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New ideas for non-animal approaches to predict repeated-dose systemic toxicity: Report from an EPAA Blue Sky Workshop

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ABSTRACT

The European Partnership for Alternative Approaches to Animal Testing (EPAA) convened a 'Blue Sky Workshop' on new ideas for non-animal approaches to predict repeated-dose systemic toxicity. The aim of the Workshop was to formulate strategic ideas to improve and increase the applicability, implementation and acceptance of modern non-animal methods to determine systemic toxicity. The Workshop concluded that good progress is being made to assess repeated dose toxicity without animals taking advantage of existing knowledge in toxicology, thresholds of toxicological concern, adverse outcome pathways and read-across workflows. These approaches can be supported by New Approach Methodologies (NAMs) utilising modern molecular technologies and computational methods. Recommendations from the Workshop were based around the needs for better

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chemical safety assessment: how to strengthen the evidence base for decision making; to develop, standardise and harmonise NAMs for human toxicity; and the improvement in the applicability and acceptance of novel techniques. “Disruptive thinking” is required to reconsider chemical legislation, validation of NAMs and the opportunities to move away from reliance on animal tests. Case study practices and data sharing, ensuring reproducibility of NAMs, were viewed as crucial to the improvement of non-animal test approaches for systemic toxicity.

1. Introduction

This report describes the main findings and conclusions of The European Partnership for Alternative Approaches to Animal Testing (EPAA) ‘Blue Sky Workshop’ which discussed new ideas for the use of non-animal approaches in the assessment of repeated-dose systemic toxicity, held on 1–2 October 2019 in Brussels, Belgium. The EPAA ‘Blue Sky Workshop’ aimed to formulate strategic research elements and derive a list of recommended actions to bring innovative approaches forward for repeated-dose systemic toxicity (RDT). The Workshop built upon various initiatives including two previous Workshops for RDT organised by the EPPA. In 2008 an EPAA Workshop pioneered ideas for systemic toxicity research by combining computational chemistry, systems biology and toxicology (Kimber et al., 2011). The ideas from this Workshop stimulated research in the European “Safety Evaluation Ultimately Replacing Animal Testing” (SEURAT-1) cluster of projects from 2011 to 2015 (Gocht et al., 2015) and latterly Cosmetics Europe's Long Range Research Strategy (LRSS) (Desprez et al., 2018) and the EU-ToxRisk Project. A second EPAA Workshop in 2018 was devoted to finding cross-sector synergies in using alternatives for RDT testing to provide non-animal solutions relating to chemical safety assessment. The Workshop concluded that there are no validated alternatives to RDT and that a direct one-to-one replacement is not appropriate (Laroche et al., 2019).

The EPAA Partners' Forum brought together over 30 participants from industry and the European Commission (EC), along with invited representatives from regulatory agencies and researchers from academia. The invited participants represented the EC Directorates-General (DGs) Environment (ENV); Internal Market, Industry, Entrepreneurship and SMEs (GROW); Joint Research Centre (JRC); and Research and Innovation (RTD); the European Chemicals Agency (ECHA); the European Food Safety Authority (EFSA); the U.S. Environmental Protection Agency; as well as companies from the chemicals, pharmaceuticals, cosmetics, soaps and detergents, fragrance and crop protection industries and their European trade associations; in addition to representatives from key EC funded projects relevant for this topic. Drs George Daston and Catherine Mahony co-chaired the ‘Blue Sky Workshop’ and moderated the discussions.

It should be noted that this report is based on short presentations and actual discussions at the EPAA ‘Blue Sky Workshop’ aiming to achieve the stated objectives of the event. These focussed on the practical issues of developing acceptable non-animal approaches for assessing repeated-dose systemic toxicity. This report should not be considered a complete or comprehensive review of research efforts in the area of alternatives to testing for repeated-dose systemic toxicity nor a detailed record of all discussions held, but rather a reflection on the strategic ideas that emerged to push the science forward and increase the pace of uptake.

1.1. Background

Systemic effects following repeated exposure to chemicals remain difficult to determine and have traditionally involved the use of whole animal testing in vertebrate species. There is an opportunity and desire for change across all industrial sectors and stakeholders to a more robust and human-relevant means of determining adverse outcomes, although a number of significant challenges have to be overcome to achieve this goal. Whilst significant progress has been made in recent years, it is acknowledged that a direct one-to-one replacement of the currently applied *in vivo* animal tests is not possible, nor necessarily desirable, and that integrated use of new technologies and approaches is more likely to succeed (Laroche et al., 2019; Thomas et al., 2019).

In order to understand some of the challenges and relatively slow rate of change in chemical safety assessment, a number of factors must be considered. The general aim of legislation to regulate chemicals is to protect human health and the environment whilst allowing for efficient and effective functioning of trade. Whereas legislation is intended to recognise the need for innovation, competitiveness and sustainability, it is often written such that changes in how safety may be evaluated, such as through implementation of New Approach Methodologies (NAMs) (defined in Section 2), are difficult. Within the European Union (EU) alone there are over 40 pieces of key chemical legislation, crossing all industrial sectors (Laroche et al., 2019). The Workshop recognised that there are no validated/standard alternative methods for RDT, although many NAM-based approaches are currently under development and/or evaluation. In addition, there are number of factors responsible for the relatively slow rate of change in regulatory acceptance of updates to RDT testing and implementing new technology and innovations. In many sectors the lack of validated methods for RDT means there is reliance on the traditional testing paradigm, this is, in part, due to the slow pace of validation and acceptance of NAMs. There has been a lack of agreement, from all sides, to consider *in silico* or *in vitro* techniques which are not fully validated and approved by the Organisation for Economic Co-operation and Development (OECD). There is also a lack of knowledge to handle and interpret new data sets, e.g. from omics technologies, amongst many “traditional” toxicology researchers. The situation of using data from the new technologies is exacerbated by a lack of coherent and transferable data resources to use the new information. In addition to issues over validation of NAMs, there is also a lack of harmonisation and consistency in data requirements in regulations between sectors and also between regions. All of these factors, and others, have resulted in the varied and limited implementation of new RDT methods in regulatory toxicology.

The Threshold of Toxicological Concern (TTC) and read-across approaches are well established. Various initiatives have demonstrated their applicability and how NAMs could be used to augment read-across. Notable amongst these (with particular reference to the cosmetics sector) were the European Union/Cosmetics Europe SEURAT-1 initiative which provided a workflow for assessing RDT (Gocht et al., 2015), taking account of low exposure through the TTC (Yang et al., 2017) as well as laying the groundwork for *ab initio* next generation risk assessment (NGRA) (Berggren et al., 2017). This led to the conception of principles for NGRA for cosmetics by a working group of the International Cooperation on Cosmetics Regulation (ICCR) (Dent et al., 2018). Both the workflow and the principles have been taken up in the Cosmetics Europe LRSS (Desprez et al., 2018) where, in partnership with the EU-ToxRisk Project, they are being used to support the capability to make decisions through read-across and from *ab initio* schemes. Currently the latter are being explored using *in vitro* bioactivity from high throughput and high content data streams as a surrogate Point of Departure (PoD) for comparison to exposure predictions (Paul Friedman et al., 2020; Pham et al., 2019). This is proving to be a useful tool for the prioritisation of substances where the bioactivity and exposure overlap but it is noted that the *in vitro* POD currently does not

Abbreviations

AOP	Adverse Outcome Pathway
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EPA	United States Environmental Protection Agency
EPAA	European Partnership for Alternative Approaches to Animal Testing
EU	European Union
FAIR	Findable, Accessible, Interoperable, and Reusable
HTS	High Throughput Screening
IATA	Integrated Approaches for Testing and Assessment
ICCR	International Cooperation on Cosmetics Regulation
JRC	Joint Research Centre
KERs	Key Event Relationships
KEs	Key Events
LRSS	Long Range Science Strategy
MIE	Molecular Initiating Event
MPS	Microphysiological Systems
MoA	Mode of Action
NAMs	New Approach Methodologies
NGRA	Next Generation Risk Assessment
OCM	Organotypic Culture Model
OECD	Organisation for Economic Co-operation and Development
PoD(s)	Point(s) of Departure
(Q)SARs	(Quantitative) Structure-Activity Relationships
RDT	Repeated Dose Toxicity
SEURAT-1	Safety Evaluation Ultimately Replacing Animal Testing
TTC	Threshold of Toxicological Concern
UVCB	Unknown or Variable Composition, complex reaction products or of Biological materials

equate to adverse effects due to the upstream nature of the molecular targets, short term duration and limited biological space of the *in vitro* platforms such as ToxCast. As well as progress in the science and technology, and development of workflows and principles, the Workshop recognised from previous and on-going work the importance of illustrating the value of NAMs through successful case studies. In this regard, the decision context in the new paradigm is essential as this puts conditions and constraints on the 'why, what, how and who', for example working in an emergency response situation, versus a controlled exposure in humans or ingredient stewardship versus registration of a new substance or drug. There are also gaps in our knowledge and the ultimate replacement of an animal test will need to ensure not only sufficient coverage of toxicological modes of action, but also appropriate consideration of toxicokinetics and metabolism and temporal aspects as well as aspirational aspects such as including population variability.

1.2. Aim of workshop: questions to be answered

In the context of the current state-of-the-art of chemical safety assessment, the Workshop participants were requested to formulate strategic ideas that would help improve and increase the applicability, implementation, familiarisation and acceptance of modern non-animal methods to determine systemic toxicity. Specifically, the following questions were addressed:

- How can the "modern safety assessment toolbox" (see Supplementary Information Table 1) be better utilised in chemical safety assessments?
- What is needed to make chemical safety assessments less reliant on animal data and more relevant to human health?
- What are the different ways of thinking required to implement the new methods and make them acceptable for all stakeholders?

The Workshop also aimed to build on previous EPAA Workshops (e.g. Kimber et al., 2011; Laroche et al., 2019) to find solutions providing information relating to chemical safety assessment. There was a particular emphasis on the need to make assessment more efficient in terms of costs, time and resources as well as making the solution more relevant to human safety. Specifically, the focus of the Workshop was on NAMs that can provide information that may be relevant to assessing RDT and/or human safety; the use of NAMs to derive PoDs for human safety assessments; strategies to use and combine synergistically the information and knowledge from NAMs and how to incentivise these; and means to implement the information from NAMs such that it may, ultimately, be acceptable for global, harmonised regulatory purposes. To assist in these goals, the Workshop considered a nominal "toolbox" for modern chemical safety assessment that comprised NAMs and strategies for implementation, the approaches are summarised below and in Supplementary Information Table S1.

In order to address these themes, the Workshop was organised into plenary presentations and discussions as well as breakout groups addressing specific questions. This report summarises the key deliberations in the Workshop, specifically the NAMs that could be applied (summarised in Section 2) as well as the strategies and workflows for their use (summarised in Section 3), the challenges and opportunities for the implementation of the NAMs (Section 4), as well as making recommendations for the way forward towards a new era of assessing systemic toxicity using NAMs (Section 6).

2. Brief overview of the state-of-the-art of current technologies including computational techniques and other new approach methodologies (NAMs)

The Workshop discussed the technologies that can be currently used in chemical safety assessment. The new technologies are described under the broad umbrella of NAMs. The term 'new approach methodologies' (NAMs) has been recently adopted in reference to any non-animal technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals (US EPA, 2018). Participants were provided with illustrations of various types of NAMs, as summarised in Supplementary Information Table S1 and below. The Workshop was not able to undertake a full review of NAMs for chemical safety, rather it selected illustrative examples, and analysed the challenges and potential solutions for their implementation. This section provides an overview of the types of NAMs that may be considered – along with examples presented in the Workshop. Challenges and solutions to their use and implementation are provided in Section 4.

2.1. Computational chemistry and in silico approaches

A variety of computational chemistry and *in silico* approaches that can be used to make predictions of hazard, kinetics and exposure were noted in the Workshop. These range from the well-established use of structural alerts, (quantitative) structure-activity relationships ((Q)SARs), and read-across (described in more detail in Section 3.2) to innovative approaches.

The Workshop noted that computational models are moving into a new era, extending what has been previously undertaken in (Q)SAR. This trend can be thought of as moving from predominantly 2D models to those being in 3D. The advantage of 3D models is the ability to model protein binding and interactions, for instance, a recent review has demonstrated the utility of artificial intelligence, through machine

learning, to model hormone receptor binding affinity (Wong et al., 2017).

2.2. Computational systems biology

Biological systems are complex, with many interacting parts in multiscale networks that ultimately determine how cells and tissues react to genomic programming and biomolecular perturbations. *In silico* approaches have been extended to develop computational systems biology and systems pharmacology models. These attempt to resolve the challenge of translating complex data into predictive models relevant to humans (Saili et al., 2019).

2.3. In vitro and systems level models

The Workshop acknowledged the potential role in chemical safety assessment of non-animal (*in vitro*) test methods ranging from relatively simple cellular systems up to more complex systems reflecting the development and function of tissues and, potentially, organs. Their contribution was seen to relate to aspects of chemical safety such as mechanisms of action and describing and quantifying key events. Particular focus was paid to organoids, which are part of the growing field of microphysiological systems (MPS) that also includes Organ-on-Chips. MPS models recapitulate the underlying biology and toxicology of key events in (Adverse Outcome Pathways) AOPs. There has been a growth in such systems for various organs which could assist in the provision of information relevant to chemical safety assessment (Marti-Figueroa and Ashton, 2017). However, the Workshop recognised that whilst spontaneous organoid morphogenesis has the capability of producing significant microscale organisation and cell phenotype diversification, it is often inconsistent and non-stereotyped at the macroscale with regard to cell phenotype composition, tissue morphology and tissue structure, anatomy and cytoarchitecture. There is also a need for even better mimicking of physiological conditions i.e. advanced microfluidics, combined with real-time monitoring of toxicity read-outs. Such limitations need to be defined and understood for their application to alternative, more public health relevant and efficient, chemical toxicity testing methods. As such, there is a need for reproducibility in organoids to increase confidence and reduce the cost of screening assays.

2.4. Omics technologies and complex data

The Workshop agreed that there was already a demonstrable value to omics (and consequently high dimensional data) in toxicology, which could assist in chemical safety assessment. Such high dimensional, or complex, data are derived from a number of sources such as toxicogenomics or high throughput screening (HTS) from ToxCast. Different types of data have different characteristics and will require translation in practical solutions. The data provided by omics can provide considerable biological coverage and consequently insights in MOA directly or allowing for hypothesis generation. Omics and big data are one key area where data sharing will be vital and should build on existing resources. As part of this, progress is being made in the interoperability of big data resources (Watford et al., 2019) and reporting standards (Viant et al., 2019).

The Workshop agreed that new methods in omics technologies could have distinct benefits and provide useful information for chemical safety assessment whilst acknowledging that current shortcomings in omics technologies inhibit progress.

3. Making a decision from NAMs data – strategies to integrate and apply information

The NAMs described in Section 2 will provide the raw data or information about the hazard or exposure to a chemical. In order to make a decision regarding the safety of a chemical in a particular exposure scenario the Workshop acknowledged the requirement for frameworks or workflows to organise and integrate the data and apply a weight-of-evidence. Thus, it is not anticipated that an individual NAM will replace an *in vivo* assay for a complex toxicological endpoint, rather a combination of data and approaches is foreseen to build consensus with an appropriate, stated, level of certainty (Laroche et al., 2019). For instance, the ICCR principles could act as a guide for further development of integrate workflows for risk assessment.

This section brings together the types of approaches discussed in the Workshop that can be used to combine information and data from NAMs such that a risk assessor and/or risk manager could make a decision.

3.1. Mechanistic understanding and organisation within Mode of Action ontologies

The Workshop agreed that for the successful use of NAMs for chemical safety assessment, there is a requirement for mechanistic understanding and the meaning of data derived from NAMs. In the context of the uncertainty associated with mechanisms of action, this can be rationalised in terms of AOPs whereby there is often much information on the molecular initiating event (MIE) e.g. from QSAR, high-throughput screening and toxicogenomics, as well as many historical data on the adverse outcome. However, much less is known – and hence there is much greater uncertainty - about the intermediate key events (KEs) and key event relationships (KERs) for many AOPs. The use of mechanistic *in vitro* models as part of a predictive toxicology workflow is underpinned by the report from the National Academy of Science (National Academies of Sciences, Engineering, and Medicine, 2017) and includes global gene expression, high throughput screening, as well as bioinformatic and biophysical approaches – a simplified workflow adapted from the National Academy of Sciences report is shown in Fig. 1. Such information can support the identification of relevant analogues for read-across (discussed in Section 3.2). Whilst much is known regarding mechanisms of action for some endpoints, there are still many unknowns. One solution will be a concerted effort to map mechanisms of toxicity. In cases where insufficient mechanistic information or knowledge is available, e.g. for new chemistries, techniques such as agent-based computational models, the range of *in vitro* systems from

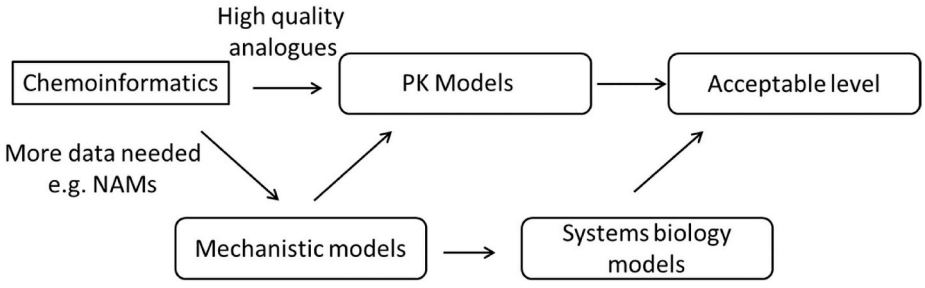

Fig. 1. A simplified workflow for predictive toxicology adapted from the National Academy of Science (National Academies of Sciences, Engineering, and Medicine, 2017). The workflow allows for identification of suitable analogues for a target chemical and integration of toxicokinetic data to establish an acceptable level of exposure, or the incorporation of NAMs data to support the decision.

simple functional assays to organotypic culture models and MPS may provide outputs to demonstrate similarity with known compounds. The integration of systems biology approaches with such models and assays will ultimately facilitate elucidation of the necessary mechanistic insights.

In addition to the protective nature (to human health) of bioactivity based assessments, there is an increasing realisation that information relating to mode-of-action can be captured within a framework, or ontology, to make it useable for chemical safety assessment. Recent and on-going work relating to Mode of Action (MOA) Ontologies has been funded through the Cosmetics Europe LRSS (Desprez et al., 2019).

Thus, the integration of mechanistic information into predictive toxicology workflows, along with knowledge of exposure and biokinetics, is considered one of the cornerstones of modern chemical safety assessment. Key to the use of this information is the appreciation of the contribution of bioactivity based assessments that are designed to be protective of human health.

3.2. Read-across

Read-across is the process by which the toxicity of a compound (the target compound) with no, or insufficient, data is read across from one with high quality data (the source compound(s)) (Berggren et al., 2015). The Workshop participants agreed that read-across is a key technique to predict the hazard of some substances and is being transformed through a better understanding of uncertainties and inclusion of NAM and other data. This process has gained some, but not universal, acceptance for regulatory and other purposes. Key to understanding the use of read-across and its acceptance is definition of the uncertainties in the process (Schultz et al., 2019). In addition, it is foreseen that read-across will develop in a number of ways:

- "traditional" read-across, the current analogue based approach focussed on hazard;
- "next-generation" read-across, with an increased input of NAM data, for instance to support the read-across hypothesis and provide evidence of toxicodynamic similarity. In certain circumstances, this may also include exposure based information to assist in making a decision regarding risk;
- "functional" read-across, when chemistry-based analogues may not be appropriate or available and the similarity concept is refined with biological or mechanistic information (this may also be referred to as "biological" read-across in some circumstances).

NAMs data can assist in all read-across approaches in the building up of lines of evidence to support an overall weight-of-evidence. These different read-across approaches should not be seen as mutually exclusive. It is likely that both functional and next-generation read-across will build on the traditional approaches. The opportunity is to develop these techniques so that they resolve current issues, such as the justification of similarity and read-across hypotheses. NAMs may also support further and better category formation i.e. development of groups of chemicals supported by NAMs data.

Read-across is increasingly becoming a data gathering and predictive exercise. As such, better and more easily accessible computational tools are required. These range from well curated chemical and biological databases, to chemoinformatic workflows capable of combining multiple sources of information.

3.3. Ab initio

Ab initio methods are necessary when there are no existing data and/or TTC or read-across is not possible for the chemical in question. Berggren et al. (2017) described an *ab initio* assessment in terms of a safety evaluation being performed on the basis of hypothesis-driven *in vitro* testing combined with *in vitro* to *in vivo* extrapolation by computational modelling. Further, Berggren et al. (2017) demonstrated the application of an *ab initio* approach as part of a workflow including TTC and read-across. In the absence of information from animal tests, data from NAMs will be relied upon to make a decision. These approaches, typified by Berggren et al. (2017), allow for a consideration of both dose and bioactivity – as such they require appropriate information to inform on these topics. This information may come from appropriate NAMs. The *ab initio* process is seen as being flexible and transparent. In many ways it can incorporate the current Integrated Approaches for Testing and Assessment (IATA) paradigm as it allows the decision maker to go further through the inclusion of exposure and dose.

4. Key opportunities, challenges and solutions to improving the assessment of systemic toxicity

The Workshop identified a number of key opportunities and challenges to the implementation of new technologies to chemical safety as well as potential solutions. These covered all aspects of the topics discussed in the Workshop and can be summarised as being relating to the following:

- Problem formulation: The current paradigm for chemical safety assessment has changed little for several decades. With the new technologies available, there is an opportunity to redefine how we pose the risk assessment or regulatory problem in ways that complement the tools we have at our disposal to answer them in the context of our protection goals.
- Legislation: Global chemical safety legislation is complex and varied, as well as allowing and/or encouraging only minor application of new technologies, as a result of current need for, and restrictions of, validation of NAMs. There is an opportunity to rethink the current legislative process, ensuring it is sufficiently conservative, to make it appropriate for the new technologies and problem formulation.
- Data sharing: Data sharing is fundamental across all technologies and approaches. Currently data sharing is incomplete, or even lacking, for many types of toxicological data and information. The opportunity is to increase the availability of data for sharing with authorities but also within industries and to implement appropriate technologies and informatics structures, doing so applying the Findable, Accessible, Interoperable, and Reusable (FAIR) principles (Wilkinson et al., 2016). A globally and freely accessible database is desirable, and free sharing of data should be incentivised. Solutions need to be identified to enable more extensive sharing of propriety/confidential data.
- Development of computational approaches and other NAMs: There are many *in silico* models and other NAMs for use in safety assessment. These are at various stages of development, validation and acceptance. The opportunity is to establish which approaches are currently suitable to chemical safety assessment and, for those not at that stage, to establish how to develop promising methods further.
- Development of decision-making frameworks: Read-across, *ab initio* approaches and IATA are frameworks to enable chemical safety decisions, at different stages of maturity. The opportunity is to develop one or more decision making frameworks that reflect the new technologies to an acceptable level.
- Acceptance of *in silico* approaches, other NAMs and Next Generation Risk Assessment (NGRA). The acceptance of new methods and decision-making frameworks is complex and variable. The opportunity is to revise how validation can be achieved and to improve the acceptance of non-animal methods. To improve acceptance, a 'safe harbour concept' may be helpful, where safety assessments based on NAMs are shared alongside typical regulatory submissions.
- Communication. Communication between stakeholders has

traditionally been poor, although better dialogue is now occurring between the regulated community and regulators. However, the general public remains sceptical of industry. The opportunity is to engage more fully across all stakeholders, including consumers, to transparently discuss how the new approaches could provide better and more relevant chemical safety assessment.

The Workshop participants acknowledged that there is no single solution to improving chemical safety assessment and to incorporating the new technologies in an appropriate legislative, decision-making framework. Effort to improve chemical safety assessment should not be considered in isolation within the topics listed in this section but should cross them. The development of case studies, which include all stakeholders and cross sectors, is seen as one crucial means of achieving this goal.

There were a large number of individual challenges and potential solutions identified by the Workshop to the improvement of chemical safety assessment. These are organised and summarised in the text below and provided in more detail in Supplementary Information Table S2.

5. Conclusions

The EPAA Blue Sky Workshop '*New Ideas for Systemic Toxicity*' recognised that we have collectively been on the right track in our approach to assessing RDT without animals. Previous EPAA Workshops (Kimber et al., 2011; Laroche et al., 2019), as well as other initiatives (e.g. SEURAT-1, LRSS, EU-ToxRisk amongst others), acknowledged and utilised existing knowledge in toxicology, including read-across workflows that build upon historical toxicology testing data supported by modern molecular and computational methods to develop the next generation of safety assessment. TTC is probably the most predominant example of non-animal approaches in safety assessment that has gained broad regulatory acceptance. The Workshop participants concluded that there are many opportunities to use all NAMs to address challenges in toxicology and risk assessment. However, using these approaches will require the systematic understanding and solving of technical and data analysis challenges, if such approaches are ever to reach broader acceptance. A wide range of tools and means to implement them will be required, spanning all possible toxicological modes of action from targeted testing to untargeted comprehensive testing. Currently grouping and read-across is relied upon in many contexts and can be developed further to include biological (functional) and exposure information. *Ab initio* approaches can be applied when read-across and *in silico* tools are not sufficient, but will require substantially more work. These will eventually lead to integrative systems modelling and the transition from decisions based mainly on *in vivo* data to human knowledge-driven decisions. Improvements will be made through both evolution of knowledge and tools, but also revolution in their application, understanding and dissemination. Key to this will be ensuring the new approaches are transparent (and mechanistically-based), reproducible, accessible (encouraging all aspects of data sharing) and in line with the desired protection goal. Partnering with regulators to achieve appropriate problem formulation, transformation of current regulations and implementation of alternatives in tiered strategies building confidence with the inclusion of further data and information will be crucial. This could be made possible through the use of case studies and will increase confidence and accelerate their application to chemical risk assessment.

6. Recommendations

It was emphasised that more could be made of biological similarity as the basis to form chemical groups for read-across and a move towards *ab initio* assessment would probably require disruptive regulatory approaches. Having said that, the regulatory agencies present were already using TTC and read-across under certain circumstances and were generally supportive of the use of NAMs, especially when they support read-across. It is acknowledged that bioactivity based assessment is generally conservative and this may be crucial for risk assessment.

There are many areas of technology that are already being developed where more work is needed. These include:

- A deep mechanistic understanding of systems toxicology, linked to human relevant toxicological outcomes, to develop a full range of computational and *in vitro* methods.
  - This would include the development of databases that use ontologies of modes of action that catalogue the known universe of MOAs, so that the degree of biological coverage needed by NAMs can be estimated and to structure the development of AOPs.
- Chemistry/biochemistry
  - Identify 3D structural features that better inform read-across and are the basis for QSARs.
  - Better predictions of physico-chemical characteristics and their relationship to toxicokinetic parameters.
- Omics assays
  - To gain insights into broad cellular and molecular mechanistic changes across different cells and tissues from chemical perturbations as a means to maximise coverage of biological space, as support for read-across and the basis for functional read-across as well as providing PoDs.
- Systems-level models
  - Microphysiological systems to associate mechanistic changes with predictions of adverse outcome within and across different target organs.
  - Computational systems – to associate mathematically the connections between molecular and cellular perturbations and adverse outcome.

In addition to basic scientific research, there were policy recommendations to facilitate progress:

- The provision of opportunities for regulators to find ways of accepting the new generation of risk assessment processes and to explore mechanisms beyond traditional validation. Specifically, the new bioactivity assays are known to be protective and conservative and methods to define this and drive acceptance are required to increase uptake.
- Deliberate targeting of data sharing and case studies practices, rewarding those with a will to share data and move towards application of non-animal methods. The regulatory processes could be used to mobilise and direct resources towards those cases where there is the will to use non-animal data.
- Risk managers should conduct a 'stock take' of problem formulations and protection goals. This should identify strengths and weaknesses in current risk management practices and opportunities to move away from reliance on animal tests.
- Harmonisation of performance standards and reference standards.
- The need for a road map on how to transform NAM development into regulatory decision-making, and the resources (e.g. public funds) to do this.

Conflicts of interest

The authors of this article participated in the workshop that was organised by the EPAA. Some of the authors received reimbursement of their travel expenses by the EPAA to make their participation in the workshop possible. If deemed necessary, a list of those people who received travel expenses support can be provided. Dr Barbara Birk is an employee of BASF. Prof Alan Boobis has no current employment. Previously Prof Boobis was employed by Imperial College London full-time until June 2017 and subsequently part-time until May 2019. Prof

Boobis is, or has, collaborated on a number of activities on chemical risk assessment through FAO/WHO JECFA, FAO/WHO JMPR, WHO-IPCS, EFSA, ILSI HESI (now HESI), ILSI Europe, ILSI Research Foundation, and UK Committees on Toxicity (COT), and the Medical Effects of Air Pollutants (COMEAP); he is a member of several scientific advisory boards for Swiss Centre for Applied Human Toxicology (SCAHT), Centre for Research on Ingredient Safety (CRIS), Michigan State University, USA, Long Range Research Strategy (LRSS) Cosmetics Europe, Agency for Innovations in Food and Chemical Safety Programme, Science, Technology and Research, Singapore (A*STAR), Owlstone Medical (https://www.owlstonemedical.com/), Evidence-Based Toxicology Collaboration, Bloomberg School of Public Health, Johns Hopkins, USA; he has served as a member of the ECETOC working group on developing an ontogeny of developmental toxicity (until 2016/17). None of Prof Boobis' collaborative activities are or were remunerated and no research funding was received as a result of them. Prof Boobis received no funding in cash or kind for his contribution to this manuscript. Drs Tom Cull and Evita Vandenbossche are employees of Unilever. At the time of the workshop, Dr Lorna Ewart was an employee of Veroli Consulting Limited; at the time of manuscript preparation she is an employee of Emulate Inc., who design and develop Organ-Chips. Dr Boris Müller is an employee of Symrise AG. Prof Pär Nordlund is co-founder of Pelago Bioscience AB.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Some of the authors received reimbursement of their travel expenses by the EPAA to make their participation in the workshop possible. If deemed necessary, a list of those people who received travel expenses support can be provided.

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Appendix A. Supplementary data

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