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Use of New Approach Methodologies (NAMs) in regulatory decisions for chemical safety: Report from an EPAA Deep Dive Workshop

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ABSTRACT

New Approach Methodologies (NAMs) are considered to include any *in vitro*, *in silico* or chemistry-based method, as well as the strategies to implement them, that may provide information that could inform chemical safety assessment. Current chemical legislation in the European Union is limited in its acceptance of the widespread use of NAMs. The European Partnership for Alternative Approaches to Animal Testing (EPAA) therefore convened a 'Deep Dive Workshop' to explore the use of NAMs in chemical safety assessment, the aim of which was to support regulatory decisions, whilst intending to protect human health. The workshop recognised that NAMs are currently used in many industrial sectors, with some considered as fit for regulatory purpose. Moreover, the workshop identified key discussion points that can be addressed to increase the use and regulatory acceptance of NAMs. These are based on the changes needed in frameworks for regulatory requirements and the essential needs in education, training and greater stakeholder engagement as well the gaps in the scientific basis of NAMs.

1. Introduction

This report describes the main findings and conclusions of The European Partnership for Alternative Approaches to Animal Testing (EPAA) 'Deep Dive Workshop', which discussed the use of New Approach Methodologies (NAMs) in regulatory decisions for chemical safety. The EPAA seeks to bridge the knowledge gaps with regard to replacing animal testing and to facilitate coordination and cooperation via its partners and project platforms. The workshop was held virtually on 23–24 November 2021. The EPAA 'Deep Dive Workshop' provided a platform to exchange information between EPAA partners regarding how NAMs are being applied and/or considered for regulatory use in safety assessment and registration of new and existing substances. The workshop was opened by Mrs Sirpa Pietikäinen, Member the European Parliament, who stated that there must be an overall commitment to the safety of consumers and workers, but also to use the best science to achieve this goal. She recognised that the traditional animal tests may

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
AOP	Adverse Outcome Pathway
APCRA	Accelerating the Pace of Chemical Risk Assessment
CMap	Connectivity Mapping
CSS	Chemical Strategy for Sustainability
DART	developmental and reproductive toxicity
DNEL	derived no-effect level of exposure
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EPAA	European Partnership for Alternative Approaches to Animal Testing
EU	European Union
httk	high-throughput toxicokinetics
IATA	Integrated Approaches for Testing and Assessment
JRC	Joint Research Centre
KE	Key Event
log P	logarithm of the octanol-water partition coefficient
NAMs	New Approach Methodologies
NGO	non-governmental organisation
NGRA	Next Generation Risk Assessment
OECD	Organisation for Economic Co-operation and Development
PBK	physiologically-based kinetics
PK	pharmacokinetics
PoD	Point of Departure
qAOP	quantitative Adverse Outcome Pathway
QIVIVE	quantitative *in vitro – in vivo* extrapolation
(Q)SARs	(quantitative) structure-activity relationships
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical substances
SAR	structure-activity relationship
SME	small and medium sized enterprise
TTC	Threshold of Toxicological Concern
US EPA	United States Environmental Protection Agency

not be the best means to obtain safety information and that the European Union (EU) has been at the forefront of developing new approaches. In order to implement new approaches, Mrs Pietikäinen emphasised the need to increase dialogue between scientists and politicians in key areas, notably to speed up the validation of new approaches, integrate animal welfare and the opinions of patient organisations, whilst recognising the inevitable need for regulatory change. Mrs Pietikäinen set the challenge for the workshop to provide a vision of how NAMs could be implemented to make regulatory decisions in future safety assessment.

In line with the call for action by Knight et al. (2021) and the EU Chemicals Strategy for Sustainability (CSS) (EC, 2020), the workshop explored how progress in the application of NAMs for human safety could be used to provide more human-relevant information for use in defined contexts to better protect EU citizens and to boost innovation for safe and sustainable chemicals. In particular, the workshop aimed to open the discussion around safety decision-making using information from NAMs that may not be direct surrogates for the output from traditional animal data, since this is perceived as a hurdle to progress with regulatory uptake. In order to meet the challenge laid to the workshop, a number of examples and case studies of the use of NAMs were considered. From the outset, the workshop acknowledged the potential for the greater use of human-relevant NAMs in a hypothesis-based manner. Hence, the workshop focussed on regulatory decision-making and aimed to investigate the following questions:

a) Are there circumstances where NAMs could be used for safety assessments in different chemical sectors and to provide information for the classification and labelling of ingredients in the EU – regardless the tonnage – across different safety endpoints? For instance, low tonnage compounds in Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH, (Regulation (EC) No 1907/2006)) where industry still needs to make decisions on safety prior to use e.g., systemic safety, carcinogenicity.
b) Could NAMs be used to provide alternate derived guidance values for protecting the health of humans exposed to chemical substances such as no-effect levels of exposure (DNELs) or acceptable daily intake (ADI) for decision-making in a different way from traditional toxicology testing whilst still providing robust information on safety?
c) Could a and b contribute significantly and rapidly to the EU Green Deal and CSS concept of "One Substance – One Assessment" (EC, 2019, 2020)?

The EPAA itself aims to replace animal testing by innovative, non-animal testing methods, to reduce the number of animals used and to refine procedures where no alternatives exist or are not sufficient to ensure the safety of substances. Its members include various Directorates-General (DGs) of the European Commission (EC), federations representing trade and industry and industrial partners themselves. EPAA is unique in allowing the EC and European industries to work together to identify areas of synergy and increase knowledge across all partners. It is within this context that the workshop brought together over 50 participants from industry and the EC, along with invited representatives from regulatory agencies and researchers from academia. The participants represented the EC 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); as well as companies from the chemicals, pharmaceuticals, cosmetics, soaps and detergents, fragrance and crop protection industries and their European trade associations. Dr Hans Bender moderated the discussions of the 'Deep Dive Workshop', which was led by Drs Federica Madia, Pilar Prieto and Carl Westmoreland.

It should be noted that this report is based on the presentations and actual discussions at the EPAA 'Deep Dive Workshop' aiming to achieve the stated objectives of the event. These focussed on the practical issues of implementing NAMs for regulatory decisions on chemical safety including, for instance, the EU REACH regulation, CLP (Classification, Labelling and Packaging of substances and mixtures) regulation (Regulation (EC) No 1272/2008) and the CSS (EC, 2020). This report should not be considered a complete or comprehensive review of research efforts in the area of NAMs, nor a detailed record of all discussions held, but rather the collection of discussion points that will help to shape practical actions to increase the use and acceptability of NAMs.

1.1. Definition of NAMs for the purposes of the workshop

The workshop recognised that, as yet, there is no formally accepted definition of the phrase "New Approach Methodology". However, two of the more recognised descriptions of NAMs are that they ' … *include in silico approaches, in chemico and in vitro assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard assessment. They also include a variety of new testing tools, such as "high-throughput screening" and "high-content methods"* e.g., *genomics, proteomics, metabolomics* ….' (ECHA, 2016) and that the term NAM is ' … *a broadly descriptive reference to any 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). For the purposes of the workshop and this report, the term "NAM" is used in a broad context going beyond *in vitro* approaches, including, for instance, *in silico* models and chemistry-based approaches that may be used to provide information on chemical hazard and exposure to support safety assessment. The workshop also considered defined approaches, Integrated Approaches for Testing and Assessment (IATA) and tiered—based approaches that can be used to apply the data from NAMs to make a decision on chemical safety without animal testing.

1.2. Use of NAMs in safety assessment

The EU has long been committed to promoting the development and validation of approaches to assure safety of chemicals that do not rely on animal testing. There is a commitment and requirement in the Directive for the protection of animals used for scientific purposes (Directive 2010/63/EU) to use non-animal approaches instead of animal tests if they can provide the result sought and are recognised under the legislation of the EU. The traditional animal-based testing paradigm has a long history of use and the scientific/regulatory community has confidence that safety assessments based on these data are protective of human health. However, it is quite possible that similar (or better) protection of human health could be provided using the modern science and understanding of human biology from NAMs, without necessarily predicting the effects seen in non-human relevant (e.g. high) dose rodent studies first used in the 1950s/60s.

Due to the benefits of their use and the desire to improve safety assessment, the use of NAMs has grown in development and adoption at the regulatory level since the early 2000s and NAMs are increasingly used within industry (and widely used in some sectors) to make decisions about the human safety of chemical exposure. These methods can provide information for use in safety decisions that is both human-relevant and can often be generated far more quickly than traditional animal toxicity studies. In addition to it being a legal requirement, the workshop agreed the importance of, and commitment to, safety in the use and disposal of chemicals across all industrial sectors, including but not limited to cosmetics and personal care products, household products, biocides, plant protection products and industrial chemicals. The safety of humans following exposure to chemicals was an overriding principle to the workshop, whether the exposure was from normal use as a consumer, occupational use during manufacture or as an operator, environmental or accidental exposure. It is also important to note that safety decision-making using information from NAMs may ultimately be a different process to that currently applied, since NAMs are generally not direct surrogates for the output from traditional animal data.

The workshop discussed the possibility of using NAMs to support safety assessments of human chemical exposure, where this current process in many industrial sectors is based predominantly on an understanding of levels of human exposure compared with hazard information including that from animal-based testing. It was further noted that the cosmetics sector does not produce new animal data, rather relying on historical animal data from before the EU bans on animal testing of cosmetic products and their ingredients (EC, 2009) as well as non-animal approaches to assuring safety. NAMs can assist in making decisions about the human safety of ingredients in cosmetic products prior to manufacturing new products (Dent et al., 2018; SCCS, 2021).

The successful application of reliable NAMs may allow great numbers of chemicals to be evaluated with confidence (Mahony et al., 2020). There is a desire to avoid replacing one set of studies directly with another, but to allow for flexible adaptation and incorporate advances in scientific knowledge and methodology. Other opportunities include the safety assessment of combined exposure scenarios.

1.3. Use of NAMs in chemical safety regulations – the need for change

The workshop appreciated that there are challenges (related to both policy/regulations and science) to the more widespread and routine use of NAMs in regulatory decisions on chemical safety. However, the bringing together of EPAA partners from a variety of backgrounds, interests and industrial sectors allowed for a review of work using NAMs. In addition to sharing knowledge and experience, the workshop aimed to identify the key challenges to the use of NAMs in regulatory decision-making and identify potential areas that require further effort to increase the uptake, use and acceptance of NAMs. Firstly, there is a need for greater understanding of the possible applications, as well as advantages and limitations, of NAMs for those undertaking and assessing chemical safety. In order to encourage use, there is also a need to change, or adapt, relevant legislation.

The overwhelming need for change and potential solutions in many areas are discussed in more detail in Section 2. To bring about these changes, the workshop recognised the need to improve the dialogue between scientists, regulators, politicians, NGOs and society as a whole.

1.4. NAMs – demonstrating utility and selected case studies

Next Generation Risk Assessment (NGRA) methodologies based on NAMs are already used for decision-making within industry in certain areas. For instance, in the cosmetics sector there are several examples of NGRA published for systemic toxicity and skin sensitisation (Rogiers et al., 2020; Bury et al., 2021; Hewitt et al., 2022; Ouedraogo et al., 2022). It is also significant that the NGRA approach is taken up in the Notes of Guidance of the EC's Scientific Committee on Consumer Safety (SCCS/1628/21) (SCCS, 2021). The need for a greater understanding of where NAMs could be applied within chemical safety regulations and where the gaps in knowledge and useable NAMs are, was appreciated. In order to develop understanding, during 2021, the EC's JRC undertook a survey to identify NAMs or NAM-based strategies that have the potential to fulfil a number of regulatory needs under REACH and CLP. The ultimate aim of the JRC survey was to collect information that could be useful in developing options for increasing REACH information requirements as one of the actions of the CSS. The focus of the survey was on the users of NAMs and it identified several key aspects of their use. The survey found that a relatively large number of NAMs are being utilised by those who responded, with the greatest use in the industry sector. With regard to human health systemic effects, the use of NAMs was most frequently reported for acute systemic toxicity, certain organ level toxicities, and mutagenicity. There was, however, less coverage in the responses to the survey of NAM use for critical hazards (with reference to the CSS) and in particular the more challenging and complex endpoints, such as carcinogenicity, immunotoxicity, developmental neurotoxicity and respiratory sensitisation.

The workshop also recognised the need for more case studies on chemical safety, showing the utility of NAMs for use in regulatory decision-making. Published case studies are valuable to promote understanding, disseminate knowledge as well as identify the strengths and limitations of NAMs and their application(s). Table 1 summarises a number of case studies that were presented on a variety of topics relating to human health. The purpose of this report is not to describe the case studies in detail, which are explained in the information sources noted in Table 1, but to articulate the discussion and main findings relating to the application of NAMs. A number of key themes emerged from the case studies that are relevant to the vision of increased use of NAMs in regulatory decisions for chemical safety. Namely, the case studies demonstrated NAMs aim to assist in decision making for chemical safety assessment, increase the numbers of chemicals that can be assessed and the confidence that can be associated with the decisions. NAMs were broadly defined as encompassing many *in vitro* and *in silico* techniques and could have many roles with regard to regulatory decisions for safety assessment, including screening and prioritisation as well as for the

Table 1
Representative examples of the use of NAMs in risk assessment as presented to the Workshop.

Type of NAM	Approach	Intended application or purpose including relevant regulation (if stated)	Comments	Further information (if any)
Threshold of Toxicological Concern (TTC); Structure-Activity Relationships (SARs), gene expression data and Connectivity Mapping (CMap) for analogue determination; *in vitro* data related to mode of action for potency adjustments	Safety assessment of parabens based on read-across and internal exposure	Safety assessment for use in across cosmetic products	A joint case study from Cosmetics Europe and the EU-ToxRisk Project	OECD (2020a); Alexander-White et al. (2022); Ouedraogo et al. (2022)
SARs, gene expression data and CMap for analogue determination; *in vitro* data related to mode of action for potency adjustments	Safety assessment of caffeine based on read-across and internal exposure	Safety assessment for use across cosmetic products and diet	A case study presented by Cosmetics Europe	OECD (2020b); Bury et al. (2021); Alexander-White et al. (2022)
Gene expression and *in vitro* exposure relating to transcriptomic activity	Exploratory study using NAMs for *ab initio* safety assessment of phenoxyethanol and a major metabolite based on internal exposure	Safety assessment for use at high exposure in a cosmetic product	A case study presented by Cosmetics Europe	OECD (2021); Dent et al. (2021); Hewitt et al. (2022)
Variety of *in vitro* activities targeting information on relevant mechanisms of action	Tiered approach incorporating NAMs for hazard in addition to exposure data for vincozolin, coumarin and phenoxyethanol	Safety assessment of ingredients for specific use scenarios	Case studies intended to meet the current requirements of REACH Annex XI, although this has yet to be verified.	European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Transformational Programme; Ball et al. (2022)
Variety of *in vitro* data including ToxCast and high-throughput toxicokinetic data	Use of *in vitro*-to-*in vivo* extrapolation using *in vitro* data to inform PoD	Development of conservative PoDs for prioritisation and screening for a variety of substances	Accelerating the Pace of Chemical Risk Assessment (APCRA) retrospective case study led by the United States Environmental Protection Agency (US EPA)	https://www.epa.gov/chemical-research/accelerating-pace-chemical-risk-assessment-apcra; Paul Friedman et al. (2020)
In vitro data to identify exposure to, and hazard of, nanomaterials	Informing a mechanistic and weight-of-evidence approach to risk assessment	Provision of relevant toxicokinetic and toxicodynamic information regarding nanomaterials	European Food Safety Authority (EFSA) guidance on the risk assessment of nanomaterials in the food and feed chain: human and animal health. OECD study report and preliminary guidance on adaptations of the micronucleus assay TG 487 for nanomaterials safety testing.	More et al. (2021a, b); OECD (2022)
Mechanistically-based *in vitro* data representing Key Events in AOPs	Assessment of *in vitro* test methods and combination of *in vitro* data through an IATA	Development of IATA for the identification of non-genotoxic carcinogens	Development of IATA by the Organisation for Economic Cooperation and Development (OECD)	Mascolo et al. (2018); Jacobs et al. (2020); Desaulniers et al. (2021); Sovadinová et al. (2021); Jacobs et al. (2022a, b); Ohmori et al. (2022)
In vitro method for inhalation risk assessment	Hazard identification with *in vitro* techniques in combination with Computational Fluid Dynamic modelling of human airways	Use of *in vitro* models to assess damage to respiratory epithelial cells following exposure to respiratory irritants	Not yet accepted, further demonstration of acceptability, e.g., through an OECD IATA case study, required	Corley et al. (2021)

implementation of the CSS. As noted elsewhere in this report, updating of the REACH regulation, by means of Standard Information Requirements and Annex XI adaptations, could assist in the greater use of NAMs. NAMs were seen to be of value when mechanistically relevant, with linkages to Adverse Outcome Pathways (AOPs) and IATAs often being useful, but not essential. In addition, there were many examples of quantification of NAMs, with efforts to demonstrate how such data may replace use of animal test data in regulatory assessments, or how they could be used directly for regulatory decision-making. Within the quantitative examples discussed, there are opportunities to incorporate quantitative AOPs (qAOPs), the prediction of internal doses and Points of Departure (PoDs).

With regard to using data from NAMs to support regulatory decision-making on chemical safety, understanding levels of human exposure and the role of exposure information in different regulatory contexts is key to ensuring the correct NAM-based decisions are made. There are a number of approaches to incorporate exposure information, ranging from knowledge of use (habits and practices) and from biomonitoring activities through to high-throughput *in vitro/in silico* approaches to understand levels of systemic exposure.

2. Summary of the main findings of the workshop

The workshop participants identified the main aspects of NAMs that need to be developed further to increase their regulatory use and application in decisions on chemical safety for human health. The text below summarises the main points discussed, with all participants given the opportunity to engage and have their opinions noted. The discussion was centred around four topics, namely (1) the recognition of the overall goals and benefits of NAMs, (2) what is required to make further scientific progress, (3) the changes needed in regulatory frameworks and (4) the essential needs in education, training and exchange between stakeholders. The key points for future actions are identified at the end of each discussion item.

2.1. Overall goals and benefits of NAMs

The goals and benefits of using NAMs to undertake and implement the best science in regulatory decisions on chemical safety were considered. It was concluded and agreed that the prime purpose of moving towards an increased use of NAMs in chemical safety assessment was to ensure the protection of humans and build upon the EC's

commitment to reduce the use of animals as well as regulatory considerations, such as the use of animal testing only as a last resort as required by Article 25 of EU REACH. There was a very strong commitment to use the best science to ensure the health of EU citizens (consumers and workers) with the vision that NAMs will allow for this. The use of NAMs is already well established to enable different means of making decisions regarding safety, particularly through the use of new methods to identify and characterise hazards and incorporate information on exposure.

A number of benefits of using NAMs were identified, these go beyond those associated with the currently used approaches, which include animal testing. A key benefit is for human health, with the possibility of making decisions based on information and data more relevant to humans, through the use of NAMs with cells derived from human origin. Specifically, applying NAMs has the potential to make innovations in terms of rapid and informed safety decisions, as well as increasing possibilities for commercialisation.

A number of case studies in the varied use and application of NAMs are summarised in Section 1.4. The value of these case studies was recognised, and particularly the openness and willingness of contributors from all sectors (industry and regulators) to share information and knowledge. There is a need to increase this knowledge-sharing and to capitalise on the learnings from such case studies, to guide the future use of NAMs to illustrate their benefits and limitations to help build confidence. A better understanding of NAMs also provides the opportunity for their use across sectors, across regulations and for different regulatory agencies. Whilst much progress has been made, sharing the lessons learned will assist in the development of new, and refinement of existing, NAMs.

From the discussion, the following key points were highlighted. There was consensus that all work on NAMs needs to be guided by:

a) A strong commitment to protection of humans and the environment.
b) A passion to realise benefits of NAMs to increase human-relevance, accelerate innovation and for animal welfare.
c) A willingness to openly share achievements and lessons learned for the benefit of all stakeholders (including SMEs).

2.2. State-of-the-art and current achievements

The Workshop recognised the scientific progress and breadth of techniques and approaches that underpin the applications of NAMs to support regulatory decisions in chemical safety assessment for human health, whilst appreciating it is essential for methods to be robust and reproducible. Examples were provided of many types of NAMs ranging from experimental to computational approaches (summarised in Section 1.4). Many, although not all, NAMs for hazard identification/ characterisation are based on mechanisms of action and specifically the molecular initiating events and early key events (KEs) of AOPs. The case studies (see Table 1) illustrated some of the diversity of methodologies and potential applications for NAMs. Such case studies are useful to demonstrate how confidence can be built in NAMs and how they may be used to support regulatory decisions. It was recognised that regulatory decisions based on NAMs require not only that appropriate methodologies are put in place but also their acceptance (see Section 2.3).

A number of current achievements were identified with NAMs being potentially useable in a number of scenarios (as identified in the EC JRC NAMs survey – see Section 1.4). This was considered to be an excellent starting point, with the use of NAMs for complex endpoints to be developed further. There was an appreciation of the need for a broad biological coverage with NAM-based assessment approaches. The future development of NAMs could attempt to address gaps in their coverage e.g., for developmental and reproductive toxicity (DART), respiratory sensitisation and immunotoxicity as examples of critical hazards mentioned in the CSS. There was also agreement that there should be greater emphasis of moving NAMs from the development to the application phase, i.e., encouraging use of NAMs as early as possible, by putting more resources into ensuring that the methods are reproducible.

It was recognised that current legislation for chemical safety often still relies on animal test data to fulfil information requirements. There are some examples in current legislation/regulatory guidance where non-animal approaches are currently used and find acceptance, for instance *in vitro* data for genotoxicity, skin irritation and skin sensitisation as well as the use of approaches such as threshold of toxicological concern (TTC) and read-across. Techniques such as -omics are technically advanced and are being investigated for regulatory applicability in case studies. However, translational barriers remain; for example, are NAMs sufficiently standardised, and is there sufficient capacity within companies and CROs to apply them? Such new techniques are potentially useful for hazard identification and/characterisation, as well as providing data to help better estimate exposure. Whilst notable successes were presented, a number of gaps in current knowledge, understanding and resources were identified that, if addressed, would increase uptake and acceptance of NAMs.

2.3. Scientific needs

A number of areas where the scientific basis of NAMs needs further development for the so-called "critical hazards" were identified to enable more widespread use in regulatory decision-making on chemical safety. The workshop agreed that benchmarking against known criteria is essential to increase confidence in the reliability of NAMs. Current benchmarking procedures mostly compare performance against the animal test data which are to be replaced, and could also be used to ensure reproducibility of the NAM. Benchmarking of NAMs can go beyond this, with a move to benchmarking against human exposure, e.g., for workers' occupational exposure, as well as against relevant endpoints, disease outcomes or regulatory decisions such as those for risk assessment. Such an approach is being used for the development of IATAs for non-genotoxic carcinogens by the OECD (Paparella et al., 2017; Jacobs et al., 2020) and IATA for developmental neurotoxicity risk assessment (Hernández-Jerez et al., 2021). The need for appropriate data sets to assist in this process was recognised. The data sets should, ideally, be well curated, standardised and peer-reviewed, including an assessment of their variability, to provide a yardstick against which to measure performance. Data could be compiled from a variety of sources and the need to collate information from *in vitro* and mechanistic assays, as well as historic *in vivo* data that could be used to provide confidence to the weight-of-evidence assessment and mechanistic assays, was appreciated. In addition, there is a great need to compile relevant qualitative and quantitative human data following a variety of exposures scenarios. Such information for human exposure includes epidemiological data, clinical data and human biomarkers of disease. The use of human data provides the opportunity to demonstrate that safety decisions based on the use of NAM data can be protective of human health and predictive of adverse effects. In order to assist with the use of human data, their uncertainties should also be identified and characterised, as is usual for hazard and risk assessment.

The use of curated data sets for benchmarking will open further opportunities to understand the performance of NAMs. For instance, there is a need to characterise performance in terms of specificity and sensitivity as well as understanding the robustness of tests in terms of within- and between-lab reproducibility. There is currently much to be learned about the sensitivity of NAMs, in terms of their characterisation and understanding. In addition, for NAMs associated with events upstream in a pathway or AOP, greater knowledge is required regarding the biokinetic processes and relationship to downstream events. It was agreed that an improved scientific approach to characterising the sensitivity of NAMs will improve transparency, as well as assisting in their potential use for classification/labelling to avoid over- and under-classification of hazards.

In addition to characterising and optimising the performance of NAMs, the need to gain as much information as possible regarding the

performance of NAMs across a wide range of substances and exposure scenarios was recognised. Further information is required with reference to the so-called "difficult" substances, for instance those at solubility or bioavailability limits, different types of nanoparticles, in addition to those substances more commonly tested. Overall, gaining a broad body of evidence for NAMs demonstrating their reproducibility and applicability will help to develop trust in these approaches. There is a need to demonstrate the applicability of NAMs to other, potentially complex, classes of substances such as for nanomaterials (which are already in EU REACH) and polymers, as well as other new classes of chemicals. To assist in their use, the workshop discussed whether NAMs should be associated with an applicability domain (which may be established by the developer or derived from a validation process), with consideration of whether it is appropriate for complex substances and potential new chemical classes. The applicability domain could be used to define ranges of relevant properties, such as the logarithm of the octanol-water partition coefficient (log P) or aqueous solubility, in which the NAM can be used. It was further noted that applicability domains vary for different NAMs. In addition, during any validation process, applicability domains may be defined solely based on the reference chemicals, which could initially be restrictive, but should not be taken to imply the NAM's applicability domain will not be more extensive as knowledge and experience with its use is expanded.

In addition to tackling challenging endpoints such as the critical hazards in the CSS, the workshop agreed there is a need to provide realistic and meaningful Point of Departure (PoD) data which could be useful not only for risk assessment, but also applied to (regulatory) hazard identification schemes. This may include the repurposing of existing NAMs to provide updated hazard and PoD data. The protection afforded by such NAM-based PoD data should be investigated, with possible reference to variability of the currently used animal studies used to provide this hazard data. Related to the PoD is the opportunity provided by human-derived NAMs to investigate the potential hazard of a larger number of, and higher, concentrations, relevant to human exposure, than is possible with the current animal tests used to derive PoDs. It is possible that some NAMs currently used primarily for hazard classification could be repurposed for hazard and potentially also risk assessment if there is a concentration-response relationship (Jacobs et al., 2022a), and for risk assessment purposes, also exposure data. This offers the possibility to move away from the current paradigm of attempting to predict or simulate NOAEL/LOAEL values from traditional animal-based tests whilst ensuring equivalent, or greater, protection.

As many approaches to using NAMs in safety assessment involve the use of *in vitro* methods, the incorporation of kinetic information and quantitative *in vitro – in vivo* extrapolation (QIVIVE) is critical for the integration of NAMs into decision-making frameworks was emphasised. QIVIVE will assist in the provision of the linkage from responses measured in cell-based systems to whole organisms. Current use of kinetic information and QIVIVE approaches for safety decision-making are relatively limited and the workshop discussed that such models are integral to using NAMs and may need further development for regulatory use. Thus, there is a need to develop improved *in vitro* models to support some aspects of QIVIVE and the assessment of absorption, distribution, metabolism and excretion (ADME) properties of chemicals. Due to the known limitations of some of the current approaches, it was considered important to demonstrate the applicability of QIVIVE and ADME to allow for extrapolations from NAMs. Such approaches should demonstrate and increase reliability, as well as applicability, for instance using the high-throughput toxicokinetic (httk) models (Breen et al., 2021). This could be supported by a framework for implementation and standardisation of *in vitro* tests that can support QIVIVE.

Levels of human exposure to a chemical are known to be a strong driver in risk assessment, as noted in several of the case studies. With regard to exposure in particular, a wide range of views were shared in the workshop. It was recognised that human exposure is not always known or well described, with surrogate measures such as production tonnage (e.g., in REACH) being relatively crude. More work is required to document, understand and assess intentional and unintentional levels of human exposure to chemicals, which in turn could lead to improvements in the use of exposure-based adaptations within REACH Annex XI. Greater emphasis could be applied to understand exposure and the quality of the data, especially with regard to realistic human exposure in various settings from intended use to chemical accidents, with decisions based on NAMs being subsequently applied. In addition to this is the need to obtain information for the complete life-cycle of chemicals e.g., exposure from water, waste etc. Tiered-based frameworks use exposure to classify risk or allow for decisions to be made (even before the use of NAM data). The greater use of exposure information, and exposure classification, will continue to bring streams of hazard and exposure assessment data together, integrate human-based data along with toxicokinetics and other sources of information such as human biomonitoring and the exposome. The range of opinions in the workshop demonstrated that the use of exposure information in chemical safety assessment, especially with regard to the application of NAMs for regulatory decisions requires further dialogue between all partners.

Given the evidence provided, especially as part of the case studies and the discussion in the workshop, the following discussion points were agreed. There was consensus that all work on NAMs needs to be guided by:

a) Building trust through defining criteria for robust, reliable and reproducible use of NAMs and level of acceptable variability.
b) Sharing NAMs experience for a wide coverage of substances/exposure situations.
c) Increasing applicability and reliability of *in vitro* ADME and QIVIVE.
d) Defining curated data sets that could be used to evaluate the performance of NAMs including qualitative/quantitative human data.
e) Taking advantage of human-based NAMs across appropriate doses vs. predicting NOAELs/LOAELs from animal studies.
f) Developing a transparent scientific approach to characterise sensitivity/specificity and avoid potential over/under-classification with NAMs.
g) Better defining exposure information across the lifecycle of chemicals and progressing work on exposure classification.
h) Building on achievements of use of NAMs (link to survey) and addressing complex areas that currently have fewer NAM approaches (e.g., DART).
i) Ensuring new approaches provide Points of Departure for risk assessments and hazard classification schemes, including repurposing existing NAM data.
j) Consider applicability domain for NAMs-based approaches including future chemical classes (e.g., nanomaterials, polymers).

2.4. Regulatory needs and opportunities

The workshop recognised the overwhelming role that relevant legislation has in dictating the acceptance, or otherwise, of NAMs. There are many pieces of chemical legislation, which cross sectors and geographic areas, and fundamental to all legislation is the need and desire to protect humans and the environment. Many pieces of EU legislation allow for the use of NAMs, although they are often not referred to as such in the legislation, in a number of different ways but there is no universal coverage. It was agreed that there are clear opportunities to consider how NAMs could be applied within existing legislation and the needs and opportunities for the future.

Examples of where progress could be made in terms of adapting chemical legislation were articulated in terms of the EU CSS, in particular the promotion of innovative assessment methods and their regulatory uptake and specifically extending of the REACH information requirements to assist with the uptake of NAMs. It was noted that guidance documents are revised regularly and are already used to assist in this regard. Updating of guidance documents was seen as being more

rapid to respond to progress in NAMs rather than attempting to change legislation. It was also noted that some guidance is very prescriptive depending on the legislation e.g., when the use of specific NAMs is detailed, others are more flexible and could more readily allow for adaptation.

With regard to chemical safety assessment, an understanding of levels of human exposure to a substance is a crucial, but complex, issue. Thus, a greater understanding of exposure to chemicals, its meaning and implications, is required. It was noted that exposure depends on many issues, e.g., lifecycle stage, transport and use, as well as other associated scenarios, which could include unintended events such as spillages or accidents. In addition, exposure is likely to be different within different sectors and uses, thus the use of the information for risk assessment will be less standardised across legislations. However, integrating exposure into regulatory decisions is already taking place, for instance, through the use of various tonnage bands, exposure is implicitly integral to EU REACH. It was agreed that, in principle and where appropriate, future chemicals legislation could place a greater emphasis on exposure considerations. An example of this could be through the EU CSS. One possibility identified to improve the use of exposure information was the use of tiered schemes, which allow for hazard and then risk assessment. As such, the workshop proposed that it would be possible that existing regulation could be revised to include tiered schemes using exposure information and NAMs without relying on animal tests as a gold standard. The difficulty in gaining consensus on the definition and application of exposure-based assessments was recognised, and indeed it was noted that this could be the subject of a future EPAA Partners' Forum.

There was an overwhelming desire to use NAMs as part of regulatory submissions, when they are viewed as fit for purpose. Being fit for regulatory needs would imply a NAM should be scientifically validated and also comply with the legal context of the legislation. Whilst there is a need to increase opportunities to use NAMs for regulatory purposes, this may require adapting the text of legislation to allow for more flexibility. For instance, there could be provision for increased use of NAMs through the Annexes of the EU REACH legislation. Whilst EU REACH is one possible place where suitable NAMs could be applied, it was stressed that NAMs could be utilised under a number of different regulations. In order to maximise the value of information derived from NAMs, it is recognised that a holistic approach is required across all legislation, such that acceptability can be facilitated once the value and scientific validity of a NAM has been demonstrated.

One approach to developing the text of legislation further that was discussed was the possibility that the text of the legislation could cover information needs, whilst being test method agnostic. Approaches to appropriate methods/approaches could be defined in guidance associated with legislation, which could include specific references to suitable tiered and weight-of-evidence approaches to addressing information needs. For instance, to understand chronic toxicity, information needs could be stated as requiring information on repeat dose toxicity, which may open the possibility of including information from NAMs with specific tests such as the 90-day study in rodents to be used only as a last resort.

The dichotomy between greater flexibility and prescriptiveness of the regulatory use of NAM methods was noted. Greater flexibility in the legislative text will allow for increased use of NAMs. However, greater flexibility may imply an increased reliance on a weight of evidence, with the lack of consistency and increased uncertainty that may bring for both authorities and registrants. Conversely, prescriptive definition of NAM use will ensure their use. Overall, there is an opportunity to explore what is defined as "recognised" NAMs as well as the delicate balance required with legal certainty, flexibility and prescription within chemical legislation.

NAMs will inevitably be used across legislative frameworks in the EU and elsewhere. The need for NAMs, or strategies incorporating NAMs (such as IATA), to be applied, when scientifically valid, horizontally crossing legislation was acknowledged. It was appreciated that whilst the same NAMs could be used in the context of various legislations, the specific use of the information obtained from them within the legislative context may be different.

The workshop appreciated the need and possibilities of using NAMs across industrial sectors. Currently little is known on this topic and there is a need to explore the possibility of NAM data being applied across sectors. As an example, a potential use could be within the revision of the REACH information requirements following the EU CSS and the move towards "one substance – one assessment". It was recognised that whilst NAM-based hazard characterisation across sectors may be achievable, risk assessment would be required for individual uses, thus risk assessment could not be considered with the "one substance – one assessment" paradigm.

In order to understand when novel NAMs and strategies could be applied, an increase in formal channels for dialogue between the registrant and authorities, especially those regulators that will potentially be making decisions on the basis of NAMs data was recommended. It was recognised that there are various forums through which dialogue can be maintained. For instance, the EPAA is a place for informal dialogue. There are also more formal channels with, for instance, the European Chemicals Agency (ECHA), which include pre-submission consultations for biocides. These channels for dialogue are seen as being highly beneficial and useful to gain feedback on when a NAM may be acceptable.

Given the importance of incorporating and encouraging the use of NAMs through regulatory frameworks, the following key discussion points were made. Looking at changes needed in the regulatory framework, the following needs to be considered:

a) Existing regulation could be revised to further explore tiered schemes that include exposure and NAMs without seeing animal studies as the gold standard.
b) Increasing opportunities to use NAMs that are fit for regulatory needs (e.g., Annexes of REACH) such as sharpening the text to better facilitate the use of NAMs.
c) Striving to seek balance between flexibility/adaptation and prescribing defined test approaches in regulations, retaining the goal of protecting humans and the environment.
d) Ensuring that scientifically valid NAMs/strategies are horizontally applied across different legislative frameworks.
e) Exploring whether a cross-sector approach for use of NAMs is conceivable for one substance - one assessment.
f) Increasing formal channels for scientific dialogue between decision-making regulators and industry on bespoke use of NAMs for filling information requirements.

2.5. Education, training and exchange

NAMs bring a new approach to toxicology and chemical safety assessment. The potential advantages and benefits to the use of NAMs have been well documented and are appreciated by many, however the value of, and need for, continued education, training and exchange was acknowledged. It appreciated that the implementation of NAMs will bring about a paradigm change and that new and/or expanded expertise will be required across industry (including contract research organisations), governmental agencies, academia, SMEs, and non-governmental organisations (NGOs).

A key need identified was raising awareness of the new technologies and their implementation. Much expertise will be required over a significant number of disciplines including, but not limited to, toxicology, *in vitro* methods, chemical analysis, read-across, (quantitative) structure-activity relationship ((Q)SAR), physiologically-based kinetic (PBK) and exposure modelling, and statistical analysis. The building of capacity and training is required at all levels from incorporation into under- and post-graduate programmes to continuing professional development of scientists working in this area.

As part of training and raising awareness, the need for industry and regulators to gain a better understanding as well as sharing experiences in assessing applying NAMs was recognised, the case studies presented at the workshop being good examples, as well as efforts from (inter) national learned societies. Learnings from these case studies will assist in the development of future regulatory submissions and to grow confidence in the use of NAMs. Another obvious advantage of building capacity and training is to develop a common language and understanding between all stakeholders. This relates not only to those directly involved, but also NGOs and wider society to inform them of the progress made and emphasise issues such as increased human-relevance. The role that could be played by the EPAA in engaging all stakeholders was recognised.

The workshop and EPAA recognised the importance of EU CSS and how the use of NAMs has the potential to help its implementation, as well as demonstrating the use of good science and technology as part of the EU Green Deal.

In order to capitalise on the innovations and improvements that NAMs may bring to chemical safety assessment, the following key discussion points were identified. Essential needs in education, training and exchange:

a) Raise awareness and provide relevant expertise and training.
b) Industry and regulators to find ways to explore more NAM assessments in regulatory submissions to increase confidence in use of NAMs in regulatory discussions.
c) Build common understanding with other stakeholders: NGOs, wider society – role for EPAA.
d) Identify opportunities to leverage NAMs for the EU Chemicals Strategy for Sustainability.

3. Conclusions

The EPAA Workshop '*Use of NAMs in regulatory decisions for chemical safety*' recognised the important role NAMs will have in future approaches to chemical safety assessment. The workshop was opened by the challenge laid down by Mrs Sirpa Pietikäinen MEP who emphasised the need for safety and how NAMs could achieve that within the context of regulatory decisions. The unique nature of EPAA, bringing together a variety of partners from across the EC and European industry sectors, provided the opportunity to learn from a number of case studies that were presented along with discussion of the future use of NAMs for regulatory decisions. A range of opinions was aired within the workshop, however, general consensus was achieved on many topics, as noted in this report. The workshop concluded that the greater implementation of NAMs would have many benefits for chemical safety assessment across all industrial sectors and for all stakeholders. The CSS was a common theme in the workshop, specifically what could be done to better leverage NAMs. A number of issues were identified that could be addressed to increase the uptake of NAMs. Specifically, the workshop identified 23 key discussion points to increase the use of NAMs. The key discussion points relate to the overall benefits of NAMs. They identified a number of gaps in science which could be addressed, notably in the need to create trust by sharing experience in NAMs, the better implementation of QIVIVE and ADME, the importance (and range of opinions) regarding exposure in chemical safety assessment as well as supporting the future possibilities of the use of NAMs for complex endpoints and provision of PoDs. There was also consideration of the changes needed in regulatory framework(s), such as implementation of tiered schemes, increasing opportunities for the use of NAMs and searching for cross-sector and cross-legislation use. The essential needs in education, training and exchange were also identified, with the needs to build capacity, gain more confidence in the use of NAMs in regulatory discussions as well as with all stakeholders. The key discussion points from the workshop provide the main topics that EPAA can work on in the future to allow for the better use of NAMs for regulatory decisions.

Conflicts of interest

The authors of this article participated in the workshop that was organised by the EPAA.

Disclaimer

The views and opinions expressed in this manuscript do not represent those of ECHA, EFSA and the UK Health Security Agency.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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