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Novel chemical hazard characterisation approaches

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Abstract

There is a fundamental change in thinking within the regulatory community due to a better understanding of the underlying biology of adverse effects to human health and the environment. The development of alternatives to use laboratory animals has become a priority. In addition, technological progress is impacting greatly on the amount of data available and on the ways to process and analyse it. Topics, such as identification of adverse outcome pathways (AOPs) and modes of action (MoA), together with integrated assessment and testing approaches (IATAs), represent fundamental tools for hazard identification and characterisation of a chemical. Complex endpoints cannot be predicted by a single standalone non-animal test; thus, a major challenge is the complex nature of biological systems. Microphysiological systems (MPS) will enable more complex *in vitro* human models that better simulate the organ's biology and function by combining different cell types in a specific three-dimensional configuration that simulates functional organs. The process of validation of new approaches needs to be considered in terms of efficiency and length. Regulators might still not have enough confidence to adopt and apply these new approaches: this phase is very challenging and the activities performed by assay developers are not yet addressing the regulatory requirements needs sufficiently. The IATAs provide a framework to consistently evaluate new approach data and could assist in understanding their relevance for specific endpoints. The data need to be reproducible, understandable and statistically sound: indeed, a major issue lies in the interpretation and integration of the results based on subjective assessment, which relies on expert judgement. A well-defined mechanistic characterisation is proposed as a way forward to ensure the relevance of new cell-based test systems.

Keywords: hazard characterisation, AOP, MoA, QSAR, integrated assessment, microphysiological systems

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Summary

Scientific and technological advances are revolutionising biology and toxicology, as well as regulatory risk assessment, by making available many new tools available for investigating the effects of chemicals on biological systems. Complex endpoints can be predicted by integrating evidence from existing information, computational modelling and data from *in vitro* methods. This is also meeting the need in current research to reduce and/or replace animal testing with alternative methods, in agreement with the 3Rs (Replacement, Reduction and Refinement) concept. The roadmaps of the new toxicology paradigm are represented by the US Tox21 initiative, as well as the European Union Safety Evaluation Ultimately Replacing Animal Testing-1 (EU SEURAT-1) initiative, which both support the need for researching, developing, validating and interpreting innovative chemical testing methods that characterise toxicity pathways.

This session presented the most up-to-date initiatives for undertaking safety assessments using new alternative methods in integrated and alternative testing strategies. The topic is of great relevance in the light of the new perspectives and developments in toxicology brought about by a paradigm shift towards predictions based on mechanistic understanding from approaches relying on observations in animals that might improve human risk assessment.

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1. Introduction

There is a fundamental change in thinking in the regulatory community due to a better understanding of the underlying biology concerning how chemicals cause adverse effects in humans and to the environment. There is a recognised need to change risk assessment from that based on standard tests using intact animal models to one that is centred on modes of action (MoA) using alternative non-animal methods (i.e. the development of alternatives to using laboratory animals has become a priority). In addition, technological progress is impacting greatly on the amount of data available and on methods for analysis; such data are currently under consideration for incorporation into the risk assessment process. Approaches, such as the development of adverse outcome pathways (AOPs) and the identification of MoA, together with the incorporation of integrated assessment and testing approaches (IATAs) as the means of combining multiple lines of evidence, are seen as the fundamental pathway to hazard identification and characterisation of a chemical. MoAs and AOPs are conceptually similar: MoAs include chemical specific elements, among which kinetics comprises a fundamental component, and describe the mechanism of action of the chemical in the human body, whereas AOPs focus on non-chemical specific biological pathways starting with a molecular initial event (e.g. binding to an enzyme), and then resulting in perturbation on different organ levels (identified as key events), leading to an adverse outcome at the level of the organism. Complex endpoints cannot be predicted by a single standalone non-animal test because it will never be possible to reproduce a whole organism, mainly due to the lack of kinetic relationships and cross-talk among cells, tissues and organs. It is instead necessary to use IATA based on a weight of evidence (WoE) approach where information and evidence from a battery of tests can be incorporated. Data can then be integrated by means of modelling. A shift is foreseen towards using more human data in terms of biologically significant perturbations in key toxicity pathways.

The article presents the outcomes of the session on 'Novel chemical hazard characterisation approaches' held at the EFSA 2nd International Conference (Milan, Italy, 14–16 October 2015). Current tools for performing hazard assessment using integrated and alternative testing strategies were presented and discussed.

2. The frontiers of predictive toxicology

Over the last few years, the scientific community has been discussing the advantages and limitations of both the animal tests and traditional cell culture work. In parallel, molecular research and cell biology knowledge have evolved, effectively relying to a large extent on methodologies that substitute or complement traditional animal tests. The biotechnology and informatics evolution made such technologies broadly available, standardised and useful. Novel approaches aim at replicating human physiology based on the use of human stem cells and lower species (e.g. zebrafish, worms) in high-throughput testing and modelling. In addition, the increasing attention paid over the last decade to the need for chemical safety combined with the very large numbers of compounds in commerce with little or no toxicological data has resulted in the need to improve and speed up the process by moving from a risk assessment based on standard animal tests to one assessing MoA. Also, there is a need to shift from a hazard-driven process to one that is exposure-driven, giving increased attention to the assessment of internal exposure levels. Regulatory toxicologists are starting to embrace these new approaches, as also pointed out by the European Union (EU) non-food Scientific Committee opinion on New Challenges in Risk assessment (European Commission, 2013).

Several frontiers will therefore challenge researchers and regulatory risk assessors in the near future, starting with the need for improved dialogue among all stakeholders, including assay developers and data producers. One of the most relevant issues for consideration is how well the current standard toxicological studies predict human health hazards. There is consensus about the need to perform standardised tests following good laboratory practice and international test guidelines; however, the currently used standardised assays need to be integrated with the new technologies in order to move towards a progressive reduction of tests using laboratory animals. The relevance to human of the current animal tests needs to be reconsidered because many developed drugs fail in clinical trials. This failure implies the waste of human and economic resources, as well as reduced attention regarding areas where there is need to deal quickly with, for example, public health issues. Efforts to improve the throughput and relevance of alternative testing methods continue. However, there are still limitations in the use of non-animal models (both *in vitro* and *in silico* models). To improve the usefulness of these alternative methods, projects such as the Human Toxome project (Bouhifd et al., 2015) are working to enhance the use of Good Cell Culture Practice (Coecke

et al., 2005), and to define and clarify the potential and limitations of these assays in order to complement and improve the currently used tools. The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) is coordinating and promoting the development of alternative approaches in research and for regulatory application. The EURL ECVAM is also responsible at the EU level for coordinating the validation of *in vitro* methods.[1] Better models using cells of human origin that preserve all the properties of their *in vivo* counterparts for prolonged periods of time are needed, in combination with methods capable of reflecting toxicokinetics properties. Critical for the prediction of human disease is the need to identify definitive *in vitro* biomarkers of adversity by establishing clear relationships between *in vitro* endpoints and adverse effects *in vivo* (Blaauboer et al., 2012). In an integrated vision, it is essential that new technologies and approaches be embraced with the purpose of developing alternative methods/approaches. For example, mapping the human toxome using system toxicology would result in a software tool that relies on 'omics' and not on literature searching only. With this amount of possibly available information, the evidence in toxicology needs to be processed differently, which is why systematic review methods are increasing in importance internationally and gaining acceptance in toxicology. In a tiered approach or IATA, a number of non-testing methods can be applied, such as grouping and read-across, (quantitative) structure–activity relationship models ((Q)SARs), biokinetic or physiologically based pharmacokinetic modelling. Optimised *in vitro* tests are included in an integrated testing strategy (Hartung et al., 2013a; Rovida et al., 2015) followed, when necessary, by optimised *in vivo* tests. Read-across and grouping are an alternative approach currently used in regulation (e.g. Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), biocide and food flavouring safety evaluation), although, in most cases, it has only partial application. However, in the case studies developed within the Safety Evaluation Ultimately Replacing Animal Testing-1 (SEURAT-1), the traditional read-across based on structural similarities has been strengthened by evidence from alternative methods, and both data from the US Environmental Protection Agency (EPA) ToxCast project (http://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data) and alternative data produced within SEURAT-1, were used to reach higher confidence in the read-across assessment. Great benefit would result from a continuous enhanced transatlantic collaboration between the USA and Europe that shares needs and optimises resources, as well as the involvement of all world regions to reach agreement and acceptance of the same new approaches in a harmonised manner.

3. Alternative and integrated testing strategies: an update

Mimicking the complexity of an *in vivo* system will likely require a battery of *in vitro* tests, each of which would cover a different *in vivo* endpoint of the assay to be replaced. The use of an integrated testing strategies (ITS) approach has been recommended by the EURL ECVAM, as recently published in 'The EURL ECVAM strategy for achieving 3Rs impact in the assessment of toxicokinetics and systemic toxicity', in which it is stated that information on toxicokinetics, although important in the safety assessment of chemicals, is only rarely included in the data requirements of the EU regulatory framework (Bessems et al., 2015). As previously indicated, information about the *in vivo* and *in vitro* kinetics of a chemical under evaluation needs to be collected up front (Coecke et al., 2013). Toxicokinetics, together with AOPs, is essential to any IATA (OECD, 2015). More recently, the AOP approach was used to analyse the major pathways underlying some toxicity endpoints and *in vitro* assays were developed for each step in each pathway.

For positioning toxicity data generated by alternative methods into a regulatory context, the AOP concept is supported by the Organisation for Economic Co-operation and Development (OECD). An AOP is a conceptual framework for organising existing knowledge concerning the predictive and/or causal links between exposure and measureable/observable biological changes that are essential to the progression from a molecular initiating event to an adverse outcome considered relevant to regulatory decision-making. The OECD adopted the AOP as a relevant step forward for regulators to use. It is also an opportunity for assay developers and users to interact with regulators. This approach has successfully been used to partially replace skin sensitisation testing in animals: two *in vitro* OECD test guidelines have been adopted, covering the molecular initiating event of the AOP for skin sensitisation (OECD TG 442C) and the intermediate key events (OECD 442D).

[1] 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. OJ L 276, 20.10.2010, 1–33.

The EU SEURAT-1 project started with the aim of superseding the traditional animal experiments with a predictive toxicology based on a comprehensive understanding of how chemicals can cause adverse effects in humans focussing on repeated dose toxicity. To achieve this goal, three levels of proof-of-concept were identified: (1) knowledge: to establish a mechanistic understanding underpinning AOP constructs; (2) prediction: to develop integrated systems including *in vitro* and computational methods to predict toxicity; and (3) application: to use predictive systems to support regulatory safety assessment. Following this conceptual approach, AOPs for liver toxicity as well as several integrated systems for toxicity prediction based on alternative methods were developed within SEURAT-1. The need to integrate the resulting *in vitro* biodynamic data with biokinetic data, as exemplified by the EU project Predict-IV (Mueller et al., 2014), was fully embraced in the SEURAT-1 case studies. Finally, based on a general conceptual framework (Daston et al., 2014) three different case studies were developed to meet requirements for regulatory safety assessment: (1) threshold of toxicological concern extended to dermal exposure; (2) strengthening a traditional read-across based on structural similarity with evidence of biological similarity from alternative methods (Berggren et al., 2014; Schultz et al., 2015); and (3) the *ab initio* case study where a hypothesis is constructed through a logic workflow and confirmed by data from alternative methods. In the frame of Horizon 2020, the project EU-ToxRisk21 (http://www.eu-toxrisk.eu) intends to build on the experiences made within SEURAT-1.

The field of *in silico* models is rapidly evolving and offering advantages, such as innovation and the reduction in costs and the use of animals. In addition, such approaches allow for the possibility of prioritising testing needs. QSAR represents therefore a means of supporting human expert assessments by offering the possibility of integrating multiple approaches (e.g. WoE), as well as the comparative extent of experimental uncertainty/variability. The challenge consists of both the correct prediction and the integration of multiple forms of information in a reproducible, understandable and statistically sound manner. Essential for the regulatory risk assessment is a quantitative assessment of the reliability of the information obtained. Data coming from *in vitro* testing are extremely useful as an input for physiologically based pharmacokinetic and pharmacodynamic models used to integrate data with human *in vivo* physiological parameters towards a better prediction of toxicity (Bessems et al., 2014).

Launched formally in 2007, the objective of the Tox21 partnership is to shift the assessment of chemical hazards from traditional animal studies to a target-specific, mechanism-based, biological observations system using high-throughput screening and high-content *in vitro* assays (Leist et al., 2014). So far, the Tox21 Program has profiled a library of > 10,000 chemicals, including mixtures, across a set of nuclear receptor and stress response pathway assays. Limitations of the high-throughput screening include the limited pathway coverage, the general lack of xenobiotic metabolism and the lack of biological complexity. Thus, Tox21 is currently trying to overcome these limitations by incorporating more physiologically relevant cell types and kinetics into the testing strategy, coupled with high-content screening and high-throughput transcriptomics platforms to assess chemical toxicity potential.

Organs-on-chips offer further new approaches to answer relevant questions in biology through the development of *in vitro* surrogates for regulatory sciences. Microfabrication approaches are applied to engineered cell culture microenvironments that go beyond conventional three-dimensional models by recapitulating the tissue–tissue interfaces, spatiotemporal chemical gradients, mechanical microenvironments and physiological function of living organs. These organs-on-chips are being combined with bioengineered devices allowing perfusion to elucidate human physiology in an organ-specific context. This technology is poised to achieve new standards in emulating human physiology for use in biological testing to advance product innovation, design and safety across a range of applications within pharmaceutical development, food safety, personalised health, agriculture and chemical-based consumer products.

4. Open issues

A major challenge is the complex nature of biological systems. Reducing the complexity of test systems to simpler cell and small model organisms enables the application of higher throughput testing strategies but, in so doing, many of the systems-level characteristics that make a human toxicological response complex (Knudsen et al., 2015) might be lost. According to Bouhifd et al. (2014), an AOP is largely a framework for referencing and assembling existing scientific knowledge into putative pathways. The goal of pathways of toxicity (Hartung and McBride, 2011), in contrast, is to develop molecular annotations of network perturbations and their causation from biological high-content phenotyping. A pathway of toxicity is a molecular definition of the cellular processes shown to mediate adverse outcomes of toxicants. Knowledge of these molecular mechanisms is crucial for understanding

chemico–biological interactions in biological systems and the perturbed normal physiology (i.e. homeostasis under stress) that is established in response (Hartung et al., 2012).

The process of validation of new approaches needs to be reconsidered in terms of efficiency and time to completion: in particular, the scientific community needs to understand if it is possible for alternative methods to meet some or all regulatory needs and not be used for prioritisation purposes only. Furthermore, the fate of the animal testing in this transitional phase towards ITS is unclear.

Regulators might still not have enough confidence to adopt and use these new approaches in legal implementation, especially for more complex systemic endpoints. This phase is very challenging and the activities performed by assay developers and users are not yet addressing the needs of regulators in terms of demonstrating adequate assay reliability and relevance. The time that it takes for international assay acceptance is generally very long and a gap between the identification of a need and fulfilling that need is often created. According to Pamies et al. (2014), microphysiological systems (MPS) promise to generate more complex *in vitro* human models that better simulate the organ's biology and function. MPS combine different cell types in a specific three-dimensional configuration to simulate organs with normal function. Their final aim is to combine different 'organoids' to generate a human-on-a-chip, an approach that would allow studies of complex physiological organ interactions. The recent advances in the area of induced pluripotent stem cells provide a range of possibilities that include cellular studies of individuals with different genetic backgrounds (e.g. human disease models). However, throughput remains a significant limitation and there will continue to be a need for 'fit-for-purpose' assays.

5. Conclusions

Regulatory toxicology has begun to embrace new hazard characterisation approaches that can be integrated into regulatory safety assessments. The vision is to fundamentally change the way we assess the safety of chemicals, by replacing or reducing the use of traditional animal experiments with a predictive toxicology based on a comprehensive understanding of how chemicals can cause adverse effects in humans (MoA and AOP) or adversely impact on the environment, as well as a 'renewed' awareness of the importance of the fate of the chemical in the body (kinetics) for induction of adverse effects. The IATAs will assist evaluators to consistently evaluate new approach data and to understand their relevance for specific endpoints, in order to obtain reproducible, understandable and statistically sound results. The concept of mechanistic validation is proposed as a way forward to quality-assure new cell-based tests (Hartung et al., 2013b). For positioning toxicity data generated by alternative methods into a regulatory framework, reliable methods have to be combined with strategies to integrate computational modelling data and existing knowledge and information, which together should provide a toxicological prediction with a level of confidence sufficiently high for acceptance in a safety assessment context.

6. Key recommendations

Key recommendations for future risk assessment approaches include:

- Combining hazard testing with toxicokinetics predictions (both *in vivo* and *in vitro*).
- Developing integrated test strategies.
- Incorporating new high-content endpoints to classical assays.
- Integrating new disciplines, such as systems biology and high-throughput screening.
- The utility of omics after approximately 20 years of developments needs to be reconsidered and integrated into the current risk assessment approaches.
- The issue of adversity vs adaptive effects, relevant to *in vivo* studies, is relevant to *in vitro* studies as well. There is clear need for identifying relevant adversity biomarkers to make the available approaches applicable to a regulatory context.
- The progress is ongoing with regard to different experimental models: three-dimensional cultures are evolving and promising. However, the extent of standardisation needs to be carefully considered in order to avoid additional uncertainty in their future uses.
- There is the need of large institutional databases to improve and increase the availability of data, also allowing better *in silico* models.
- The transition to alternative testing methods needs to proceed faster and more effective solutions for alternative methods and the possibility to link them in ITS have to be identified.
- Enhanced dialogue and collaboration between all stakeholders (e.g. regulators, researchers, assay developers and users, bioinformaticians) has to be further developed and maintained.

References

Berggren E, Amcoff P, Benigni R, Blackburn K, Carney E, Cronin M, Deluyker H, Gautier F, Judson RS, Kass GN, Keller D, Knight D, Lilienblum W, Mahony C, Rusyn I, Schultz T, Schwarz M, Schüürmann G, White A, Burton J, Lostia AM, Munn S and Worth A, 2014. Chemical safety assessment using read-across: assessing the use of novel testing methods to strengthen the evidence base for decision making. Environmental Health Perspectives, 123, 1232–1240. doi: 10.1289/ehp.1409342

Bessems JG, Loizou G, Krishnan K, Clewell HJI, Bernasconi C, Bois F, Coecke S, Collnot EM, Diembeck W, Farcal LR, Geraets L, Gundert-Remy U, Kramer N, Küsters G, Leite SB, Pelkonen OR, Schröder K, Testai E, Wilk-Zasadna I and Zaldívar-Comenges JM, 2014. PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment: recommendations from a joint EURL ECVAM – EPAA workshop. Regulatory Toxicology and Pharmacology, 68, 119–139. doi: 10.1016/j.yrtph.2013.11.008

Bessems J, Coecke S, Gouliarmou V, Whelan M and Worth A, 2015. JRC Science and Policy Report: EURL ECVAM strategy for achieving 3Rs impact in the assessment of toxicokinetics and systemic toxicity. Available online: http://publications.jrc.ec.europa.eu/?repository/?bitstream/JRC96418/?eurl%20ecvam%20toxicokinetics%20strategy.pdf doi: 10.2788/197633

Blaauboer BJ, Boekelheide K, Clewell HJ, Daneshian M, Dingemans MML, Goldberg AM, Heneweer M, Jaworska J, Kramer NI, Leist M, Seibert H, Testai E, Vandebriel R, Yager J and Zurlo J, 2012. The use of biomarkers of toxicity for integrating *in vitro* hazard estimates into risk assessment for humans. ALTEX, 29, 411–425. doi: 10.14573/altex.2012.4.411

Bouhifd M, Hogberg HT, Kleensang A, Maertens A, Zhao L and Hartung T, 2014. Mapping the human toxome by systems toxicology. Basic and Clinical Pharmacology and Toxicology, 115, 24–31. doi: 10.1111/bcpt.12198

Bouhifd M, Andersen ME, Baghdikian C, Boekelheide K, Crofton KM, Fornace AJ Jr, Kleensang A, Li H, Livi CB, Maertens A, McMullen PD, Rosenberg M, Thomas R, Vantangoli M, Yager JD, Zhao L and Hartung T, 2015. The Human Toxome Project. ALTEX, 32, 112–124. doi: 10.14573/altex.1502091

Coecke S, Balls M, Bowe G, Davis J, Gstraunthaler G, Hartung T, Hay R, Merten O-W, Price A, Schechtman L, Stacey G and Stokes W, 2005. Guidance on good cell culture practice. ATLA –Alternatives to Laboratory Animals, 33, 261–287.

Coecke S, Pelkonen O, Batista Leite S, Bernauer U, Bessems JGM, Bois FY, Gundert-Remy U, Loizou G, Testai E and Zaldívar JM, 2013. Toxicokinetics as a key to the integrated toxicity risk assessment based primarily on non-animal approaches. Toxicology In Vitro, 27, 1570–1577. doi: 10.1016/j.tiv.2012.06.012

Daston G, Knight D, Schwarz M, Gocht T, Thomas R, Mahony C and Whelan M, 2014. SEURAT: safety evaluation ultimately replacing animal testing – recommendations for future research in the field of predictive toxicology. Archives of Toxicology, 89, 15–23. doi: 10.1007/s00204-014-1421-5

European Commission, 2013. Addressing the New Challenges for Risk Assessment. Available online: http://ec.europa.eu/health/scientific_committees/emerging/?docs/scenihr_?o_037.pdf

Hartung T and McBride M, 2011. Food for thought . . . on mapping the human toxome. ALTEX, 28, 83–93.

Hartung T, van Vliet E, Jaworska J, Bonilla L, Skinner N and Thomas R, 2012. Food for thought . . . systems toxicology. ALTEX, 29, 119–128.

Hartung T, Luechtefeld T, Maertens A and Kleensang A, 2013a. Food for thought. . . Integrated testing strategies for safety assessments. ALTEX, 30, 3–18.

Hartung T, Stephens M and Hoffmann S, 2013b. Mechanistic validation. ALTEX, 30, 119–130. doi: 10.14573/altex.2013.2.119

Knudsen TB, Keller DA, Sander M, Carney EW, Doerrer NG, Eaton DL, Fitzpatrick SC, Hastings KL, Mendrick DL, Tice RR, Watkins PB and Whelan M, 2015. FutureTox II: in vitro data and in silico models for predictive toxicology. Toxicological Sciences, 143, 256–267.

Leist M, Hasiwa N, Rovida C, Daneshian M, Basketter D, Kimber I, Clewell H, Gocht T, Goldberg A, Busquet F, Rossi A-M, Schwarz M, Stephens M, Taalman R, Knudsen TB, McKim J, Harris G, Pamies D and Hartung T, 2014. Consensus report on the future of animal-free systemic toxicity testing 2014, doi: 10.14573/altex.1406091

Mueller S, Dekant W, Jennings P, Testai E and Bois F, 2014. Comprehensive summary – Predict-IV: a systems toxicology approach to improve pharmaceutical drug safety testing. Toxicology in Vitro, 30, 4–6. doi: 10.1016/j.tiv.2014.09.016

OECD (Organisation for Economic Co-operation and Development), 2015. Report No 215 Report of the Workshop on a Framework for the Development and Use Of Integrated Approaches to Testing and Assessment. Available online: http://www.oecd.org/officialdocuments/?publicdisplaydocumentpdf/??cote=ENV/JM/MONO(2015)22&doclanguage=en

Pamies D, Hartung T and Hogberg HT, 2014. Biological and medical applications of a brain-on-a-chip. Experimental Biology and Medicine, 239, 1096–1107. doi: 10.1177/1535370214537738

Rovida C, Asakura C, Daneshian M, Hofman-Huether H, Leist M, Meunier L, Reif D, Rossi A, Schmutz M, Valentin J-P, Zurlo J and Hartung T, 2015. Toxicity testing in the 21st century beyond environmental chemicals. ALTEX, 32, 25–40. doi: 10.14573/altex.1506201

Schultz TW, Amcoff P, Berggren E, Gautier F, Klaric M, Knight DJ, Mahony C, Schwarz M, White A and Cronin MTD, 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regulatory Toxicology and Pharmacology, 72, 586–601. doi: 10.1016/j.yrtph.2015.05.016

Abbreviations

AOP	adverse outcome pathway
EPA	Environmental Protection Agency
EURL ECVAM	European Union Reference Laboratory for alternatives to animal testing
IATA	integrated assessment and testing approach
ITS	integrated testing strategies
MoA	modes of action
MPS	microphysiological systems
QSAR	quantitative structure–activity relationship model
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
WoE	weight of evidence