECD website of the TGP.

The following chapters mainly focus on TGs for which the EC (through JRC/EURL ECVAM) has the lead or co-lead. However, some selected projects with relevance to the alternative field led by other Member Countries are also briefly described. Beside 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. The JRC acts as a National Coordinator for the OECD Test Guidelines Programme, representing the European Commission and EU, and as such is a member of the Working Group of National Coordinators of the OECD Test Guidelines Programme.

#### READ MORE

- ▶ OECD Test Guidelines Programme: [www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm](http://www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm)

#### 5.6.2 Use and Analysis of Control Fish in Toxicity Studies

This project was initiated by the International Council on Animal Protection in OECD Programmes (ICAPO) and is co-led by the EC (through JRC/EURL ECVAM) and ICAPO. The overall aim is to reduce the number of control fish in fish toxicity studies. At present, OECD test guidelines require the use of a water and a solvent control group, when a solvent is used.

As a first step, GD 23 on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals was revised and includes now the experience gained in handling difficult test chemicals in aquatic exposures as well as progress made in developing methods for testing these chemicals (OECD, 2019a). Specific attention was paid to updating exposure methods that do not employ a solvent in order to eliminate the need for a solvent control, and thus, reducing the number of animals used in aquatic toxicity tests.

For the second part of the project, data generated according to OECD test guidelines in the presence of a solvent have been collected. Data analysis should determine if the use of only one control would have had an impact on the outcome of the study. A Detailed Review Paper will be drafted summarising results, conclusions and recommendations.

#### READ MORE

- ▶ International Council on Animal Protection in OECD Programmes (ICAPO): [www.icao.org](http://www.icao.org)

#### 5.6.3 OECD Guidance Document on IATA for Fish Acute Toxicity Testing

This OECD project started in 2015 under the lead of Austria and ICAPO and aims at developing an IATA for fish acute toxicity testing. The IATA will guide the combination of existing information, computational tools, mechanistic information from the molecular and cellular levels, information from *in vitro* assays (e.g. RTgill-W1 cytotoxicity assay; see Section 5.6.6) and fish embryo acute toxicity tests (OECD TG236; OECD, 2013), to derive acute fish toxicity information. Moreover, the IATA is placed into the framework of

OECD GD 126, the threshold approach for acute fish toxicity (OECD, 2010) taking into consideration the three trophic levels (daphnia, algae and fish).

#### 5.6.4 Revision of OECD Test Guideline 203

Under the lead of Switzerland and the UK, OECD finalised the revision of OECD TG 203, the acute fish toxicity test (OECD, 2019c).

OECD TG 203, fish acute toxicity test, determines the concentration of a chemical at which 50% of the fish die (LC50) and is one of the few guidelines still using death as an endpoint. The revised guideline includes possibilities to reduce and refine fish testing whenever possible and appropriate. For example, before any fish acute toxicity test is carried out it should be considered whether reliable information could be derived from a weight of evidence approach using methods such as QSAR, read-across, fish embryos (OECD, 2013), fish cell lines, and others, if reliable and fulfilling the regulatory testing requirements. In case an acute fish test needs to be carried out, the OECD GD 126 (Threshold Approach for Acute Fish Toxicity; OECD, 2010) or the limit test, as described in OECD TG 203, may be sufficient.

The above listed alternative approaches can be used for range finding tests. Moreover, testing at concentrations causing 0 and 100% mortality is no longer a mandatory requirement, *i.e.*, there is no need to test additional concentration only to demonstrate these mortality values. Guidance is given on the use of solvents, solvent control and possible omission of the water control group. The guideline includes a list of visible abnormalities or sublethal clinical signs to be recorded during exposure of the fish, which may help to establish humane endpoints in acute fish tests.

#### 5.6.5 OECD Test Guidelines with Transgenic Fish and Amphibian Models to Identify Endocrine Disruptors

Under the lead of France, the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) (OECD, 2019b) has been validated and included in a new OECD test guideline 248 in June 2019. The purpose of the test is to measure the capacity of a chemical to activate or inhibit the transcription of a genetic construct (THb/ZIP-GFP *X. laevis* eleutheroembryos) either by binding to the thyroid hormone receptor or by modifying the amount of thyroid hormone available for transcription.

Two other projects under the lead of France and co-lead of UK and France, respectively, focus on assays for screening the endocrine disruption potential using genetically modified organisms. The EASZY assay is based on the use of the transgenic cyp19a1b-GFP zebrafish embryos for the detection of endocrine active substances acting through the estrogen pathway (Brion *et al.*, 2012). After the first commenting round, the EASZY protocol and validation report will be amended.

The Rapid Androgen Disruption Adverse outcome Reporter (RADAR) Assay aims at screening potential androgen and steroidogenesis disrupting chemicals using fluorescent medaka fry (Spiggin-GFP Medaka) immediately after hatch. The ring test is ongoing.

#### 5.6.6 New Test Guideline on a Fish Cell Line Acute Toxicity Test

This new project is co-led by Switzerland and Norway and aims at drafting a test guideline using the RTgill-W1 (rainbow trout gill cell line) cytotoxicity assay for fish acute toxicity testing. The assay has been developed within the Cefic LRI ECO8 project (CELLSens project; Tanneberger *et al.*, 2013) and a ring trial evaluating the transferability and within-laboratory reproducibility of the test method has been organised by the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) within the follow-up Cefic LRI project ECO8.3-NC3Rs-EAWAG (Fischer *et al.*, 2019). Recently, Natsch *et al.*, 2018 further demonstrated the suitability of the RTgill-W1 cell line for fish acute toxicity predictions of fragrance chemicals.

An ISO guideline (ISO/DIS 21115) using the RTgill-W1 cells for water quality testing was adopted in 2019 (ISO, 2019).

#### READ MORE

- ▶▶ ECO8: Development of a strategy to predict acute fish lethality using fish cell lines and fish embryos: [cefic-lri.org/projects/eco8-development-of-a-strategy-to-predict-acute-fish-lethality-using-fish-cell-lines-and-fish-embryos](https://cefic-lri.org/projects/eco8-development-of-a-strategy-to-predict-acute-fish-lethality-using-fish-cell-lines-and-fish-embryos)
- ▶▶ ECO8.3-NC3Rs-EAWAG: Round-robin test of the RTgill-W1 cell line assay to study its robustness in establishment and inter-laboratory comparability: [cefic-lri.org/projects/eco8-3-nc3rs-eawag-round-robin-test-of-the-rtgill-w1-cell-line-assay-to-study-its-robustness-in-establishment-and-inter-laboratory-comparability](https://cefic-lri.org/projects/eco8-3-nc3rs-eawag-round-robin-test-of-the-rtgill-w1-cell-line-assay-to-study-its-robustness-in-establishment-and-inter-laboratory-comparability)

### 5.6.7 OECD Test Guideline on Cytochrome P450 (CYP) Enzyme Activity Induction

A chemical may have inherent toxicity or become toxic due to its metabolites produced by biotransformation enzymes (*e.g.* CYPs) or its ability to induce biotransformation enzymes that affect its rate of metabolism (Tsaion *et al.*, 2016). Besides detoxifying chemicals or increasing their toxicity due to formation of toxic metabolites, CYP enzymes play a key role in the biosynthesis of endogenous substrates (*e.g.*, steroid hormones, prostaglandins and bile acids; Coecke *et al.*, 1999; Hakkola *et al.*, 2018).

Chemical CYP enzyme activity induction may therefore cause dysregulation of normal metabolism and homeostasis, with potential toxicological effects (Amacher, 2010; Staudinger *et al.*, 2013). For example, induction of CYP enzymes involving PXR, CAR and/or AhR activation has been demonstrated to be indirectly related to increased clearance of thyroid hormones (metabolised by other biotransformation enzymes), perturbing thyroid functions (OECD, 2017b; ECHA and EFSA, 2018). CYP enzyme activity induction alone does not provide information on the full spectrum of the metabolic processes, but it may support integrated approaches to testing and assessment (IATA) where standardised data from various sources are used and interpreted in a structured way to better predict the toxicokinetic and toxicodynamic profile of chemicals (Coecke *et al.*, 2013; OECD, 2016).

In September 2019, an updated draft TG was submitted to OECD based on a validation study coordinated by EURL ECVAM. Details on the method validation can be found in the EURL ECVAM Tracking System for Alternative methods towards Regulatory acceptance (TSAR), including a link to the DB-ALM Protocol No. 194 (SOP and related forms).

The validation study demonstrated the reliability and relevance of the CYP enzyme activity induction method using differentiated human hepatic cells (*i.e.*, cryopreserved differentiated HepaRG cells) by testing

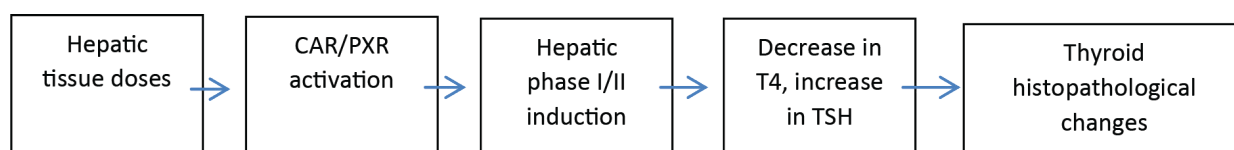
ten chemicals and three reference chemicals (one for each CYP isoform) in three laboratories. To avoid the need for species extrapolation when evaluating the obtained *in vitro* CYP enzyme activity induction validation study data (Bernasconi *et al.*, 2019; EURL ECVAM, 2014) only chemicals with high quality *in vivo* human CYP enzyme activity induction data (available from clinical studies) were selected.

The test guideline on the Cytochrome P450 (CYP) enzyme induction *in vitro* method using human hepatic metabolic cells could play a role in regulatory risk assessment by providing information on *e.g.*, metabolism, chemical-chemical interactions, thyroid disruption, and as indicator of nuclear-receptor mediated dysregulation of biochemical pathways. In particular, when there is evidence that these receptors are involved in pathways for which specific measurement methods are lacking (*e.g.*, induction of Phase II enzymes for glucuronidation and sulfation; Kodama & Negishi, 2013), the human CYP induction *in vitro* method could be used as an initial surrogate/indicator method until validated methods for Phase II enzyme induction become available.

As an example, the induction of enzyme activity in the liver is considered a potential key event in endocrine (thyroid) disruption (ECHA and EFSA, 2018). To study thyroid hormone metabolism following chemical exposure, a battery of validated *in vitro* methods is needed to investigate the effect of induction of biotransformation enzymes and on the clearance levels of thyroid hormones (OECD, 2017b; Figure 5.1). Induction of CYP enzymes has been demonstrated to be indirectly related to increased clearance of thyroid hormones (metabolised by other biotransformation enzymes), perturbing thyroid functions (OECD, 2017b, ECHA and EFSA, 2018).

#### READ MORE

- ▶ Cytochrome P450 (CYP) enzyme induction *in vitro* method using cryopreserved differentiated human HepaRG™ cells: [tsar.jrc.ec.europa.eu/test-method/tm2009-14](https://tsar.jrc.ec.europa.eu/test-method/tm2009-14)



**Figure 5.1:** Example of a postulated mode of action for increase in thyroid hormone metabolism (from ECHA and EFSA, 2018, *EFSA Journal*, 16(6), p. 103, under CC BY-ND 4.0).

### 5.6.8 OECD Draft Updated TG 458 on Androgen Transactivation Assays

EURL ECVAM has taken the lead in developing an OECD test guideline for Androgen Receptor Transactivation Assays (ARTAs). This TG includes one ARTA already validated (the AR STTA using the AR-EcoScreen cell line, developed and validated by Japan and issued as TG 458) and two ARTAs for which the validation was recently completed: an ARTA using the 22Rv1/MMTVGR- cell line (developed and validated by South Korea) and an ARTA using the AR-CALUX cell line. The latter method was submitted by the Dutch company BioDetection Systems (BDS, The Netherlands) to EURL ECVAM for a validation study (see [Section 4.1](#)).

This study, carried out with three participating laboratories of EU-NETVAL, and BDS, was finalised in 2019 and the validation report reviewed by ESAC (see [Section 4.6.2](#)). The draft updated TG 458, encompassing three ARTAs, is currently being reviewed by WNT at OECD and its acceptance and publication is envisioned for 2020.

#### READ MORE

- ▶▶ Draft TG 458: Stably Transfected Androgen Receptor Transactivation Assays for Detection of Androgenic Agonist and Antagonist Activity of Chemicals: [www.oecd.org/env/ehs/testing/DRAFT-TG458-10-07-2019v2.pdf](http://www.oecd.org/env/ehs/testing/DRAFT-TG458-10-07-2019v2.pdf)

### 5.6.9 OECD Guideline on Defined Approaches on Skin Sensitisation

Skin sensitisation is a complex toxicological endpoint that, as yet, cannot be addressed by individual non-animal methods. In 2017, the OECD included in its work program a project on the development of a Guideline on combinations of non-animal methods. These defined combinations of *in chemico*, *in vitro* and *in silico* methods using a fixed data interpretation procedure are called "Defined Approaches" (DAs) (OECD, 2017a,b).

The project, led by EC (through JRC's EURL ECVAM), US (NICEATM/EPA/CPSC) and Canada (Health Canada) (Casati *et al.*, 2018), is supported by an OECD expert group composed of more than 68 members representing 16 member countries.

In 2018, a draft guideline on three DAs (the "2 out of 3" integrated testing strategy from BASF, the sequential testing strategy (STS) and the integrated testing strategy (ITS) for sensitising potency classification,

both from Kao Corporation; OECD 2017c), based on the simpler data interpretation procedures, was circulated to the WNT for commenting. In addition to the guideline, a supporting document reporting the analyses performed to address a number of scientific aspects (see section 5.3.8 of Zuang *et al.*, 2018) was circulated to the WNT.

The feedback provided formed the basis for discussion at a face-to-face meeting of the expert group in December 2018. On that occasion a decision was taken to not include the Kao STS DA in the next draft of the guideline since the Kao ITS DA appears to have better predictive performance and provides information that may be used for potency categorisation. Discussions at the meeting highlighted also the need for a more thorough consideration of the mouse local lymph node assay (LLNA) and human reference data and for a more detailed characterisation of the applicability domain and predictive performance.

The project leads are working on those aspects in view of revising the draft guideline and supporting document for discussion and eventual approval at the 2020 WNT 32 meeting.

The development of a guideline for DAs involves novel aspects that need to be cautiously considered. One example is the definition of transparency and quality assurance requirements for *in silico* data that, as part of DAs described in a guideline, would be covered by Mutual Acceptance of Data, as well as the need to guarantee consistency in the predictions when updates of the software versions are used.

Discussions on the specific issues described above took place in occasion of the Joint meeting of the OECD IATA Case Studies Work Group and the OECD QSAR Toolbox Management Group in November 2018. In particular, quantitative prediction reporting formats (QPRF) of different software packages are being evaluated by OECD experts in order to determine the level of detail that will be required for those DAs that use *in silico* predictions.

### 5.6.10 OECD Feasibility Study for Establishing Test Guidelines for In Vitro Human Hepatic Metabolic Clearance and Metabolite Formation

In 2018, EURL ECVAM published a technical report (Lostia *et al.*, 2016) containing the 'preliminary experimental work towards a representative *in vitro* human



hepatic metabolic clearance method' using a method that was based on knowledge gathered through literature search, an EURL ECVAM call for procedures on *in vitro* human hepatic clearance methods, and input through expert consultation. The method that was proposed in the report as the most suitable method to use, based on the collection of available methods, was similar to the protocol used by Zanelli *et al.* (2012) for many methodological aspects.

The method provided similar clearance values for selected reference chemicals when the results of the two laboratories were compared. These two very similar protocols are considered to be an optimal starting point for the establishment of a test guideline for *in vitro* human hepatic metabolic clearance. Plated primary hepatocytes, plated HepaRG cells and the co-culture methods have been shown to be more reliable and relevant approaches (Bonn *et al.*, 2016) for the determination of clearance values of slowly metabolised chemicals. Some of the above test systems (plated primary human hepatocytes and plated HepaRG cells) have been used successfully in the EURL ECVAM validation study looking at enzyme induction (Bernasconi *et al.*, 2019).

A collaborative effort between the Wageningen Food Safety Research (WFSR), the National Institute for Public Health and the Environment (RIVM), and EURL ECVAM, shows that differences in methodological aspects have impact on intrinsic clearance values obtained with rat or human hepatocytes (Louisse *et al.*, 2019). This would indicate the need for harmonisation of *in vitro* hepatic clearance methods, especially if data derived from these studies are to be used in a regulatory context. The clearance values obtained in different laboratories with variations in method components diverge greatly, spanning more than an order of magnitude for the majority of the chemicals. The results of this analysis emphasise the need for harmonisation of *in vitro* hepatic clearance methods, which is of importance for their use in current regulatory requests (EFSA, 2019; Gouliarmou *et al.*, 2018; Lostia *et al.*, 2016). Such harmonisation in method components is necessary, as well as compliance with the recently issued OECD guidance document on good *in vitro* method practices (GIVIMP; OECD, 2018b).

The insights obtained can be used as a starting point in developing a harmonised protocol that can be integrated in guidance/guideline documents. An OECD project on a feasibility study for establishing

test guidelines for *in vitro* human hepatic metabolic clearance and metabolite formation led by the Netherlands is currently ongoing.

#### 5.6.11 OECD Guidance Document on Developmental Neurotoxicity In Vitro Assays

In most regulatory sectors, including the ones dealing with pesticides and industrial chemicals registration, historical use of the *in vivo* developmental neurotoxicity (DNT) test guideline has been limited. Current challenges include a lack of DNT data and mechanistic information for thousands of chemicals, as well as difficulty in interpreting relevance of animal results to humans. Therefore, EURL ECVAM together with international partners (EFSA, OECD, US EPA, and academia) is developing a strategy to enhance DNT testing using a battery of *in vitro* assays (proliferation, differentiation, synaptogenesis, etc.; see Annex 2) applied to human neuronal/glial models. Preferably, these *in vitro* assays should be anchored to critical neurodevelopmental processes and key events identified in DNT AOPs, facilitating their mechanistic relevance for applying an integrated approach to testing and assessment (IATA).

Specific activities have been initiated to facilitate this OECD project, including the selection of *in vitro* test methods, the selection of a reference set of chemicals that are currently tested using the battery of *in vitro* assays; the development of case studies exemplifying how DNT *in vitro* data can be interpreted. EFSA has created a DNT working group which is intended to develop IATA case studies for hazard identification and characterization where the outcome of the *in vitro* studies will be used as a part of the available evidence (see Section 5.4). These activities will support the development of an OECD guidance document on the use of alternative methods for DNT testing, including guidance on data interpretation (Sachana *et al.*, 2019).

#### 5.6.12 OECD Guidance Document on 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. In 2016, the OECD established an expert group led by the UK to address the development of an integrated approach to the testing and assessment (IATA) of non-genotoxic carcinogens, with the objective of addressing this gap (Jacobs *et al.*, 2016).



Initially, the working group agreed on a definition of non-genotoxic carcinogens in a regulatory context. Moreover, it carried out an analysis of uncertainties related to the two-year cancer bioassay (Paparella *et al.*, 2017). A major endeavor of the working group was to apply the adverse outcome pathway (AOP) concept to various types of cancers, leading to the identification of common mechanisms and modes of action of carcinogenicity.

This has set the basis for the construction of an overarching IATA that would accommodate the different available assays previously collected in a database (Jacobs *et al.*, submitted). This assay database has been assembled through literature search, existing databases investigation, followed by an open call for assays published by the OECD in 2018. At present, the assays are being evaluated for their level of readiness following well defined and agreed assessment criteria.

### 5.6.13 OECD Detailed Review Paper on the Miniaturised Ames Test

An OECD expert working group on the development of the miniaturised Ames test was established in November 2016 with the aim to conduct a retrospective

analysis of data obtained from the different miniaturised versions of the Ames test and a comparative evaluation with Ames results from the classic test method. The ultimate goal of the project, proposed by three co-lead countries Belgium, the Netherlands and the US, is to draft a Detailed Review Paper that provides for a decision following the evaluation of data as to whether the mini Ames test should be considered for inclusion in a revised test guideline (OECD TG 471) or for a separate new test guideline.

Based on a decision from the kick-off meeting held in February 2017, the expert working group launched first an exploratory survey (May 2017) in order to obtain better insights in (i) the use and the amount of data available for the different miniaturised Ames tests; and (ii) the extent to which these data can ultimately be shared. The outcome of the exploratory survey has served a first open call for data sharing in March 2018. For the purpose of the call, the expert working group developed a template for data collection, definition of the test result calls, collection of data and data curation.

In July (2019), a second face-to-face meeting (Figure 5.2) was held to discuss the overall progress of the project and to evaluate and share results of the data



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**Figure 5.2:** Second Meeting of the Expert group for the development of a Detailed Review Paper on the miniaturised versions of the bacterial mutation test (OECD Headquarters, Paris, France, 17-18 July 2019).

analysis. Based on the curated analysis of the data received, the expert group has agreed on a second call for data from specific miniaturised versions of the Ames test in order to conclude on their readiness. Currently, a number of chapters within the DRP are being drafted, covering information on performance; dynamic range of each miniaturised version of Ames test; advantages of various assays; acceptability criteria; quantitative vs qualitative analysis and applicability domain. A draft DRP including the results of the data analysis is expected by June 2020.

#### 5.6.14 OECD Detailed Review Paper on the Retinoid System

A Detailed Review Paper on the retinoid system is currently being drafted under the co-lead of Sweden and the EC. This DRP responds to the need to extend our knowledge on endocrine disruption beyond the estrogen, androgen, and thyroid hormone signalling pathways. The document will present a review of retinoid biology and biochemistry, as well as the evolutionary conservation of the retinoid system across phyla, and cross-species comparisons of retinoid function. It will also depict the opportunities for quantitative measurements of different types of retinoids in different tissues that could be incorporated into new and existing test guidelines. It will consider the role of the retinoid system in organ formation and function.

A first part of this document focusing on male and female reproductive systems will be published as a Nordic Report before being incorporated into the OECD DRP. The project will be further developed through funding by Directorate-General for Environment, under the coordination of the OECD secretariat, to include chapters on the central nervous system and skeletal/craniofacial development.

#### 5.6.15 OECD Guiding Principles on Good Practices for the Distribution/Availability of Protected Elements in OECD Test Guidelines

The Guiding Principles on Good Practices for the Distribution/Availability of Protected Elements in OECD Test Guidelines was approved at the WNT 31 meeting (see Section 5.6.1) and published as number 298 in the OECD Series on Testing and Assessment (<http://www.oecd.org/chemicalsafety/testing/series-testing-and-assessment-publications-number.htm>).

As mentioned in Zuang *et al.* (2018), the document describes good practices for the licensing of protected elements included in OECD TGs and specifies the information required from a test method developer when submitting a proposal for a new TG that contains protected elements. It serves as a guide for organisations (*e.g.*, private companies, universities, research institutes) who developed and claimed intellectual property (IP) on material and techniques that could be readily used to fulfil a regulatory need, if it was integrated in an OECD TG.

By following the guiding principles, test developers join the OECD Test Guideline Programme with increased awareness of expectations and requirements. For instance, an important feature of the Guiding Principles is that the test method developer/proponent (*i.e.*, the declarant) will need to sign a declaration form guaranteeing that he/she will grant licenses to all users of his/her protected material necessary for the execution of the test guideline. The declarant will also ensure that (sub)licenses can be granted on a non-exclusive basis to developers of (*e.g.*, similar) test methods using the protected material covered by Intellectual property. Such (sub-)licences will be on terms and conditions that are fair, reasonable and non-discriminatory (FRAND) as described in the Guiding Principles for Licensing. If the declarant fails to comply with this declaration, the OECD may cancel the test guideline.

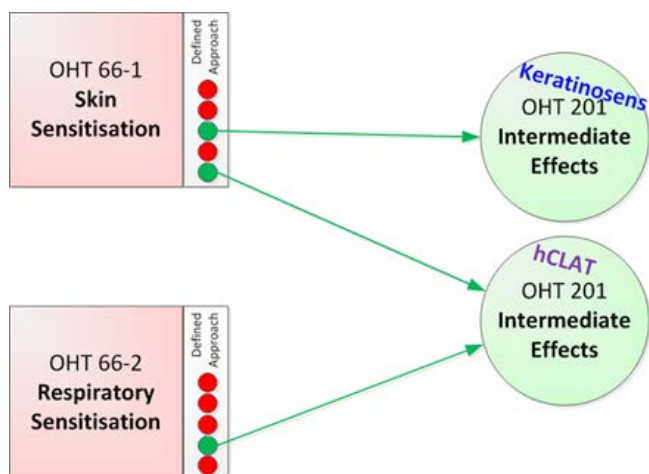
With the FRAND principles in place, market monopoly should not on its own be a driver for requesting the development of performance standards anymore. The WNT considered that there are other cases where performance standards might be useful, *e.g.*, to enable geographical sustainability of supply; thus their development should be decided on a case-by-case basis. It was suggested that criteria be developed for inclusion/exclusion of performance standards, in order to harmonise decisions related to their development.

#### 5.6.16 OECD Harmonised Template 201

The OECD Harmonised Template (OHT) 201 is used to report non-apical observations during *in vitro* testing, *i.e.*, intermediate effects on molecular, subcellular, cell, tissue or organ level that can be relevant to the hazard assessment (and possibly inform adverse outcome pathways). The template is implemented in the IUCLID application, the International Uniform Chemical Information Database, a software application to record,

store, maintain and exchange data on intrinsic and hazard properties of chemical substances. IUCLID is a key software application for both regulatory bodies and the chemical industry who use it in the implementation of various regulatory programmes.

As the entity that originally created the OHT 201 template, EURL ECVAM initiated, and now manages, an OECD/ECHA/JRC process to update and streamline the template with naming and ontology conventions in other initiatives like the AOP Knowledge Base (see Section 5.7.1). Another aim of the OHT 201 review is to facilitate, and ultimately enforce, the reporting of mechanistic data independently from apical considerations, as shown in Figure 5.3 *In vitro* observations made with, e.g., the KeratinoSens and the h-CLAT assays, are reported in OHT 201 and can then be linked to any apical endpoint they serve to underpin. Thus, mechanistic data become reusable in many contexts, just as key events are in AOPs.



**Figure 5.3:** OHT 201 allowing the re-use of mechanistic data across apical endpoints.

#### READ MORE

- ▶▶ OECD Harmonised Template 201: [www.oecd.org/ehs/templates/harmonised-templates-intermediate-effects.htm](http://www.oecd.org/ehs/templates/harmonised-templates-intermediate-effects.htm)

#### 5.6.17 eChemPortal

The OECD eChemPortal provides free public access to information on properties of chemicals:

- Physical Chemical Properties
- Ecotoxicity
- Environmental Fate and Behaviour
- Toxicity



eChemPortal allows simultaneous searching of reports and datasets by chemical name and number, by chemical property, and by classification according to the Globally Harmonised System of Classification and Labelling of chemicals (GHS). Direct links to collections of chemical hazard and risk information prepared for government chemical programmes at national, regional and international levels are obtained. Classification results according to national/regional hazard classification schemes or to GHS are provided when available. In addition, eChemPortal provides also exposure and use information on chemicals.

The JRC/EURL ECVAM is a member of the Steering Group for the management and further development of the eChemPortal, and as such provides expertise in both the subject matter (e.g., chemical risk assessment and study results) and the Information and Communications Technology (ICT) aspect.

In 2019, EURL ECVAM started to prepare the application for its Endocrine Active Substances Information System (EASIS, see Section 3) to become eChemPortal participant. In parallel, EURL ECVAM volunteered to draft an OECD guidance document for other potential participants, which will help them to address administrative and technical issues when applying for participant status.

#### READ MORE

- ▶▶ eChemPortal: [www.echemportal.org](http://www.echemportal.org)

#### 5.7 Activities in the 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 Adverse Outcome Pathway (AOP) programme in 2012.

Currently the AOP programme is the dominant activity of EAGMST, with four different sub-groups working on: AOP development methodology and practice; scientific peer review; AOP knowledge-base; and education, training and outreach.

The EAGMST work programme also has ongoing projects 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 biokinetic (PBK) models for regulatory applications (jointly with the WPHA; see [Section 5.5](#)). In recent years, EAGMST has increased its engagement and cooperation with the OECD's Working Party on Hazard Assessment (see [Section 5.5](#)) and the OECD Test Guidelines Programme (see [Section 5.6](#)).

### 5.7.1 Update on the AOP-Knowledge Base

An Adverse Outcome Pathway (AOP) is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect. AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning. The OECD had launched a programme for the development of AOPs in 2012, and, in parallel to the instigation of the overall AOP initiative, started the AOP-Knowledge Base (KB) project. Within this project, JRC/EURL ECVAM contributes to ICT design and analysis know-how, and co-manages the project together with the US EPA.

The main AOP-KB module available to the public is the AOP-KB Wiki, a system that organises, via crowd-sourcing, the available knowledge and published research into a verbal description of individual pathways, via a user friendly wiki interface.

The current version of the AOP-KB Wiki is fully functional, and a complete review and possible redesign of the AOP-KB as version 2.0 is foreseen for 2020-2021. To bridge the gap between the current version and the future version 2.0, EURL ECVAM decided in 2018 to issue and manage an evolutive maintenance contract for the current AOP Wiki: Bug fixes, adaptations to the ever changing system environment,

as well as quick improvements by introducing new functionalities, will make sure that the AOP Wiki user experience will not deteriorate.

#### READ MORE

▶▶ AOP Knowledge base: [aopkb.oecd.org](http://aopkb.oecd.org)

### 5.7.2 Development of Adverse Outcome Pathways and Networks Relevant to Neurotoxicity

During the last year EURL ECVAM finalised an AOP related to the disruption of the synthesis of thyroid hormone entitled "Inhibition of Na<sup>+</sup>/I<sup>-</sup> symporter (NIS) leads to learning and memory impairment". This AOP passed an external OECD reviewing process, was endorsed by WNT/WPHA and published by OECD (Rolaki *et al.*, 2019).

Assembly of single AOPs into networks through interconnected pathways and identification of common key events in several AOPs are likely to represent more realistic descriptions of the complexity of disease pathophysiology. Such AOPs network(s) reflect more realistically that single molecular initiating events (MIE) can trigger multiple adverse outcomes and that multiple MIEs can lead to the same adverse outcome (Bal-Price *et al.*, 2018).

Recently, EURL ECVAM together with Liverpool John Moores University (UK) developed an AOP network for human neurotoxicity (see [Box 5.1](#)). A four-step workflow which describes general design principles was established linking nine linear AOPs into an AOP network (Spinu *et al.*, 2019). The developed AOP network was also published in the AOPXplorer repository. The AOP network provides a basis for further development of *in vitro* test methods and guides the quantitative modelling of key events (KEs) and key event relationships (KERs), facilitating diverse regulatory applications.

#### READ MORE

▶▶ AOP 54 - Inhibition of Na<sup>+</sup>/I<sup>-</sup> symporter (NIS) leads to learning and memory impairment: [aopwiki.org/aops/54](http://aopwiki.org/aops/54)

▶▶ AOPXplorer repository: [apps.cytoscape.org/apps/aopexplorer](http://apps.cytoscape.org/apps/aopexplorer)

### 5.7.3 Transcriptomics and Metabolomics Reporting Framework

To facilitate the use of transcriptomics data in regulatory decision-making in the absence of a test



guideline, a comprehensive reporting framework is necessary to thoroughly document the components of a transcriptomic study (Sauer *et al.*, 2017). For this reason, under the auspices of EAGMST at the OECD, a project was initiated, which aims to develop frameworks for the standardisation of reporting of 'omics data generation and analysis, to ensure that all information required to evaluate transcriptomic

study design, quality and applicability to regulatory decision-making processes is available.

The project has initially focused on the development of a guidance document for reporting of transcriptomics data. The transcriptomics reporting framework (TRF) is characterised by a modular structure including an introductory and an experimental module that

## Box 5.1

### Biological networks – the key to a better understanding of chemical neurotoxicity

Studying the effects of chemicals on the brain provides the basis for reducing disorders of learning and memory in children, as well as cognitive disorders in adults, such as Alzheimer's and Parkinson's.

In the fight against these diseases, EURL ECVAM scientists are involved in multiple collaborative projects aimed at better assessing the possible effects of chemicals on the nervous system. Early life exposures to certain chemicals, such as pesticides, may have long-term adverse health consequences for the adult brain. In addition, aging and neurodegenerative diseases such as Alzheimer's and Parkinson's pose major challenges for societies. Animal models are limited. Relevant and reliable non-animal approaches, such as cell-based (*in vitro*) methods and computational (*in silico*) models, provide a promising means of mechanistic understanding the chemical contribution to these complex disease.

#### Challenge

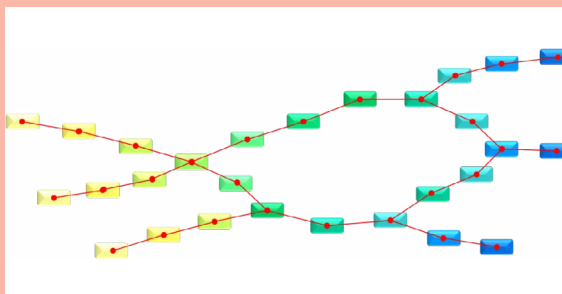
A special issue of *Toxicology and Applied Pharmacology* (Fritsche & Price, Eds., 2018), co-authored by a EURL ECVAM scientist, describes a range of non-animal methods for assessing the potential of chemicals to cause developmental neurotoxicity (DNT). Biological processes underlying neurotoxicity are complex. It is unlikely though that any of these alternative methods will provide a standalone solution for DNT testing. A similar challenge applies to the assessment of chemical neurotoxicity in adults. It is therefore necessary to develop solutions, called Integrated Approaches to Testing and Assessment (IATA), based on the combined use of multiple non-animal methods. One way of guiding the optimal design of IATA for neurotoxicity is to leverage existing knowledge on Adverse Outcome Pathways (AOPs) that describe how chemical perturbations at the molecular level may cause a series of cascading events that ultimately result in neurotoxicity.

#### Collaboration

In a collaboration with Liverpool John Moores University, EURL ECVAM has combined existing AOPs to form an AOP network for human neurotoxicity. The study illustrates how established concepts from network science can be used to identify the most common key events underlying neurotoxicity. Non-animal methods that model these pathways are therefore expected to be the most informative and useful in chemical safety assessment.

The work was carried out in the context of the in3 project, an EU-funded Innovative Training Network of early stage researchers developing *in vitro* and *in silico* tools for chemical safety assessment.

Source: EU Science Hub (<https://europa.eu/Rn49Ft>.)





will be independent of the 'omics platform used. In addition, 'omics' platform-dependent modules on the description of sample processing procedures, methods used to collect raw data and methods used to generate processed data and identify differentially expressed genes. A first draft of several of the modules has been produced. Among others, this includes the experimental module, which drafting was coordinated by EURL ECVAM, and a module on the use of microarrays to which EURL ECVAM contributed. A similar activity related to the reporting of metabolomics data has also started.

#### 5.7.4 AOP Framework Analysis Study

The goal of the Adverse Outcome Pathway (AOP) framework is to compile and synthesise the wealth of biological information collected in recent years, such that it can be transparently and efficiently employed for decision-making. The AOP Framework was originally introduced and defined in 2010:

*An Adverse Outcome Pathway (AOP) is a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment (Ankley et al., 2010).*

The AOP Framework gained momentum in recent years, especially in the regulatory environment, driven by the OECD. One main motivation for the further development of the AOP framework was to steer toxicology away from mainly observing (in animal tests) towards understanding and predicting (through *in vitro* and *in silico* tests). The Framework was, for example, instrumental in the replacement of animal tests for the testing of skin sensitisation under the EU REACH regulation. While the concept is now widely known and appreciated in regulatory and scientific circles, some shortcomings of the framework, such as the need for harmonisation, re-assessment and continuous updating, as well as for alerting about pitfalls, misuses and limits of applicability, are also recognised.

In other words, nine years after its introduction, the AOP framework needs to be reviewed from the standpoint of multiple stakeholder communities in order to make sure that it is still, and will remain, fit for purpose. To this end, the JRC/EURL ECVAM, in collaboration with the OECD secretariat, is managing

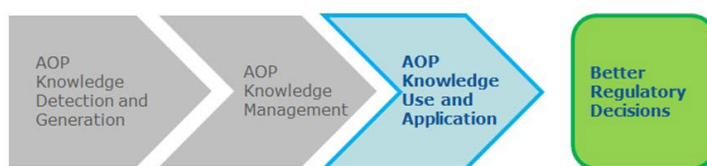
a study to assess the framework's ability to arrive at better regulatory decisions. This study is executed in collaboration with an external service provider.

AOP knowledge is identified, generated, processed, managed, used and applied in three steps to improve regulatory decisions, as shown in Figure 5.4:



**Figure 5.4:** The three steps in the AOP workflow.

While the AOP knowledge generation and management communities are equally important for the success of the framework, the study concentrates on stakeholders belonging to the AOP Knowledge Use and Application population, see Figure 5.5:



**Figure 5.5:** Focus on the AOP knowledge use and application.

The principal user group for the AOP Framework and associated knowledge in the context of this study consists of (regulatory) toxicologists, risk assessors and risk managers, directly involved in evaluating chemical safety and implementing risk mitigation measures. The responsibilities and needs within this group can be very diverse, depending upon jurisdiction, policy context, legislative mandate, industry sector and company size, so it was decided to analyse the AOP Framework status in the six user groups (*i.e.*, regulatory toxicologists, risk assessors and risk managers from both industry and governments, respectively) shown in Figure 5.6:



**Figure 5.6:** Six stakeholder groups relevant for the AOP Framework Study.

The main purpose of the study is to ensure the framework's sustainability, increase stakeholder involvement and engagement and increase the actual applicability and regulatory use.

This purpose will be achieved via four tasks, see also [Figure 5.7](#):

1. describing the AOP Framework from the perspective of the relevant stakeholders, market analysis-like
2. reviewing relevant literature (peer-reviewed and not)
3. gathering evidence, engagement with relevant stakeholders via surveys, interviews and focus groups
4. delivering a final report describing the outcome of tasks 1, 2, and 3, drawing conclusions and giving suggestions for prospects and opportunities.



**Figure 5.7:** Deliverables of the AOP Framework Study.

Tasks 1 and 2 were concluded in June 2019, task 3 is ongoing (a large-scale survey among affected stakeholders was launched at the time of writing), and the final report is expected in 2020.

### 5.8 VICH Guideline on Vaccines: Harmonisation of Criteria for Waiving of Laboratory Animal Batch Safety Testing of Vaccines for Veterinary Use

Since many years, EURL ECVAM is working on behalf of EMA with experts of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) on the development of VICH guidelines on harmonisation of criteria to waive the target animal / laboratory animal batch safety testing for veterinary vaccines (see section 5.7 in *Zuang et al., 2018*).

The draft VICH GL59 on Harmonisation of Criteria for Waiving of Laboratory Animal Batch Safety Testing of Vaccines for Veterinary Use entered public consultation in 2019 and it is planned to publish the final guideline in 2020.

In line with two VICH guidelines addressing the target animal batch safety test for inactivated (VICH GL50(R); VICH, 2017b) and live vaccines (VICH GL55; VICH, 2017a), manufacturers can apply for waivers after having demonstrated that safe and consistent production has been established.

It should be noted that general safety tests for batch release of human and veterinary vaccines are no longer required in Europe and were deleted from European Pharmacopoeia monographs several years ago (abnormal toxicity test; *Schwanig et al., 1997*) or recently (target animal batch safety test; EDQM, 2012). Since these tests may still be required outside of Europe, European manufacturers may need to carry out these tests when exporting to third countries.

#### READ MORE

- ▶ Draft guidelines - Harmonisation of criteria to waive Laboratory Animal Batch Safety Testing for vaccines for veterinary use (VICH GL 59): [vichsec.org/en/activities/concept-papers/active-draft-guidelines.html](http://vichsec.org/en/activities/concept-papers/active-draft-guidelines.html)

### 5.9 Promoting the Regulatory Acceptance of 3Rs Approaches in the Frame of EMA

EMA suspended/reduced the work of all non-product related working groups, *e.g.*, the activities of the J3Rs Working Group, due to the preparation for the Brexit and move of the agency to Amsterdam. Further information on the accomplished work of J3Rs Working Group is available here: <https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/expert-group-3rs>.

Nevertheless, in 2019, EMA has developed a regulatory strategy to 2025 covering both human and veterinary medicines (EMA, 2018). The draft strategy aims to build a more adaptive regulatory system that will encourage innovation in human and veterinary medicine and keep pace with scientific and technological advances while ensure the sound assessment of ground-breaking, more complex therapies. Key messages from the analysis of the public consultation were discussed in two multi-stakeholder workshops, for human and veterinary medicines, held in November and December

2019, respectively. One full session of the veterinary medicine workshop was dedicated to “Reinforce and further embed application of the 3Rs principles”, which was among the core recommendations prioritised by the stakeholders.

#### READ MORE

- ▶▶ EMA draft 'Regulatory Science to 2025': [www.ema.europa.eu/en/about-us/how-we-work/regulatory-science-2025](http://www.ema.europa.eu/en/about-us/how-we-work/regulatory-science-2025)

### 5.10 Activities of EPAA to Promote 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.



## The European Partnership for Alternative Approaches to Animal Testing

The partners are committed to pooling knowledge and resources to accelerate the development, validation and acceptance of alternative approaches to animal use in regulatory 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. JRC, represented by EURL ECVAM, is one of the Commission services together with DG GROW, DG ENV, DG SANTE and DG RTD, that are members of the EPAA. 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 described in the sections below. The partnership also engages in training and dissemination activities (see [Section 6.4](#)). An overview of the EPAA activities in 2018 and 2019 is given in the EPAA Annual Reports (EPAA, 2018,2019).

#### READ MORE

- ▶▶ EPAA, European Partnership for Alternative Approaches to Animal Testing (EPAA): [europa.eu/!yf93TH](http://europa.eu/!yf93TH)

#### 5.10.1 Optimised Evaluation of Skin Sensitisation

The results of the EPAA skin sensitisation team's collaborative project (see section 5.7.1, Zuang *et al.*, 2018) to explore the performance of three methods based on 3D skin models (Reconstructed human Epidermis (RhE) IL-18 test method, SensCeeTox and SENS-IS) for predicting skin sensitisation hazard and potency categorisation by testing a set of 12 difficult substances have been published (Mehling *et al.*, 2019). Overall, these preliminary results indicate that test methods using RhE can assist in a weight of evidence approach to identify the skin sensitisation potential of substances for which testing in aqueous medium proves difficult. Despite the limited dataset, the study provided also an indication of which tests appear to have better accuracy.

Moreover the team held a workshop in partnership with Cefic LRI and Cosmetics Europe at the ECHA premises in Helsinki in February 2019 to share experience on the latest available methods and approaches, and their applicability or limitations in meeting regulatory safety requirements for skin sensitisation. The experience shared by industry and regulators, as well as the conclusions/recommendations from the workshop were published (Basketter *et al.*, 2019).

#### READ MORE

- ▶▶ Training and Knowledge-sharing Workshop : "Applying non-animal strategies for assessing skin sensitisation": [europa.eu/!xk94ww](http://europa.eu/!xk94ww)

#### 5.10.2 Waiving of the Two-year Carcinogenicity Studies

The EPAA is funding a project to provide evidence that data from three-month repeated dose toxicity studies can be leveraged with Mode-of-Action (MoA) information to enhance the prediction of carcinogenic potential of agrochemicals. Literature search for more than 400 agrochemicals was conducted which resulted in approximately 160 agrochemicals that have been shown to induce cancer in rodents via non-genotoxic mechanisms. A subsequent review of mechanistic information of these chemicals provided in carcinogenicity reports led to the identification of the most

common MoA networks involved in tumour formation which include liver enzyme induction, changes to the hormone system, oxidative stress and sustained cytotoxicity (Heusinkveld *et al.*, in preparation).

In collaboration with industrial partners, the proposed novel approach was evaluated using case studies with one of the MoA networks. These formed the basis for the discussions held at a workshop aiming at discussing a mechanism-based approach for cancer risk assessment of agrochemicals so that, ultimately, the need for the two-year carcinogenicity bioassay would be reduced or even removed. This EPAA workshop was organised by the project co-chairs (scientists from the Dutch National Institute for Public Health and the Environment (RIVM), industry and EURL ECVAM) and the EPAA Secretariat in June 2019 to seek feedback from regulatory, industry and academic experts on the proposed approach.

Take-home messages from various break-out group discussions indicated a consensus view that it is worth pursuing with this approach, which also complements other international initiatives, such as those driven by OECD on an IATA for non-genotoxic carcinogens and by US EPA in relation to agrochemicals. This workshop represented the first event that brought together representatives from the different ongoing international initiatives in the area.

Participants were confident that a toolbox of *in silico*, *in vitro*, and short-term *in vivo* models could be used to identify most common MsAs involved in carcinogenesis, the results of which could be used instead of the two-year bioassay. Furthermore, the participants discussed the outline of a roadmap for improving, evaluating and implementing the mechanism-based approach.

#### READ MORE

- ▶▶ Flash report of EPAA expert workshop on “Mechanism-based approach to cancer risk assessment of agrochemicals incorporating 3Rs principles”: [europa.eu/FB88TY](https://europa.eu/FB88TY)

### 5.10.3 Acute Toxicity

As previously reported (see section 5.7.3 in Zuang *et al.*, 2018), a data mining exercise is carried out as part of the EPAA acute toxicity project, which aims to identify clinical signs predictive of mortality at higher dose levels. The project was initiated in 2015 and is done in close collaboration with the UK National Centre for the

Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and the UK Chemicals Regulation Directorate (CRD). In order to increase the number of studies that meet the acceptability criteria, the team continue to access data of the UK CRD. New studies have been coded and analysed. The need to expand the dataset is under consideration including the option of exploring additional data sources (*i.e.*, from EPAA member companies).

### 5.10.4 Clostridial Vaccine Project

This project evaluates Vero cell based assays to replace the *in vivo* Minimum Lethal Dose (MLD) and Total Combining Power (TCP) assays required for in-process control of *Clostridium septicum* vaccines.

The initial collaborative study (EDQM BSP I/II) demonstrated that the *in vitro* assays are repeatable and reproducible and that there is excellent overall concordance with the mouse tests (Sinitskaya *et al.*, 2016). The validation work is carried out under the EDQM Biological Standardisation programme (see Section 4.3).

During a follow-up study (BSP130 III) with 14 participants the Vero cell assays were optimised and three *in vitro* assays are now available to replace the three *in vivo* tests carried out during in-process testing, which are the minimum lethal dose (MLD) for antigen quantification and residual toxicity testing, as well as the total combining power (TCP) for quantification of the toxoid antigenicity. The final report of the study will be published soon and a dissemination workshop is planned for October 2020.

The European Pharmacopoeia experts responsible for veterinary vaccines (Group 15V) are currently revising monographs for vaccines based on cytotoxic clostridial antigens to introduce the Vero cell line based methods.

#### READ MORE

- ▶▶ EDQM Biological Standardisation programme (BSP): [www.edqm.eu/en/work-programme-bsp](http://www.edqm.eu/en/work-programme-bsp)

### 5.10.5 Human Rabies Vaccine Project

The project aims at replacing the *in vivo* test carried out for batch potency testing of human rabies vaccines with an *in vitro* antigen quantitation test [*e.g.*, an enzyme-linked immunosorbent assay (ELISA)



quantifying the glycoprotein-G content in rabies vaccines; (Morgeaux *et al.*, 2017)].

The BSP148 project "Validation of a rabies *in vitro* potency assay" was launched in 2017 (see Section 4.3) and has attracted global interest during 2018. There are 28 participants in the study from the European Union, China, India, Indonesia, Africa, Japan, and America. Phase I of the study (transfer of the method to lead laboratory, identification of laboratories, collection of appropriate samples, identification and availability of critical reagents as monoclonal antibodies, material transfer agreements, etc.) was finalised in 2019. Phase II, the actual testing of the samples started in autumn 2019.

The ELISA and the study were presented at a NICEATM and International Alliance for Biological Standardization – North America (IABS) workshop on "Achieving scientific and regulatory success in implementing non-animal approaches to human and veterinary rabies vaccine testing" (Washington, 16-17 October 2018; Poston *et al.*, 2019).

#### READ MORE

- ▶▶ EDQM Biological Standardisation programme (BSP): [www.edqm.eu/en/work-programme-bsp](http://www.edqm.eu/en/work-programme-bsp)

### 5.10.6 Harmonisation on Biologicals

This EPAA project aims at progressing harmonisation of requirements for batch testing of vaccines and other biological products at a global level.

Following up on the recommendations of a workshop held in 2015 (Schutte *et al.*, 2017) in collaboration with workshop participants and relevant stakeholders, achievements are to be reported for general batch safety tests such as the abnormal toxicity test (ATT) and the target animal batch safety test (TABST). For example, the ATT has been deleted from 49 European Pharmacopoeia monographs (EDQM, 2017). Revised monographs are in force since 2019. References to VICH GL50 and 55 (VICH, 2017a,b) establishing criteria for waiving the TABST have been included into the "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018".

On several occasions, members of the project team addressed the deletion of the innocuity/abnormal toxicity test from WHO recommendations. Eventually, at the 69<sup>th</sup> meeting of the WHO Expert Committee

on Biological Standardization (ECBS), WHO experts recommended "*immediate discontinuation of the inclusion of the innocuity test in all future WHO Recommendations, Guidelines and manuals for biological products published in the Technical Report Series...*" (WHO, 2019).

At present, the group is working on the promotion of alternatives to the rabbit pyrogenicity test, which are the bacterial endotoxin test or monocyte activation test, and possible barriers to their use.

Linked to general safety tests, the Humane Society International organised a seminar in March 2019 summarising the progress achieved so far and addressing further steps towards their deletion (Viviani *et al.*, 2020).

#### READ MORE

- ▶▶ Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018: [www.oie.int/standard-setting/terrestrial-manual/access-online](http://www.oie.int/standard-setting/terrestrial-manual/access-online)
- ▶▶ WHO Expert Committee on Biological Standardization (ECBS): 69<sup>th</sup> Meeting – deletion of innocuity/abnormal toxicity test: [apps.who.int/iris/bitstream/handle/10665/325184/9789241210256-eng.pdf?ua=1](https://apps.who.int/iris/bitstream/handle/10665/325184/9789241210256-eng.pdf?ua=1)

### 5.10.7 Physiologically Based Kinetic (PBK) Modelling in Safety Assessment

A new project entitled "Tools to Support Application of Physiologically Based Kinetic (PBK) Modelling in Safety Assessment" was proposed by EURL ECVAM and endorsed by EPAA. The main project aims are to: (1) undertake and publish a complete systematic review of existing published PBK models in rats and humans in order to provide an updated resource for PBK model developers and users; (2) characterise the chemical space coverage of existing PBK models and compare this with the chemical space of food additives, drugs, cosmetics, pesticides and industrial chemicals, which will help to identify where gaps exist in current knowledge and availability of PBK models; (3) investigate a range of similarity assessment metrics (*e.g.*, chemical fingerprints) to determine which individual or consensus method leads to the most appropriate selection of source chemicals to predict the behaviour/properties of target chemicals and support PBK model development; (4) develop a freely available software tool to assist the identification of appropriate chemicals to use as templates via an automated workflow.



The project will run for three years and is led by scientists at the Liverpool John Moores University, and follows up on recommendations made by Laroche *et al.* (2018), Madden *et al.* (2019), and Paini *et al.* (2019). These last two references are part of a Special Issue in the Computational Toxicology journal entitled Development and Applications of Physiologically-Based Kinetic (PBK) Models, for which JRC was invited as guest editor.

#### READ MORE

- ▶ Computation Toxicology Special Issue - Development and Applications of Physiologically-Based Kinetic (PBK) Models: [www.sciencedirect.com/journal/computational-toxicology/special-issue/104KOMVT820](http://www.sciencedirect.com/journal/computational-toxicology/special-issue/104KOMVT820)

### 5.10.8 Quantitative *In Vitro* to *In Vivo* extrapolation (QIVIVE)

Testing an algorithm for quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) is a new EPAA project that will start in 2020 and will be led by the UK Health and Safety Executive (HSE) Science and Research Centre. EURL ECVAM will be part of the monitoring expert team with ECHA, EFSA and industry.

The aim of this project is to test the effectiveness of a computational algorithm developed to convert *in vitro* concentration-response data to *in vivo* dose-response data (known as quantitative *in vitro* to *in vivo* extrapolation, QIVIVE) and will be applicable to a wide range of chemicals from different regulatory areas.

The project will translate *in vitro* concentration-response relationships to *in vivo* dose-responses, determine *in vivo* benchmark dose (BMD) values from the translated data, and compare the predicted *in vivo* BMD to existing experimental BMD values used in chemical safety assessments by a regulatory agency. This project builds on an earlier EPAA and Cefic LRI supported project that developed a mathematical modelling software application (R-Vis) for retrospective exposure prediction from biological monitoring data (see [Section 2.12.5](#)).

### 5.10.9 EPAA Blue Sky Workshop on New Ideas for Systemic Toxicity

The EPAA organised a Blue Sky Workshop, titled “New ideas for systemic toxicity” in Brussels, Belgium, on 1 to 2 October 2019. The workshop brought together 31 experts from Europe and North America representing cross-sector industries and regulatory agencies, the European Commission and academia. The overall

goal of the Workshop was to formulate strategic research elements to derive a list of recommended actions to identify new ideas for repeated dose systemic toxicity. The primary focus was on how specific scientific areas and new approaches could be applied in a regulatory context.

A number of New Approach Methodologies (NAMs) that have evolved over the last decade that can provide insights into the systemic effects of chemicals were discussed and these included: computational chemistry and biochemistry methods (capable of handling the 3D structure of potential ligands); high throughput and content techniques; big data from omics including “deep proteomics”; microphysiological systems (including organ-on-chip); and *in silico* physiological models and virtual organs.

The workshop recognised that much progress has been made in recent years on many fronts. It was felt overall therefore, that research programmes are essentially on the right track. Successful NAM-based assessment workflows will likely be based around mechanistic thinking that can optimally combine computational and *in vitro* methods in a fit-for-purpose manner. Participants also acknowledged the significant level of stakeholder involvement and cooperation required to ensure proper context-specific problem formulation and a collective approach to gaining experience and confidence in emerging solutions.

#### READ MORE

- ▶ “Blue sky” workshop on systemic toxicity - flash report: [europa.eu/ITd79Hp](http://europa.eu/ITd79Hp)

### 5.11 UN Sub-Committee on the Transport of Dangerous Goods (TDG) and on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS)

#### 5.11.1 Revisions of the UN TDG Model Regulations

In the 21<sup>st</sup> revision of the UN TDG Model Regulations, the application of non-animal methods for the classification of corrosive chemicals and mixtures in different packing groups for safe transport worldwide, was introduced based on the revised OECD TG No 431 using reconstructed human epidermis.

The European Union, on the initiative of JRC/EURL ECVAM, presented further revisions to the same

**Box 5.2****No more animal tests needed to guarantee safe transport of corrosives**

The United Nations (UN) subcommittee on the Transport of Dangerous Goods has decided to allow testing on engineered skin rather than on animals to identify correct packing requirements for corrosive chemicals. The proposal was made by the JRC/EURL ECVAM on behalf of the European Union.

Corrosives are chemicals that cause irreversible damage to skin. They can also destroy goods to which they come in contact or even the means that transports them. The most severely corrosive chemicals can only be transported in very limited quantities and when the packaging rules set by the UN Model Regulations are respected. Animal testing has typically been required to distinguish between the potency of corrosive chemicals, to ensure safety of people, property and the environment while transporting goods worldwide. The JRC/EURL ECVAM has been involved for many years in the development, validation and regulatory acceptance of *in vitro* methods, such as the use of engineered skin models, allowing classification of corrosives while avoiding painful animal testing.

*The UN decision*

Based on a JRC/EURL ECVAM proposal presented on the behalf of the European Union, the UN subcommittee on the Transport of Dangerous Goods agreed in December 2018 to include non-animal testing in the criteria for classification of corrosives in the 21<sup>st</sup> revision of the UN Model Regulations. In the EU, as in the vast majority of countries worldwide, the text will be directly transposed into national legislation. This is because worldwide transport must follow the same rules to facilitate international trade and guarantee agreed levels of safety as goods pass national borders.

*Decrease in animal use without decrease in safety*

The revision of the classification of corrosives in the UN Model Regulations opens the door for replacement of animal testing with more reliable *in vitro* methods, safe-guarding transport by road, rail, sea and air.

Source: EU Science Hub (<https://europa.eu/!HR34BF>)

classification criteria in 2019 (see Box 5.2). Firstly, application of packing group 1, the most severe one, was proposed, if the test results indicate that a substance or mixture is corrosive, but the test method does not allow discrimination between packing groups and no other test results indicate that a less severe packing group should be applied.

This revision allows for no further testing, disregarding the method applied, once a substance or mixture has shown to be corrosive. Secondly, it was proposed that a negative result from an *in vitro* skin irritation test should be conclusive for no classification as corrosive.

The members of the UN subcommittee on TDG agreed to the two proposals and these revisions will be included in the 22<sup>nd</sup> revision of the UN TDG Model Regulations.

**READ MORE**

- ▶ UN TDG (2019) 21<sup>st</sup> revision of the United Nations Transport of Dangerous Goods Model Regulations (UN TGD): [www.unece.org/ru/trans/danger/publi/unrec/rev21/21files\\_e.html](http://www.unece.org/ru/trans/danger/publi/unrec/rev21/21files_e.html)

**5.11.2 Activities of the GHS Informal Working Group on the Use of Non-animal Test Methods**

The Working Group started its activities in 2017, and during the first biennium the GHS chapter on skin corrosion/irritation was revised to include criteria on how to apply non-animal methods when classifying substances and mixtures. The resulting text is included in the 8<sup>th</sup> revision of GHS (UN GHS, 2019).

### Box 5.3

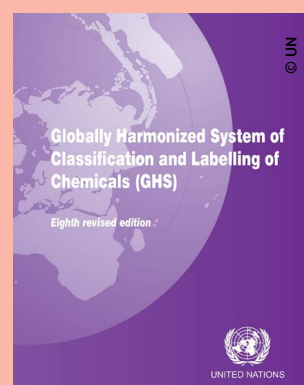
#### Better ways to identify chemicals that can damage our skin

Under the United Nations (UN) Globally Harmonised System of classification and labelling of chemicals (GHS), data from non-animal test methods can now be used to classify chemicals that can corrode or irritate skin on contact.

Non-animal approaches based on *in vitro* methods provide stand-alone solutions to determine if a chemical is corrosive or irritating to the skin. They have gained international acceptance for regulatory application, primarily as OECD test guidelines, due to their high degree of reliability and human relevance. The recognition of these internationally accepted alternative methods within the GHS will have an impact worldwide in both protecting people from harmful chemicals while avoiding unnecessary animal testing.

##### *The non-animal working group at the UN*

A non-animal working group was established by the UN subcommittee on GHS almost 3 years ago. Since then, the JRC has been an active member representing the European Union together with the European Chemicals Agency (ECHA) and the Commission Directorates-General for Environment (DG ENV) and for Internal Market, Industry, Entrepreneurship and SMEs (DG GROW). The group is co-chaired by The Netherlands and the United Kingdom. After publication of the revised classification criteria for skin effects in the 8<sup>th</sup> revision of GHS, the working group is now elaborating the criteria for serious eye damage and eye irritation, aiming for a final agreement on the introduction of non-animal methods for classification of ocular effects in the 9<sup>th</sup> revision.



##### *GHS & EU legislation*

The EU Regulation on Classification, Labelling and Packaging of substances and mixtures (CLP) implements the GHS within the EU. It is one of the most important pieces of crosscutting chemicals legislation to ensure a high level of protection of human health and the environment, because many other legal acts such as authorisation of pesticides or workers protection refer to its classifications. The revision of the GHS has an impact worldwide and when implemented in the EU it becomes the most important legal act for the safe use of chemicals in the workplace and in the home.

Source: EU Science Hub (<https://europa.eu/!CQ64gT>)

In the current biennium the revision of the next chapter, severe eye damage/eye irritation, is planned under the lead of JRC/EURL ECVAM in collaboration with NL and UK, the Working Group co-chairs. In July 2019, the Working Group discussed issues related to this specific endpoint and based on the outcome, EURL ECVAM started to draft the text of the revised chapter aiming on final agreement by December 2020. One of the issues that will need further consideration is the lack of *in vitro* methods directly identifying Category 2, eye irritation, while there are several methods to determine whether a substance or mixture warrants classification in Category 1, severe eye irritation or no classification for eye effects. Therefore integrated

testing approaches, combining information from more than one *in vitro* method in a purposeful way, considering the predictive capacity of the different methods, can be applied for classification in Category 2. The Working Group will evaluate examples together to reach a common understanding prior to finalise the text on this specific issue.

##### READ MORE

- ▶▶ UN GHS (2019) Globally harmonised system for classification and labelling for chemicals (GHS): [www.unece.org/index.php?id=51896](http://www.unece.org/index.php?id=51896)

### 5.12 ICCR Joint Regulators-Industry Working Group on "Integrated Strategies for Safety Assessments of Cosmetic Ingredients"

Animal testing for the safety assessment of cosmetic ingredients and products has been completely banned in the EU. The safety of cosmetics therefore needs to be ensured by new methods and strategies. This is in line with a general shift in toxicology from testing in whole organisms as a “black box” towards understanding the mechanisms behind adverse effects, allowing for a more informed and targeted evaluation of chemicals.

The *ad hoc* ICCR Joint Regulators-Industry Working Group (JWG) on "Integrated Strategies for Safety Assessments of Cosmetic Ingredients" has described in recent publications the overarching principles that underpin the integration of new approach methodologies (NAMs) and data for the safety assessment of cosmetic ingredients (Dent *et al.*, 2018; ICCR, 2017), as well as additional guidance to safety assessors on the types of NAMs that may be used in this ‘next generation’ risk assessment (ICCR, 2018).

In June 2019, the ICCR Integrated Strategies JWG organised a workshop at the margins of the ICCR meeting in Montreal, Canada, to investigate how these principles are currently being applied to next generation risk assessment through the discussion of case studies. The main purpose was to encourage discussion on how the ICCR principles can aid in the application of NAMs for decision making and to identify and discuss gaps that may prevent decision making. During the workshop the participants tried to address the following questions:

- Can we agree that *in vitro* risk assessment for cosmetics can work and is likely protective?
- What are the common components of the case studies? Can we agree on what a next generation risk assessment looks like for cosmetics?
- Where do we go from here to make next generation risk assessment a reality for day-to-day decision-making?

EURL ECVAM participated in the workshop and will continue contributing to the JWG. The outcome of the workshop will be published in a peer-reviewed workshop report, building on the previous work of this JWG by providing further real-life examples showing how NAMs can be used in safety decision making relevant to cosmetics.

#### READ MORE

- ▶ International Cooperation on Cosmetics Regulation (ICCR): [www.iccr-cosmetics.org](http://www.iccr-cosmetics.org)

### 5.13 In Silico Medicine

EURL ECVAM continued to explore opportunities to promote non-animal methods in the field of *in silico* medicine. This involved, in particular, continued collaboration with the Avicenna Alliance, an association of industry, academia and healthcare organisations who have a commercial or research interest in the development of *in silico* medicine.



**Avicenna Alliance**  
Association for Predictive Medicine

The Association, established in 2015, has its origins in the Virtual Physiological Human Initiative, a European Commission endorsed research area on computer modelling and simulation. Tasked by the European Commission with developing a “Roadmap for *in silico* medicine”, the Association now seeks to put this roadmap into policy and ensure the development of a regulated *in silico* market.

#### READ MORE

- ▶ Avicenna Alliance: [avicenna-alliance.com](http://avicenna-alliance.com)







# 6

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## **Dissemination of Information on Alternatives**

# 6.

## Dissemination of Information on Alternatives

### 6.1 EURL ECVAM Databases and Tools

Readily accessible information on alternative approaches plays an important role in the uptake of the Three Rs in different areas and contexts (*e.g.*, knowledge sharing, regulatory applications, education and training, etc.; Holley *et al.*, 2016).

The mandate of EURL ECVAM includes the dissemination of information by providing public databases and information systems on alternative approaches. [Figure 6.1](#) and [Figure 6.2](#) give an overview of the EURL ECVAM datasets, databases and tools currently available. The new ones and those that were recently updated are described in more detail in the following sections.

**Figure 6.1:** EURL ECVAM datasets and tools available in the JRC Data Catalogue.

**Computational Models for the Safety Assessment of Nanomaterials**

This dataset contains the supplementary materials of the Nanocomput report.

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- Inventories of QSAR/QSPR
- Model inventories for PBK models
- Environmental fate models for nanomaterials
- Collected data for multi-walled carbon nanotubes (MWCNT) analogues

[europa.eu/DV83uk](https://europe.eu/DV83uk)

**DB-ALM - EURL ECVAM Database on Alternative Methods to Animal Experimentation**

DB-ALM dataset is a collection of alternative method summaries and protocols. Search criteria include topic areas, biological endpoints, experimental systems, and others.

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- Registration not required
- 184 protocols

[europa.eu/Cp69pH](https://europe.eu/Cp69pH)

### EURL ECVAM Fish In Vitro Intrinsic Clearance Database

The Fish In Vitro Intrinsic Clearance database includes fish in vitro intrinsic clearance rates derived with in vitro methods using fish S9 fraction, hepatocytes, or microsomes.

- 960 entries for 232 unique chemicals
- The DB supports the two OECD test guidelines using cryopreserved hepatocytes (OECD TG 319A) or S9 fractions (OECD TG 319B) from rainbow trouts to determine in vitro intrinsic clearance

[europa.eu/!gR36Dk](http://europa.eu/!gR36Dk)

### EURL ECVAM Fish In Vivo Biotransformation Database

The Fish In Vivo Biotransformation database includes fish in vivo biotransformation data (whole body, biotransformation rate constant (k<sub>B</sub>) estimates).

- 1535 entries for 702 organic chemicals
- The data support chemical assessments and the development of non-animal methods

[europa.eu/!xV83uw](http://europa.eu/!xV83uw)

### EURL ECVAM Genotoxicity and Carcinogenicity Consolidated Database of Ames Positive Chemicals

This database contains available genotoxicity and carcinogenicity data for Ames positive chemicals.

- 726 chemicals
- Available here is also the Carcinogenicity Genotoxicity eXperience (CGX) dataset, previously hosted in the Lhasa Limited Site
- The DB is also linked to two other JRC databases, CheLIST and ChemAgora, which provide supplementary information on the Ames positive chemicals

[europa.eu/!RX73pD](http://europa.eu/!RX73pD)

### EURL ECVAM Rodent In Vitro Intrinsic Clearance Database

The rodent In Vitro Intrinsic Clearance database includes rodent (mouse, rat) in vitro intrinsic clearance rates derived with in vitro methods using rodent S9 fraction, hepatocytes, or microsomes.

- 8648 entries for approximately 6100 unique chemicals

[europa.eu/!ux69cq](http://europa.eu/!ux69cq)

### EURL ECVAM Rodent In Vivo Biotransformation Database

The Rodent In Vivo Biotransformation database includes rodent (mouse, rat) in vivo biotransformation data (clearance or elimination or half-life, and in most entries additional TK parameters relating to the reported clearance value).

- 2442 entries for approximately 568 unique chemicals

[europa.eu/!Tc98yT](http://europa.eu/!Tc98yT)

### HTS Database of Nanomaterials on HepaRGs

The dataset contains 14 different read outs including viable cell count, cell membrane permeability, apoptotic cell death, mitochondrial membrane potential and steatosis of the human hepatoma HepaRG cell line treated with a large set of nanomaterials, coatings and supernatants at different concentrations.

- The database can be utilised for the development of in silico hazard assessment and prediction tools or can be combined with toxicity effect on other in vitro test systems

[europa.eu/!mK49yD](http://europa.eu/!mK49yD)

### Inventory of the 3Rs Knowledge Sources

A detailed inventory of 800 knowledge sources relevant to the Three Rs.

- 800 knowledge sources identified
- The inventory covers different types of knowledge sources both explicit (e.g. websites, publications, databases, etc.) and more tacit (e.g. organisations, events, expert groups, etc.)

[europa.eu/!kN83xc](http://europa.eu/!kN83xc)

### Three Rs Education and Training Courses and Resources

This catalogue provides a snapshot overview of education and training courses and resources on the Three Rs principles offered at secondary school, university and professional levels worldwide.

- 569 courses and resources
- Data collected between June and September 2018

[europa.eu/!Mc76wP](http://europa.eu/!Mc76wP)

**Figure 6.2:** EURL ECVAM Databases and tools.

## ChemAgora

ChemAgora is a portal to retrieve chemical, physical and toxicological information on chemical substances from existing publicly available databases.

### Main features

- Search by identifier (name, CAS, InChIKey, etc.) or by structure
- Data from 13 third-party collections
- FP7 funded project

The search result page visualises the molecular formula, the InChI, and an image of the chemical structure, a list of synonyms and provides clickable links leading to the page with information about the chemical.

[chemagora.jrc.ec.europa.eu](http://chemagora.jrc.ec.europa.eu)

## CheLIST\*

The Chemical Lists Information System (CheLIST) helps determine if a chemical has been used in a research or validation project, and if it is regulated and listed under a specific regulatory inventory.

### Main features

- Search by identifier (name, CAS, InChIKey, etc.) or by structure
- Comparison of various datasets and inventories
- Download of lists and associated references

Lists can be downloaded and the references provided for each list allow traceability back to source.  
\* CheLIST is currently being updated and a new version will be released during the first half of 2020.

[checlist.jrc.ec.europa.eu](http://checlist.jrc.ec.europa.eu)

## CRAFT and METIS

The Chemical Reactivity and Fate Tool (CRAFT) allows modelling and evaluation of the chemical reactivity, persistence and biodegradation of chemicals in the environment. The Metabolic Information Input System Editor (METIS) can be used for input and storage of information about metabolism and degradation reactions.

### Main features

- Open source software tools
- Visual representation of (bio)chemical reactions
- METIS accessible from CRAFT

Case study available: "In silico modelling of microbial and human metabolism: a case study with the fungicide carbendazim".

[europa.eu/!nx76Tf](http://europa.eu/!nx76Tf)

## DART

The Decision Analysis by Ranking Techniques (DART) is designed for the ranking of chemicals according to their environmental and toxicological concern based on the most recent ranking theories.

### Main features

- Different kinds of order ranking methods, e.g. total and partial-order ranking (Hasse diagram)
- The ranking methods can be used to order chemicals based on more than one variable

Case studies available: "A Set of Case Studies to Illustrate the Applicability of DART (Decision Analysis by Ranking Techniques) in the Ranking of Chemicals".

[europa.eu/!NG97PX](http://europa.eu/!NG97PX)

## EASIS

The Endocrine Active Substances Information System (EASIS) is a tool to search for results from scientific studies on potential endocrine activity or adverse effects of chemicals.

### Main features

- 629 substances & 9940 studies on Endocrine Disruptors and Endocrine Active Substances
- To be featured in eChemPortal
- Based on IUCLID 6

Information is reported using OECD Harmonised Templates allowing a rapid search for health effects, effects on biotic systems or biomarkers of endocrine activity.

[easis.jrc.ec.europa.eu](http://easis.jrc.ec.europa.eu)

## IPCHEM

The Information Platform for Chemical Monitoring (IPCHEM) is the European Commission access point for searching, accessing and retrieving chemical occurrence data.

### Main features

- 145 data sets covering >3000 chemicals
- More than 265 million records
- Hosting aggregated and single measurement data
- Search by chemical or location

IPCHEM is structured into four modules, according to the chemical monitoring data categories: Environmental monitoring, Human Biomonitoring, Food and Feed, Products and Indoor Air.

[ipchem.jrc.ec.europa.eu](http://ipchem.jrc.ec.europa.eu)

## Search Guide

The EURL ECVAM Search Guide provides search principles and procedures, suggested search terms and user guidance to support untrained database users in finding information on relevant alternative strategies and methods on the Internet.

### Main features

- Portuguese and Korean versions available
- Checklist for searching
- Many useful search tips including the Seven Golden Steps

The Seven Golden Steps allow for searches in a structured and systematic way and to document them - a fundamental step in preparing a research project in biomedical sciences and toxicology.

[europa.eu/!fy74VF](http://europa.eu/!fy74VF)

## JRC QSAR Database\*

The JRC QSAR Model Database is a database of quantitative/qualitative structure activity relationship models (QSARs) typically used for predicting chemical properties related to hazards and risk.

### Main features

- Information based on the internationally adopted QMRF format
- It includes models for prediction of physicochemical properties, human health and environmental effects

Developers and users of QSAR models can submit information by using the QSAR Model Reporting Format (QMRF).  
\* The DB is currently not available and will be available through the JRC Data Catalogue.

[qsardb.jrc.ec.europa.eu](http://qsardb.jrc.ec.europa.eu)

## Toxmatch

Toxmatch encodes several chemical similarity indices to facilitate the grouping of chemicals into categories and read-across.

### Main features

- Search by identifier (name, CAS, InChIKey, etc.) or by structure
- Data from 13 third-party collections
- FP7 funded project

Toxmatch can compare datasets based on various structural and descriptor-based similarity indices and calculate pairwise similarity between compounds or aggregated similarity of a compound to a set.

[europa.eu/!jQ73Uc](http://europa.eu/!jQ73Uc)

## ToxRTool

The Toxicological data Reliability Assessment Tool (ToxRTool) provides criteria and guidance for assessing the reliability of toxicological data.

### Main features

- It consists of two parts to evaluate in vivo and in vitro data.
- Can be used to evaluate reliability of in vivo and in vitro data
- Can support weight-of-evidence scoring in toxicological assessments and systematic reviews

ToxRTool makes the decision process of assigning reliability categories more transparent and harmonised.

[europa.eu/!IPg34Pr](http://europa.eu/!IPg34Pr)

## Toxtree

Toxtree places chemicals into categories and predicts various kinds of toxic effects by applying decision tree approaches.

### Main features

- Open source software tool
- Online version also available
- To implement transparent decision trees for human health and environmental effects

Predictions are based on chemical structure (e.g. SMILES or by using the built-in 2D structure diagram editor).

[europa.eu/!YU84Rh](http://europa.eu/!YU84Rh)

## TSAR

The EURL ECVAM Tracking System for Alternative methods towards Regulatory acceptance (TSAR) provides an overview and the status of alternative (non-animal) methods proposed for regulatory purposes.

### Main features

- 99 tracked test methods
- Colour coding system for tracking progress
- Test methods from ICATM partners

Where available, TSAR includes documents associated with a method for each step of the process: submission, validation, peer-review, recommendations and regulatory acceptance (e.g. international standards).

[tsar.jrc.ec.europa.eu](http://tsar.jrc.ec.europa.eu)

### 6.1.1 DB-ALM — EURL ECVAM Database on Alternative Methods to Animal Experimentation

DB-ALM was created in the early 2000s to satisfy the increasing need for information about the alternative methods available in that period and to fulfil one of the duties of ECVAM defined in the Communication of the Commission to the Council and the European Parliament on the establishment of a European Centre for the Validation of Alternative Methods (EC, 1991), namely to set up, maintain and manage a database on alternative procedures. Over the following years, DB-ALM continuously grew in terms of content, functionality and user community.

During the following decade, there was considerable investment made in the alternatives domain, which resulted in a substantial increase in the number of alternative methods being developed and applied for scientific purposes including biomedical research and regulatory testing. Moreover, during this period many new dissemination channels appeared including scientific journals dedicated to *in vitro* and *in silico*

methods. To maintain its relevance and added-value, the strategy behind DB-ALM was shifted from a 'catch all' approach to one focused primarily on methods submitted to EURL ECVAM for validation and those identified in *ad hoc* reviews of the literature and end-users in specific application areas.

In recent years, not only has the amount of alternative methods being developed grown rapidly, but the paradigm of knowledge dissemination has changed significantly also due to the digital transformation of our society. Thus, large 'one-stop-shop' databases being the most prominent and effective channel of dissemination are not the preferred option anymore. EURL ECVAM has therefore instigated a process to adapt once more its strategy with which it can facilitate the sharing of knowledge about non-animal models and methods across communities.

A first visible step of this modernisation is the recent publication of a lightweight DB-ALM version, which is available in the JRC Data Catalogue (see [Section 6.1.3](#))



and which provides all the information that the original system came with (ca. 350 methods and their descriptions), but offering a more intuitive user experience and a search facility optimised for quicker access to relevant information. In addition, the whole database can be downloaded for easier perusal and further analysis on a local PC. Access to this new DB-ALM is free for anyone and no user ID or password is required.

The new-look DB-ALM is only a first step in the EURL ECVAM initiative to overhaul its models and methods dissemination strategy and to move from a one-directional publication paradigm to a bi-directional, more interactive and social approach. In the coming months and years, stakeholders will be consulted when this new strategy of method knowledge sharing is drafted and implemented.

#### READ MORE

►► EURL ECVAM Database on Alternative Methods to Animal Experimentation (DB-ALM): [europa.eu/!Cp69pH](http://europa.eu/!Cp69pH)

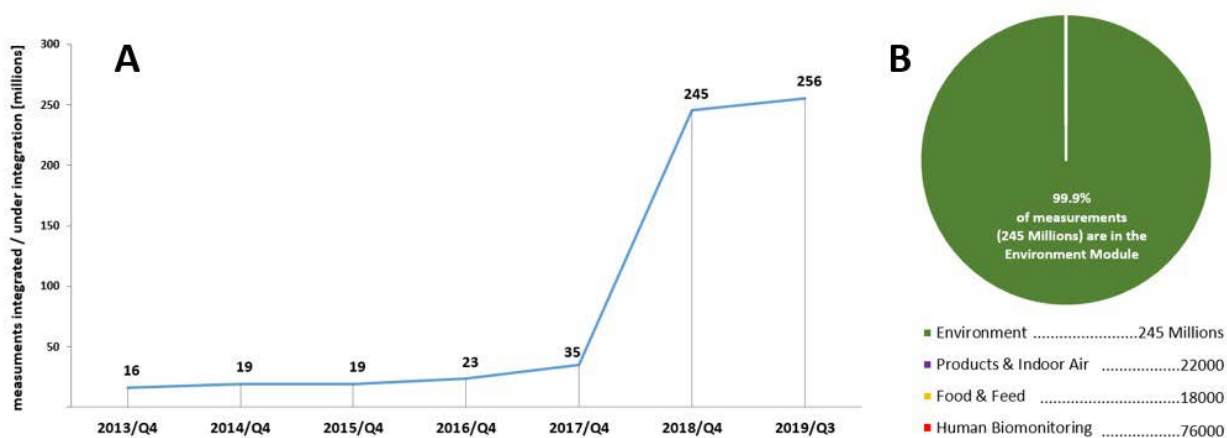
### 6.1.2 IPCHEM

IPCHEM is the European Commission's reference platform for chemical monitoring data collected across various media by the European Commission bodies, Member States, international and national organisations and research communities. Monitoring data is available via four thematic modules: environmental monitoring, food & feed monitoring, human biomonitoring, and consumer products and indoor air monitoring. The Platform aims to support a coordinated approach

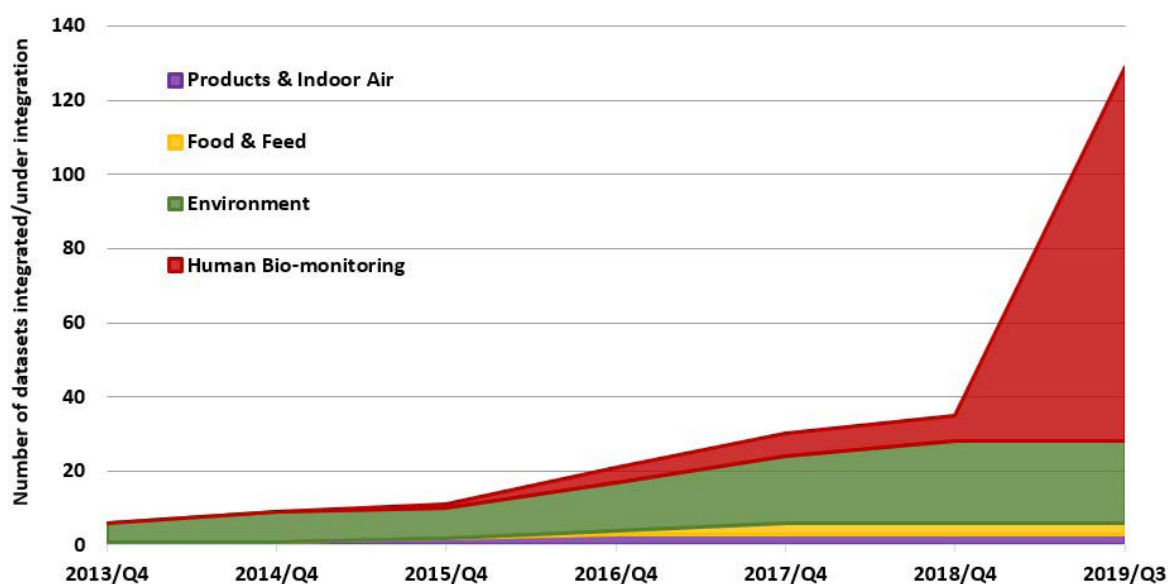
for collecting, storing, accessing and comparing data related to the occurrence of chemicals, their metabolites, and chemical mixtures, in relation to humans and the environment (see [Figure 6.3](#) and [Figure 6.4](#)). The platform is focused on data quality and usability and the basis for facilitating exposure and risk assessment and management practices in support of EU policies.



The primary objectives of IPCHEM are focused on: (1) Assisting policy makers and scientists to discover and access chemical monitoring data covering a range of matrices and media; (2) Offering safe and secure data storage for data currently not readily accessible; (3) Boosting data harmonisation and comparison, by integrating quality control rules and procedures into the platform; (4) Facilitating exposure and risk assessment practices in support of EU policies (Bopp *et al.*, 2018).



**Figure 6.3:** Status of IPCHEM data integration 2013-2019. A) Total number of measurements integrated (in millions) over time. B) Distribution of the number of measurements per module in October 2019.



**Figure 6.4:** Number of data collections in the four IPCHEM modules, for which at least metadata are available in IPCHEM.

One of the main activities in 2019 is to support the Human Biomonitoring Initiative HBM4EU by hosting metadata and data (see [Section 2.2](#)), in order to make existing HBM data findable and accessible.

**READ MORE**

▶▶ IPCHEM: [europa.eu/jjk38YF](https://europa.eu/jjk38YF)

### 6.1.3 JRC Data Catalogue: Open Access Datasets

[Figure 6.1](#) gives an overview of the datasets and tools available in the EURL ECVAM collection of the JRC Data Catalogue.

**READ MORE**

▶▶ EURL ECVAM Collection in the JRC Data Catalogue [data.jrc.ec.europa.eu/collection/id-0088](https://data.jrc.ec.europa.eu/collection/id-0088)

- **DB-ALM—EURL ECVAM’s Database on Alternative Methods to Animal Experimentation**

DB-ALM is a publicly accessible collection of alternative method summaries and protocols (see [Section 6.1.1](#)). It focuses primarily on methods submitted to EURL ECVAM for validation, and those identified in *ad hoc* reviews of the literature and end-users in specific application areas between 2000 and 2019. All method summaries and protocols can be searched in a web interface and then retrieved individually, but they can also be downloaded for flexible perusal on a

local PC. Search criteria include topic areas, biological endpoints, experimental systems, and others.

**READ MORE**

▶▶ DB-ALM: [europa.eu/!Cp69pH](https://europa.eu/!Cp69pH)

- **EURL ECVAM Fish *In Vitro* Intrinsic Clearance Database**

The Fish *In Vitro* Intrinsic Clearance database is an Excel™ workbook and includes fish *in vitro* intrinsic clearance rates derived with *in vitro* methods using fish S9 fractions, hepatocytes, or microsomes. There are approximately 960 entries for 232 unique chemicals. The database has been compiled by ARC Arnot Research and Consulting Inc (Toronto, Canada) for EURL ECVAM (Joint Research Centre, F.3; contract number: CCR.F.C931336.X0) applying quality criteria as outlined in the spreadsheets.

The EURL ECVAM Fish *In Vitro* Intrinsic Clearance database supports the two OECD test guidelines using cryopreserved hepatocytes (OECD TG 319A; OECD, 2018d) or S9 fractions (OECD TG 319B; OECD, 2018c) from rainbow trout to determine *in vitro* intrinsic clearance.

**READ MORE**

▶▶ EURL ECVAM Fish *In Vitro* Intrinsic Clearance Database: [europa.eu/!gR36Dk](https://europa.eu/!gR36Dk)

- **EURL ECVAM Fish *In Vivo* Biotransformation Database**

The Fish *In Vivo* Biotransformation database is an Excel™ workbook and includes fish *in vivo* biotransformation data (whole body, biotransformation rate constant (k<sub>B</sub>) estimates). There are 1535 entries for 702 organic chemicals. The database has been compiled by ARC Arnot Research and Consulting Inc (Toronto, Canada) for EURL ECVAM (Joint Research Centre, F.3; contract number: CCR.F.C931336.X0) applying quality criteria as outlined in the spreadsheets. The data support chemical assessments and the development of non-animal methods.

**READ MORE**

▶▶ EURL ECVAM Fish *In Vivo* Biotransformation Database: [europa.eu/lxV83uw](http://europa.eu/lxV83uw)

- **EURL ECVAM Rodent *In Vitro* Intrinsic Clearance Database**

The EURL ECVAM Rodent *In Vitro* Intrinsic Clearance database provides information on metabolic clearance in rodents (mouse, rats) derived with *in vitro* methods using rodent S9 fraction, hepatocytes, or microsomes. It supports chemical assessments and the development of non-animal methods. There are 8648 entries for approximately 6100 unique chemicals. The database has been compiled by ARC Arnot Research and Consulting Inc (Toronto, Canada) for EURL ECVAM (Joint Research Centre, F.3; contract number: CCR.F.C931336.X0) applying quality criteria as outlined in the spreadsheets.

**READ MORE**

▶▶ EURL ECVAM Rodent *In Vitro* Intrinsic Clearance Database: [europa.eu/lux69cq](http://europa.eu/lux69cq)

- **EURL ECVAM Rodent *In Vivo* Biotransformation Database**

The EURL ECVAM Rodent *In Vivo* biotransformation database provides information on biotransformation (clearance, elimination, half-life, and in most entries additional toxicokinetics parameters relating to the reported clearance value) in rodents (mice, rats). It supports chemical assessments and the development of non-animal methods. There are 2442 entries for approximately 568 unique chemicals. The database has been compiled by ARC Arnot Research and Consulting Inc (Toronto, Canada) for EURL ECVAM (Joint Research Centre, F.3; contract

number: CCR.F.C931336.X0) applying quality criteria as outlined in the spreadsheets.

**READ MORE**

▶▶ EURL ECVAM Rodent *In Vivo* Biotransformation Database: [europa.eu/!Tc98yT](http://europa.eu/!Tc98yT)

- **HTS Database of Nanomaterials on HepaRGs**

The database contains *in vitro* measurements of effects of a large set of nanomaterials on the human hepatoma HepaRG cell line within the FP7 Project NanoMILE. It contains 14 different read outs such as *e.g.*, the viable cell count, cell membrane permeability, apoptotic cell death, mitochondrial membrane potential, steatosis, and intracellular accumulation of neutral lipids. A set of 89 nanomaterials (29 cerium dioxides, 17 silica, 12 titanium dioxides, eight zinc oxides, eight silver, five gold, three polystyrene, three calcium, two copper oxide, one barium, and one iron oxide, all with different sizes and coatings, was tested at ten different concentrations in three independent biological replicates (Joossens *et al.*, 2019).

**READ MORE**

▶▶ HTS DB of Nanomaterials on HepaRGs: [europa.eu/!mK49yD](http://europa.eu/!mK49yD)

- **Three Rs Education and Training Courses and Resources**

This collection provides a snapshot overview of education and training courses and resources on the Three Rs principles offered at secondary school, university and professional levels worldwide. The results yielded 569 courses and resources that were available between June and September in 2018. This collection was the basis of the study carried out by EURL ECVAM to review the education and training courses and resources relating to the Three Rs (see [Section 6.3.1](#)). It is featured on two websites (Edelweiss Connect and Norecopa) where the source can be browsed, filtered and visualised.

**READ MORE**

▶▶ Three Rs Education and Training Courses and Resources: [europa.eu/!Mc76wP](http://europa.eu/!Mc76wP)

▶▶ Inventory of 3Rs Knowledge Sources by Edelweiss Connect: [3rs.douglasconnect.com](http://3rs.douglasconnect.com)

▶▶ 3R Guide by Norecopa: [norecopa.no/3r-guide](http://norecopa.no/3r-guide)

## 6.2 Knowledge Sharing Activities

### 6.2.1 Bridging Across Methods in the Biosciences (BeAMS)

With its project "Bridging across Methods in the Biosciences (BeAMS)", EURL ECVAM has initiated a cross-cutting undertaking, addressing one of the main challenges of biosciences, *i.e.*, the lack of crossdisciplinarity (see section 6.2.1 in Zuang *et al.*, 2018).

Biosciences are rich in innovation, and sophisticated methods based on new technologies are constantly emerging such as gene editing, organ-on-a-chip and super-resolution imaging. Crossdisciplinary research is essential if science is to properly inform policy and address societal needs. However, compartmentalisation within the biosciences is limiting the potential for new methods to translate from one domain to another. It also hinders scientific communities from interacting and collaborating to tackle serious health issues such as cancer. All scientific methods have their associated knowhow and practices, which demand high levels of specialisation on the part of researchers. On the one hand, this high degree of method-related expertise enables scientists to dig deeper in the pursuit of new knowledge. On the other hand however, such specialisation can also contribute to the emergence of a new type of scientific silo.

While scientists often refer to themselves as physicists, chemists, biologists etc., these traditional disciplines can sometimes be an arbitrary demarcator of scientific activity and knowledge, while it is actually the methods that scientists use that are becoming more important in defining scientific domains. The most commonly cited challenge to crossdisciplinarity remains the absence of a common language. Thus, it proves difficult to appropriately describe methods in an equivalent way and to compare the different types of scientific evidence they generate. More acknowledgement and understanding of this issue are needed if scientific communities are to devise ways to bridge knowledge and practices.

BeAMS is a multi-annual endeavour, in which stakeholders from many diverse disciplines are listened to, with the aim of arriving at a joint approach to overcome the silo dilemma. In 2019, EURL ECVAM published a first BeAMS report (Carusi *et al.*, 2019), which sets out four key recommendations:

- Gain a better understanding of cases where scientific methods do succeed in becoming bridgeable

across disciplines and sectors, through analysis of historical and current paradigm cases, and extract lessons from these;

- Create new opportunities to facilitate and experiment with ways to bridge across methods;
- Analyse existing policies and initiatives regarding open access, open science and others, and see what gaps there are between data and method complementarity;
- Include more explicit criteria for crossdisciplinary research and bridgeability across methods in funding calls, and in assessments of research and innovation projects.

As Horizon Europe paves the way for a missions-orientated approach to research policy in the next EU framework programme, it will be important to consider these recommendations to facilitate meaningful crossdisciplinarity to ensure success. EURL ECVAM will continue to investigate the topic.

#### READ MORE

► Missions in Horizon Europe: [europa.eu/!JF79qd](https://europa.eu/!JF79qd)

### 6.2.2 Indicators of Alternative Methods or Approaches to Animal Experimentation

Monitoring frameworks can help to identify progresses, impactful trends and future opportunities for the level of development and uptake of alternative methods or approaches to animal testing. In recent years, many indicators have been developed to measure science and innovation using both quantitative and qualitative data. However, only a few attempts have been specifically made to monitor the level of uptake of alternative methods or approaches.

In 2018, EURL ECVAM promoted a feasibility study carried out with the support of an external contractor. This study aimed to investigate a suitable methodological approach and examples of indicators relevant for non-animal experimentation in basic and applied biomedical research, as well as in a regulatory and educational context. The feasibility study identified prototypical indicators, such as bibliometric indicators, metrics related to patents or funding strategies, which can allow gathering information on knowledge dissemination, scientific and technological progress, and the economical impact of alternatives to animal testing. It also showed the need for an in-depth analysis of available data sources and the development of robust data collection strategies. Findings from the feasibility

study could support the creation of a community of practice where different stakeholders can co-contribute to the establishment of sustainable indicators to monitor trends in the area of alternatives to animal testing.

Following the outcome of the feasibility study, a new initiative was launched by EURL ECVAM in 2019 to investigate indicators that could be suitable to measure return of investment in biomedical research addressing non-communicable diseases, which are becoming highly prevalent in European countries. In particular, despite conspicuous research and economical endeavors, the

clinical failure rate in drug development still remains very high, with an overall likelihood of approval from Phase I of about 9.6% (Thomas *et al.*, 2016). On the other hand, the expanding toolbox of non-animal methods, including for instance induced pluripotent stem cells derived from patients, next-generation sequencing, 'omics and integrated computer modelling, can be used to study human diseases in human-based settings, identifying new potential druggable targets and evaluating treatment effects, as recently advocated (Herrmann *et al.*, 2019; see [Box 6.1](#)).

## Box 6.1

### Moving beyond the Three Rs in Biomedical Research

A new study co-authored by EURL ECVAM prioritises human relevant methods and the replacement of animal models in biomedical research (Herrmann *et al.*, 2019).

This year marks the 60<sup>th</sup> anniversary of the Three Rs, aimed at promoting the 'Replacement' of animal use in science, the 'Reduction' of the number of animals used per experiment, and the 'Refinement' of experimental procedures to minimise suffering and improve welfare. These principles were first described in 1959 by the UK scientists Russell and Burch and have contributed considerably ever since to progressing humane research methods and excellence in science. However, the 21<sup>st</sup> Century has already seen the development of a wide range of non-animal methods incorporating complex cell cultures, organs-on-a-chip and computer modelling. These methods are more relevant to human biology and are already enabling the replacement of animals as the default option in life science, particularly in the areas of toxicology and regulatory testing, but also in biomedical research. This recently published study says the time has now come to prioritise the Replacement of animals used for scientific purposes, over refinement and reduction strategies. The emerging paradigm in research likely foreshadows an era in which the Three Rs are increasingly perceived as a solution to a receding problem.

#### *Replacing animal experimentation in biomedical research*

In the European Union, basic and applied research accounts for about two-thirds of the animals used in science. To date however, replacement of animal methods with human-based models has been mainly discussed in the context of regulatory toxicology and chemical safety. This can be linked to several factors including the relatively limited number of standard studies performed and significant public concern over this use of animals. Moreover, biomedical research is traditionally more diverse and decentralised compared to toxicity testing, encouraging originality and combinations of both animal and non-animal approaches, despite the limited capacity of current preclinical animal models to accurately predict the safety and efficacy of new drugs.

#### *Promoting Human Relevance in Biomedical Research*

Prioritising animal-free methods of high human relevance is a sensible way to avoid the limited translational value of animal models of human biology. Non-animal approaches and technologies, such as patient-derived cells and biological samples, large clinical data repositories, computational and imaging tools, machine learning and micro-dosing approaches, are already enabling scientists to incorporate human relevance as a primary design criterion of biomedical research models and approaches. Such a human-oriented perspective is particularly relevant to the study of chronic, degenerative, non-communicable diseases, which are characterized by complex interactions between environmental and genetic factors. It is important to prioritize human relevant methods and the replacement of animal models in biomedical research in order to deepen our understanding of human pathologies and increase the likelihood of success in the development of drugs that are truly effective in humans.

Source: EU Science Hub (<https://europa.eu/!BF99dx>)



While research efforts based on the use of both animal and/or non-animal approaches to tackle non-communicable diseases (such as Alzheimer's disease, breast and prostate cancer) have been extensively supported at European level, systematic assessment of return on investment of research funding strategies by means of suitable indicators should be implemented to retrospectively assess public health trends, and re-address funding strategies when needed.

### 6.2.3 Statistics on Animal Use in Science

The first report of statistical information on the use of animals in procedures has become available (EC, 2020) in accordance with the provisions of article 57(2) of Directive 2010/63/EU regarding the protection of animals used for scientific purposes.

EURL ECVAM was requested by Directorate-General for Environment to support the preparation of this EC report based on data provided by Member States in accordance with article 54(2). For this purpose, EURL ECVAM performed the statistical analysis, providing a comprehensive overview on the use of animals in procedures in the European Union between 2015 and 2017.

The first section of this report focuses on the numbers of animals used for the first time and their origins. These animals can be both conventional animals or those that have been genetically altered (but excludes animals that have been used for the maintenance or creation of new genetically altered animal lines).

The second section focuses on the way in which animals are used in scientific procedures, covering both the first and any subsequent reuse (Figure 6.5), so that a global picture can be drawn of all uses of animals. This section takes into account the nature of the procedures, their legislative context, reuse of animals, their genetic status and the actual severities experienced by the animal having undergone a procedure.

The third section focuses on genetically altered animals providing information on the numbers and type of purposes of genetically altered animals needed to support scientific research in the Union. It reports on the animals used for the creation of new genetically altered animal lines and the maintenance of colonies of existing genetically altered animals.

## 6.3 Education and Training Activities

Education and training are fundamental for driving the progress in the development and uptake of alternative methods and approaches. EURL ECVAM has recently been involved in several education and training activities aiming at increasing the awareness of the Three Rs and alternative methods.

These activities include, among others, a study to review available education and training resources on the Three Rs (see Section 6.3.1), a guidance document and freely available teaching resources (see Section 6.3.2), a summer school on non-animal approaches held in 2017 and 2019 (see Section 6.3.6) and the participation in university open days on the Three Rs (Urani et al., 2019; see Box 6.2).

More details on all the education and training activities are available in the following sections.

### 6.3.1 Review of the Three Rs Education and Training Resources

A EURL ECVAM study Holley *et al.* (2016) showed that Three Rs sharing can be improved through better coordination, communication and outreach, and by more emphasis on targeted education and training initiatives. As a follow up, a review of existing education and training (E&T) courses and resources relating to the Three Rs was carried out. The aim was to map the existing E&T provision available within and beyond the European Union and across three levels of learning: professional, university and secondary school. The inventory is freely available in the JRC Data Catalogue (see Section 6.1.3). This study also included an analysis of current trends as well as areas of strength and demand to inform further actions in accelerating the development and uptake of the Three Rs within an E&T context.

The identification of E&T courses and resources with an implicit or explicit mention of the Three Rs was carried out between June and September 2018 through an online survey. The results were then combined with a web-based search using PubMed and Google employing a combination of keywords. The resulting entries were characterised using several descriptors such as course title, language, format, education level, program accreditation and type of access (free or fee-based), Three Rs relevance.

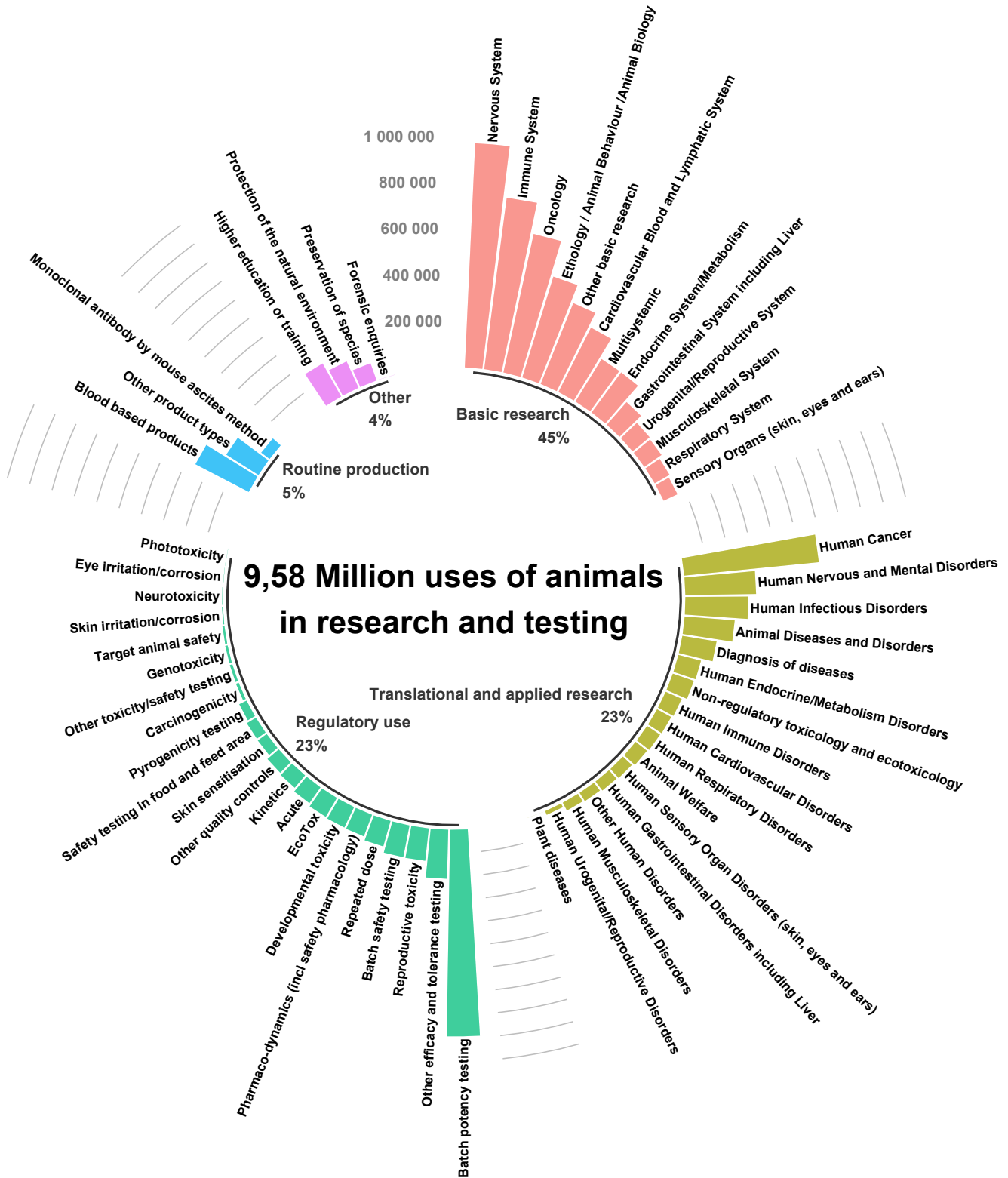


Figure 6.5: All uses of animals for research and testing in the European Union in 2017.

**Box 6.2****University Open Day on using alternative methods in research**

The Open Day was co-organised by the EURL ECVAM and the University of Milano (Bicocca) as part of an initiative to introduce principles underpinning the Replacement, Reduction, and Refinement (the Three Rs) of animal experiments into university curricula.

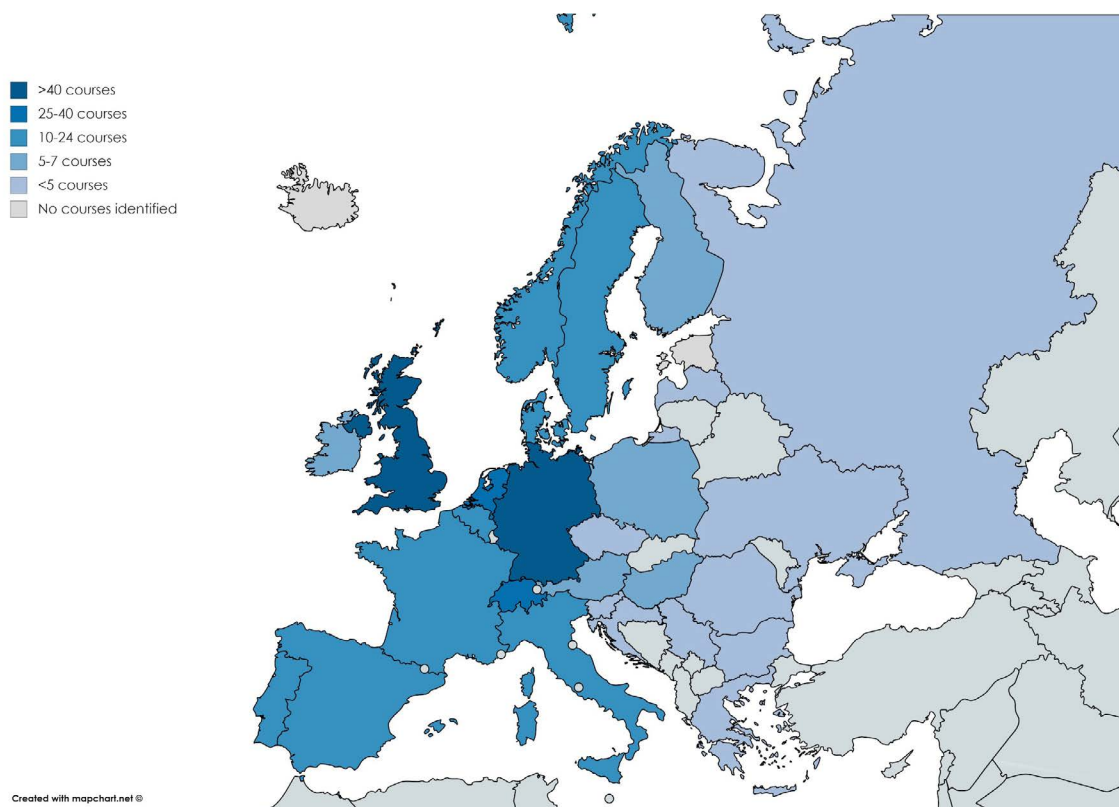
A report of the Open Day, on the "Use of Alternative Methods: From Fundamental to Industrial Research", has also been published (Urani et al., 2019). Intended as a series of related events, this first workshop was held on the 31 October 2018 at the University of Milano (Bicocca). The purpose of the initiative is to equip future researchers, regulators and decision makers with the necessary understanding of alternative approaches to enable them to address research and testing needs with modern tools and techniques. The overall aim is to accelerate the uptake of the Three Rs and to reduce the reliance on animal testing, in line with EU policy set out in Directive 2010/63/EU on the protection of animals used for scientific purposes. The relevance and novelty of the initiative was highlighted by the Rector of the University, Maria Cristina Messa, who opened the day. She spoke about both the educational content of the 3Rs Open Day and its cultural implications. There is a growing recognition at the university that ethics is fundamental in all fields of research. Considerable efforts are being made by the university in this direction through the commitment and efforts of the ethical committee and the work and collaboration with EURL ECVAM.

In this context, the need to move from the concept of the Three Rs to that of 4Rs (Reduction, Refinement, Replacement and Responsibility) was stressed. The history of the Three Rs and their significance, and an overview of *in vivo* and *in vitro* models in neurosciences and their potential applications and limitations were introduced by EURL ECVAM scientists. In the morning, examples of reduction, integrated testing strategies, and innovative *in vitro* assays in the context of environmental monitoring were given by speakers from academia and industry. The practical experience of applying the Three Rs principles to animal experimentation was described by the President of the Animal Welfare Body as well. Computational methods were the central topic of the afternoon, with a brief introduction to the use of quantitative structure-activity relationship (QSAR) models, an overview of different case studies and collaborative projects, and advanced computational methods to investigate molecular mechanisms associated with toxicity effects.

The final part of the Open Day included a round table on "The business future working with alternative methods" in which representatives from academia and industry described their experience, and gave an idea of the future opportunities for careers involving alternative methods, especially addressed to the young students and researchers.



Source: EU Science Hub (<https://europa.eu/!kB46fm>)



**Figure 6.6:** Distribution of the Three Rs E&T courses and resources in Europe 8 from Dura & Holloway, 2019, *Biomedical Science and Engineering*, 3(2s), p. 18. under CC BY-NC 4.0).

A total of 569 E&T courses and resources with Three Rs relevance were identified in 52 countries worldwide (Dura & Holloway, 2020), the majority located in Europe (72%) and North America (14%). In Europe, more than 80 courses were delivered in the United Kingdom, whilst Germany, Switzerland and the Netherlands provided 41, 29 and 26 courses respectively (Figure 6.6). English was the most common teaching language (80%) but several courses were also taught in more than one language, which usually includes English.

Several E&T courses and resources combined both classroom and distance learning formats. Face-to-face education, in the form of lectures and hands-on-training, was identified as the most common format of delivery (64%). On the other hand, distance learning through, for example, webinars or interactive online resources represented approximately one third (30%) of the total courses and resources.

Seventy percent of the identified E&T courses and resources targeted university level (undergraduate, postgraduate and postdoctoral). Professionals were addressed by 26% of the courses and only 3% focused

on high school-goers. Around 50% of the E&T courses and resources were delivered exclusively in the form of fee-based access. Approximately one third of the courses and resources focused on the Replacement principle and the second most addressed principle was Refinement (20%), whilst a few courses are offered on Reduction (8%). 18% of the courses and resources addressed all Three Rs principles.

The current study shows that there is a significant and rich number of Three Rs E&T courses and resources available across six continents and accessible in different formats such as distance and/or classroom learning. However, certain methodologies and education levels dominate, as does the English language showing an uneven distribution around the globe. Massive open online courses and summer schools are underrepresented as teaching formats. In addition, high-school goers and professionals are the least targeted audience, while E&T resources are mostly fee-based.

#### READ MORE

▶▶ JRC launches survey on alternatives to animal testing: [europea.eu/ltJ78mT](https://europea.eu/ltJ78mT)

### 6.3.2 European Parliament Pilot Project on Promoting Alternatives to Animal Testing

In 2018, the European Parliament (EP) made one million euro available to the Directorate-General for Environment to promote the use of alternatives to animal testing in the EU through information sharing and education activities. This pilot project should play a pivotal role for the implementation of Directive 2010/63/EU. It aims to actively promote existing alternatives, facilitate development and validation of new alternatives, foster exchanges of information, knowledge and best practices, and provide tools for education and training to facilitate the application of the principle of Three Rs - to Replace, Reduce and Refine the use of animals used for scientific purposes - in line with the Directive.

To achieve those objectives, the following three actions are being carried out:

1. Development of six open access e-learning modules;
2. Support the Education and Training Platform for Laboratory Animal Science (ETPLAS);
3. Development of guidance to facilitate the incorporation of the Three Rs into education curricula and production of teaching resources.

EURL ECVAM is coordinating action 3 and participating in action 1.

#### • Action 1- Six open access e-learning modules

Six open access and interactive e-learning modules are being developed by Directorate-General for Environment. It is expected that these modules will provide much needed consistency across the EU on some of the key elements, crucial for the correct application of the legislation.

Two modules focus on non-animal alternatives, whilst the other four modules focus on the implementation of the Three Rs under the Directive:

1. "Searching for and identification of existing alternative non-animal methods and approaches"
2. "Developing reliable and relevant alternative non-animal approaches for regulatory use"
3. "Design of procedures and projects (level I)"
4. "Design of procedures and projects (level II)"
5. "Project Evaluator"
6. "Implementation of the severity assessment framework within projects using live animals"

The six modules will be ready in 2020 and will be free of charge and available for any individuals or course

providers to be used as stand-alone training tools or as part of a curriculum.

EURL ECVAM is collaborating on two modules, namely module 1 "Searching for and identification of existing alternative non-animal methods and approaches" and module 2 "Developing reliable and relevant *in vitro* methods and approaches for scientific purposes and regulatory use".

#### *Module 1: Searching for non-animal alternatives*

This interactive e-learning module provides training to enable participants to understand the concept and the importance of replacement of animal use, and how this can be achieved through project design and thorough searches for alternative non-animal methods and approaches. EURL ECVAM is collaborating closely with Directorate-General for Environment and external partners.

It covers the identification of suitable information sources, a stepwise approach for developing effective searches for those sources and for combining them into an overall search strategy. Moreover, it teaches how to document and report a search, and how to use this search as the basis for a complete review.

When following this module, students will learn about the meaning of replacement and the application of the Three Rs according to Directive 2010/63/EU. Furthermore, they will learn how to identify sources for replacement information, categorise them and learn how to judge their reliability. Finally, they will understand good search practices, principles and procedures including the best ways to approach research questions.

#### *Module 2: Developing reliable and relevant in vitro methods and approaches for scientific purposes and regulatory use*

This e-learning module will enable students to understand the context and need for developing reliable and relevant *in vitro* methods, the pathway from development, evaluation of *in vitro* methods towards regulatory acceptance, based on the OECD guidance document on Good *In Vitro* Method Practices (GIVIMP; OECD, 2018b) and how the reliability and completeness of a test method for a specific purpose can be improved. The aim of the GIVIMP is to reduce the uncertainties in cell and tissue-based *in vitro* method derived predictions by applying good scientific, technical and quality practices from method development



to method implementation for regulatory use (see section 5.3.7 of Zuang *et al.*, 2018).

- **Action 2 – Support the Education and Training Platform for Laboratory Animal Science (ETPLAS)**

ETPLAS pools together Member State authorities, course providers and course accreditors, as a one-stop-shop in the EU. The key aim of ETPLAS is the provision of information and tools for the delivery and assessment of high quality laboratory animal science training in Europe, in line with the EU Education and Training Framework guidance (DG ENV, 2014). ETPLAS will also host the six e-learning modules of action 1.

Under this pilot project the objective is to facilitate the process of mutual recognition of, and access to quality education and training in laboratory animal science in Europe to support the attainment of competence as now legally required by legislation. ETPLAS will develop an IT platform that will deliver tools to enable course organisers to assess whether those persons carrying out procedures on animals have met the required level of competence in theoretical and practical skills.

- **Action 3 - Guidance to facilitate the incorporation of the Three Rs into education curricula and production of teaching resources**

Under the EP pilot project and in collaboration with external partners (Ecorys, Syrcle and European Schoolnet), EURL ECVAM will develop:

- a) guidance that targets decision-makers in educational organisations on how to introduce the Three Rs in education programmes and curricula and
- b) Three Rs teaching resources for secondary school students, universities, and early career scientists.

In 2016, the report "Accelerating progress in the replacement, reduction and refinement of animal testing through better knowledge sharing" (Holley *et al.*, 2016) suggested that education and training opportunities relating to the Three Rs should be increased and improved, extending across three levels of learning: professional, undergraduate and school-goers. Furthermore, educators need more dedicated teaching resources and these should be freely available to them and their students. In line with these two recommendations, action three of the EP pilot project addresses the provision of future educators with guidance and practical tools at three key education levels with the aim of having long-term impact on the uptake of Three Rs.

On 3 to 4 October 2019, selected experts in the Three Rs and in education attended a workshop at the JRC Ispra to discuss the guidance and the teaching resources (Figure 6.7). Both guidance and teaching resources will be available in 2020.

- a) *Guidance on how to introduce the Three Rs in education programmes and curricula*

The guidance targets decision-makers and educational influencers, who are pioneers within the education



**Figure 6.7:** Participants of the workshop "Education and training for the Three Rs" held on 3 to 4 October 2019 at JRC Ispra.

system innovating in teaching and learning, such as deans of university departments, teachers, heads of schools, education organisations, etc.

The guidance stresses the importance of introducing the Three Rs in the education curricula, including scientific, career and societal aspects, and proposes a dual approach:

1. Introducing the Three Rs during the development of curriculum frameworks. Guidelines for this process already exist, for instance the one developed by the International Bureau of Education of UNESCO (UNESCO-IBE, 2017). It suggests a 5-step process, which is quite flexible and can be adapted to meet

the needs of each Member State and their teaching methodology. Education falls under Member State competence as defined by the Treaty of the Functioning of the European Union (EC, 2012).

2. Influencing the existing curricula by targeting specific groups within the education system, such as teachers, lecturers, and their hierarchy, since the development and implementation of a curriculum framework can be a complex and slow process. This can be done compiling teaching resources and activities in learning scenarios, which are 'standard operating procedures' containing activities, required resources and tool for delivering effective lesson plans (see [Box 6.3](#)).

### Box 6.3

#### Teaching the Three Rs in secondary schools

In collaboration with European Schoolnet (a network of 34 ministries of education in Europe), a group of secondary school teachers have developed learning scenarios that can be readily implemented by teachers or adapted according to their needs to introduce the Three Rs into the biology curricula.

The pilot teachers are from six European countries, namely Belgium (LAB-Gedreven Onderwijs - Sint Amands and Sint Donatus Bovenbouw - Merchtem), Italy (I.I.S. Mazzini - Vittoria and Liceo Statale Guglielmo Marconi - Pescara), Malta (St. Paul's Missionary College - Rabat and De La Salle College Sixth Form - Birgu/Vittoriosa), Portugal (AE Rodrigues de Freitas - Porto and Escola Secundária Quinta do Marquês - Oeiras), Spain (Escola Secundária Quinta do Marquês - Madrid and IES Bilingüe Cervantes - Madrid) and Turkey (İstanbul Fuat Sezgin Bilim ve Sanat Merkezi - Istanbul and Küçükkuşu Fernur Sözen Ortaokulu - Canakkale).



The learning scenarios 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, emotions and science literacy.

These learning scenarios will be validated in at least two schools to prove the value of the materials created as well as to further refine them. Ultimately, they will be available through a public platform ([www.scientix.eu](http://www.scientix.eu)).

A massive open online course (MOOC) for teachers is also being developed under the umbrella of the European Schoolnet Academy (<https://www.europeanschoolnetacademy.eu>). The aim is to provide life science teachers with all the materials, support and tutorials to integrate elements connected to the Three Rs in their teaching. The MOOC, which will be available from January 2020 from the European Schoolnet Academy platform, will include four modules and last five and a half weeks.

### b) Learning scenarios and Three Rs teaching resources

Under this project, new and already existing education activities on the Three Rs are being developed or contextualised in learning scenarios for the three levels of education, *i.e.*, secondary schools, university and continuing education. Some practical teaching resources, such as slide sets and posters/infographics on the Three Rs, will complement the learning scenarios.

All the resources and learning scenarios will be available in 2020 and to everyone in the education system who would like to introduce the Three Rs as a subject in their education curriculum.

#### READ MORE

- ▶▶ European Schoolnet: [www.eun.org](http://www.eun.org)
- ▶▶ Scientix: [www.scientix.eu](http://www.scientix.eu)
- ▶▶ 3Rs MOOC: [www.europeanschoolnetacademy.eu/courses/course-v1:3Rs+AnimalsInScience+2020/about](http://www.europeanschoolnetacademy.eu/courses/course-v1:3Rs+AnimalsInScience+2020/about)

### 6.3.3 Training Activities related to Adverse Outcome Pathways

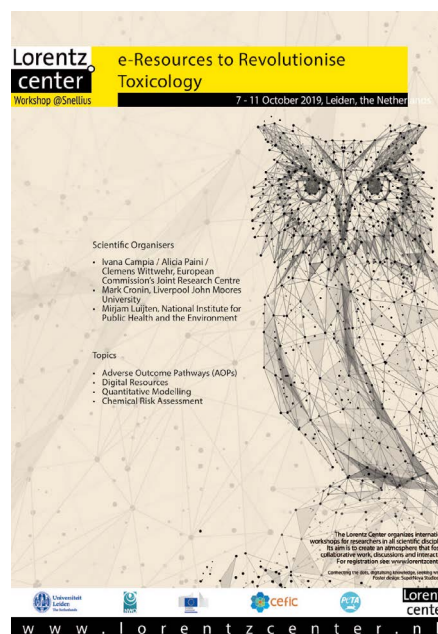
In the pre-conference continuing education program of the 4<sup>th</sup> International Conference on Toxicity Testing Alternatives and Translational Toxicity & the 2<sup>nd</sup> Asian Congress on Alternatives in Guangzhou, China (9-11 October 2018), EURL ECVAM gave a presentation about the AOP-Wiki. Approximately 50 (mostly Chinese) participants heard about how to capture an AOP in the Wiki, but also learned what motivations could lead them to actually do so. A discussion with participants after the lecture on their views on the Wiki and the AOP framework gave interesting insights. It became obvious that, while EURL ECVAM or even OECD first-hand training and education interventions in strategically important countries like China are crucial, they will never be scalable to really make a lasting impact. Local ambassadors need to be identified, recruited and trained to spread AOP knowledge, something that will be taken in consideration in the ongoing AOP framework sustainability efforts.

### 6.3.4 Lorentz Workshop on Computational Modelling

The last decade has seen an enormous growth in digital resources to capture data related to the toxicological effects of chemicals as well as to support predictive chemical risk assessment. Digital resources can thus help to revolutionise toxicology through the development and quantification of AOPs and AOP networks. Quantification is necessary for a more reliable

prediction of chemical effects, including potency, which is a prerequisite for risk assessment.

A workshop entitled “e-Resources to Revolutionise Toxicology: Linking Data to Decisions”, held in October 2019, was co-organised by EURL ECVAM, the Dutch National Institute for Public Health and the Environment (RIVM, NL) and Liverpool John Moores University (LJMU, UK). The aim was to explore ways of enhancing the development and uptake of quantitative models of AOPs and AOP networks by using digital resources. The five-day workshop was hosted at the Lorentz Center in Leiden (NL), which promotes international workshops in all scientific disciplines with support from the Netherlands Organisation for Scientific Research (NWO) and the University of Leiden with additional sponsorship from EURL ECVAM, the European Chemical Industry Council (CEFIC) and the PETA International Science Consortium. The workshop brought together around 25 participants, including toxicologists, biologists, computational modelers, bioinformaticians and risk assessors from industry, academia and governmental organisations.



Presentations included the state-of-the-art in quantitative AOPs (qAOPs) development, existing resources for computational modelling, role of the AOP Knowledge Base, use of weight-of-evidence in AOP development, and the validation of qAOP models. Following discussions in break-out groups, participants developed a series of qAOP case studies for three endpoints (skin sensitisation, neurotoxicity and carcinogenicity). These case studies captured lessons learned in quantifying AOPs using currently available digital resources, and identified needs for further research and development.



The interdisciplinary setting of the workshop provided a unique and lively opportunity to foster collaboration and engage several scientific communities. A report on the outcome of the workshop is foreseen.

#### READ MORE

- ▶ Lorentz Center's Workshops: [www.lorentzcenter.nl/all-workshops.html](http://www.lorentzcenter.nl/all-workshops.html)
- ▶ European Chemical Industry Council (Cefic): [cefic.org](http://cefic.org)
- ▶ Peta International Science Consortium Ltd. (PETA): [www.piscltd.org.uk](http://www.piscltd.org.uk)

### 6.3.5 Traineeships at EURL ECVAM

During 2018–2019, EURL ECVAM continued to host and train early career scientists, including several PhD students, via the JRC traineeship programme, which offers paid visits for periods between three and five months. The topics of the traineeships are listed in [Table 6.1](#).

#### READ MORE

- ▶ JRC Traineeship scheme: [europa.eu/!yn83Xx](http://europa.eu/!yn83Xx)

### 6.3.6 JRC Summer School on Non-animal Approaches in Science

EURL ECVAM organised a JRC Summer School entitled: "Non-Animal Approaches in Science: Challenges and Future Directions", that took place at the JRC site in Ispra from 21 to 24 May 2019 (see [Box 6.4](#) and [Figure 6.8](#)). The aim of the JRC Summer School was to share

knowledge and experience on the latest non-animal approaches used in research and testing, and explore the role of the Three Rs in science today through discussion and debate.

The JRC Summer School was specifically tailored for postgraduate students and early-career scientists focused on non-animal methods and technologies and on the opportunities and challenges associated with their application in various fields, such as regulatory toxicology and biomedical research. The program combined lectures by experts in the field with plenty of interactive sessions to encourage exchange of views and facilitate networking among participants. Each participant presented a poster describing his/her own studies and interests or work area related to the topics of the JRC Summer School. The programme and the proceedings of the JRC Summer School are now publicly available (EURL ECVAM, 2019).

A Summer School on Innovative Approaches in Science will take place on 22 to 25 June 2020 at the Johns Hopkins University Baltimore, USA. EURL ECVAM is a member of the organising committee.

#### READ MORE

- ▶ JRC Summer School 2019 on Non-Animal Approaches in Science: [europa.eu/!VK84rn](http://europa.eu/!VK84rn)
- ▶ Summer School on Innovative Approaches in Science: [www.ascctox.org/innovativescience2020](http://www.ascctox.org/innovativescience2020)



**Figure 6.8:** Participants of the JRC Summer School on Non-animal Approaches in Science (JRC Ispra, Italy, 21–24 May 2019).

**Table 6.1:** List of topics of the traineeships hosted at EURL ECVAM during 2018-2019.

Topic	Aim and description
Mathematical modelling with the Virtual Cell Based Assay (VCBA)	To support and collaborate to the development of an extension of the VCBA to include active transport processes. This included a literature search to gather information and understand the current state of science of active transporter systems. Then mathematical equations were developed (as part of the already existing R code of the VCBA), and relevant datasets were found to calibrate the model, run simulations and understand the possible implications of the new model for application in toxicology and risk assessment.
Physiologically Based Kinetic (PBK) modelling of chemical mixtures	To apply several PBK models in the context of an in-house project on chemical mixtures and human biomonitoring to understand the absorption, distribution, metabolism and excretion for different classes of chemicals. This was done by retrieving the parameters (physiological and anatomical, physicochemical and biokinetic) needed to parameterise the models, running two models, and use of human biomonitoring data to evaluate model performance.
Development of adverse outcome pathways: Endocytic lysosomal uptake to liver fibrosis and retinoid related cardiotoxicity	To familiarise with the concept and related tools in the AOP Knowledgebase and contribute to the elaboration and finalisation of AOPs related to liver or heart toxicity.
Quantitative modelling of adverse outcome pathways	Exploring approaches to the development of quantitative adverse outcome pathways (AOPs) for human health effects, including the use of modelling approaches to quantify key event relationships.
Analysis of relationships across toxicological endpoints	Development of methodology for the evaluation of systemic toxicity effects. The overall aim was to allow a better use of existing data and provide a framework to introduce data from alternative methods in the regulatory decision process.
Analysis of regulatory protection levels, with reference to the GHS system	Support the development of a framework to investigate the protection level based on the classification of chemicals implementing the CLP Regulation. The aim was to explore how the current classification criteria covers the chemical space of regulated chemicals, and how it could be possible to estimate the market and societal consequences of changes in the criteria.
Evaluation of within-lab reproducibility of developmental neurotoxicity readouts using human neuronal/glia culture derived from induced pluripotent stem cells	To support intra-laboratory transferability and variability evaluation of human neuronal/glia test system derived from human induced pluripotent stem cells, exposed to the persistent organic pollutants (POP) mixture and single compounds.
Standardisation of microelectrode arrays (MEA) measurements using human neuronal networks	To establish the most relevant conditions for neuronal differentiation on a MEA chip, resulting in a robust and reproducible measurements of electrical activity.
Analysis of data related to Three Rs education and training	To analyse and visualise existing and new data related to the availability of Three Rs information and resources globally, with attention to how alternative methods and approaches are incorporated into education and training at three levels, high school, university and professional.
Modelling the diffusion behaviour of nanoparticles	To contribute to our understanding of the diffusion behaviour of nanomaterials in various multiphase (liquid) media with a view to elucidating how different media/nanomaterial properties affect diffusion and sedimentation behaviour. Attention was given to how nanomaterial diffusion can be measured using optical techniques and how the knowledge gained can be applied in various contexts, such as predicting nanomaterial behaviour in biological media relevant to <i>in vitro</i> cell-based assays.
Definition of indicators evaluating the impact of biomedical research funding	Identification and definition of a set of indicators potentially suitable to measure return on investment of funded biomedical research. As the ultimate goal of biomedical research on (noncommunicable) diseases should be the improvement of health and well-being, such indicators should specifically enable societal impact assessment.



**Box 6.4****JRC Summer School on Non-Animal Approaches in Science**

The JRC hosted more than 100 international postgraduate students and young professionals to explore the latest developments in non-animal approaches and their application in various fields such as regulatory toxicology and biomedical research.

The Summer School on "Non-Animal Approaches in Science - Challenges & Future Directions" took place at the JRC in Ispra (Italy) from 21 to 24 May 2019. The primary aim was to share knowledge and experience on the latest non-animal approaches used in research and testing including *in vitro* methods and computational modelling. In addition, participants explored the evolving role of the Three Rs in science and policy today within the European Union and beyond.

The action-packed programme combined lectures from top-level experts with a variety of interactive sessions including world cafés, participant debates, polls, a quiz and visits to the *in vitro* methods facility of the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM). Participants came from 34 countries worldwide (including India, Bangladesh, Korea, China, Brazil and Iran) and were selected on the basis of a letter of motivation, and an abstract describing their own work that was presented in poster sessions during the school. A number of supporting organisations generously provided 13 students with travel grants. "These very talented young scientists and professionals represent key enablers and decision-makers of the future", comments EURL ECVAM scientist Brigitte Landesmann, who organised the event. "We want to encourage and help them to become champions in shifting the paradigm so that society can benefit from excellent, relevant and impactful science that doesn't need animals."

*EURL ECVAM activities on education & training*

The first JRC Summer School on the theme of alternative methods took place in May 2017. These are part of a series of initiatives undertaken by EURL ECVAM in the field of education and training relevant to the Three Rs. A EURL ECVAM study showed indeed that although much knowledge exists on non-animal approaches, there is a clear need for better education, communication and outreach to enhance knowledge sharing and promote their uptake. EURL ECVAM has completed an overview of the Three Rs education and training landscape by mapping currently available courses and resources worldwide (see Section 6.3.1). The inventory is freely available through the JRC Data Catalogue (see Section 6.1.3).



Source: EU Science Hub (<https://europa.eu/!XH86gX>)

## 6.4 Dissemination Activities of EPAA

### 6.4.1 EPAA Partners Forum on Repeated Dose Toxicity (RDT) Testing

The European Partnership for Alternative Approaches to Animal Testing (EPAA) organised a Partners Forum on the topic of repeated dose toxicity (RDT) testing with the ultimate aim of identifying potential research gaps and synergies between industry sectors and ultimately to progress alternative approaches for RDT. The main findings and conclusions are reported in a scientific publication (Laroche *et al.*, 2019).

In brief, synergies between sectors were identified and it was recommended to explore them further. While the need to share data, information and methodologies across sectors was highlighted, it was recognised that for that to happen, there is a need for a clear benefit for the different parties.

Given the complexity of the animal test and the detailed knowledge that it provides, direct replacement of RTD tests by non-animal approaches is currently not possible. Depending on the regulatory context, the outcome of the animal test offers answers to different questions. Thus, it was agreed that formulating properly the questions and decision-making context would be the first step when addressing the challenging task of finding alternative approaches to RTD testing. Different approaches could be envisaged depending on the question/problem (*e.g.*, point of departure, identification of target organs) to be tackled.

It was further recognised that refinements and improvements to RTD could reduce and optimise animal use. In this regard, the adaptation of *in vivo* standard tests for systemic toxicity was highlighted as a possible way to improve the value of information coming from current guideline tests and to possibly reduce and waive other higher-tier studies.

As a follow up, a proposal for a blue sky workshop on RDT was presented and discussed (see [Section 5.10.9](#)).

### 6.4.2 EPAA Awards

The EPAA grants 3Rs Science Prizes to young scientists or 3Rs Refinement Prizes to laboratory technicians and animal caretakers (3Rs Refinement Prize), respectively, whose work has brought an outstanding contribution to the development and

implementation of alternatives to animal testing. Both the Science and the Laboratory technician prize are awarded alternating every other year. To this end, each year a jury of representatives from the EPAA partners is established, among them a staff member of EURL ECVAM.

In 2018, the Science prize was awarded to Dr Antje Appelt-Menzel from the University Hospital Würzburg of Germany. Her study addressed the “Need of robust and standardized test systems - Stem cell derived human *in vitro* models to determine blood-brain barrier penetration and neurotoxicity”.

In 2019, the 3Rs Refinement Prize was granted to Mrs Yvonne Armbrrecht from the University of Veterinary Medicine Hannover of Germany. Her study addressed the “Effect of positive conditioning on stress induced heart rate increase in sheep used in a research and veterinary education facility”. This is a simple and effective approach to train animals to cooperate in routine procedures, such as weighing or blood sampling.

Beginning of 2018, a new award programme was launched, the “EPAA 3Rs Student Grants”, with the aim to support students and young scientists conducting outstanding work in the area of alternative approaches to attend high-profile scientific events.

In 2018 and 2019, EPAA 3Rs student grants were awarded to attend the ESTIV, EUSAAT and EUROTOX annual congresses.

#### READ MORE

▶ EPAA 3Rs AWARDS: [europa.eu/lxx44cF](http://europa.eu/lxx44cF)

### 6.4.3 Other Dissemination Activities

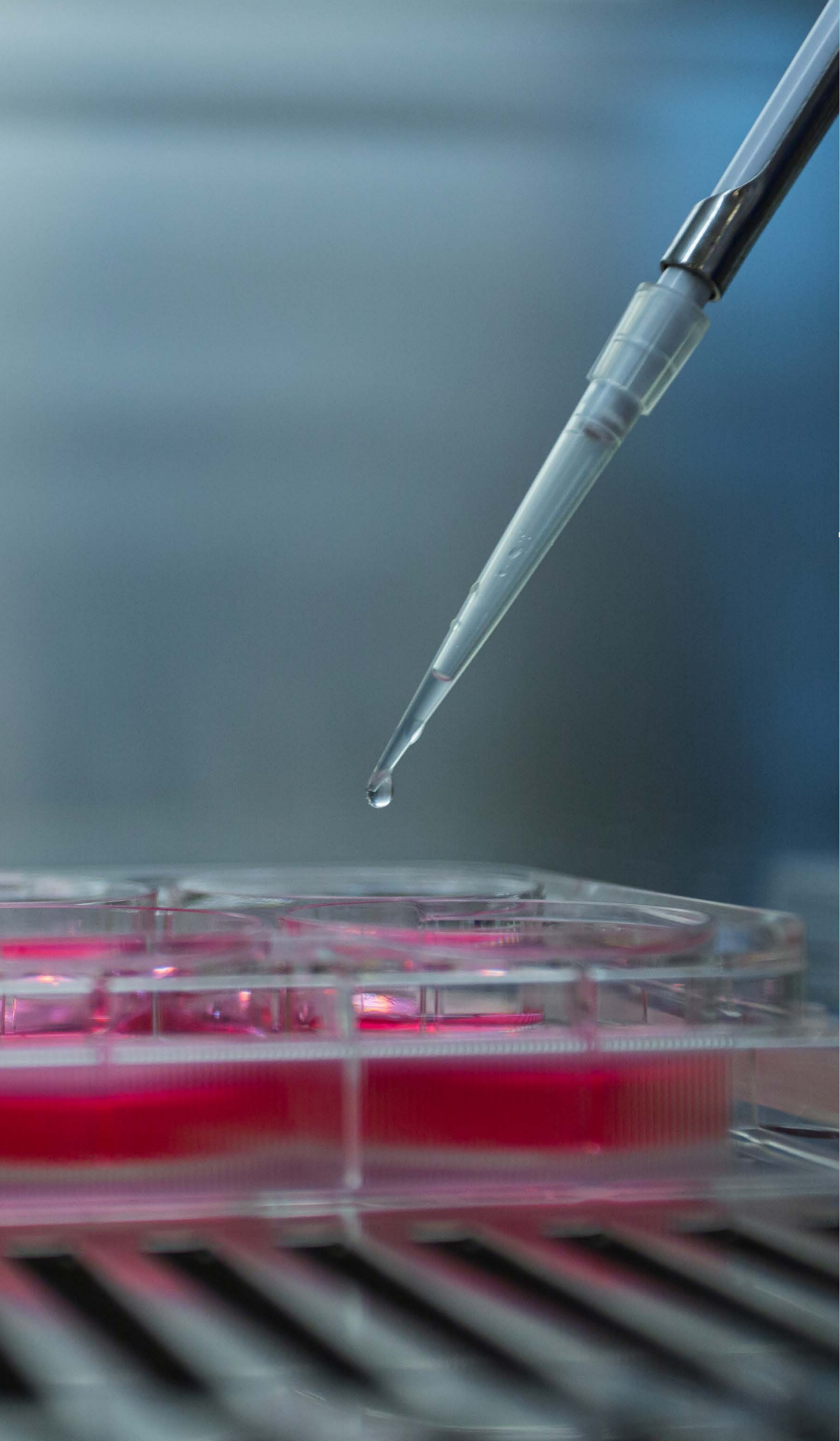
The 14<sup>th</sup> EPAA Annual Conference, which took place in Brussels, Belgium, on 20 November 2018, focused on “Pooling resources to promote the use of alternative methods for advancing safety assessment”, while the 15<sup>th</sup> EPAA Annual Conference took place in Brussels on 29 October 2019 and focused on “Building confidence for the use of 3Rs”.

During the two conferences, the overall EPAA achievements were presented, plus an in depth description of two selected projects: “Harmonisation of 3Rs in biologicals” (see [Section 5.10.6](#)) and “Prediction of carcinogenicity of agrochemicals” (see [Section](#)

5.10.2). The presentation sessions were followed with lively panel discussions. The recordings of the Conferences and the presentations were published on the EPAA website.

**READ MORE**

- ▶▶ European Partnership for Alternative Approaches to Animal Testing (EPAA): [europa.eu/!rq76qf](https://europa.eu/!rq76qf)
- ▶▶ EPAA Report of Annual Conference 2018: [europa.eu/!pw86cx](https://europa.eu/!pw86cx)
- ▶▶ EPAA Annual Conference 2019: [europa.eu/!Yv47kV](https://europa.eu/!Yv47kV)



# 7

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## **International Collaborations on Alternative Test Methods**



# 7.

## International Collaborations on Alternative Test Methods

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

EURL ECVAM organised an ICATM workshop on “The Future of Alternative Methods for Regulatory Testing and their Contribution to Public Health” which was held at the JRC in Ispra, Italy on 22 October 2019. This follows a series of successful ICATM workshops which had been organised by EURL ECVAM in collaboration with its ICATM partners the two preceding years, and which dealt with the international regulatory applicability and acceptance of alternative approaches to skin sensitisation assessment of chemicals (Casati *et al.*, 2018), and with the validation of alternative methods towards internationally recognised standards for regulatory application, respectively.

The outcome of these meetings were each time shared and presented at the OECD WNT meetings and also led to an OECD project on a Guideline for defined approaches on skin sensitisation (see [Section 5.6.9](#)) and to a proposal to shift to standards that classes of methods, rather than one single or very similar methods, should cover.

At the 2019 ICATM workshop, besides the ICATM partners, participants included the EURL ECVAM networks PARERE and ESTAF (see [Box 7.1](#) and [Section 4.8](#)). The participants shared their knowledge and experience

on the use and acceptance of alternative approaches from across the globe and identified challenges and opportunities to progress the field of alternatives at global level. The workshop celebrated also the 10<sup>th</sup> anniversary of ICATM and discussed how ICATM could contribute to reinforce the ingredients of success and meet the challenges in a future of alternative methods for regulatory testing.

The workshop was followed by an ICATM meeting on 23 October ([Figure 7.1](#)), which involved the ICATM partners only. Some proposals on the way forward were discussed as follow-up to the workshop. For example, ICATM partners suggested to prioritise problematic health and environmental effects from a public health perspective, to continue to work on standardisation and harmonisation but at a higher level, to provide guidance resources for validation and to be active in case studies that also allow the progression of non-standard methods. Some concrete follow-up actions were also agreed, such as to revisit OECD TG 439 in terms of standards, to devise the peer review of new complex methods, and to conceive new ways to approach chemical selection for complex toxicological endpoints.

#### READ MORE

▶ International Cooperation on Alternative Test Methods (ICATM): [europa.eu/Vt69mb](http://europa.eu/Vt69mb)



**Figure 7.1:** Members of ICATM at the ICATM meeting of 23 October 2019 at the JRC Ispra, Italy.

## 7.2 Global Consortium on *In Silico* Toxicology Protocols: Update on the Carcinogenicity Protocol

The Global Consortium on *in silico* toxicology protocols, led by Leadscope® Inc. US, is an international initiative, founded by NIEHS grant, aimed to create *in silico* protocols for major toxicological endpoints, similar to test guidelines routinely used in the application of *in vitro* or *in vivo* methods and to improve the efficiency, quality, and acceptance of such assessments. It includes over 60 international organisations (regulatory or governmental agencies, academics, industry, model developers and consultants across different sectors and regions) in view to achieve a broad consensus. EURL ECVAM is also part of the consortium.

The first publication of the consortium (Myatt *et al.*, 2018) described the general framework of the *in silico* toxicology protocols and how they could guide the international acceptance of computational predictions with experimental data to support the assessment of a chemical for adverse health effects. Different working groups are supporting protocol development for various toxicological endpoints. A genetic toxicology *in*

*silico* protocol has recently been completed (Hasselgren *et al.*, 2019) and a number of other protocols on skin sensitisation, acute toxicity, neurotoxicity, endocrine activity, and environmental toxicity are currently being drafted.

The work on the carcinogenicity protocol has started in spring 2019 and it is conducted using the *In Silico* Toxicity Consortium Wiki. The overall aim is to assemble the information available on different aspects of carcinogenicity. The genotoxicity protocol became a model template for customising the approach to the carcinogenicity framework. Sub-teams, including EURL ECVAM, have been established to work on different aspects of carcinogenicity. The latter link to the ten key characteristics of carcinogens, as defined by Smith *et al.* (2016), which are serving as backbone for the framework. Furthermore, they inform on the existing technologies and platforms (*in vivo*, *in vitro*, and *in silico*) that should be applied to a human-relevant carcinogenicity evaluation strategy, and in what combination. Work is ongoing and it is expected to be completed by the end of 2020.

## Box 7.1

## The International Cooperation on Alternative Test Methods celebrates 10 year anniversary

The JRC hosted a workshop to celebrate the 10<sup>th</sup> anniversary of the International Cooperation on Alternative Test Methods (ICATM). Invited participants from validation bodies, European agencies, national regulatory authorities and stakeholder organisations reflected on past achievements and looked to the future.

The ICATM workshop, titled "The Future of Alternative Methods for Regulatory Testing and their Contribution to Public Health", was organised by EURL ECVAM on 22 October 2019. It was held in conjunction with meetings of ECVAM's regulatory advisory network (PARERE) and its stakeholder forum (ESTAF). ICATM partners work together to enhance international cooperation and coordination on the scientific development, validation and regulatory application of alternative approaches to animal testing. "International collaboration is so important. We've been working closely these last 10 years to ensure a harmonised approach across different regions and regulated sectors. We want to be as efficient and impactful as possible in what we do together", says Maurice Whelan, head of EURL ECVAM.



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. In 2011, the National Institute of Environmental Health Sciences in South Korea formally joined the cooperation. Since then, other governmental institutions from Brazil, Singapore and China have been participating in ICATM initiatives on an *ad hoc* basis. The long established collaboration between ICATM partners for the validation and peer review of alternative methods has led to their international adoption as OECD Test Guidelines and has helped expedite their regulatory uptake within the respective countries.



One example of the effectiveness of this cooperation is the ICATM workshop that brought together ICATM partners and international regulators to decide the best way forward to bring new non-animal approaches to assessing skin sensitisation into the regulatory domain, to give them the same status as the traditional animal tests. This led to a project at the OECD to develop the first Guideline for skin sensitisation that combines *in vitro* and computational methods within the context of Integrated Approaches to Testing and Assessment.

Source: EU Science Hub (<https://europa.eu/gY93MQ>)



### 7.3 Prioritising Activities to Meet Regulatory Needs for Acute Systemic Toxicity

In order to progress the development of alternative approaches for acute systemic toxicity via mechanisms of action (see previous efforts reported in sections 2.9 and 2.4 in Zuang *et al.*, (2017; 2018), respectively, a workshop that focused on mechanisms of acute systemic toxicity associated with chemicals after oral, dermal and inhalation exposure was held in October 2019 at the National Institute of Health (NIH) in Bethesda (USA). The ultimate aim was to prioritise the activities to meet the regulatory needs for acute systemic toxicity. The workshop was jointly organised by PCRM (Physicians Committee for Responsible Medicine), NICEATM (The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods), PETA (People for the Ethical Treatment of Animals), EPA (the United States Environmental Protection Agency), JRC and Dow Chemical.

It is expected that the discussions will help to a) define how to calculate the LD50 of a chemical mixture/formulated product; b) identify gaps where model (or assay) development or optimisation is needed; c) understand regulatory needs for model (or assay) outputs; d) pinpoint the types of mechanistic information that would be useful, and e) establish the feasibility of using artificial intelligence in model development.

#### READ MORE

- ▶▶ Workshop Mind the Gaps: Prioritizing Activities to Meet Regulatory Needs for Acute Systemic Lethality: [ntp.niehs.nih.gov/whatwestudy/niceatm/3rs-meetings/past-meetings/at-2019/atwksp-2019.html?utm\\_source=direct&utm\\_medium=prod&utm\\_campaign=ntpgolinks&utm\\_term=atwksp-2019](http://ntp.niehs.nih.gov/whatwestudy/niceatm/3rs-meetings/past-meetings/at-2019/atwksp-2019.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=atwksp-2019)

### 7.4 Collaboration with the US Interagency Coordinating Committee on the Validation of Alternative Methods Workgroups

The US Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) has established *ad hoc* workgroups to perform specific tasks identified by ICCVAM as being important for the development or validation of new approach methods, and the implementation of the “Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States” (ICCVAM, 2018). The workgroups are chaired by representatives from agencies that use or require data from the topic of

interest. ICCVAM member agencies and ICATM partners (EURL ECVAM, the Japanese Centre for the Evaluation of Alternative Methods, the Korean Centre for the Evaluation of Alternative Methods, and Health Canada) are invited to participate in the workgroups.

Some of the workgroups described below were converted into expert groups in 2019, as they fulfilled their charges in the context of the ICCVAM strategic roadmap.

#### READ MORE

- ▶▶ Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM): [ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam](http://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam)

#### 7.4.1 ICCVAM Ocular and Dermal Irritation Workgroup

ICCVAM’s efforts to establish alternatives to eye and skin irritation testing are led by the ICCVAM Ocular and Dermal Irritation Workgroup (ODIWG), which is comprised of experts from multiple member agencies and is supported by NICEATM. The ODIWG’s main activity is to evaluate and promote the use of alternative test methods for regulatory use in eye and skin irritation hazard assessments.

In June 2019, the ODIWG published a scoping document that identifies the United States agencies’ regulatory requirements, needs and decision contexts for skin and eye irritation testing (Choksi *et al.*, 2019).

The ICCVAM ODIWG members also served as the Validation Management Team for the interlaboratory validation study of the OptiSafe Method. The study comprised the testing of 90 chemicals in two independent phases following a training and transferability phase, and was completed in 2019. A manuscript describing the conduct and outcome of the study has been submitted for publication.

NICEATM and the PETA International Science Consortium are conducting a study with stakeholders from ICCVAM, ODIWG, EURL ECVAM, PMRA, and industry, to evaluate the eye irritation potential of agrochemical formulations using non-animal methods. Prospective testing was conducted with the Bovine Corneal Opacity and Permeability method (BCOP - TG 437 with and without histopathology), the Isolated Chicken Eye method (ICE - TG 438 with and without histopathology), the Neutral Red Release (NRR), the EpiOcular™ assay (TG 492 Eye Irritation Test (EIT) and the ET50

time-to-toxicity protocol) and the Porcine Corneal Ocular Reversibility Assay (PorCORA) on 16 formulations (7 EPA Cat. I, 1 EPA Cat. II, 1 EPA Cat. III and 7 EPA Cat. IV) to fill data gaps and complete a dataset of existing data provided by five companies. No single test method could be used to assign a correct classification for all 16 pesticide formulations relative to their *in vivo* classifications, but the results suggest that combining results of multiple tests in an integrated approach may be useful in classification of these formulations. Next steps in this project are currently being discussed.

No additional activities have been suggested to address the strategy and roadmap for using non-animal approaches to replace animal use in ocular or dermal irritation/corrosion testing and therefore, ICCVAM members agreed that the ODIWG should be sunset as a formal ICCVAM workgroup and be converted into an expert group. The members of the expert group will receive relevant information and may be asked to review projects and documents, but no further formal activities will be conducted. EURL ECVAM participated as liaison in the ODIWG and will continue its participation in the ICCVAM Ocular and Dermal Irritation Expert Group (ODIEG).

#### 7.4.2 ICCVAM Skin Sensitisation Workgroup

EURL ECVAM is also participating as liaison in the ICCVAM Skin Sensitisation Workgroup (SSWG) coordinating the implementation of the roadmap goals for skin sensitisation testing. The workgroup has members from seven ICCVAM agencies and representation from ICATM.

Among other activities (<https://ntp.niehs.nih.gov/pub-health/evalatm/natl-strategy/rdmp-imp/imp-sensit/index.html>) the Workgroup is also supporting the development of the OECD guideline on defined approaches for skin sensitisation. In April 2018, EPA released a draft Science Policy to reduce animal use through defined approaches to identify potential skin sensitizers. Under this policy, EPA's Office of Pesticide Products and Office of Pollution Prevention and Toxics will accept submissions for single chemicals that can be tested using non-animal methods integrated in two specific defined approaches. This draft policy is the result of national and international collaboration between ICCVAM, NICEATM, EURL ECVAM and Health Canada's Pest Management Regulatory Agency. In addition, the US National Toxicology Program is currently testing approximately 200 chemicals of interest to ICCVAM

agencies to further support the evaluation of defined approaches. All testing was completed in 2019 and data will be made publicly available.

In July 2019, ICCVAM members agreed that the SSWG should be converted into an expert group having completed the SSWG charges.

#### 7.4.3 ICCVAM Acute Toxicity Workgroup

EURL ECVAM is participating as liaison in the ICCVAM Acute Toxicity Workgroup (ATWG) since 2016. The main focus of ATWG is the regulatory implementation of non-animal methods for acute systemic toxicity testing (Kleinstreuer *et al.*, 2018; Strickland *et al.*, 2018). The activities of the Working Group contribute to implementing the goals of the ICCVAM strategic roadmap (ICCVAM, 2018). The group has been working on the identification, collection and curation of high quality rat oral acute toxicity LD50 data using multiple existing sources. A manuscript summarising this work and the analyses carried out to assess the variability of the data is in preparation. This large dataset of rodent studies was further used in the context of an international collaborative project aimed to develop *in silico* models of acute oral systemic toxicity.

#### 7.4.4 ICCVAM Developmental and Reproductive Toxicology Workgroup

Acknowledging the lack of alternative methods for developmental and reproductive toxicology testing, ICCVAM has established a workgroup aiming at drafting an ICCVAM strategy and roadmap for reproductive and developmental toxicity testing. The aim is to develop and evaluate alternative approaches to classifying chemicals for reproductive and developmental toxicity hazards using *in vitro* and/or *in silico* methods. The initial focus of the group is on developmental toxicity (reproductive effects will be addressed in a further step). The first task of the group is to identify international regulatory requirements for developmental toxicity testing, mapping commonalities and differences between agencies. The workgroup was converted into an expert group in September 2019.

#### 7.4.5 ICCVAM Ecotoxicology Workgroup

The ICCVAM Ecotoxicology Workgroup was established in 2018 to provide expertise in identifying and evaluating alternative approaches to identify ecological and environmental hazards using *in vitro*



and/or *in silico* methods. For this purpose the group is identifying the US agencies needs for assessment of ecotoxicity, emerging technologies for ecotoxicity and environmental safety, and their utility in regulatory testing. This summary will then be used to advance the development and evaluation of defined approaches for screening, testing and assessment of relevant endpoints. These evaluations and supporting documentation will be made available to the public and may also be submitted to OECD to facilitate international harmonisation and global implementation of alternative approaches for ecotoxicity testing.

#### 7.4.6 ICCVAM In Vitro to In Vivo Extrapolation Workgroup

The ICCVAM-IVIVE Workgroup was established to bring together views on the term *In Vitro to In Vivo* Extrapolation (IVIVE). EURL ECVAM is part of this international effort to agree on the IVIVE definition, to harmonise relevant nomenclature and identify applications of IVIVE for regulatory needs.

#### 7.4.7 ICCVAM Read-across Workgroup

The initial work of the Read-Across Workgroup (RAWG), has focused on understanding what read-across approaches US agencies require, routinely apply, or are familiar with, and what read-across tools are being routinely used or are under development. The findings of this work, to which a member of EURL ECVAM staff contributed, have been published (Patlewicz *et al.*, 2019).

### 7.5 Contributions to Health and Environmental Sciences Institute Projects

#### 7.5.1 HESI Genetic Toxicology Technical Committee

The Health and Environmental Sciences Institute (HESI) is a non-profit organisation whose mission is to collaboratively identify and help to resolve global health and environmental challenges through the engagement of scientists internationally from academia, government, industry, NGOs and other partners. Its Genetic Toxicology Technical Committee (GTTC) is committed to integrating genetic toxicology into risk assessment with a specific focus on improving new and existing test guidelines, strategies, interpretation of results and examining non-traditional modalities, including novel technologies. Current activities are also in line with the

aim to enhance the performance of the *in vitro* testing battery and to reduce and optimise the use of animals in *in vivo* testing previously drawn in the EURL ECVAM Genotoxicity Strategy (Corvi *et al.*, 2013).

The status of GTTC projects have been discussed during the annual meeting that took place in Washington DC, US, in May 2019. Significant progress has been made on a number of projects such as data interpretation of the *in vitro* micronucleus assay; quantitative analysis for the setting of genotoxicity thresholds and benchmark dose (BMD) calculations across genotoxicity *in vivo* studies with characterisation of critical effect size (CES); analysis of the most appropriate *in vivo* follow-up testing; development of AOPs related to specific genotoxic mode of action pathways, etc. In this context, methods to determine the mode of action of genotoxic agents are investigated, as well as the application of new technologies to establish the mode of action of genotoxicity for new chemical entities. During the year, EURL ECVAM also contributed to the application of the recent 'clean sheet' conceptual framework (Dearfield *et al.*, 2017) for a next-generation testing strategy for assessment of genomic damage for risk assessment, using the industrial chemical benzene as a case study. The results of this study which demonstrate the utility of the new framework to quantitatively model human risk on the basis of genetic damage, have recently been published (Luijten *et al.*, 2020).

#### READ MORE

▶ HESI Genetic Toxicology (GTTC) Committee: [hesiglobal.org/genetic-toxicology-gttc](https://hesiglobal.org/genetic-toxicology-gttc)

#### 7.5.2 HESI Physiologically based Pharmacokinetic Working Group

The HESI Physiologically based Pharmacokinetic (PBPK) Working Group brings together an international pool of experts in PBK modelling. The goal is to facilitate the use of PBK modelling approaches in various risk assessment applications. Two main efforts are ongoing: a) the establishment of a harmonised template to report information, and provide recommendations to model reviewers to facilitate the uptake of PBK models in regulatory risk assessment and b) the development of a framework and decision tree on PBK applications based on different degrees of data availability.

#### READ MORE

▶ HESI PBPK Models Committee: [hesiglobal.org/pbpb-models/#4](https://hesiglobal.org/pbpb-models/#4)

### 7.5.3 HESI Animal Alternatives in Environmental Risk Assessment Committee

The work of the HESI Animal Alternatives in Environmental Risk Assessment committee focused over the past years on effluents testing (Norberg-King *et al.*, 2018) and the development of the ecological Threshold of Toxicological Concern (eco-TTC) concept for environmental risk assessment (Belanger *et al.*, 2015).

EURL ECVAM has contributed to this committee for many years and worked mainly on the eco-TTC concept as part of an international collaboration/working group established by HESI. This group developed

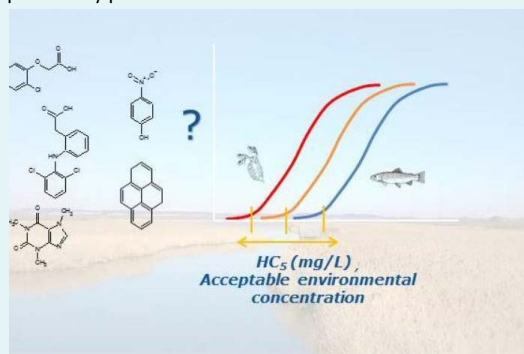
the EnviroTox database with approximately 91,000 unique ecotoxicological records, 4,000 chemicals and 1,500 aquatic species from three trophic levels (fish, invertebrates, algae/plants). The EnviroTox database (Connors *et al.*, 2019) is publicly available since November 2018 (see Box 7.2). It includes an analytical tool allowing the calculation of environmental concentration of no concern based on statistical distributions of two types, Predicted No Effect Concentration (PNEC) distribution or ecotoxicological data distribution, according to particular research criteria. EURL ECVAM worked in particular on the evaluation of the existing aquatic mode of action classification

## Box 7.2

### New online tool to improve the environmental risk assessment of chemicals

EURL ECVAM scientists have contributed to an international project to improve the environmental risk assessment of chemicals which has built a high quality ecotoxicological database called EnviroTox and developed new methodology for identifying chemicals that may pose a threat to the aquatic environment.

Environmental risk assessment of chemicals typically requires toxicity data for fish, algae and crustaceans. To carry out more rigorous assessments additional data are often needed. However, it is not practically possible to test all chemicals for effects on all wildlife species and it is even more challenging to assess risks posed by combined exposure to multiple chemicals, such as chemical mixtures. A pragmatic and scientifically credible way of filling data gaps is to assign chemicals to groups and examine the distribution of toxicological effects across chemicals in the group. The groups can be composed in various ways, for example based on species, chemical class or mode of toxicological action. Since the chemicals within each group usually have a range of toxic potencies, the most toxic values can be used to derive a safe concentration, known as the ecological Threshold of Toxicological Concern (eco-TTC). The ecoTTC approach can be used to fill data gaps while reducing the need for additional animal testing.



#### Case study published

A recently published case study demonstrated that eco-TTC values can facilitate a screening level mixture assessment if data are missing for a limited number of chemicals (Kienzler *et al.*, 2019a). Additional case studies are ongoing to better understand how the safe exposure levels derived by the ecoTTC approach compare with regulatory threshold values based on experimental data.

#### New database

A valuable resource produced by this international collaborative project, led by the Health and Environmental Sciences Institute (HESI, USA), is a new public database of ecotoxicological data, the EnviroTox database, which brings together more than 91,000 curated records for more than 4000 chemicals across 1500 species.

Source: EU Science Hub (<https://europa.eu/IPQ68FR>)

frameworks, their overlap and possibilities to move forward (Kienzler *et al.*, 2017); the implementation of a consensus mode of action in the database to help the user to quickly distinguish narcotics from non-narcotics chemicals (Kienzler *et al.*, 2019b) and the possible use of those environmental threshold concentrations of no concern for data gap filling in the context of mixtures risk assessment (Kienzler *et al.*, 2019a).

#### READ MORE

- ▶▶ EnviroTox database: [envirotoxdatabase.org](http://envirotoxdatabase.org)
- ▶▶ HESI Animal Alternatives in Environmental Risk Assessment Committee: [hesiglobal.org/animal-alternatives-in-environmental-risk-assessment](http://hesiglobal.org/animal-alternatives-in-environmental-risk-assessment)

#### 7.5.4 HESI Bioaccumulation Technical Committee

The Bioaccumulation Technical Committee aims at developing methods needed for a tiered approach to assess potential bioaccumulation of organic chemicals. The activities cover terrestrial and aquatic bioaccumulation and are described in more than 25 publications (see “read more” below).

EURL ECVAM contributes mainly to the work on development of *in vitro* methods for bioaccumulation testing. Thus, USA (represented by HESI) and EC (represented by JRC/EURL ECVAM) co-led an OECD project to establish new test guidelines on determination of *in vitro* fish intrinsic hepatic clearance. The two OECD TGs 319A (OECD, 2018d) and 319B (OECD, 2018c) were published last year as well as the results of the multi-laboratory ring trial (coordinated by HESI during 2014-2016; Nichols *et al.*, 2018; OECD, 2018f). The clearance values can be either used directly in a PBTK model or extrapolated to the whole animal to calculate a “whole body” biotransformation rate constant (kM), which is used as an input to *in silico* bioaccumulation prediction models (OECD, 2018a).

In order to enhance uptake, application, and advancement of these approaches, HESI organised a workshop on “Fish Biotransformation in Bioaccumulation: Technical Workshop” (Washington, USA, 1 to 3 October 2019), followed by a training workshop for regulators and manufacturers informing on the available tools and their application. A summary of the workshop discussion will be published in 2020.

#### READ MORE

- ▶▶ Development of Methods for a Tiered Approach to Assess

Bioaccumulation of Chemicals Committee: [hesiglobal.org/development-of-methods-for-a-tiered-approach-to-assess-bioaccumulation-of-chemicals](http://hesiglobal.org/development-of-methods-for-a-tiered-approach-to-assess-bioaccumulation-of-chemicals)

#### 7.6 International Collaborations on the Implementation of GIVIMP

The recently approved OECD Guidance Document, Good *In Vitro* Method Practices (GIVIMP; OECD, 2018b), coordinated by EURL ECVAM, provides a framework of technical and quality practices to help ensure that the overall development and implementation of *in vitro* methods is of the highest possible quality and contributes as such to scientific integrity. While the guidance is intended for all OECD member countries and encompasses a wide range of audiences including method developers, validation bodies and end users, its greatest impact may be in regions where *in vitro* methods are just beginning to take root.

China and Brazil are continuing to adopt and implement non-animal testing approaches for the safety assessment of cosmetics and ingredients. Collaborative efforts between industry and the Institute for *In Vitro* Sciences (IIVS, Gaithersburg, USA) have focused on the transfer of several OECD test guideline methods to government laboratories in China and have supported the creation of an *in vitro* toxicology testing laboratory within the Zhejiang Institute for Food and Drug Control (Hangzhou, China). Recently BASF SE (Ludwigshafen, Germany) and IIVS have partnered to introduce a cell based *in vitro* skin sensitisation test, LuSens method, into China using the principles of GIVIMP. This case study exemplifies the practical way in which the GIVIMP guidance can assist interested parties in the development, transfer and establishment of *in vitro* approaches.

Since 2016, EURL ECVAM and the Brazilian Centre for the validation of alternative methods (BraCVAM) agreed on joint actions for cooperation on alternative methods to animal use. Specific areas addressed are: i) growing the collaboration between the Brazilian laboratory network supporting the validation and deployment of alternative methods, RENAMA [supported by the Ministry of Science, Technology, Innovation and Communication (MCTIC)], InMetro, and the EU equivalent, EU-NETVAL (coordinated by JRC/EURL ECVAM, see Section 4.7), with particular focus on GIVIMP ii) sharing of knowledge related to the Three Rs of animal testing across jurisdictions and sectors and iii) promoting the use of alternative approaches for regulatory safety assessment.

# 8

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## Conclusions

In the context of regulatory testing, research initiatives at EU level aim to progress mechanism-based toxicity testing and risk assessment of chemicals with non-animal methods and approaches for complex endpoints such as repeated dose toxicity, and developmental and reproductive toxicity. Other EU-funded research projects focus on new testing and screening methods to identify endocrine disrupting chemicals or on chemical mixtures.

The very interesting and fast evolving field of organ-on-chip devices and methods is closely monitored through the EU funded project ORCHID. EURL ECVAM has been collaborating with ORCHID on exploring the impact of organ-on-chip technology in different areas, in particular in the field of chemicals toxicity.

Regarding EURL ECVAM's own in-house research, EURL ECVAM has investigated new ways for carcinogenicity testing by integrating data across toxicity endpoints in an attempt to avoid redundant *in vivo* studies and minimise reliance on apical endpoint tests. In the same spirit, EURL ECVAM has initiated an analysis of existing mechanistic knowledge related to the toxicological effects on target organs observed in animal models after repeated exposure to a test chemical for a better understanding of the relevance of repeated-dose toxicity studies.

EURL ECVAM also analysed the relevance and possible gaps in the current way of testing for carcinogenicity, taking into consideration the increasing global trend of chemical production and the prevalence of certain cancers.

EURL ECVAM organised an exploratory workshop to discuss how Digital Transformation and the emerging field of Artificial Intelligence could impact the scientific-technical evaluation and the decision-making processes of chemical risk assessment.

In the framework of the Cefic Long-Range Research Initiative, R&D projects related to fish toxicity and bioaccumulation continued to be funded. These include the development of a weight of evidence approach integrating the fish embryo test to predict potential acute fish toxicity; the development of a tiered testing strategy based on *in vitro* approaches for the assessment of the bioaccumulation potential of chemicals; the development of a toxicokinetic

modelling framework for bioaccumulation assessment in mammals and; the improvement of *in vitro* approaches to predict fish bioconcentration.

During 2019, test methods were submitted and evaluated by EURL ECVAM in the areas of skin and respiratory sensitisation.

The androgen receptor transactivation assay AR-CALUX, as well as the Bioelution test method that measures the relative bioaccessibility of metals in inorganic metal compounds and metal-containing materials using a simulated gastric fluid, were both peer reviewed positively by ESAC.

ESAC also reviewed the scientific validity of non-animal-derived antibodies and non-antibody affinity reagents used for research, diagnostics, and regulatory applications with a successful outcome. The aim is to replace the current use of animals for the generation and production of monoclonal and polyclonal antibodies as well as other affinity reagents.

EURL ECVAM continued its validation efforts with EU-NETVAL on a series of *in vitro* methods for the detection of chemicals that disrupt normal thyroid hormone function. During the initial phase of the validation study, these efforts mainly concentrated on guaranteeing the quality and long-term availability of the test systems and of the performance of the validation study according to GIVIMP, the selection of relevant reference and control items and the comprehensive definition of the 17 methods.

External validation efforts mainly focused on transcriptomics/bioinformatics-based *in vitro* methods for skin sensitisation and, in the area of genotoxicity, on *in vitro* genotoxicity tests for dermal exposure using 3D skin models (*i.e.*, RSMN and 3D Skin Comet) and the hen's egg test for micronucleus induction (HET-MN).

Validation of analytical methods for the quality control of biologicals, including Three Rs methods, as well as establishment of reference preparation, are taking place in the Biological Standardisation Programme (BSP) of EDQM at the Council of Europe. These projects focus on the development of new European Pharmacopoeia (Ph. Eur.) methods, several of these are dedicated to applying the Three Rs principles to the field of quality control of biologicals.



The regulatory use of standardised methodology accepted at international level to determine the hazardous properties of chemicals is crucial to make the EU Chemicals Policy framework, including more than 40 pieces of legislation, operational.

EURL ECVAM has therefore continued to work closely with international standardisation organisations in the field such as the OECD and the UN to promote the international adoption of standardised non-animal methods and criteria for chemicals safety testing or safe transport of goods.

International harmonisation is also crucial in the area of vaccines, where EURL ECVAM is working on behalf of EMA, with experts of the VICH on the development of VICH guidelines on harmonisation of criteria to waive the target animal / laboratory animal batch safety testing for veterinary vaccines.

With a view to promote the regulatory acceptance and use of alternative approaches, EURL ECVAM also continued to collaborate closely with regulators and stakeholders through its networks PARERE and ESTAF as well as through the EPAA partnership and international cooperations (*e.g.*, ICATM, ICCR).

Regarding EURL ECVAM's mandate on coordinating and promoting the development and use of alternatives to procedures in the areas of basic and applied research, several reviews on models and methods already in use in the areas of respiratory tract diseases, neurodegenerative disorders, cardiovascular

diseases, breast cancer, immunogenicity testing for advanced therapy medicinal products, autoimmune diseases and immune oncology models, were undertaken and will be published in 2020.

Similarly, knowledge sharing activities that encourage cross-disciplinarity in the biosciences and that monitor the level, or lack of uptake of alternative approaches in various contexts are meant to support the wider and more rapid application of such approaches.

Accelerating the development and uptake of the Three Rs within an E&T context was another important activity of EURL ECVAM in 2019. For example, a review of E&T courses and resources with a mention of the Three Rs was carried out. This allowed to identify 569 E&T courses and resources with Three Rs relevance in 52 countries worldwide. In the framework of an EP pilot project and in collaboration with external partners (Ecorys, Syrcle and European Schoolnet), EURL ECVAM also developed guidance for decision-makers in educational organisations on how to introduce the Three Rs in education programmes and curricula, and Three Rs teaching resources for secondary school students, universities, and early career scientists. The guidance document and the teaching resources will become available in 2020.

Finally, EURL ECVAM continued to share and disseminate information on alternative approaches through workshops, trainings and its specialised database services.

## References

- Amacher, D.E. (2010). The effects of cytochrome P450 induction by xenobiotics on endobiotic metabolism in pre-clinical safety studies. *Toxicology Mechanisms and Methods*, 20(4), 159-166. doi:10.3109/15376511003690307
- Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrano, J.A., Tietge, J.E., & Villeneuve, D.L. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry*, 29(3), 730-741. doi:10.1002/etc.34
- Bal-Price, A., Pistollato, F., Sachana, M., Bopp, S.K., Munn, S., & Worth, A. (2018). Strategies to improve the regulatory assessment of developmental neurotoxicity (DNT) using *in vitro* methods. *Toxicology and Applied Pharmacology*, 354, 7-18. doi:10.1016/j.taap.2018.02.008
- Basketter, D., Azam, P., Casati, S., Corvaro, M., Ezendam, J., Griem, P., Hubesch, B., Irizar, A., Kern, P., Manou, I., Mehling, A., & Rossi, L.H. (2019). Applying non-animal strategies for assessing skin sensitisation report from an EPAA/cefic-LRI/IFRA Europe cross sector workshop, ECHA helsinki, February 7<sup>th</sup> and 8<sup>th</sup> 2019. *Regulatory Toxicology and Pharmacology*, 109, 104477. doi:10.1016/j.yrtph.2019.104477
- Belanger, S.E., Sanderson, H., Embry, M.R., Coady, K., Dezwart, D., Farr, B.A., Gutsell, S., Halder, M., Sternberg, R., & Wilson, P. (2015). It is time to develop ecological thresholds of toxicological concern to assist environmental hazard assessment. *Environmental Toxicology and Chemistry*, 34(12), 2864-2869. doi:10.1002/etc.3132
- Bergman, Å., Rüegg, J., Söder, O., Öberg, M., Drakvik, E., Jönsson, M., Rudén, C., Bornehag, C.-G., Sturve, J., Lindh, C., Eewa, N., Demeneix, B., Fini, J.-B., Kiess, W., Kitraki, E., Kiviranta, H., Testa, G., & Gennings, C. (2019). EDC-MixRisk Policy Brief. Available at <https://edcmixrisk.ki.se/wp-content/uploads/sites/34/2019/03/Policy-Brief-EDC-MixRisk-PRINTED-190322.pdf>
- Bernasconi, C., Pelkonen, O., Andersson, T.B., Strickland, J., Wilk-Zasadna, I., Asturiol, D., Cole, T., Liska, R., Worth, A., Muller-Vieira, U., Richert, L., Chesne, C., & Coecke, S. (2019). Validation of *in vitro* methods for human cytochrome P450 enzyme induction: Outcome of a multi-laboratory study. *Toxicology in Vitro*, 60, 212-228. doi:10.1016/j.tiv.2019.05.019
- Bonn, B., Svanberg, P., Janefeldt, A., Hultman, I., & Grime, K. (2016). Determination of Human Hepatocyte Intrinsic Clearance for Slowly Metabolized Compounds: Comparison of a Primary Hepatocyte/Stromal Cell Co-culture with Plated Primary Hepatocytes and HepaRG. *Drug Metabolism and Disposition*, 44(4), 527-533. doi:10.1124/dmd.115.067769
- Bopp, S., Barouki, R., Brack, W., Dalla Costa, S., Dorne, J.C.M., Drakvik, P.E., Faust, M., Karjalainen, T.K., Kephelopoulos, S., van Klaveren, J., Kolossa-Gehring, M., Kortenkamp, A., Lebre, 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.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
- Carusi, A., Whelan, M., & Wittwehr, C. (2019). Bridging Across Methods in the Biosciences - BeAMS. EUR 29852 EN. Publications Office of the European Union, Luxembourg. JRC116305. Available at: <https://publications.jrc.ec.europa.eu/repository/handle/JRC116305>
- Casati, S., Aschberger, K., Barroso, J., Casey, W., Delgado, I., Kim, T.S., Kleinstreuer, N., Kojima, H., Lee, J.K., Lowit, A., Park, H.K., Regimbald-Krnel, M.J., Strickland, J., Whelan, M., Yang, Y., & Zuang, V. (2018). Standardisation of defined approaches for skin sensitisation testing to support regulatory use and international adoption: position of the International Cooperation on Alternative Test Methods. *Archives of Toxicology*, 92(2), 611-617. doi:10.1007/s00204-017-2097-4
- Choksi, N.Y., Truax, J., Layton, A., Matheson, J., Mattie, D., Varney, T., Tao, J., Yozzo, K., McDougal, A.J., Merrill, J., Lowther, D., Barroso, J., Linke, B., Casey, W., & Allen, D. (2019). United States regulatory requirements for skin and eye irritation testing. *Cutaneous and Ocular Toxicology*, 38(2), 141-155. doi:10.1080/15569527.2018.1540494

- Coecke, S., Rogiers, V., Bayliss, M., Castell, J., Doehmer, J., Fabre, G., Fry, J., Kern, A., & Westmoreland, C. (1999). The Use of Long-term Hepatocyte Cultures for Detecting Induction of Drug Metabolising Enzymes: The Current Status. *ATLA*, 27(4), 579-638. doi:10.1177/026119299902700408
- Coecke, S., Pelkonen, O., Leite, S.B., Bernauer, U., Bessems, J.G., Bois, F.Y., Gundert-Remy, U., Loizou, G., Testai, E., & Zaldivar, J.M. (2013). Toxicokinetics as a key to the integrated toxicity risk assessment based primarily on non-animal approaches. *Toxicology in Vitro*, 27(5), 1570-1577. doi:10.1016/j.tiv.2012.06.012
- Connors, K.A., Beasley, A., Barron, M.G., Belanger, S.E., Bonnell, M., Brill, J.L., de Zwart, D., Kienzler, A., Krailler, J., Otter, R., Phillips, J.L., & Embry, M.R. (2019). Creation of a Curated Aquatic Toxicology Database: EnviroTox. *Environmental Toxicology and Chemistry*, 38(5), 1062-1073. doi:10.1002/etc.4382
- 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, Luxembourg. JRC86616. Available at: <https://publications.jrc.ec.europa.eu/repository/handle/JRC86616>
- Corvi, R., Madia, F., Guyton, K.Z., Kasper, P., Rudel, R., Colacci, A., Kleinjans, J., & Jennings, P. (2017). Moving forward in carcinogenicity assessment: Report of an EURL ECVAM/ESTIV workshop. *Toxicology in Vitro*, 45(3), 278-286. doi:10.1016/j.tiv.2017.09.010
- Dearfield, K.L., Gollapudi, B.B., Bemis, J.C., Benz, R.D., Douglas, G.R., Elespuru, R.K., Johnson, G.E., Kirkland, D.J., LeBaron, M.J., Li, A.P., Marchetti, F., Pottenger, L.H., Rorije, E., Tanir, J.Y., Thybaud, V., van Benthem, J., Yauk, C.L., Zeiger, E., & Luijten, M. (2017). Next generation testing strategy for assessment of genomic damage: A conceptual framework and considerations. *Environmental and Molecular Mutagenesis*, 58(5), 264-283. doi:10.1002/em.22045
- Dent, M., Amaral, R.T., Da Silva, P.A., Ansell, J., Boisleve, F., Hatao, M., Hirose, A., Kasai, Y., Kern, P., Kreiling, R., Milstein, S., Montemayor, B., Oliveira, J., Richarz, A., Taalman, R., Vaillancourt, E., Verma, R., Posada, N.V.O.R.C., Weiss, C., & Kojima, H. (2018). Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients. *Computational Toxicology*, 7, 20-26. doi:10.1016/j.comtox.2018.06.001
- DG ENV. (2014). Directive 2010/63/EU - Education and Training Framework. DG Environment. Available at: [https://ec.europa.eu/environment/chemicals/lab\\_animals/pdf/Endorsed\\_E-T.pdf](https://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_E-T.pdf)
- DG Environment and Ministry of Environment and Food of Denmark. (2019). *EU Chemicals Policy 2030: Building on the past, moving to the future - Brussels, 27-28 June 2019 - Report of the conference*. Publications Office of the European Union, Luxembourg. doi:10.2779/127571. Available at: <https://euchemicalspolicy2030.teamwork.fr/docs/report.pdf>
- Dura, A., & Holloway, M. (2019). Characterising the current provision of education and training courses relevant to the Replacement, Reduction and Refinement (Three Rs) of animals used for scientific purposes. *Biomedical Science and Engineering*, 3(2s):88, 17-18. doi:10.4081/bse.2019.88
- EC. (1991). Communication from the Commission to the Council and the European Parliament on the Establishment of a European Centre for the Validation of Alternative Methods. SEC (91) 1794final, 29 June 1991.
- EC. (2012). Consolidated version of the Treaty on the Functioning of the European Union. *Official Journal of the European Union*. C 326, 26/10/2012 P. 0001-0390. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A12012E%2FTXT>
- EC. (2020). 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. (2016). *New Approach Methodologies in Regulatory Science - Proceedings of a scientific workshop*. European Chemicals Agency (ECHA), Helsinki. ECHA-16-R-21-EN. Available at: [https://echa.europa.eu/documents/10162/22816069/scientific\\_ws\\_proceedings\\_en.pdf](https://echa.europa.eu/documents/10162/22816069/scientific_ws_proceedings_en.pdf)
- ECHA, EFSA, with the technical support of the Joint Research Centre, Andersson, N., Arena, M., Auteri, D., Barmaz, S., Grignard, E., Kienzler, A., Lepper, P., Lostia, A.M., Munn, S., Parra Morte, J.M., Pellizzato, F., Tarazona, J., Terron, A., & Van der Linden, S. (2018). Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. *EFSA Journal*, 16(6), 5311. doi:10.2903/j.efsa.2018.5311
- EDQM. (2012). Press release of 17 April 2012 "Vaccines for veterinary use adopted by the European Pharmacopoeia Commission at the 142<sup>nd</sup> Session: international harmonisation with VICH guidelines 41 and 42, deletion of the TABST and 3Rs". Available at: [https://www.edqm.eu/medias/fichiers/vaccines\\_for\\_veterinary\\_use\\_adopted.pdf](https://www.edqm.eu/medias/fichiers/vaccines_for_veterinary_use_adopted.pdf)

- EDQM. (2017). Press release of 8 December 2017 "Suppression of the Test for Abnormal Toxicity from the European Pharmacopoeia". Available at: [https://www.edqm.eu/sites/default/files/pr\\_pheur159sess\\_abnormal\\_toxicity\\_2017.pdf](https://www.edqm.eu/sites/default/files/pr_pheur159sess_abnormal_toxicity_2017.pdf)
- EDQM. (2018). The EDQM on the move - 2018 Annual Report. Council of Europe, Strasbourg. Available at: [https://www.edqm.eu/sites/default/files/medias/fichiers/AboutUs/annual\\_report\\_2018.pdf](https://www.edqm.eu/sites/default/files/medias/fichiers/AboutUs/annual_report_2018.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
- EFSA. (2019). EFSA Workshop on *in vitro* comparative metabolism studies in regulatory pesticide risk assessment. *EFSA Supporting publication*. 16(4), EN-1618, 1616 pp. doi:10.2903/sp.efsa.2019.EN-1618
- EMA. (2018). *EMA Regulatory Science to 2025 - Strategic reflection*. EMA/872479/2018. European Medicines Agency. Available at: [https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ema-regulatory-science-2025-strategic-reflection\\_en.pdf](https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ema-regulatory-science-2025-strategic-reflection_en.pdf)
- EPAA. (2018). Annual Report 2018. European Partnership for Alternative Approaches to Animal Testing. Available at: <https://ec.europa.eu/docsroom/documents/32325/attachments/1/translations/en/renditions/native>
- EPAA. (2019). Annual Report 2019. European Partnership for Alternative Approaches to Animal Testing. Available at: <https://ec.europa.eu/docsroom/documents/38804/attachments/1/translations/en/renditions/native>
- 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, L 276, 20.10.2010, p. 33–7. Available at: <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063>
- EURL ECVAM. (2014). Multi-study validation trial for cytochrome P450 induction providing a reliable human metabolically competent standard model or method using the human cryopreserved primary hepatocytes and the human cryopreserved HepaRG™ cells. Validation project report. Available at: [https://tsar.jrc.ec.europa.eu/system/files/Uploaded/CYP\\_validation%20project%20report\\_FINAL%2020140314\\_0.pdf](https://tsar.jrc.ec.europa.eu/system/files/Uploaded/CYP_validation%20project%20report_FINAL%2020140314_0.pdf)
- EURL ECVAM. (2019). *Proceeding and programme of JRC Summer School 2019: Non-Animal Approaches in Science-Challenges & Future Directions*. Publications Office of the European Union, Luxembourg. JRC117137. Available at: <https://publications.jrc.ec.europa.eu/repository/handle/JRC117137>
- Fischer, M., Belanger, S.E., Berckmans, P., Bernhard, M.J., Blaha, L., Coman Schmid, D.E., Dyer, S.D., Haupt, T., Hermens, J.L.M., Hultman, M.T., Laue, H., Lillicrap, A., Mlnarikova, M., Natsch, A., Novak, J., Sinnige, T.L., Tollefsen, K.E., von Niederhausen, V., Witters, H., Zupanic, A., & Schirmer, K. (2019). Repeatability and Reproducibility of the RTgill-W1 Cell Line Assay for Predicting Fish Acute Toxicity. *Toxicological Sciences*, 169(2), 353-364. doi:10.1093/toxsci/kfz057
- Fritsche, E., & Price, A. (Eds.). (2018). Alternative Approaches to Developmental Neurotoxicity Evaluation. *Toxicology and Applied Pharmacology*. 354, 1-224. Special Issue. Available at: <https://www.sciencedirect.com/journal/toxicology-and-applied-pharmacology/vol/354/suppl/C>
- Gouliarmou, V., Lostia, A.M., Coecke, S., Bernasconi, C., Bessems, J., Dorne, J.L., Ferguson, S., Testai, E., Remy, U.G., Brian Houston, J., Monshouwer, M., Nong, A., Pelkonen, O., Morath, S., Wetmore, B.A., Worth, A., Zanelli, U., Zorzoli, M.C., & Whelan, M. (2018). Establishing a systematic framework to characterise *in vitro* methods for human hepatic metabolic clearance. *Toxicology In Vitro*, 53, 233-244. doi:10.1016/j.tiv.2018.08.004
- Gray, A.C., Sidhu, S.S., Chandrasekera, P.C., Hendriksen, C.F., & Borrebaeck, C.A. (2016a). Animal-based antibodies: Obsolete. *Science*, 353(6298), 452-453. doi:10.1126/science.aag3305
- Gray, A.C., Sidhu, S.S., Chandrasekera, P.C., Hendriksen, C.F.M., & Borrebaeck, C.A.K. (2016b). Animal-Friendly Affinity Reagents: Replacing the Needless in the Haystack. *Trends in Biotechnology*, 34(12), 960-969. doi:10.1016/j.tibtech.2016.05.017
- Hakkola, J., Bernasconi, C., Coecke, S., Richert, L., Andersson, T.B., & Pelkonen, O. (2018). Cytochrome P450 Induction and Xeno-Sensing Receptors Pregnane X Receptor, Constitutive Androstane Receptor, Aryl Hydrocarbon Receptor and Peroxisome Proliferator-Activated Receptor alpha at the Crossroads of Toxicokinetics and Toxicodynamics. *Basic & Clinical Pharmacology & Toxicology*, 123 Suppl 5, 42-50. doi:10.1111/bcpt.13004
- Halder, M., Depraetere, H., Delannois, F., Akkermans, A., Behr-Gross, M.E., Bruysters, M., Dierick, J.F., Jungback, C., Kross, I., Metz, B., Pennings, J., Rigsby, P., Riou, P., Balks, E., Dobby, A., Leroy, O., & Stirling, C. (2018). Recommendations of the VAC2VAC workshop on the design of multi-centre validation studies. *Biologicals*, 52, 78-82. doi:10.1016/j.biologicals.2018.01.003

- 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
- Herrmann, K., Pistollato, F., & Stephens, M.L. (2019). Beyond the 3Rs: Expanding the use of human-relevant replacement methods in biomedical research. *ALTEX*, 36(3), 343-352. doi:10.14573/altex.1907031
- Heusinkveld, H., Braakhuis, H., Gommans, R., Botham, P., Corvaro, M., Fotakis, G., van der Laan, J.W., Lewis, D., Madia, F., Manou, I., Melching-Kollmus, S., Schorsch, F., Terron, A., Wolterink, G., Woutersen, R.A., Corvi, R., Mehta, J., & Luijten, M. (Manuscript in preparation). Identification of mechanisms underlying the non-genotoxic potential of agrochemicals.
- 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>
- ICCR. (2017). Report for the International Cooperation on Cosmetics Regulation, Joint Regulators-Industry Working Group Integrated Strategies for Safety Assessments of Cosmetic Ingredients - Part 1. International Cooperation on Cosmetics Regulation. Available at: [http://www.iccr-cosmetics.org/files/4715/0824/0761/ICCR-11\\_JWG\\_Integrated\\_Strategies\\_Integrated\\_Strategies\\_for\\_Safety\\_Assessments\\_of\\_Cosmetic\\_Ingredients\\_-\\_Part\\_1.pdf](http://www.iccr-cosmetics.org/files/4715/0824/0761/ICCR-11_JWG_Integrated_Strategies_Integrated_Strategies_for_Safety_Assessments_of_Cosmetic_Ingredients_-_Part_1.pdf)
- ICCR. (2018). Report for the International Cooperation on Cosmetics Regulation, Joint Regulators-Industry Working Group Integrated Strategies for Safety Assessments of Cosmetic Ingredients - Part 2. International Cooperation on Cosmetics Regulation. Available at: [https://www.iccr-cosmetics.org/files/8315/4322/3079/ICCR\\_Integrated\\_Strategies\\_for\\_Safety\\_Assessment\\_of\\_Cosmetic\\_Ingredients\\_Part\\_2.pdf](https://www.iccr-cosmetics.org/files/8315/4322/3079/ICCR_Integrated_Strategies_for_Safety_Assessment_of_Cosmetic_Ingredients_Part_2.pdf)
- ICCVAM. (2018). A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. Interagency Coordinating Committee on the Validation of Alternative Methods. Available at: <https://ntp.niehs.nih.gov/go/iccvam-rdmp>
- ISO. (2019). Water quality - Determination of acute toxicity of water samples and chemicals to a fish gill cell-line (RT gill-W1). ISO/DIS 21115.
- Jacobs, M.N., Colacci, A., Louekari, K., Luijten, M., Hakkert, B.C., Paparella, M., & Vasseur, P. (2016). International regulatory needs for development of an 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. (Manuscript submitted). Developing an integrated approach for the testing and assessment of chemical non-genotoxic carcinogens for global regulatory purposes. *Nature Reviews Cancer*.
- Joossens, E., Macko, P., Palosaari, T., Gerloff, K., Ojea-Jimenez, I., Gilliland, D., Novak, J., Fortaner Torrent, S., Gineste, J.M., Romer, I., Briffa, S.M., Valsami-Jones, E., Lynch, I., & Whelan, M. (2019). A high throughput imaging database of toxicological effects of nanomaterials tested on HepaRG cells. *Scientific Data*, 6(1), 46. doi:10.1038/s41597-019-0053-2
- Kienzler, A., Barron, M.G., Belanger, S.E., Beasley, A., & Embry, M.R. (2017). Mode of Action (MOA) Assignment Classifications for Ecotoxicology: An Evaluation of Approaches. *Environmental Science & Technology*, 51(17), 10203-10211. doi:10.1021/acs.est.7b02337
- Kienzler, A., Bopp, S., Halder, M., Embry, M., & Worth, A. (2019a). Application of new statistical distribution approaches for environmental mixture risk assessment: A case study. *The Science of the Total Environment*, 693, 133510. doi:10.1016/j.scitotenv.2019.07.316
- Kienzler, A., Connors, K.A., Bonnell, M., Barron, M.G., Beasley, A., Inglis, C.G., Norberg-King, T.J., Martin, T., Sanderson, H., Vallotton, N., Wilson, P., & Embry, M.R. (2019b). Mode of Action Classifications in the EnviroTox Database: Development and Implementation of a Consensus MOA Classification. *Environmental Toxicology and Chemistry*, 38(10), 2294-2304. doi:10.1002/etc.4531



- Kirkland, D., Zeiger, E., Madia, F., & Corvi, R. (2014a). Can *in vitro* mammalian cell genotoxicity test results be used to complement positive results in the Ames test and help predict carcinogenic or *in vivo* genotoxic activity? II. Construction and analysis of a consolidated database. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, 775-776, 69-80. doi:10.1016/j.mrgentox.2014.10.006
- Kirkland, D., Zeiger, E., Madia, F., Gooderham, N., Kasper, P., Lynch, A., Morita, T., Ouedraogo, G., Parra Morte, J.M., Pfuhrer, S., Rogiers, V., Schulz, M., Thybaud, V., van Benthem, J., Vanparrys, P., Worth, A., & Corvi, R. (2014b). Can *in vitro* mammalian cell genotoxicity test results be used to complement positive results in the Ames test and help predict carcinogenic or *in vivo* genotoxic activity? I. Reports of individual databases presented at an EURL ECVAM Workshop. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, 775-776, 55-68. doi:10.1016/j.mrgentox.2014.10.005
- Kleinstreuer, N.C., Karmaus, A., Mansouri, K., Allen, D.G., Fitzpatrick, J.M., & Patlewicz, G. (2018). Predictive Models for Acute Oral Systemic Toxicity: A Workshop to Bridge the Gap from Research to Regulation. *Computational Toxicology*, 8(11), 21-24. doi:10.1016/j.comtox.2018.08.002
- Kodama, S., & Negishi, M. (2013). Sulfotransferase genes: regulation by nuclear receptors in response to xeno/endo-biotics. *Drug Metabolism Reviews*, 45(4), 441-449. doi:10.3109/03602532.2013.835630
- Krebs, A., Waldmann, T., Wilks, M.F., Van Vugt-Lussenburg, B.M.A., Van der Burg, B., Terron, A., Steger-Hartmann, T., Ruegg, J., Rovida, C., Pedersen, E., Pallocca, G., Luijten, M., Leite, S.B., Kustermann, S., Kamp, H., Hoeng, J., Hewitt, P., Herzler, M., Hengstler, J.G., Heinonen, T., Hartung, T., Hardy, B., Gantner, F., Fritsche, E., Fant, K., Ezendam, J., Exner, T., Dunkern, T., Dietrich, D.R., Coecke, S., Busquet, F., Braeuning, A., Bondarenko, O., Bennekou, S.H., Beilmann, M., & Leist, M. (2019). Template for the description of cell-based toxicological test methods to allow evaluation and regulatory use of the data. *ALTEX*, 36(4), 682-699. doi:10.14573/altex.1909271
- Laroche, C., Aggarwal, M., Bender, H., Benndorf, P., Birk, B., Crozier, J., Dal Negro, G., De Gaetano, F., Desaintes, C., Gardner, I., Hubesch, B., Irizar, A., John, D., Kumar, V., Lostia, A., Manou, I., Monshouwer, M., Muller, B.P., Paini, A., Reid, K., Rowan, T., Sachana, M., Schutte, K., Stirling, C., Taalman, R., van Aerts, L., Weissenhorn, R., & Sauer, U.G. (2018). Finding synergies for 3Rs - Toxicokinetics and read-across: Report from an EPAA partners' Forum. *Regulatory Toxicology and Pharmacology*, 99, 5-21. doi:10.1016/j.yrtph.2018.08.006
- Laroche, C., Anny, E., Bender, H., Botelho, D., Botham, P., Brendler-Schwaab, S., Clayton, R., Corvaro, M., Dal Negro, G., Delannois, F., Dent, M., Desaintes, C., Desprez, B., Dhalluin, S., Hartmann, A., Hoffmann-Doerr, S., Hubesch, B., Irizar, A., Manou, I., Muller, B.P., Nadzialek, S., Prieto, P., Rasenberg, M., Roggeband, R., Rowan, T.G., Schutte, K., van de Water, B., Westmoreland, C., Whelan, M., Wilschut, A., Zvonimir, Z., & Cronin, M.T.D. (2019). Finding synergies for the 3Rs - Repeated Dose Toxicity testing: Report from an EPAA Partners' Forum. *Regulatory Toxicology and Pharmacology*, 108, 104470. doi:10.1016/j.yrtph.2019.104470
- Lostia, A.M., Zorzoli, M.C., Torrent, S.F., Mendoza, E., Bouhifd, M., & Coecke, S. (2016). Development of an *in vitro* metabolic hepatic clearance method. EUR 28354 EN. Publications Office of the European Union, Luxembourg. JRC104213. Available at: <https://publications.jrc.ec.europa.eu/repository/handle/JRC104213>
- Louisse, J., Alewijn, M., Peijnenburg, A., Cnubben, N.H.P., Heringa, M.B., Coecke, S., & Punt, A. (2019). 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., Ball, N.S., Dearfield, K.L., Gollapudi, B.B., Johnson, G.E., Madia, F., Peel, L., Pfuhrer, S., Settivari, R.S., Ter Burg, W., White, P.A., & van Benthem, J. (2020). Utility of a next generation framework for assessment of genomic damage: A case study using the industrial chemical benzene. *Environmental and Molecular Mutagenesis*, 61(1), 94-113. doi:10.1002/em.22346
- Madden, J.C., Pawar, G., Cronin, M.T.D., Webb, S., Tan, Y.-M., & Paini, A. (2019). *In silico* resources to assist in the development and evaluation of physiologically-based kinetic models. *Computational Toxicology*, 11, 33-49. doi:10.1016/j.comtox.2019.03.001
- Madia, F., Worth, A., Whelan, M., & Corvi, R. (2019). Carcinogenicity assessment: Addressing the challenges of cancer and chemicals in the environment. *Environment International*, 128, 417-429. doi:10.1016/j.envint.2019.04.067
- Madia, F., Kirkland, D., Morita, T., White, P., Asturiol, D., & Corvi, R. (Manuscript in preparation-a). EURL ECVAM Genotoxicity and Carcinogenicity Database with substances giving negative Ames test results: Construction of the Database.
- Madia, F., Pillo, G., Prieto, P., Worth, A., & Corvi, R. (Manuscript in preparation-b). Integration of data across toxicity endpoints to explore new ways for carcinogenicity safety assessment of chemicals: a case study.
- Madia, F., Corvi, R., Worth, A., Mathys, I., & Prieto, P. (Manuscript submitted). Making better use of toxicity studies for human health by extrapolating across endpoints. *Regulatory Toxicology and Pharmacology*.

- Mastrangeli, M., Millet, S., Mummery, C., Loskill, P., Braeken, D., Eberle, W., Cipriano, M., Fernandez, L., Graef, M., Gidrol, X., Picollet-D'Hahan, N., Van Meer, B., Ochoa, I., Schutte, M., & Van den Eijnden-van Raaij, J. (2019a). Building blocks for a European Organ-on-Chip roadmap. *ALTEX*, *36*(3), 481-492. doi:10.14573/altex.1905221
- Mastrangeli, M., Millet, S., partners, T.O., & van den Eijnden-van Raaij, J. (2019b). Organ-on-Chip In Development: Towards a roadmap for Organs-on-Chip. *Preprints*, 2019030031. doi:10.20944/preprints201903.0031.v1
- Mehling, A., Adriaens, E., Casati, S., Hubesch, B., Irizar, A., Klaric, M., Letasiova, S., Manou, I., Muller, B.P., Roggen, E., van Vliet, E., & Basketter, D. (2019). *In vitro* RHE skin sensitisation assays: Applicability to challenging substances. *Regulatory Toxicology and Pharmacology*, *108*, 104473. doi:10.1016/j.yrtph.2019.104473
- Morgeaux, S., Poirier, B., Ragan, C.I., Wilkinson, D., Arabin, U., Françoise, G.-M., Levis, R., Meyer, H., Riou, P., Shaid, S., Volokhov, D., Tordo, N., & Chapsal, J.-M. (2017). Replacement of *in vivo* human rabies vaccine potency testing by *in vitro* glycoprotein quantification using ELISA - Results of an international collaborative study. *Vaccine*, *35*(6), 966-971. doi:10.1016/j.vaccine.2016.12.039
- Myatt, G.J., Ahlberg, E., Akahori, Y., Allen, D., Amberg, A., Anger, L.T., Aptula, A., Auerbach, S., Beilke, L., Bellion, P., Benigni, R., Bercu, J., Booth, E.D., Bower, D., Brigo, A., Burden, N., Cammerer, Z., Cronin, M.T.D., Cross, K.P., Custer, L., Dettwiler, M., Dobo, K., Ford, K.A., Fortin, M.C., Gad-McDonald, S.E., Gellatly, N., Gervais, V., Glover, K.P., Glowienke, S., Van Gompel, J., Gutsell, S., Hardy, B., Harvey, J.S., Hillegass, J., Honma, M., Hsieh, J.H., Hsu, C.W., Hughes, K., Johnson, C., Jolly, R., Jones, D., Kemper, R., Kenyon, M.O., Kim, M.T., Kruhlak, N.L., Kulkarni, S.A., Kummerer, K., Leavitt, P., Majer, B., Masten, S., Miller, S., Moser, J., Mumtaz, M., Muster, W., Neilson, L., Oprea, T.I., Patlewicz, G., Paulino, A., Lo Piparo, E., Powley, M., Quigley, D.P., Reddy, M.V., Richarz, A.N., Ruiz, P., Schilter, B., Serafimova, R., Simpson, W., Stavitskaya, L., Stidl, R., Suarez-Rodriguez, D., Szabo, D.T., Teasdale, A., Trejo-Martin, A., Valentin, J.P., Vuorinen, A., Wall, B.A., Watts, P., White, A.T., Wichard, J., Witt, K.L., Woolley, A., Woolley, D., Zwickl, C., & Hasselgren, C. (2018). *In silico* toxicology protocols. *Regulatory Toxicology and Pharmacology*, *96*, 1-17. doi:10.1016/j.yrtph.2018.04.014
- Natsch, A., Laue, H., Haupt, T., von Niederhausen, V., & Sanders, G. (2018). Accurate prediction of acute fish toxicity of fragrance chemicals with the RTgill-W1 cell assay. *Environmental Toxicology and Chemistry*, *37*(3), 931-941. doi:10.1002/etc.4027
- Neagu, D., & Richarz, A. (Eds.). (2019). *Big Data in Predictive Toxicology*. Royal Society of Chemistry, London. doi:10.1039/9781782623656
- Nichols, J., Fay, K., Bernhard, M.J., Bischof, I., Davis, J., Halder, M., Hu, J., Johanning, K., Laue, H., Nabb, D., Schlechtriem, C., Segner, H., Swintek, J., Weeks, J., & Embry, M. (2018). Reliability of *In Vitro* Methods Used to Measure Intrinsic Clearance of Hydrophobic Organic Chemicals by Rainbow Trout: Results of an International Ring Trial. *Toxicological Sciences*, *164*(2), 563-575. doi:10.1093/toxsci/kfy113
- Norberg-King, T.J., Embry, M.R., Belanger, S.E., Braunbeck, T., Butler, J.D., Dorn, P.B., Farr, B., Guiney, P.D., Hughes, S.A., Jeffries, M., Journal, R., Leonard, M., McMaster, M., Oris, J.T., Ryder, K., Segner, H., Senac, T., Van Der Kraak, G., Whale, G., & Wilson, P. (2018). An International Perspective on the Tools and Concepts for Effluent Toxicity Assessments in the Context of Animal Alternatives: Reduction in Vertebrate Use. *Environmental Toxicology and Chemistry*, *37*(11), 2745-2757. doi:10.1002/etc.4259
- OECD. (2010). *Short guidance on the threshold approach for acute fish toxicity*. Series on Testing and Assessment, No. 126. OECD Publishing, Paris. ENV/JM/MONO(2010)17. Available at: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2010\)17&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2010)17&doclanguage=en)
- OECD. (2012). *Detailed Review Paper on the State of the Science on Novel In Vitro and In Vivo Screening and Testing Methods and Endpoints for Evaluating Endocrine Disruptors*. OECD Series on Testing and Assessment, No. 178. OECD Publishing, Paris. doi:10.1787/9789264221352-en
- OECD. (2013). Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. doi:10.1787/9789264203709-en
- OECD. (2016). *Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA)*. OECD Series on Testing and Assessment No. 260. OECD Publishing, Paris. ENV/JM/MONO(2016)67. Available at: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2016\)67&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)67&doclanguage=en)
- OECD. (2017a). *Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation*. OECD Series on Testing and Assessment, No. 256. OECD Publishing, Paris. doi:10.1787/9789264279285-en

- OECD. (2017b). *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. (2017c). *Guidance Document on the Reporting of Defined Approaches to be Used Within Integrated Approaches to Testing and Assessment*. OECD Series on Testing and Assessment, No. 255. OECD Publishing, Paris. doi:10.1787/9789264274822-en
- OECD. (2018a). *Validation Report of the two new Test Guidelines on Determination of In Vitro Intrinsic Clearance Using Cryopreserved Rainbow Trout Hepatocytes or Liver S9 Sub-Cellular Fractions*. Series on Testing and Assessment, No. 281. OECD Publishing, Paris. ENV/JM/MONO(2018)13. Available at: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO\(2018\)13&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2018)13&doclanguage=en)
- OECD. (2018b). *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. (2018c). *Test No. 319B: Determination of in vitro intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)*. OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing, Paris. doi:10.1787/9789264303232-en
- OECD. (2018d). *Test No. 319A: Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP)*. OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing, Paris. doi:10.1787/9789264303218-en
- OECD. (2018e). *Test No. 443: Extended One-Generation Reproductive Toxicity Study*. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. doi:10.1787/9789264185371-en
- OECD. (2018f). *Guidance Document on the Determination of In Vitro Intrinsic Clearance Using Cryopreserved Hepatocytes (RT-HEP) or Liver S9 Sub-Cellular Fractions (RT-S9) From Rainbow Trout and Extrapolation to In Vivo Intrinsic Clearance*. Series on Testing and Assessment, No. 280. OECD Publishing, Paris. ENV/JM/MONO(2018)12. Available at: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO\(2018\)12&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2018)12&doclanguage=en)
- OECD. (2019a). *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures*. OECD Series on Testing and Assessment. OECD Publishing, Paris. doi:10.1787/Oed2f88e-en
- OECD. (2019b). *Test No. 248: Xenopus Eleutheroembryonic Thyroid Assay (XETA)*. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. doi:10.1787/a13f80ee-en
- OECD. (2019c). *Test No. 203: Fish, Acute Toxicity Test*. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. doi:10.1787/9789264069961-en
- Oredsson, S., Coecke, S., van der Valk, J., & Vinken, M. (2019). What is understood by "animal-free research"? *Toxicology in Vitro*, 57, 143-144. doi:10.1016/j.tiv.2019.03.001
- Paini, A., Leonard, J.A., Joossens, E., Bessems, J.G.M., Desalegn, A., Dorne, J.L., Gosling, J.P., Heringa, M.B., Klaric, M., Kliment, T., Kramer, N.I., Loizou, G., Louise, J., Lumen, A., Madden, J.C., Patterson, E.A., Proenca, S., Punt, A., Setzer, R.W., Suci, N., Troutman, J., Yoon, M., Worth, A., & Tan, Y.M. (2019). Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making. *Computational Toxicology*, 9, 61-72. doi:10.1016/j.comtox.2018.11.002
- Paparella, M., Colacci, A., & Jacobs, M.N. (2017). Uncertainties of testing methods: What do we (want to) know about carcinogenicity? *ALTEX*, 34(2), 235-252. doi:10.14573/altex.1608281
- Patlewicz, G., Lizarraga, L.E., Rua, D., Allen, D.G., Daniel, A.B., Fitzpatrick, S.C., Garcia-Reyero, N., Gordon, J., Hakkinen, P., Howard, A.S., Karmaus, A., Matheson, J., Mumtaz, M., Richarz, A.N., Ruiz, P., Scarano, L., Yamada, T., & Kleinstreuer, N. (2019). Exploring current read-across applications and needs among selected U.S. Federal Agencies. *Regulatory Toxicology and Pharmacology*, 106, 197-209. doi:10.1016/j.yrtph.2019.05.011
- Pfuhler, S., van Benthem, J., Curren, R., Doak, S., 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. (2020). Use of in vitro 3D tissue models in genotoxicity testing: strategic fit, validation status and way forward. Report of the working group from the 7<sup>th</sup> International workshop on genotoxicity testing (IWGT). *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 503135. doi:<https://doi.org/10.1016/j.mrgentox.2020.503135>
- Pistollato, F., Mendoza de Gyves, E., Carpi, D., Bopp, S., Nunes, C., Worth, W., & Price-Bal, A. (Manuscript submitted). Assessment of developmental neurotoxicity induced by chemical mixtures using an adverse outcome pathway concept. *Environmental Health*.

- Poston, R., Hill, R., Allen, C., Casey, W., Gatewood, D., Levis, R., Mallet, L., Smith, D., Srinivas, G., Stirling, C., & Allen, D. (2019). Achieving scientific and regulatory success in implementing non-animal approaches to human and veterinary rabies vaccine testing: A NICEATM and IABS workshop report. *Biologicals*, 60, 8-14. doi:10.1016/j.biologicals.2019.06.005
- Reisinger, K., Dony, E., Wolf, T., & Maul, K. (2019). Hen's Egg Test for Micronucleus Induction (HET-MN). *Methods in Molecular Biology*, 2031, 195-208. doi:10.1007/978-1-4939-9646-9\_10
- Rolaki, A., Pistollato, F., Munn, S., & Bal-Price, A. (2019). "Adverse Outcome Pathway on inhibition of Na<sup>+</sup>/I<sup>-</sup> symporter (NIS) leads to learning and memory impairment". *OECD Series on Adverse Outcome Pathways*, No. 14. OECD Publishing, Paris. doi:10.1787/7ca86a34-en
- Sachana, M. (2019). An international effort to promote the regulatory use of PBK models based on non-animal data. *Computational Toxicology*, 11, 23-24. doi:10.1016/j.comtox.2019.01.002
- Sachana, M., Bal-Price, A., Crofton, K.M., Bennekou, S.H., Shafer, T.J., Behl, M., & Terron, A. (2019). International Regulatory and Scientific Effort for Improved Developmental Neurotoxicity Testing. *Toxicological Sciences*, 167(1), 45-57. doi:10.1093/toxsci/kfy211
- Sauer, U.G., Deferme, L., Gribaldo, L., Hackermuller, J., Tralau, T., van Ravenzwaay, B., Yauk, C., Poole, A., Tong, W., & Gant, T.W. (2017). The challenge of the application of 'omics technologies in chemicals risk assessment: Background and outlook. *Regulatory Toxicology and Pharmacology*, 91 Suppl 1, S14-s26. doi:10.1016/j.yrtph.2017.09.020
- Schutte, K., Szczepanska, A., Halder, M., Cussler, K., Sauer, U.G., Stirling, C., Uhrich, S., Wilk-Zasadna, I., John, D., Bopst, M., Garbe, J., Glansbeek, H.L., Levis, R., Serreyn, P.-J., Smith, D., & Stickings, P. (2017). Modern science for better quality control of medicinal products "Towards global harmonization of 3Rs in biologicals": The report of an EPAA workshop. *Biologicals*, 48, 55-65. doi:10.1016/j.biologicals.2017.05.006
- Schwanig, M., Nagel, M., Duchow, K., & Kramer, B. (1997). Elimination of abnormal toxicity test for sera and certain vaccines in the European pharmacopoeia. *Vaccine*, 15(10), 1047-1048. doi:10.1016/S0264-410X(97)00074-1
- Sinitskaya, N., Redhead, K., Daas, A., Bruckner, L., & Behr-Gross, M. (2016). Validation of alternative/3Rs methods for the in-process quality control of Clostridium septicum vaccines. EDQM Council of Europe, Strasbourg. Available at: [https://www.edqm.eu/sites/default/files/report\\_workshop\\_bsp\\_130\\_alternative\\_methods\\_qc\\_clostridium\\_septicum\\_vaccines.pdf](https://www.edqm.eu/sites/default/files/report_workshop_bsp_130_alternative_methods_qc_clostridium_septicum_vaccines.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., Coglianò, 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., Bal-Price, A., Cronin, M.T.D., Enoch, S.J., Madden, J.C., & Worth, A.P. (2019). Development and analysis of an adverse outcome pathway network for human neurotoxicity. *Archives of Toxicology*, 93(10), 2759-2772. doi:10.1007/s00204-019-02551-1
- Stadnicka-Michalak, J., Weiss, F.T., Fischer, M., Tanneberger, K., & Schirmer, K. (2018). Biotransformation of Benzo[a]pyrene by Three Rainbow Trout (*Onchorhynchus mykiss*) Cell Lines and Extrapolation To Derive a Fish Bioconcentration Factor. *Environmental Science & Technology*, 52(5), 3091-3100. doi:10.1021/acs.est.7b04548
- Stadnicka-Michalak, J., Arnot, J.A., Bramaz, N., Bury, N., Embry, M.R., Fitzgerald, J.A., Hogstrand, C., Kropf, C., Segner, H., Schoenenberger, R., & Schirmer, K. (2019). A tiered testing strategy for rapid estimation of bioaccumulation by a combined modelling - *in vitro* testing approach: derivation of kinetic rate constants in different *in vitro* models. Paper presented at the SETAC Europe 29<sup>th</sup> Annual Meeting, Helsinki.
- Staudinger, J.L., Woody, S., Sun, M., & Cui, W. (2013). Nuclear-receptor-mediated regulation of drug- and bile-acid-transporter proteins in gut and liver. *Drug Metabolism Reviews*, 45(1), 48-59. doi:10.3109/03602532.2012.748793
- Strickland, J., Clippinger, A.J., Brown, J., Allen, D., Jacobs, A., Matheson, J., Lowit, A., Reinke, E.N., Johnson, M.S., Quinn, M.J., Jr., Mattie, D., Fitzpatrick, S.C., Ahir, S., Kleinstreuer, N., & Casey, W. (2018). Status of acute systemic toxicity testing requirements and data uses by U.S. regulatory agencies. *Regulatory Toxicology and Pharmacology*, 94, 183-196. doi:10.1016/j.yrtph.2018.01.022
- Tanneberger, K., Knöbel, M., Busser, F.J.M., Sinnige, T.L., Hermens, J.L.M., & Schirmer, K. (2013). Predicting Fish Acute Toxicity Using a Fish Gill Cell Line-Based Toxicity Assay. *Environmental Science & Technology*, 47(2), 1110-1119. doi:10.1021/es303505z
- Thomas, D.W., Burns, J., Audette, J., Carroll, A., Dow-Hygelund, C., & Hay, M. (2016). Clinical Development Success Rates 2006-2015. Available at: <https://www.bio.org/sites/default/files/legacy/bioorg/docs/Clinical%20Development%20Success%20Rates%202006-2015%20-%20BIO,%20Biomedtracker,%20Amplion%202016.pdf>

- Tsaioun, K., Blaauboer, B.J., & Hartung, T. (2016). Evidence-based absorption, distribution, metabolism, excretion (ADME) and its interplay with alternative toxicity methods. *ALTEX*, *33*(4), 343-358. doi:10.14573/altex.1610101
- UNESCO-IBE. (2017). *Training tool for Curriculum Development: Developing and implementing curriculum frameworks*. International Bureau of Education-UNESCO, Geneva. IBE/2017/OP/CD/02. Available at: <https://unesdoc.unesco.org/ark:/48223/pf0000250052>
- Urani, C., Bruschi, M., Casati, S., & Gribaldo, L. (2019). Use of alternative methods: From fundamental to industrial research. *ALTEX*, *36*(2), 320-321. doi:10.14573/altex.1812172
- VAC2VAC. (July 2019). Newsletter Vol. I. Available at: [http://www.vac2vac.eu/sites/default/files/%20content/news/files/VAC2VAC%20Newsletter%20Vol.%20I%20July%202019\\_FINAL\\_190710.pdf](http://www.vac2vac.eu/sites/default/files/%20content/news/files/VAC2VAC%20Newsletter%20Vol.%20I%20July%202019_FINAL_190710.pdf)
- van der Valk, J., Bieback, K., Buta, C., Cochrane, B., Dirks, W.G., Fu, J., Hickman, J.J., Hohensee, C., Kolar, R., Liebsch, M., Pistollato, F., Schulz, M., Thieme, D., Weber, T., Wiest, J., Winkler, S., & Gstraunthaler, G. (2018). Fetal Bovine Serum (FBS): Past - Present - Future. *ALTEX*, *35*(1), 99-118. doi:10.14573/altex.1705101
- VICH. (2017a). *VICH GL55 Harmonisation of criteria to waive target animal batch safety testing for live vaccines for veterinary use*. International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products Available at: <http://www.vichsec.org/guidelines/biologicals/bio-safety/target-animal-batch-safety.html>
- VICH. (2017b). *VICH GL50(R) Harmonisation of criteria to waive target animal batch safety testing for inactivated vaccines for veterinary use*. International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products. Available at: <http://www.vichsec.org/guidelines/biologicals/bio-safety/target-animal-batch-safety.html>
- Viviani, L., Halder, M., Gruber, M., Bruckner, L., Cussler, K., Sanyal, G., Srinivas, G., Goel, S., Kaashoek, M., Litthauer, D., Lopes da Silva, A.L., Sakanyan, E., Aprea, P., Jin, H., Vandeputte, J., Seidle, T., & Yakunin, D. (2020). Global harmonization of vaccine testing requirements: Making elimination of the ATT and TABST a concrete global achievement. *Biologicals*, *63*, 101-105. doi:10.1016/j.biologicals.2019.10.007
- WHO. (2019). *WHO Expert Committee on Biological Standardization: sixty-ninth report*. World Health Organization, WHO Technical Report Series, No. 1016. Geneva. Available at: <https://apps.who.int/iris/bitstream/handle/10665/325184/9789241210256-eng.pdf?ua=1>
- Wittwehr, C., Blomstedt, P., Gosling, J.P., Peltola, T., Raffael, B., Richarz, A.-N., Sienkiewicz, M., Whaley, P., Worth, A., & Whelan, M. (2020). Artificial Intelligence for Chemical Risk Assessment. *Computational Toxicology*, *13*, 100114. doi:10.1016/j.comtox.2019.100114
- Zanelli, U., Caradonna, N.P., Hallifax, D., Turlizzi, E., & Houston, J.B. (2012). Comparison of cryopreserved HepaRG cells with cryopreserved human hepatocytes for prediction of clearance for 26 drugs. *Drug Metabolism and Disposition*, *40*(1), 104-110. doi:10.1124/dmd.111.042309
- Zuang, V., Barroso, J., Belz, S., Berggren, E., Bernasconi, C., Bopp, S., M., B., Bowe, G., Campia, I., Casati, S., Coecke, S., Corvi, R., Dura, A., Gribaldo, L., Grignard, E., Halder, M., Holley, T., Janusch Roi, A., Kienzler, A., Lostia, A., Madia, F., Milcamps, A., Morath, S., Munn, S., Paini, A., Pistollato, F., Price, A., Prieto-Peraita, P., Richarz, A., Triebe, J., van der Linden, S., Wittwehr, C., Worth, A., & Whelan, M. (2017). *EURL ECVAM status report on the development, validation and regulatory acceptance of alternative methods and approaches (2017)*. EUR 28823 EN. Publications Office of the European Union, Luxembourg. JRC108831. Available at: <https://publications.jrc.ec.europa.eu/repository/handle/JRC108831>
- Zuang, V., Dura, A., Asturiol Bofill, D., Barroso, J., Batista Leite, S., Belz, S., Berggren, E., Bernasconi, C., Bopp, S., Bouhifd, M., Bowe, G., Campia, I., Casati, S., Coecke, S., Corvi, R., Gribaldo, L., Grignard, E., Halder, M., Holloway, M., Kienzler, A., Landesmann, B., Madia, F., Milcamps, A., Morath, S., Munn, S., Paini, A., Pistollato, F., Price, A., Prieto Peraita, P., Richarz, A., Sala Benito, J., Wilk-Zasadna, I., Wittwehr, C., Worth, A., & Whelan, M. (2018). *EURL ECVAM status report on the development, validation and regulatory acceptance of alternative methods and approaches (2018)*. EUR 29455 EN. Publications Office of the European Union, Luxembourg. JRC113594. Available at: <https://publications.jrc.ec.europa.eu/repository/handle/JRC113594>



## List of abbreviations and definitions

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AD	Alzheimer's disease
AI	Artificial Intelligence
AOP	Adverse Outcome Pathway
AOP-KB	Adverse Outcome Pathway-Knowledge Base
AR	Androgen Receptor
ARTA	Androgen Receptor Transactivation Assay
ATT	Abnormal Toxicity Test
ATWG	Acute Toxicity Workgroup (ICCVAM)
BCF	Bioconcentration Factor
BCOP	Bovine Corneal Opacity and Permeability
BDNF	Brain Derived Neurotrophic Factor
BDS	BioDetection Systems (NL)
BEAMS	Bridge Across Methods in the bioSciences (EURL ECVAM)
BMD	Benchmark Dose
BraCVAM	Brazilian Centre for the validation of alternative methods
BSP	Biological Standardisation Programme
CALUX	Chemically Activated Luciferase Gene Expression
CBI	Confidential Business Information
Cefic	The European Chemical Industry Council
CES	Critical Effect Size
CF	Conceptual Framework
CheLIST	Chemical Lists Information System
CHO	Chinese Hamster Ovary
CLP	Classification, Labelling and Packaging
CM&S	Computational Modelling and Simulation
CMR	Carcinogenic, Mutagenic or Toxic to reproduction
CPSC	The Consumer Product Safety Commission (USA)
CRA	Chemical Risk Assessment
CRD	Chemicals Regulation Directorate (UK)
CSF	Cerebrospinal Fluid
CYP	Human cytochrome P450
DA	Defined Approach
DART	Developmental and Reproductive Toxicity
DB-ALM	DataBase on ALternative Methods
DG	Directorate-General (EU)
DG ENV	Directorate-General for Environment (EU)
DG GROW	Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs (EU)
DG SANTE	Directorate-General for Health and Food Safety (EU)
DNA	Deoxyribonucleic Acid
DNT	Developmental Neurotoxicity
DRP	Detailed Review Paper
E&T	Education & Training
EAGMST	Extended Advisory Group for Molecular Screening and Toxicogenomics
EASIS	EASIS
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
EC	European Commission

ECHA	European Chemicals Agency
EDC	Endocrine Disruptive Chemical
EDQM	European Directorate for the Quality of Medicines & HealthCare (Council of Europe)
EFPIA	European Federation of Pharmaceutical Industries and Associations
EFSA	European Food Safety Authority
EIT	Eye Irritation Test
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
EP	European Parliament
EPAA	European Partnership for Alternatives to Animal Testing
ER	Estrogen Receptor
ESAC	ECVAM Scientific Advisory Committee
ESTAF	ECVAM Stakeholder Forum
ESTIV	European Society of Toxicology <i>In Vitro</i>
ETPLAS	Education and Training Platform for Laboratory Animal Science
EU	European Union
EU-NETVAL	European Union Network of Laboratories for the Validation of Alternative Methods
EURION	European Cluster to Improve Identification of Endocrine Disruptors
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
EUROoCS	Society Organ-on-Chip
EUROTOX	Federation of European Toxicologists and European Societies of Toxicology
EUSAAT	European Society for Alternatives to Animal Testing
FDA	Food and Drug Administration (USA)
FET	Future and Emerging Technologies
FP7	Framework Programme 7 (EU)
FRAND	Fair, Reasonable and Non-discriminatory
GARD	Genomic Allergen Detection Test
GD	Guidance Document (OECD)
GHS	Globally Harmonised System of Classification and Labelling of chemicals
GIVIMP	Good <i>In Vitro</i> Method Practices
GLP	Good Laboratory Practice
GMO	Genetically Modified Organism
GTTC	Genetic Toxicology Technical Committee (HESI)
HBM4EU	European Human Biomonitoring Initiative
h-CLAT	human Cell Line Activation Test
HESI	Health and Environmental Sciences Institute (US)
HET-MN	Hen's Egg Test for Micronucleus Induction
HSE	Health and Safety Executive (UK)
HTS	High-Throughput Screening
IABS	International Alliance for Biological Standardisation
IATA	Integrated Approaches to Testing and Assessment
ICAPO	International Council on Animal Protection in OECD Programmes
ICATM	International Cooperation on Alternative Test Methods
ICCR	International Cooperation on Cosmetics Regulation
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods (NIEHS)
ICE	Isolated Chicken Eye
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICT	Information and Communications Technology
IIVS	Institute for <i>In Vitro</i> Sciences (US)

IMI	Innovative Medicines Initiative
IP	Intellectual Property
IPCHEM	Information Platform for Chemical Monitoring
IPF	Idiopathic Pulmonary Fibrosis
IPR	Intellectual Property Right
iPSC	induced Pluripotent Stem Cell
ISO	International Organization for Standardization
ITN	Innovative Training Network
ITS	Integrated Testing Strategy
IUCLID	International Uniform Chemical Information Database
IVIVE	<i>In vitro</i> to <i>in vivo</i> extrapolation
IWGT	International Workshops on Genotoxicity Testing
J3Rs WG	Joint Committee for Medicinal Products for Veterinary Use (CVMP)/Committee for Medicinal Products for Human Use (CHMP) Working Group on the Application of the 3Rs in Regulatory Testing of Medicinal Products (Joint 3Rs Working Group)
JRC	Joint Research Centre (EC)
JWG	Joint Working Group (ICCR)
KE	Key Event
KER	Key Event Relationship
LD50	lethal dose, 50%
LJMU	Liverpool John Moores University
LLNA	Local Lymph Node Assay
LRI	Cefic's Long-Range Research Initiative
MEA	Microelectrode Array
MLD	Minimum Lethal Dose
MoA	Mode of Action
MOOC	Massive Open Online Course
MWCN	Multi-walled Carbon Nanotubes
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)
NIH	National Institute of Health (US)
NIPH	Norwegian Institute of Public Health
NL	the Netherlands
NOW	Netherlands Organisation for Scientific Research
NRR	Neutral Red Release
NRU	Neutral Red Uptake
NTP	National Toxicology Programme (US)
ODIEG	Ocular and Dermal Irritation Expert Group (ICCVAM)
ODIWG	Ocular and Dermal Irritation Workgroup (ICCVAM)
OECD	Organisation for Economic Co-operation and Development
OHT	OECD Harmonised Template
OoC	Organ-on-Chip
ORCHID	Organ-on-chip in Development
PARERE	Preliminary Assessment of Regulatory Relevance network
PBK	Physiologically Based Kinetic (also PBPK, PBBK, PBTk)
PC	Personal Computer

PCRM	Physicians Committee for Responsible Medicine
PD	Parkinson's disease
Ph. Eur.	European Pharmacopoeia
PISC	PETA International Science Consortium
PMRA	Pest Management Regulatory Agency (Canada)
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PorCORA	Porsine Corneal Ocular Reversibility Assay
QIVIVE	Quantitative <i>In Vitro</i> to <i>In Vivo</i> Extrapolation
QMRF	QSAR Model Reporting Formats
QPRF	Quantitative Prediction Reporting Format
QSAR	Quantitative Structure Activity Relationship
R&D	Research & Development
RADAR	Rapid Androgen Disruption Adverse outcome Reporter
RAWG	Read-Across Working Group (ICCVAM)
RAWG	Read-across Workgroup (ICCVAM)
REACH	European Regulation (EC) no 1907/2006 Registration, Evaluation, Authorisation and Restriction of Chemicals
RhE	Reconstructed human Epidermis
RIVM	National Institute for Public Health and the Environment (the Netherlands)
RSMN	Reconstructed Skin Micronucleus
RTD	Repeated Dose Toxicity
SCSS	Scientific Committee on Consumer Safety (EU)
SME	Small and Medium-sized Enterprises
SOP	Standard Operating Procedure
SSWG	Skin Sensitisation Workgroup (ICCVAM)
STS	Sequential Testing Strategy
TABST	Target Animal Batch Safety Test
TCP	Total Combining Power
TDG	Transport of Dangerous Goods (United Nations)
TG	Test Guideline (OECD)
TGP	Test Guidelines Programme (OECD)
TRF	transcriptomics reporting framework
TSAR	EURL ECVAM Tracking System on Alternative Methods towards Regulatory acceptance
TTC	Threshold of Toxicological Concern
UK	United Kingdom
UN	United Nations
UNESCO	United Nations Educational, Scientific and Cultural Organisation
US EPA	United States Environmental Protection Agency
US(A)	United States (of America)
VAC2VAC	"Vaccine batch to vaccine batch comparison by consistency testing"
VCBA	Virtual Cell Based Assay
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WFSR	Wageningen Food Safety Research
WHO	World Health Organisation
WNT	Working Group of the National Coordinators of the Test Guidelines Programme (OECD)
WoE	Weight of evidence
WPHA	Working Party on Hazard Assessment (OECD)
XETA	Xenopus Embryonic Thyroid Assay

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## Annex 1 — Summary status of the adoption of Test Guidelines based on *in vitro* methods in the OECD TG programme

Table 1 summarises the status of adoption of OECD test guidelines based on *in vitro* methods. It should be noted that beside TGs, also Guidance Documents and new projects on alternative methods were respectively adopted and included in the OECD Work programme. For additional information, please consult the OECD website of the Test Guidelines Programme: <https://www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm>

**Table 1.** Status of adoption of OECD Test Guidelines based on *in vitro* methods.

Nr.	Human health/ environmental effect	Test method description	Acceptance status
1	Skin corrosion	Reconstructed human Epidermis (RhE) test methods, as included in OECD TG 431/EU TM B.40 bis	<p>Adopted as a new TG in 2004; updated version (sub-categorisation, inclusion of performance standards, inclusion of SkinEthic™ RHE and epiCS®) adopted in 2013. Revised version including sub-categorisation with the epiCS® test method adopted in 2014.</p> <p>Updated in 2015 for the deletion of the performance standards (published separately in the Series on Testing and Assessment No. 219), inclusion of paragraphs referring to the IATA for Skin Corrosion and Irritation (OECD GD No. 203) and inclusion of the use of HPLC/UPLC-spectrophotometry as an alternative procedure to measure tissue viability (increasing the applicability domain of the test methods to coloured substances interfering with the measurement of MTT-formazan).</p> <p>Updated in 2016 for improving the predictive capacity of the three validated <i>in vitro</i> skin corrosion test methods (EpiDerm™, SkinEthic™ and EpiCS®) for the correct prediction of Sub-Cat.1A. Updated version to include the LabCyte EPIMODEL-24 test method adopted in 2019.</p>

Nr.	Human health/ environmental effect	Test method description	Acceptance status
2	Skin corrosion	Transcutaneous Electrical Resistance (TER) test method, as included in OECD TG 430/EU TM B.40	Adopted as a new TG in 2004; updated version (inclusion of performance standards) adopted in 2013. Updated in 2015 for the deletion of the performance standards (published separately in the Series on Testing and Assessment No. 218) and the inclusion of paragraphs referring to the IATA for Skin Corrosion and Irritation (OECD GD No. 203).
3	Skin corrosion	<i>In vitro</i> Membrane Barrier Test Method for Skin Corrosion, as included in OECD TG 435/EU TM B.40	Adopted as a new TG in 2006; Updated in 2015 for the inclusion of the Corrositex® prediction model, the deletion of the performance standards (still available from the 2006 version of the TG), the inclusion of paragraphs referring to the IATA for Skin Corrosion and Irritation (OECD GD No. 203) and the updating of the list of proficiency substances.
4	Skin irritation	Reconstructed human Epidermis (RhE) test methods, as included in OECD TG 439/EU B.46	Adopted as a new TG in 2010; updated version (inclusion of LabCyte EPI-model24 SIT) adopted in 2013. Updated in 2015 for the deletion of the performance standards (published separately in the Series on Testing and Assessment No. 220), inclusion of paragraphs referring to the IATA for Skin Corrosion and Irritation (OECD GD No. 203) and inclusion of the use of HPLC/UPLC-spectrophotometry as an alternative procedure to measure tissue viability (increasing the applicability domain of the test methods to coloured substances interfering with the measurement of MTT-formazan); updated version (inclusion of the epiCS® and Skin+® test methods) adopted in 2019.
5	Phototoxicity	3T3 Neutral Red Uptake Phototoxicity Test, as included in OECD TG 432/EU TM B.41	Adopted as a new TG in 2004. Updated version (removal of DMSO as a recommended solvent, revision of threshold limits for chemical UV absorption to be consistent with ICH S10 guidance and more specific recommendations regarding solar simulators) adopted in 2019.
6	Phototoxicity	<i>In chemico</i> test method based on reactive oxygen species (ROS) and photostability, as included in OECD TG 495	Adopted as new TG in 2019.

Nr.	Human health/ environmental effect	Test method description	Acceptance status
7	Serious eye damage/ eye irritation	Fluorescein Leakage (FL) test method, as included in OECD TG 460/EU TM B.61	Adopted as a new TG in 2012; updated version to include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation adopted in 2017.
8	Serious eye damage/ eye irritation	Bovine Corneal Opacity and Permeability (BCOP) test method, as included in OECD TG 437/EU TM B.47	Adopted as a new TG in 2009; updated version (revision of positive controls, use to identify non-classified chemicals and several other revisions) adopted in 2013; updated version to include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation adopted in 2017. Updates to the TG have been proposed to include: 1) a laser light-based opacitometer (LLBO) as an option for measuring corneal opacity; 2) histopathology and associated decision criteria; 3) replacement of the proficiency chemical dibenzoyl-L-tartaric acid.
9	Serious eye damage/ eye irritation	Isolated Chicken Eye (ICE) test method, as included in OECD TG 438/EU TM B.48	Adopted as a new TG in 2009, updated version (use to identify non-classified chemicals and several other revisions) adopted in 2013; updated version to include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation adopted in 2017; updated version to include histopathological examination as additional endpoint to identify UN GHS cat.1 non-extreme pH (2<pH<11.5) detergents and surfactants and modified decision criteria for chemicals requiring classification for eye hazard (taking into consideration the variability of the Draize eye test) adopted in 2018.
10	Serious eye damage/ eye irritation	Cytosensor Microphysiometer (CM) test method	New draft TG first discussed at WNT in 2013 but not adopted, pending further clarification on its use to identify non-classified chemicals. The additional data requested by the WNT that should have been submitted by US to support the project were not received and thus the project has been discontinued because of lower priority for the EC (lead of project).
11	Serious eye damage/ eye irritation	Short Time Exposure (STE) test method for the detection of chemicals causing serious eye damage and chemicals not requiring classification for serious eye damage or eye irritation, as included in OECD TG 491/EU TM B.68	Adopted as a new TG in 2015; updated version to include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation adopted in 2017. Updated version has been proposed to expand the applicability domain to include highly volatile test chemicals.



Nr.	Human health/ environmental effect	Test method description	Acceptance status
12	Serious eye damage/ eye irritation	Reconstructed human Cornea-like Epithelium (RhCE) test methods for the detection of chemicals not requiring classification and labelling for eye irritation or serious eye damage, as included in OECD TG 492/EU TM B.69	Adopted as a new TG in 2015; updated version to include SkinEthic™ HCE EIT and a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation adopted in 2017; updated version to include the LabCyte CORNEA-MODEL 24 EIT adopted in 2018; updated version to include the MCTTTM EIT adopted in 2019.
13	Serious eye damage/ eye irritation	Vitrigel-Eye Irritancy Test Method for Identifying Chemicals not requiring Classification and Labelling for Eye Irritation or Serious Eye Damage, as included in OECD TG 496	Adopted as new TG in 2019.
14	Serious eye damage/ eye irritation	<i>In vitro</i> Macromolecular Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, as included in OECD TG 494	Adopted as new TG in 2019.
15	Skin sensitisation	<i>In Chemico</i> Skin Sensitisation Assays addressing the Adverse Outcome Pathway key event on covalent binding to proteins, as included in OECD TG 442C/EU TM B.59	Adopted as a new TG in 2015. Updated version to include the Amino acid Derivative Reactivity Assay (ADRA) adopted in 2019. The TG was revised to an "OECD Test Guideline for the Testing of Chemicals Based on Key events", grouping the two adopted test methods addressing key event 1 within the existing AOP into one single TG.
16	Skin sensitisation	Key-event based Test Guideline 442D: <i>In vitro</i> skin sensitisation assays addressing the AOP key event on keratinocyte activation.	Adopted as a new TG in 2015; updated version to include the ARE-Nrf2 Luciferase KeratinoSens™ test method using animal-free serum and the ARE-Nrf2 Luciferase LuSens test method adopted in 2018.

Nr.	Human health/ environmental effect	Test method description	Acceptance status
17	Skin sensitisation	<i>In Vitro</i> Skin Sensitisation assays addressing the Key Event on activation of dendritic cells in the Adverse Outcome Pathway for Skin Sensitisation, as included in OECD TG 442E/EU TM B.71	Adopted as a new TG in 2016 as " <i>In Vitro</i> Skin Sensitisation: human Cell Line Activation Test (h-CLAT)"; updated version to include U-SENS™ and IL-8 Luc test methods adopted in 2017. The TG was revised to an "OECD Test Guideline for the Testing of Chemicals Based on Key events", grouping the three adopted test methods addressing key event 3 within the existing AOP into one single TG.
18	Skin absorption	Skin absorption: <i>in vitro</i> method, as included in OECD TG 428/EU TM B.45	Adopted as a new TG in 2004. Revision of accompanying Guidance Notes 156 (parts on pesticides) currently ongoing.
19	Carcinogenicity	<i>In vitro</i> Syrian Hamster Embryo (SHE) Cell Transformation Assay (CTA) as included in OECD GD No. 214 <sup>1</sup> .	Adopted as a new GD in 2015.
20	Carcinogenicity	<i>In vitro</i> Bhas 42 Cell Transformation Assay (CTA) as included in OECD GD no 231 <sup>1</sup> .	Adoption as a new GD in 2016.
21	Genotoxicity	Bacterial Reverse Mutation Test	Adopted as a new TG in 1997.
22	Genotoxicity	<i>In vitro</i> Mammalian Chromosome Aberration Assay as included in OECD TG 473/EU TM B.10	Updated OECD TG 473 (originally adopted in 1983) adopted in 2014. Updated in 2016 to reference the Guidance Document on genetic toxicology Test Guidelines.
23	Genotoxicity	<i>In vitro</i> Mammalian Cell Micronucleus Assay as included in OECD TG 487/EU TM B.49	Updated OECD TG 487 (originally adopted in 2010) adopted in 2014. Updated in 2016 to reference the Guidance Document on genetic toxicology Test Guidelines.

1 These test methods were initially proposed to be included in Test Guidelines. It was later decided to include them in Guidance Documents.

Nr.	Human health/ environmental effect	Test method description	Acceptance status
24	Genotoxicity	<i>In vitro</i> Mammalian Cell Gene Mutation Test using <i>Hprt</i> and <i>xprt</i> genes as included in OECD TG 476/EU TM B.17	OECD TG 476 (originally adopted in 1984) " <i>In vitro</i> Mammalian Cell Gene Mutation Test" has been split up into two TGs: 1. The updated TG 476 now using the <i>Hprt</i> and <i>xprt</i> genes was adopted in 2015; 2. OECD TG 490 using <i>thymidine kinase</i> Gene was adopted in 2015. Both TGs were updated in 2016 to reference the Guidance Document on genetic toxicology Test Guidelines and TG 490 was also corrected (see below).
25	Genotoxicity	<i>In vitro</i> Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene as included in OECD TG 490/EU TM B.67.	Adopted as TG 490 in 2015 (see above). Updated in 2016 to reference the Guidance Document on genetic toxicology Test Guidelines and to correct a paragraph related to the maximum concentration that is based on cytotoxicity.
26	Endocrine disruption	H295R Steroidogenesis Assay as included in OECD TG 456/EU TM B.57	Adopted as new TG in 2011.
27	Endocrine disruption	Estrogen receptor transactivation assay (BG1Luc ER TA; agonist and antagonist protocols) as included in OECD TG 457	Adopted in 2012. OECD TG 457 was deleted in 2015. The method was included in OECD TG 455 in 2012 (agonist part) and 2015 (antagonist part) (see table entry below).
28	Endocrine disruption	Performance-Based Test Guideline for Stably Transfected Transactivation <i>In Vitro</i> Assays to Detect Estrogen Receptor Agonists and Antagonists as included in OECD TG 455/EU TM B.66	OECD 455 adopted in 2009 (STTA assay using the hER $\alpha$ -HeLa-9903 cell line); updated version (PBTG, inclusion of VM7Luc ER TA assay using the VM7Luc4E2 cell line) adopted in 2012; Second updated version, including the antagonist part of both methods was adopted in 2015. This update led to the deletion of OECD TG 457 in parallel as it is no longer needed (see above). Third updated version to include the ER-CALUX method (using a U2OS cell line) was adopted in 2016.

Nr.	Human health/ environmental effect	Test method description	Acceptance status
29	Endocrine disruption	Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) <i>In Vitro</i> Assays to Detect Chemicals with ER Binding Affinity as included in OECD TG 493/EU TM B.70	Adopted as new TG in 2015. It includes two reference test methods: <ul style="list-style-type: none"> <li>• <i>In Vitro</i> Estrogen Receptor (ER) Binding Assay Using a Full Length Human Recombinant ER<math>\alpha</math>;</li> <li>• <i>In Vitro</i> Estrogen Receptor Binding Assay Using a Human Recombinant Ligand Binding Domain Protein.</li> </ul>
30	Endocrine disruption	Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity as included in OECD TG 458/EU TM B.72	Adopted as new TG in 2016. The method uses the AR-EcoScreen™ cell line. Currently being updated to include two other ARTA assays: AR-CALUX assay and 22Rv1/MMTV_GR-KO STTA.
31	Acute fish toxicity	Fish Embryo Acute Toxicity (FET) Test <sup>2</sup> as included in OECD TG 236/EU TM C.49	Adopted in 2013.
32	Fish bioaccumulation	Determination of <i>in vitro</i> intrinsic clearance using cryopreserved rainbow trout hepatocytes as included in OECD TG 319A.	Adopted in 2018. <i>In vitro</i> derived clearance data can be used to improve the prediction of <i>in silico</i> methods to derive a bioconcentration factor as a means for bioaccumulation.
33	Fish bioaccumulation	Determination of <i>in vitro</i> intrinsic clearance using rainbow trout liver S9 sub-cellular fraction as included in OECD TG 319B.	Adopted in 2018. <i>In vitro</i> derived clearance data can be used to improve the prediction of <i>in silico</i> methods to derive a bioconcentration factor as a means for bioaccumulation.

2 The Fish Embryo Acute Toxicity Test is, strictly speaking, not an *in vitro* test, as it uses fish embryos which are not covered by Directive 2010/63/EU.

## Annex 2 — ICATM Alternative Test Methods Validation and Status of Regulatory Acceptance

**Table 2.** This table describes the status of alternative methods which have been validated and/or peer reviewed by ICATM partners and their status of international acceptance.

Method	Current Status	Lead Organisation	International Acceptance
<b><i>Dermal Corrosion Test Methods</i></b>			
<b>CORROSITEX Skin Corrosion Test</b>	<b>Completed</b>		<b>OECD TG 435 (2006)</b> Updated version (including the Corrositex® prediction model, the deletion of the performance standards (to be published separately in the Series on Testing and Assessment), including paragraphs referring to the IATA for Skin Corrosion and Irritation in OECD GD No. 203 and the updating of the list of proficiency substances) <b>adopted in 2015</b> .
<b>EpiSkin™, EpiDerm™ SCT, SkinEthic™ RHE, epiCS® Skin Corrosion Tests; Lab-Cyte EPI-MODEL24 SCT</b>	<b>Completed</b>		<b>OECD TG 431 (2004)</b> Updated version (sub-categorisation, inclusion of performance standards, inclusion of SkinEthic™ RHE and epiCS®) adopted in 2013. Revised version including the sub-categorisation with the epiCS® test method adopted in 2014. Updated version [deleting the performance standards (published separately in the Series on Testing and Assessment No. 219), including paragraphs referring to the IATA for Skin Corrosion and Irritation in OECD GD No. 203 and including the use of HPLC/UPLC-spectrophotometry as an alternative procedure to measure tissue viability (increasing the applicability domain of the test methods to coloured substances interfering with the measurement of MTT-formazan)] adopted in 2015. Updated in 2016 for improving the predictive capacity of the three validated <i>in vitro</i> skin corrosion test methods (EpiDerm™ SCT, SkinEthic™ RHE and epiCS®) for the correct prediction of Sub-Cat.1A. <b>Updated version</b> (including the Labcyte EPI-MODEL24 SCT) <b>adopted in 2019</b> .



Method	Current Status	Lead Organisation	International Acceptance
<b>Rat TER Skin Corrosion Test</b>	<b>Completed</b>		<b>OECD TG 430 (2004)</b> Updated version (inclusion of performance standards) adopted in 2013. <b>Updated version</b> [deleting the performance standards (published separately in the Series on Testing and Assessment No. 218) and including paragraphs referring to the IATA for Skin Corrosion and Irritation in OECD GD No. 203] <b>adopted in 2015</b> .
<b>Dermal Irritation Test Methods</b>			
<i>In vitro</i> Reconstructed human Epidermis (RhE) test methods: EpiSkin™, EpiDerm™ SIT, SkinEthic™ RHE and LabCyte EPI-MODEL24 SIT, epiCS®, Skin+®	<b>Completed</b>		<b>OECD TG 439 (2010)</b> Updated version (including the LabCyte EPI-MODEL24 SIT) adopted in 2013. <b>Updated version</b> [deleting the performance standards (published separately in the Series on Testing and Assessment No. 220), including paragraphs referring to the IATA for Skin Corrosion and Irritation in OECD GD No. 203 and including the use of HPLC/UPLC-spectrophotometry as an alternative procedure to measure tissue viability (increasing the applicability domain of the test methods to coloured substances interfering with the measurement of MTT-formazan)] adopted in 2015. Updated version to include the epiCS® and Skin+® test methods <b>adopted in 2019</b> .
<i>In vitro</i> reconstructed human epidermis (RhE) test methods: Korean epidermis model	KoCVAM sponsored validation study was completed in November 2019. An SPSF was submitted in November 2019.	KoCVAM	
<i>In vitro</i> reconstructed human full-thickness model test methods: LbL model	JSAAE sponsored validation study is ongoing	JaCVAM	

Method	Current Status	Lead Organisation	International Acceptance
<b>Phototoxicity Test Methods</b>			
<b>3T3 NRU Phototoxicity Test</b>	<b>Completed</b>		<b>OECD TG 432 (2004)</b> <b>Updated version</b> , removing DMSO as a recommended solvent, revising threshold limits for chemical UV absorption to be consistent with ICH S10 guidance and providing more specific recommendations regarding solar simulators, <b>adopted in 2019.</b> <b>ICH S10 (2014)</b>
Test method battery to predict phototoxicity (yeast growth inhibition phototoxicity assay and red blood cell photohemolysis assay)	Japanese Regulatory Acceptance Board recommended additional work be performed	JaCVAM	
<b><i>In vitro</i> test method based on reactive oxygen species (ROS) and photostability</b>	<b>Completed</b>		<b>ICH S10 (2014)</b> <b>OECD TG 495 (2019)</b>
<b>Ocular Toxicity Test Methods</b>			
<b>Bovine Corneal Opacity and Permeability (BCOP) Test Method</b>	<b>Completed</b>		<b>OECD TG 437 (2009)</b> Updated version (positive control, use in a bottom-up approach to identify non-classified chemicals and several other revisions) adopted in 2013. <b>Updated version</b> to include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation <b>adopted in 2017</b> . Updated version under consideration to include: 1) a laser light-based opacitometer; 2) histopathological examination to revise the Decision Criteria for classification of chemicals requiring classification for eye hazard; 3) replacement of the proficiency chemical dibenzylol-L-tartaric acid.

Method	Current Status	Lead Organisation	International Acceptance
<b>Isolated Chicken Eye (ICE) Test Method</b>	<b>Completed</b>		<p><b>OECD TG 438 (2009)</b> Updated version (use in a bottom-up approach to identify non-classified chemicals and several other revisions) adopted in 2013.</p> <p><b>Updated version</b> to include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation adopted in 2017. Updated version to include histopathological examination as additional endpoint to identify UN GHS cat.1 non-extreme pH (2&lt;pH&lt;11.5) detergents and surfactants and modified decision criteria for chemicals requiring classification for eye hazard (taking into consideration the variability of the Draize eye test) <b>adopted in 2018</b>.</p>
<b>Use of Histopathology as an additional endpoint in Ocular Safety Testing</b>	<b>Completed</b>		<p><b>OECD GD 160 (2011)</b> Updated version [including: (i) the recommendation for having an internal peer-review process when evaluating histopathological effects, (ii) the use of semi-quantitative scoring systems for <i>e.g.</i> the ICE histopathology, and (iii) inclusion of an Atlas describing typical ICE histopathological effects] adopted in 2017.</p> <p><b>Updated version</b> to provide more specific and detailed guidance on the preparation of corneas, the scoring of histopathological findings and the peer-review process of the histopathological evaluation, in particular for Test Guideline 438, where this is an additional endpoint in certain well-defined cases, <b>adopted in 2018</b>.</p>
Cytotoxicity test: SIRC CVS	Peer review coordinated by JaCVAM is ongoing	JaCVAM	
Cytotoxicity test: three-dimensional dermal model (MATREX)	JaCVAM-sponsored validation study stopped	JaCVAM	
<b>Cytotoxicity test: Short Time Exposure (STE) test</b>	<b>Completed</b>		<p><b>OECD TG 491 (2015)</b> <b>Updated version</b> to include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation <b>adopted in 2017</b>. TG is being updated to expand the applicability domain for highly volatile substances.</p>

Method	Current Status	Lead Organisation	International Acceptance
<b>Use of anaesthetics, analgesics, and humane endpoints for routine use in TG 405</b>	<b>Completed</b>		<b>OECD updated TG 405 (2012)</b> <b>Updated version</b> to delete the "Testing and Evaluation Strategy for Eye Irritation/Corrosion" and include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation <b>adopted in 2017</b> .
<i>In vitro</i> approach for categorisation of anti-microbial cleaning products: recommendations for further studies	Completed. EPA/OPP <sup>3</sup> has concluded from submission and review of alternative eye irritation tests conducted on antimicrobial pesticide products with cleaning claims (AMCPs) that the proposed testing approach is acceptable for determining the appropriate eye hazard classification and labelling for AMCPs (see <a href="https://www.epa.gov/pesticides">https://www.epa.gov/pesticides</a> for the details of the scope of the policy).	ICCVAM	
<i>In vitro</i> approach for categorisation of agrochemical formulations	Phase 1 and 2 testing completed (n=16 formulations) to demonstrate proof-of-concept and provide justification for further testing using six <i>in vitro</i> assays [BCOP, ICE, Neutral Red Release assay, PoCORA and EpiOcular (OECD TG 492 and time-to-toxicity protocols). Testing will continue into 2020.	NICEATM	

3 Environmental Protection Agency/Office of Pesticide Programme.

Method	Current Status	Lead Organisation	International Acceptance
Cytosensor Microphysiometer® (CM) Test method		EURL ECVAM	New draft TG discussed at WNT in 2013, 2015 and 2016 but not adopted. The additional data requested by the WNT that should have been submitted by US to support the project were not received and thus the project has been discontinued because of lower priority for the EC. The project is no longer on the OECD Work Plan.
<b>Fluorescein Leakage (FL) test method</b>	<b>Completed</b>		<b>OECD TG 460 (2012)</b> <b>Updated version</b> to include a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation <b>adopted in 2017</b> .
<b>Reconstructed human Cornea-like Epithelium (RhCE)</b> <b>EpiOcular™ EIT; SkinEthic™ HCE EIT, Labcyte CORNEA MODEL 24 EIT; MCCT_HCE test method</b>	<b>Completed</b>		<b>OECD TG 492 (2015)</b> Updated version to include SkinEthic™ HCE EIT and a new reference to OECD GD 236 on an IATA for Serious Eye Damage and Eye Irritation adopted in 2017. Updated version to include Labcyte CORNEA MODEL 24 EIT adopted in 2018; <b>Updated version</b> to include the Korean Cornea-model (MCCT_HCE test method) <b>adopted in 2019</b> .
<b>Vitrigel-EIT</b>	<b>Completed</b>		<b>OECD TG 494 (2019)</b>
<b>Ocular Irritation</b>	<b>Completed</b>		<b>OECD TG 496 (2019)</b>
OptiSafe	Validation Study coordinated by NICEATM is complete. Manuscript describing study results submitted for peer reviewed publication in September 2019.	NICEATM	
Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) Test Method	Validation study sponsored by Brazilian Ministry of Science, Technology Innovation and Communication (MCTIC). Preliminary phase of validation study finalised. Substances selection for phase 2 validation started.	BraCVAM	



Method	Current Status	Lead Organisation	International Acceptance
<b><i>Immunotoxicity (Allergic Contact Dermatitis) Test Methods</i></b>			
<b>Murine local lymph node assay (LLNA) for skin sensitization</b>	<b>Completed</b>		<b>OECD TG 429 (2002)</b> <b>ISO (2002)</b>
<b>Updated Murine local lymph node assay (LLNA) for skin sensitization (20% reduction)</b>	<b>Completed</b>		<b>Update to TG 429 (2010)</b> <b>ISO (2010)</b>
<b>Reduced LLNA (rLLNA)</b>	<b>Completed</b>		<b>Update to TG 429 (2010)</b>
<b>Nonradioactive LLNA protocol</b> using BrdU ELISA or BrdU FCM method	<b>Completed</b>		<b>OECD TG 442B (2010)</b> Updated version to include the BrdU FCM method adopted in 2018.
<b>Nonradioactive LLNA protocol, LLNA:DA</b>	<b>Completed</b>		<b>OECD TG 442A (2010)</b>
<b>Harmonized performance standards for the LLNA</b>	<b>Completed</b>		<b>Update to TG 429 (2010)</b>
<b><i>In vitro</i> skin sensitisation assay (DPRA)</b>	<b>Completed</b>		<b>OECD TG 442C (2015)</b>
<b><i>In vitro</i> skin sensitisation assay (h-CLAT)</b>	<b>Completed</b>		<b>OECD TG 442E (2016)</b> Draft updated TG442E under consideration to include an adaptation to use human serum/antibodies.

Method	Current Status	Lead Organisation	International Acceptance
<b><i>In vitro</i> skin sensitisation assay KeratinoSens™</b>	<b>Completed</b>		<b>OECD TG 442D (2015).</b> Updated version to include an adaptation to animal-free conditions adopted in 2018
<b><i>In vitro</i> skin sensitisation assay IL-8 Luc assay</b>	<b>Completed</b>		<b>OECD TG 442E (2017)</b>
<b><i>In vitro</i> skin sensitisation assay U937 Cell Line Activation Test (U-SENS™)</b>	<b>Completed</b>		<b>OECD TG 442E (2017)</b>
<b><i>In vitro</i> skin sensitisation assay LuSens</b>	<b>Completed</b>		<b>OECD TG 442D (2018)</b>
<i>In vitro</i> skin sensitisation assay SENS-IS	External validation study finalised	EURL ECVAM evaluation finalised. Revised submission awaited	Included in the OECD TG work plan in 2016
<i>In vitro</i> sensitisation assay Genomic Allergen Rapid Detection (GARD)	External validation study finalised	EURL ECVAM evaluation stopped.	Included in the OECD TG work plan in 2016
<i>In vitro</i> skin sensitisation assay Vitrigel-SST	MAFF <sup>4</sup> -sponsored validation study is pending	JaCVAM	
<b><i>In vitro</i> skin sensitisation assay, Amino acid derivative reactivity assay (ADRA)</b>	<b>Completed</b>		<b>OECD TG 442C (2019)</b> Updated version of OECD TG 442C to include the ADRA adopted in 2019

Method	Current Status	Lead Organisation	International Acceptance
IL-2 Luc assay for the evaluation of the immunotoxic potential of chemicals	Peer review coordinated by JaCVAM is ongoing	JaCVAM	
Electrophilic allergen screening assay (EASA)	Validation study coordinated by NICEATM is currently ongoing; converted assay to 96-well format; testing will continue into 2020.	NICEATM	
Defined approaches for skin sensitisation	ICATM partners developed and/or evaluated defined approaches for skin sensitisation to support the development of an OECD guideline on Defined Approaches.	NICEATM/EURL ECVAM/Health Canada	Included in the OECD TG work plan in 2017.
IL-1 $\beta$ Luc assay for the evaluation of the immunotoxic potential of chemicals	JaCVAM validation study is ongoing	JaCVAM	
<i>In vitro</i> skin sensitisation assay: EpiSensA	JaCVAM validation study is ongoing	JaCVAM	
<b>Acute Toxicity Test Methods</b>			
<b>Up and Down Procedure (UDP)</b>	<b>Completed</b>		<b>OECD TG 425 (2008)</b>
<b><i>In vitro</i> cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity tests</b>	<b>Completed</b>		<b>OECD GD 129 (2010)</b>

Method	Current Status	Lead Organisation	International Acceptance
<i>In vitro</i> cytotoxicity test (3T3 Neutral Red Uptake) for identifying substances with acute oral LD50 > 2000 mg/kg b.w.	EURL ECVAM ESAC peer review completed, and EURL ECVAM Recommendation published in 2013	EURL ECVAM	
EpiAirway human reconstructed lung epithelium for identifying acute inhalation toxicity	Validation study is ongoing. Testing in the lead laboratory underway and chemical selection for testing in two additional laboratories is in progress. ICCVAM agency representatives serving on the VMT	NICEATM/ ICCVAM	
The Collaborative Acute Toxicity Modeling Suite (CATMoS)	The ICCVAM Acute Toxicity Workgroup organised a global project to develop <i>in silico</i> models of acute oral systemic toxicity that predict five specific endpoints needed by regulatory agencies. Thirty-two international groups across government, industry, and academia built and submitted models. Outputs from the different models were combined to generate consensus predictions (via CATMoS).	NICEATM/ ICCVAM	

Method	Current Status	Lead Organisation	International Acceptance
<b>Toxicokinetic Test Methods</b>			
<i>In vitro</i> hepatic biotransformation – CYP induction: Hepa RG and cryopreserved human hepatocytes [surrogate for Block 4 methods (upregulation of glucuronidation) until methods for glucuronidation induction become available] <sup>5</sup>	EURL ECVAM ESAC peer review completed, and EURL ECVAM manuscript finalised.	EURL ECVAM	SPSF for a PBTG approved in April 2013. Draft PBTG underwent a first commenting round in 2014. An OECD expert meeting was held in March 2015. A TG on “Determination of cytochrome p450 (CYP) enzyme activity induction using differentiated human hepatic cells” including input of the 1 <sup>st</sup> commenting round was submitted to OECD in September 2019.
<b>Endocrine Disruptor Test Methods</b>			
<b>Stably transfected human estrogen receptor-<math>\alpha</math> transcriptional activation assay for detection of estrogenic agonist-activity of chemicals (STTA and BG1-Luc assays)</b>	<b>Completed</b>		<b>OECD TG 455 (2009), updated 2012 and 2015</b> , inclusion of the antagonist protocols in addition to the agonist protocols, deletion of OECD TG 457 in parallel as it is no longer needed.
<b>H295R Steroidogenesis assay</b>	<b>Completed</b>		<b>OECD TG 456 (2011)</b>
<b>VM7Luc Test Method human estrogen receptor transcriptional activation assay: agonist and antagonist protocols</b>	<b>Completed</b>		<b>OECD TG 457 (2012)</b> TTG 457 has been deleted in parallel to TG 455 updates (see previous table entry)

<sup>5</sup> OECD, 2014. New scoping document on *in vitro* and *ex vivo* assays for the identification of modulators of thyroid hormone signalling. Series on Testing and Assessment No. 207 ENV/JM/MONO(2014)23. <http://www.oecd.org/chemicalsafety/oecd-encourages-development-of-non-animal-test-methods-for-detection-of-thyroid-disrupters.htm>



Method	Current Status	Lead Organisation	International Acceptance
CertiChem MCF-7 cell proliferation assay for the detection of human estrogen receptor agonists and antagonists	International validation study completed. Protocol must be revised for adequate transferability.	NICEATM	Not in the OECD TGP work plan anymore.
CertiChem MDA-Kb2 assay for the detection of human androgen receptor agonists and antagonists	NICEATM coordinated single lab validation study and summary report completed.	NICEATM	
<b>Stably transfected CHO Androgen receptor-<math>\alpha</math> transcriptional activation assay for detection of androgenic agonist and antagonist activity of chemicals (AR-STTA)</b>	<b>Completed</b>		<b>OECD TG 458 (2016)</b> TG is being updated to include two other ARTA assays: AR-CALUX assay and 22Rv1/MMTV_GR-KO STTA.
MELN <sup>®</sup> human estrogen receptor transcriptional activation assay: agonist and antagonist protocols	Validation stopped		
AR-CALUX Stably Transfected Transactivation <i>in vitro</i> Assay to detect Androgen Receptor Agonists and Antagonists	EU-NETVAL validation study finalised	EURL ECVAM	Included in the OECD TGP work plan in April 2013. Included in the draft TG458 together with the AR STTA and the 22Rv1/MMTV_GR-KO STTA. Review process at OECD ongoing. Adoption expected for 2020.

Method	Current Status	Lead Organisation	International Acceptance
Transactivation assay for the detection of compounds with (anti)androgenic potential using 22Rv1/MMTV GR-KO cells	Validation study finalised	Ministry of Food and Drug Safety (MFDS) South Korea	Included in the draft TG458 together with the AR STTA and the AR-CALUX assay. Review process at OECD ongoing. Adoption expected for 2020.
<b>Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) <i>In Vitro</i> Assays to Detect Chemicals with ER Binding Affinity</b>	<b>Completed</b>		<b>OECD TG 493 (2015)</b>
CHO-K1 cells thyrotropin-releasing hormone (TRH) receptor activation (beta-galactosidase) measuring agonist and antagonist activities (Block 1 method a)	EU-NETVAL Validation study ongoing	EURL ECVAM	In the context of the detection of chemicals with thyroid disrupting potential as called for by the OECD <sup>6</sup> .
CHO-K1 cells thyrotropin-stimulating hormone (TSH) receptor activation based on cAMP measurement (Block 1 method b)			
Thyropoxidase (TPO) inhibition based on oxidation of Amplex UltraRed (Block 2 method a)			

6 OECD, 2014. New scoping document on *in vitro* and *ex vivo* assays for the identification of modulators of thyroid hormone signalling. Series on Testing and Assessment No. 207 ENV/JM/MONO(2014)23. <http://www.oecd.org/chemicalsafety/oecd-encourages-development-of-non-animal-test-methods-for-detection-of-thyroid-disrupters.htm>

Method	Current Status	Lead Organisation	International Acceptance
Thyroperoxidase (TPO) inhibition based on oxidation of Luminol (Block 2 method b)	EU-NETVAL Validation study ongoing	EURL ECVAM	In the context of the detection of chemicals with thyroid disrupting potential as called for by the OECD <sup>6</sup> .
Tyrosine iodination using liquid chromatography (Block 2 method c)			
Activation of the sodium iodide symporter (NIS) based on Sandell-Kolthoff reaction (Block 2 method d)			
Thyroxine-binding prealbumin (TTR) / thyroxine-binding globulin (TBG) using fluorescence displacement (ANSA) (Block 3 method a)			
Thyroxine-binding prealbumin (TTR) using fluorescence displacement (T4-FITC) (Block 3 method b)			
Deiodinase 1 activity based on Sandell-Kolthoff reaction (Block 4 method a)			
Inhibition of thyroid hormones (THs) glucuronidation using liquid chromatography/mass spectrometry (LC/MS) (Block 4 method b)			

Method	Current Status	Lead Organisation	International Acceptance
Inhibition of THs sulfation using liquid chromatography (Block 4 method c)	EU-NETVAL Validation study ongoing	EURL ECVAM	In the context of the detection of chemicals with thyroid disrupting potential as called for by the OECD <sup>6</sup> .
Inhibition of monocarboxylate transporter 8 (MCT8) based on Sandell-Kolthoff reaction (Block 5 method a)			
Human thyroid hormone receptor alpha (TR $\alpha$ ) and Human thyroid hormone receptor beta (TR $\beta$ ) reporter gene transactivation measuring agonist and antagonist activities (Block 6 method a)			
CALUX human thyroid hormone receptor beta (TR $\beta$ ) reporter gene transactivation measuring agonist and antagonist activities (Block 6 method b)			
Measurement of intrafollicular thyroxine (T4) using zebrafish eleutheroembryos (Block 7 method a)			
Measurement of proliferation of rat pituitary-derived cell line GH3 (Block 8 method a)			

Method	Current Status	Lead Organisation	International Acceptance
Proliferation, migration and oligodendrocyte differentiation of human neural progenitor cells (Block 8 method b)	EU-NETVAL Validation study ongoing	EURL ECVAM	In the context of the detection of chemicals with thyroid disrupting potential as called for by the OECD <sup>6</sup> .
<b>Genetic Toxicity Test Methods</b>			
<b><i>In vitro</i> mammalian cell micronucleus test</b>	<b>Completed</b>		<b>OECD TG 487 (2010), updated TG adopted in 2014 and again in 2016</b> further to an extensive revision of the genetic toxicology TGs including a comprehensive harmonisation of recommendations across the TGs (OECD, 2016) <sup>7</sup> .
<b><i>In vivo</i> comet assay</b>	<b>Completed</b>		<b>OECD TG 489 (201), updated TG adopted in 2016</b> further to an extensive revision of the genetic toxicology TGs including a comprehensive harmonisation of recommendations across the TGs (OECD, 2016) <sup>7</sup> .
<i>In vitro</i> comet assay	Validation study for the <i>in vitro</i> comet assay stopped	JaCVAM	
Genotoxicity assays (micro-nucleus and comet) in 3D skin models	Validation study completed	Cosmetics Europe (lead); EURL ECVAM support	Included in the OECD TGP work plan in April 2019 (lead Germany).
<b>Carcinogenicity Test Methods</b>			
<b><i>In vitro</i> Bhas 42 cell transformation assay (CTA)</b>	<b>Completed</b>		<b>OECD GD 231 (2016)</b>

7 OECD, 2016. Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015. Series on Testing & Assessment No. 238 -2<sup>nd</sup> edition ENV/JM/MONO(2016)33/REV1. [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-JM-MONO\(2016\)33/rev1&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-JM-MONO(2016)33/rev1&doclanguage=en)



Method	Current Status	Lead Organisation	International Acceptance
<b><i>In vitro</i> Syrian hamster embryonic cells (SHE) cell transformation assays (CTAs)</b>	<b>Completed</b>		<b>OECD GD 214 (2015)</b>
<b><i>Reproductive Test Methods</i></b>			
Hand-1 Luc assay	METI <sup>8</sup> -sponsored validation is completed.	JaCVAM	Included in the OECD TGP work plan in 2017. The 1 <sup>st</sup> step of the project consists in drafting a Detailed Review Paper of available methods and the evaluation of utility and application of the Hand-1 Luc assay; The 2 <sup>nd</sup> step consists in a feasibility study on the development of a Test Guideline.
Mouse Embryoid Bodies (mEBT) assay	KoCVAM sponsored validation study is ongoing	KoCVAM	
<b><i>Developmental Neurotoxicity Test Methods</i></b>			
Pluripotent Stem Cells differentiation into neural precursor cells/neural stem cells NPC/ NSC (embryonic phase)	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.	EFSA/EC (through EURL ECVAM)/OECD	Development of an OECD Guidance Document (GD) on the use of an <i>in vitro</i> testing battery for DNT. Included in the OECD TGP work plan in 2017. Generation of new data for some test methods is ongoing and will be followed by the drafting of the GD.
Human neural precursor cells (hNPC) proliferation	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.		
hNPC migration	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.		

Method	Current Status	Lead Organisation	International Acceptance
hNPC neuronal differentiation	Retrospective validation according to defined readiness criteria.	EFSA/EC (through EURL ECVAM)/OECD	Development of an OECD Guidance Document (GD) on the use of an <i>in vitro</i> testing battery for DNT. Included in the OECD TGP work plan in 2017. Generation of new data for some test methods is ongoing and will be followed by the drafting of the GD.
hNPC differentiated neurons	Retrospective validation according to defined readiness criteria		
hNPC oligodendrocyte different	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.		
hNPC oligodendrocyte maturation and thyrod hormone disruption	Retrospective validation according to defined readiness criteria.		
Neural Crest Cells (NCC) proliferation and migration (cMINC)	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.		
Morphological differentiation of embryonic stem cells (ESC to neurons)	Retrospective validation according to defined readiness criteria.		
Differentiation towards astrocytes, oligodendrocytes, myelination, microglia in 3D rat model	Retrospective validation according to defined readiness criteria.		
Differentiation towards astrocytes, oligodendrocytes, myelination, microglia in 3D human foetal phase model	Retrospective validation according to defined readiness criteria.		

Method	Current Status	Lead Organisation	International Acceptance
Neurite outgrowth of central neurons	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.	EFSA/EC (through EURL ECVAM)/OECD	Development of an OECD Guidance Document (GD) on the use of an <i>in vitro</i> testing battery for DNT. Included in the OECD TGP work plan in 2017. Generation of new data for some test methods is ongoing and will be followed by the drafting of the GD.
Neurite outgrowth of peripheral neurons	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.		
Neuronal sub-type ratio, neuronal maturation	Retrospective validation according to defined readiness criteria.		
Synaptogenesis	Retrospective validation according to defined readiness criteria.		
Neuronal network formation and function	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.		
Zebrafish assays	Retrospective validation according to defined readiness criteria. Prospective validation ongoing.		
<b>Acute Aquatic Toxicity Test Methods</b>			
<b>Zebrafish Embryo Acute Toxicity test (ZFET)</b>	<b>Completed</b>		<b>OECD TG 236 (2013)</b>

Method	Current Status	Lead Organisation	International Acceptance
<b>Fish Bioaccumulation Test Methods</b>			
<b>Determination of <i>in vitro</i> intrinsic clearance using cryopreserved rainbow trout hepatocytes as included in OECD TG 319A</b>	<b>Completed</b>	EURL ECVAM and US EPA	<b>OECD TG 391A and 391B (2018)</b>
<b>Determination of <i>in vitro</i> intrinsic clearance using rainbow trout liver S9 sub-cellular fraction as included in OECD TG319B</b>			
<b>Various</b>			
Zebrafish assays	NTP SEAZIT: the Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) program, jointly led by NTP and NICEATM scientists. Summarized below are four key SEAZIT program activities: <ul style="list-style-type: none"> <li>• Zebrafish information gathering</li> <li>• A webinar series focused on the use of informatics to improve data analysis for zebrafish screening studies (2017)</li> <li>• An interlaboratory zebrafish study (2018)</li> <li>• A zebrafish best practices workshop</li> </ul>	NICEATM	





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