



Report on the European Partnership for Alternative Approaches to Animal Testing (EPAA) “New Approach Methodologies (NAMs) User Forum Kick-Off Workshop”

Mark T.D. Cronin^{a,*}, Maria T. Baltazar^b, Tara S. Barton-Maclaren^c, Ofelia Bercaru^d, K. Nadira De Abrew^e, Christian Desaintes^f, Sylvia E. Escher^g, Petra Kern^h, Gavin Maxwell^b, Vera Rogiersⁱ, Katrin Schutte^j, Tomasz Sobanski^d

^a School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, United Kingdom

^b Safety & Environmental Assurance Centre (SEAC), Unilever, Colworth Science Park, Sharnbrook, Bedford, MK44 1LQ, United Kingdom

^c Existing Substances Risk Assessment Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, K2K 0K9, Canada

^d European Chemicals Agency, Telakkakatu 6, Helsinki, Finland

^e Fabric and Home Care Innovation Center, The Procter & Gamble Company, Cincinnati, OH, 45217, USA

^f European Commission, DG Research & Innovation (DG RTD), Brussels, Belgium

^g Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs Str. 1, 30625, Hannover, Germany

^h Procter & Gamble Services NV/SA, Temselaan/ Boechoutlaan, 1853, Strombeek-Bever, Belgium

ⁱ Faculty of Medicine and Pharmacy, In vitro Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090, Brussels, Belgium

^j European Commission, B-1049, Brussels, Belgium

ARTICLE INFO

Handling Editor: Dr. Martin Van den berg

Keywords:

New approach methodology (NAM)
Next generation risk assessment (NGRA)
Chemical safety assessment
Hazard identification
Exposure assessment

ABSTRACT

The European Partnership for Alternative Approaches to Animal Testing (EPAA) held the “New Approach Methodologies (NAMs) User Forum Kick-Off Workshop”, at the European Chemicals Agency (ECHA), Helsinki, Finland on 7–8 December 2023. The aim of the User Forum was to gain insight into the regulatory use of NAMs, with a particular reference to Next Generation Risk Assessment (NGRA), for chemical safety assessment. To achieve this, presentations summarised the learnings and experiences of previous EPAA Skin Sensitisation User Forums as well as that of the European Commission’s Scientific Committee on Consumer Safety (SCCS). The findings of five case studies were summarised that illustrated the use of NAMs. The presentations and subsequent discussions allowed for learnings and insights to be compiled from all stakeholders with regard to the use of NAMs. Recommendations for the regulatory use of NAMs in NGRA were made, namely for exposure assessment; hazard identification; using tiered and targeted testing strategies; performing risk assessment using NAM data; the practical implementation of NAMs; the use of -omics technologies; and the needs for capacity building and training. The EPAA User Forum provided an open platform for safety assessors to share learnings and experiences. Recommendations for the format and topics of future EPAA User Forums were also made.

1. Introduction and workshop aims

This report summarises the presentations from, and the main findings of, the European Partnership for Alternative Approaches to Animal Testing’s (EPAA’s) “New Approach Methodologies (NAMs) User Forum Kick-Off Workshop”. The workshop was a hybrid event held at the

European Chemicals Agency (ECHA) in Helsinki, Finland and on-line over two days (8–9 December 2023). It was attended by approximately 50 participants representing regulatory agencies, industry, non-governmental organisations (NGOs) and academia, as well as European Union (EU) competent authorities.

The aim of the User Forum was to gain insight into, and share experiences with, the use of New Approach Methodologies (NAMs) in

* Corresponding author.

E-mail addresses: m.t.cronin@ljmu.ac.uk (M.T.D. Cronin), Maria.Baltazar@unilever.com (M.T. Baltazar), tara.bartonmaclaren@hc-sc.gc.ca (T.S. Barton-Maclaren), Ofelia.BERCARU@echa.europa.eu (O. Bercaru), deabrew.kn@pg.com (K.N. De Abrew), Christian.DESAINTE@ec.europa.eu (C. Desaintes), sylvia.escher@item.fraunhofer.de (S.E. Escher), kern.ps@pg.com (P. Kern), Gavin.Maxwell@unilever.com (G. Maxwell), Vera.Rogiers@vub.be (V. Rogiers), katrin.schutte@ec.europa.eu (K. Schutte), Tomasz.SOBANSKI@echa.europa.eu (T. Sobanski).

<https://doi.org/10.1016/j.yrtph.2025.105796>

Received 3 January 2025; Received in revised form 17 February 2025; Accepted 27 February 2025

Available online 28 February 2025

0273-2300/© 2025 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Abbreviations	
ADME	Absorption, Distribution, Metabolism and Excretion
AED	Administered Equivalent Dose
AOP	Adverse Outcome Pathway
BER	Bioactivity-Exposure Ratio
BHT	Butylated Hydroxytoluene
BP-4	Benzophenone-4
BPA	Bisphenol A
C&L	Classification and Labelling
CMap	Connectivity Mapping
Cmax	Maximum Concentration
CMP	Canadian Chemicals Management Plan
DA	Defined Approach
DART	Developmental and Reproductive Toxicity
DASS	Defined Approach for Skin Sensitisation
EC	European Commission
ECHA	European Chemicals Agency
ED	Endocrine Disruption
EPAA	European Partnership for Alternative Approaches to Animal Testing
EU	European Union
HDAC	Histone Deacetylase
HTTK	High-Throughput Toxicokinetics
IATA	Integrated Approaches to Testing and Assessment
IVIVE	<i>In Vitro-In Vivo</i> Extrapolation
KE	Key Event
LOAEL	Lowest Observed Adverse Effect Level
MoE	Margin of Exposure
NAM	New Approach Methodology
NGRA	Next Generation Risk Assessment
NGO	Non-Governmental Organisation
NOAEL	No Observed Adverse Effect Level
NoG	Notes of Guidance
OECD	Organisation for Economic Cooperation and Development
PBK	Physiologically-Based Kinetic
PoD	Point of Departure
(Q)SAR	(Quantitative) Structure-Activity Relationship
RAAF	Read-Across Assessment Framework
SAR	Structure-Activity Relationship
SCCS	Scientific Committee on Consumer Safety
TG	Test Guideline
TTC	Threshold of Toxicological Concern

chemical safety assessment, with a particular reference to Next Generation Risk Assessment (NGRA). This was achieved through presentations from stakeholders describing their experiences and through case studies illustrating the regulatory use of NAMs. The purpose was not only to share learnings and experiences, but also to find recommendations to increase the use of NAMs, and discuss future possibilities for EPAA NAM User Forums.

No strict definitions of NAMs and NGRA were stipulated in the User Forum. NAMs were considered in a broad sense to include *in silico*, *in chemico* and *in vitro* approaches, -omics approaches or omic-enhanced *in vivo* studies combined as Defined Approaches (DAs) and/or Integrated Approaches to Testing and Assessment (IATA). NGRA was described in a number of contexts throughout the User Forum; it can be summarised as an exposure-led, hypothesis-driven, tiered strategy integrating NAM data from *in silico*, *in chemico* and *in vitro* approaches that allows for non-animal, human-relevant, risk assessment of chemical substances. Various examples of the use of NAMs and NGRA were presented in the User Forum and summarised in this report.

The purpose of this workshop report is not to provide detailed minutes of the workshop, rather to summarise the presentations in Section 2. A summary of learnings and experiences from all presentations and discussion, providing recommendations for further action, is provided in Section 3.

2. Experience from stakeholders and reporting of case studies

The NAM User Forum was informed by presentations from two stakeholders, representing the outputs from previous organised EPAA User Forums and from the Scientific Committee on Consumer Safety (SCCS), a scientific advisory committee to the European Commission (EC). Further, five case studies utilising NAMs were presented. The experiences described in these presentations are summarised in Table 1.

3. Summary of the learnings and insights from the NAMs user forum

Section 3 details the main findings of the User Forum with regard to the use of NAMs, with particular examples drawn from the contributions in Table 1 and subsequent discussion. The examples are included to illustrate the findings as well as give evidence of the practical use of

NAMs.

3.1. Overarching themes and comments relating to the use of NAMs

i) Broad Support for the Regulatory Use of NAMs

All contributions to the User Forum supported the use of NAMs as an integral component of the future safety assessment of chemicals. The particular advantages of NAMs have been highlighted elsewhere by the EPAA, through User and Partner Forums, Workshops, the Annual Meeting, etc., and recorded, for example by Westmoreland et al. (2022). One particular advantage was highlighted in the NAMs User Forum, namely that NAMs allow for the more efficient testing of greater numbers of compounds.

ii) Need for Standardised Definitions

Whilst knowledge of the term 'new approaches' is becoming widespread, a fundamental issue was identified in that agreed definitions are required for terms such as NAM, NGRA, etc. For instance, with regard to the term NAM, the cosmetics sector is considering this to be completely non-animal approaches, whilst other stakeholders may include NAM-augmented animal tests in the definition. NGRA is a broad concept, with a variety of interpretations. There is also a need to standardise the reporting of NAMs approaches and the data from them to ensure uniform methodology and interpretation.

3.2. Exposure assessment

Knowledge of exposure is fundamental in chemical risk assessment, with the crucial role that NAMs play in exposure assessment approaches having been the focus of a recent EPAA Partner Forum (Cronin et al., 2023).

i) Estimating Exposure

Exposure assessment of chemicals was seen by the participants as being crucial to NGRA. For impurities or compounds at very low concentration, the threshold of toxicological concern (TTC) may be applied. Within NGRA, a variety of methods to determine exposure assessment

Table 1
Summary of presentations at the EPAA NAMs User Forum.

Topic and Presenter	Contributions relevant to NAMs
<p>EPAA Workshops and other activities relating to development of alternatives to skin sensitisation. Presented by Drs Petra Kern (Procter and Gamble) and Katrin Schutte (European Commission, DG Environment).</p>	<ul style="list-style-type: none"> Progress in developing NAMs for skin sensitisation benefits from clear mechanistic understanding rationalised into a well-established adverse outcome pathway (AOP). The AOP has been used to organise a variety of Organisation for Economic Cooperation and Development (OECD) validated <i>in vitro</i> assays into an OECD endorsed “Defined Approach for Skin Sensitisation” (DASS) as Test Guideline (TG) No. 497 (OECD, 2023a). NAM data for skin sensitisation, as applied in the Defined Approach (DA), are used for hazard identification. NAM data also support NGRA for skin sensitisation (Gilmour et al., 2020, 2023) which are recognised by the EC’s SCCS (SCCS, 2023). The EPAA Skin Sensitisation User Forums provided the opportunity to identify and discuss a number of issues with the implementation of NAMs for different classes of substances (Basketter et al., 2012, 2013, 2015, 2019, 2020).
<p>Use of NAMs in submissions to the EC SCCS. Personal insights and opinions of Prof. Em. Vera Rogiers (Vrije Universiteit, Brussels, Belgium).</p>	<ul style="list-style-type: none"> <i>In silico</i> and validated <i>in vitro</i> NAMs are available for local toxicity endpoints relating to skin corrosion and irritation, eye irritation, skin sensitisation and phototoxicity. In addition, NAMs are available for dermal absorption, mutagenicity and genotoxicity. Many of these NAMs are OECD validated <i>in vitro</i> methods. <i>In silico</i> methods are not sufficient on their own and should be used as part of a weight-of-evidence. There are fewer NAMs available for systemic effects such as pharmacokinetic properties (other than absorption), repeated dose toxicity, reproductive toxicity and non-genotoxic carcinogenicity. The SCCS will accept data from non-TG methods where they can be demonstrated to be scientifically justified and robust. The SCCS Notes of Guidance (NoG) provide guidance on the use of NAMs as well as NGRA for endpoints such as skin sensitisation (SCCS, 2023). The SCCS states the importance of the evaluation of the NAMs assays in terms of how the method is developed, the underlying training sets and the rationale for the interpretation of data. The Threshold of Toxicological Concern (TTC) is a pragmatic solution to justify the safety of impurities and cosmetic ingredients added to the final product at very low concentrations.
<p>NGRA using NAMs to evaluate systemic safety for consumers using benzophenone-4 (BP-4) as a UV-filter in a sunscreen product. Presented by Dr Maria Baltazar (Unilever).</p>	<ul style="list-style-type: none"> The case study aimed to assess the systemic toxicity of BP-4 without using any <i>in vivo</i> animal data, adhering to NGRA principles for a chemical with regulatory interest due to potential endocrine activity. A NAM systemic toxicity toolbox consisting of <i>in silico</i> tools (read-across and (Q)SARs) and <i>in vitro</i> assays (cell stress panel, pharmacological profiling, transcriptomics) was utilised to generate and explore hypotheses and provide an estimate of bioactivity. An initial exposure assessment was performed based on the external dose, absorption, distribution, metabolism and excretion (ADME) parameters and the kinetic profile of BP-4. Sensitivity analysis was performed to identify the parameters that have the largest influence on the Physiologically-Based Kinetic (PBK) model. Statistical distributions were generated for plasma maximum concentration (C_{max}) representing various European populations with associated uncertainty and variability analyses (Moxon et al. (2020)). NAM data allowed for the calculation of a Point of Departure (PoD) which informed the risk characterisation through the Bioactivity-Exposure Ratio (BER), the ratio between PoD and in plasma C_{max}. The NAM-based NGRA workflow was found to be protective of human health (Middleton et al., 2022).
<p>Integrating NAMs to prioritise and assess data poor alternatives to bisphenol A. Presented by Dr Tara Barton-Maclaren (Health Canada).</p>	<ul style="list-style-type: none"> A case study as part of the Canadian Chemicals Management Plan (CMP) demonstrated the integration of <i>in silico</i> and <i>in vitro</i> methods to provide a weight of evidence assessment of oestrogenic activity of chemicals that are structurally similar to bisphenol A (BPA) and evaluate the ability to distinguish from those that are functional alternatives (Environment and Climate Change Canada (2020); OECD (2022)). NAM data were analysed within a tiered workflow to support hazard identification and the evaluation of different approaches to determine <i>in vitro</i> PoDs based on data type (i.e., high throughput screening and transcriptomics data). Transcriptomic data were used to assist in the derivation of PoDs, including the application of an ER biomarker and general bioactivity approaches (Corton et al., 2022; Matteo et al., 2023). Consensus predictions from <i>in silico</i> models were made on oestrogen receptor binding (Collins and Barton-Maclaren, 2022; Collins et al., 2024). Generally, there was agreement across approaches used to estimate the minimal bioactivity concentration which were converted to administered equivalent dose (AED) values through high-throughput toxicokinetics (HTTK) modelling and <i>in vitro-in vivo</i> extrapolation (IVIVE). Notably, the analysis identified some exceptions where different NAMs resulted in a broad range of values highlighting areas for further consideration. The BER was calculated from the AED and upper limit of median population exposure for purposes of illustration revealing the NAM data to be protective, robust and reproducible.
<p>A Connectivity Mapping (CMap) based assessment of butylated hydroxytoluene (BHT) for endocrine disruption (ED) potential. Presented by Dr Nadira De Abrew (Procter and Gamble).</p>	<ul style="list-style-type: none"> The endocrine disruption potential of BHT was investigated through the Connectivity Mapping (CMap) of gene expression data allowing for functional read-across analysis with structural analogues (De Abrew et al. (2022)).

(continued on next page)

Table 1 (continued)

Topic and Presenter	Contributions relevant to NAMs
A read-across case study on branched carboxylic acids for repeated dose toxicity. Presented by Dr Sylvia Escher (Fraunhofer ITEM).	<ul style="list-style-type: none"> • CMap utilises “biological signatures” which are unique to a biological system and its perturbation by a particular dose of a chemical, the CMap Signature identifies the genes with greatest over- and under-expression, applying a CMap Score (De Abrew et al., 2019). • Five doses were tested in four cell lines relevant to endocrine disruption. • BHT did not connect to known endocrine disruptors in a public database (clue.io) • A structure-activity relationship (SAR) analysis of BHT was performed using a further 15 potential structural analogues. • CMap supported association of close read-across analogues but not to less suitable analogues (Wu et al., 2010). • Read-across from valproic acid to a group of branched carboxylic acid analogues was described for chronic toxicity based on liver steatosis (Escher et al., 2022a, 2022b; Vrijenhoek et al., 2022). • A read-across workflow was applied which integrated NAM testing and evaluation to support the read-across hypothesis. • <i>In vitro</i> testing was informed by assays for the molecular initiating events and early key event (KE) from a novel AOP network for liver steatosis, providing a targeted battery for testing. • TXG-Mapper data analyses were performed using weighted correlation gene networks on gene expression data. • IVIVE and PBK analysis was performed across all analogues to make estimates of human plasma concentration. • The NAM data based on the AOP network were able to illustrate a shared mode of action between toxic compounds and supported read-across approaches and the similarity concept (Escher et al., 2019). • Valproic acid and eight structurally similar analogues were compared using NAM data to identify SAR trends relating to chain length (Wu et al., 2023). • A toxicogenomic analysis was performed using four cell types with the development of gene signatures from the CMap approach showing that valproic acid and two analogues had a similar gene expression pattern consistent with histone deacetylase (HDAC) inhibition activity. HDAC inhibition is a known Developmental and Reproductive Toxicity (DART) mode of action. • Other chemicals showed a different gene expression pattern without HDAC inhibition, some of which do not have DART effects. • CMap analysis supported better definition of SAR patterns. • SARs for binding to the HDAC receptor were investigated further using molecular docking and modelling simulations. • PBK modelling allowed for comparison of <i>in silico</i> estimates of ADME parameters with experimental data and to use models to simulate No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) values.
Use of NAMs to refine and strengthen Structure-Activity Relationship (SAR) read-across for the Developmental and Reproductive Toxicity (DART) effects of branched-alkyl carboxylic acids. Presented by Dr Petra Kern (Procter and Gamble).	

can be applied and there is a need to optimise how this is performed. Typically, exposure assessment will start with an understanding of the external exposure which will be then converted to an internal exposure using approaches such as physiologically-based kinetic (PBK) modelling. Overall, exposure assessment should be suitably conservative. Whilst general methods are known (e.g., PBK modelling), further effort on estimating exposure is required, with more work on internal exposure being especially important.

ii) Further Development of Physiologically-Based Kinetic (PBK) Models

The User Forum heard various applications of PBK modelling, for the estimation of the internal exposure assessment of chemicals. As a component of NGRA, PBK modelling is instrumental and drives hypothesis generation to investigate specific endpoints. PBK modelling allows for a focused assessment of hazard in particular organs.

There is a need to develop practical and pragmatic generic PBK models that can be applied widely within an NGRA framework. To apply PBK models successfully, greater understanding is required of their function and particularly the confidence that can be associated with an estimate through the analysis of the uncertainties. This may be achieved by generating experimental chemical-specific ADME data. Uncertainty and PBK modelling were highlighted with the use of sensitivity analyses to identify the parameters that have the largest influence on the model outputs. There were uncertainties related to population variability, parameter uncertainty, and model reliability that needed to be addressed to estimate a robust range of biologically plausible exposures (i.e., plasma Cmax) as suggested by OECD (2021).

3.3. Hazard identification and characterisation

i) Ensuring NAMs have Broad Coverage, as Well as Focusing on Specific Endpoints

Many case studies in the User Forum presented data and knowledge of NAMs associated with specific, known mechanisms of action. This is vital for focused risk assessment when the mechanism of action is known. For instance, case studies illustrated that NAM data can support a mode of action-based hypothesis. Despite progress made with well-studied modes of action, the coverage of modes of action, and implicitly also AOPs, is not yet complete (or may never be fully complete) and more work is required to understand the coverage that may be necessary from an *in vitro* battery. As such, there is a need to continue to develop AOPs that cover a broad range of human health effects.

In addition to focusing on known, specific modes of action, future use of NAM data from *in vitro* assays should also ensure a broad coverage of mechanisms and, where possible, AOPs including non-specific effects. The value of transcriptomic data was demonstrated with examples showing that bioactivity concentrations could be derived from such analyses, both for chemicals with specific modes of action, as evaluated using a biomarker, and also for those where the mode of action is not known thereby representing non-specific toxicity, or protective bioactivity concentrations. These bioactivities were converted to Administered Equivalent Doses (AEDs) to inform the BER.

ii) Defining an Appropriate Battery of NAMs and *In Vitro* Assays

With the exception of skin sensitisation, i.e., the OECD Test Guideline 497 for the Defined Approaches for Skin Sensitisation (DASS) (OECD, 2023a), there are only a few standardised batteries of *in vitro* assays so far. The variety of case studies indicated that the selection of NAM test batteries is context (both effect and chemical) dependent. However, development of fit-for-purpose test batteries that cover specific and non-specific effects is still required.

When there is no knowledge of mode of action, i.e., in an *ab initio* approach to risk assessment, it may be possible to use batteries of *in vitro* tests designed to measure perturbation to biological pathways, interaction with proteins and enzymes and general key cellular processes (e.g. mitochondrial function) in a range of different cell models. Such *in vitro* approaches may be supported by *in silico* predictions that may indicate on which mechanisms or assays to focus. Early tier batteries are intended to either derive PODs based on bioactivity or provide data for mode of action hypothesis generation. The bioactivity observed might not necessarily be linked to an adverse outcome, and therefore it might be possible to refine further to distinguish this bioactivity from adversity. Some of the approaches, such as gene expression signatures, are useful to support functional read-across.

3.4. Application of NAMs: Tiered and targeted testing strategies

The User Forum agreed that the practical application of NAM data within a chemical risk assessment context required strategies to implement them; these are typically based within tiered or targeted testing strategies that may either incorporate information sequentially, e.g., NGRA or IATA, or as part of a DA.

i) Further Development of Tiered and Targeted Testing Strategies

A number of tiered and targeted testing strategies were presented by the stakeholders and within case studies, including the use of NAM data within NGRA, IATA and DAs. The strategies were presented for a number of different endpoints and for different regulatory uses e.g., classification and labelling (C&L), hazard and risk assessment, etc. The advantages and disadvantages of their use should be evaluated, e.g., from the users' experiences of tiered strategies.

There is considerable knowledge in the application of testing strategies for skin sensitisation. The DASS is seen as being important in this regard. It was recognised that there is a requirement and opportunity to evaluate (and validate) NAM and DAs against known standards. For instance, the DASS has been evaluated both the against local lymph node assay and human reference data for skin sensitisation. Partnerships and international collaboration were seen as critical for making progress. The workflow presented by Health Canada is suitable for the assessment of multiple chemicals. A well-established AOP is of great benefit to develop and justify the use of *in vitro* NAMs, especially within tiered strategies.

Other types of workflows are also being developed. For instance, the ASPA workflow within the ASPIS cluster (<https://aspis-cluster.eu/>) is being developed to provide a means to integrate exposure and hazard information to make risk assessment decisions.

There is a clear need to understand the information or evidence required to improve the possibilities for acceptance of a negative decision from NAM data. Technologies such as toxicogenomics require further effort to determine how negatives, or the lack of a specific mode of action, can be confirmed.

3.5. Risk assessment using NAM data

NAM data can form the building blocks to estimate exposure and PODs (hazard) in NGRA. The key discussions and conclusions in the User Forum related to examples of the application of these data to allow for safety decisions to be made.

i) Understanding and Improving the Bioactivity-Exposure Ratio (BER) Concept

The BER is a fundamental concept to apply NAM data for exposure and the PoD to enable the derivation of a risk-based assessment metric. This is analogous to currently applied concepts in risk assessment such as the Margin of Exposure (MoE). Whilst BER is central to the application of NAM data in NGRA, there are a number of areas where further consideration and information is required.

There are many approaches to assess bioactivity and hence calculate BER e.g. from individual cellular biomarkers, gene pathways POD, receptor binding, etc. As such, at the current time, there is no standardised means of identifying the bioactivity endpoint, or result, to be used in deriving the BER. Currently, a pragmatic approach is used to ensure a conservative PoD to inform BER. Where a specific mode or mechanism of action is identifiable, this should be the driver for deriving BER. Whilst there are many unanswered questions on how to determine and define the acceptability of bioactivity in NGRA, some basic principles for its use were identified. For risk assessment, protection (i.e., conservatism in the PoD) is preferable. Multiple sources of information including transcriptomics and *in vitro* NAMs could be applied to determine a PoD. In some cases, large variations in bioactivity were observed between methods, while for others there was good agreement (within an order of magnitude) when comparing diverse approaches. However, the minimal values were found to be protective as compared to *in vivo* animal data.

It was considered that it is more important to set a pragmatic threshold for the PoD than obtaining a precise target or mechanism of action. However, it was acknowledged that information on mechanism of action or target will increase the level of precision in the PoD. With regard to PoD determination, it was noted that much more work needs to be performed in endpoints, such as DART, and in the use case scenarios, an example being stated for industrial chemicals (to be confident in protecting workers as well as consumers where exposure, and routes of exposure, may be different).

ii) Improving Confidence in the BER in NGRA

The BER concept is one of several approaches that is fundamental to the use of NAM data to make safety decisions, for instance in NGRA. In addition to applying appropriately conservative bioactivity data, there is a need to better understand how to determine when the BER is acceptable for a particular purpose. One proposal was that a BER > 1 would indicate a low risk of adverse effects to consumers, providing the *in vitro* measures of bioactivity provide appropriate biological coverage, there is confidence that the test systems are at least as sensitive to perturbation as human cells *in vivo*, and that the exposure estimate is conservative for the exposed population (as demonstrated in a number of exposure scenarios) (Middleton et al., 2022). It is acknowledged, however, that the use of BER continues to be a topic of discussion and that the threshold may change between industrial sectors and will be dependent on the context of use. Whilst improvements are required, the NAMs' test systems described in the User Forum were at least as sensitive to perturbation as human cells *in vivo*, thus providing a conservative PoD and thus conservatism in the BER.

In addition to bioactivity assessment, the relevance of NAM data to making decisions for human exposure requires further knowledge and experience to improve confidence in the approaches. The use of NAM data also provides an opportunity to address various aspects of uncertainty – particularly related to population variability that may not be characterised sufficiently in existing models. Skin sensitisation is an area where there is considerable experience and data relating to human exposure which could be capitalised upon. It is clear that the estimate of exposure for use in the BER should be protective for all of the population.

3.6. Practical implementation of NAMs

All stakeholders and case study presenters provided comments on the practical use of data from NAMs. These comments are summarised in this Section.

i) Assessing NAMs and Their Applicability Domain

A number of criteria can be applied to assess the quality and relevance of NAMs. It is established that criteria to assess the quality of *in silico* models such as (Quantitative) Structure-Activity Relationships ((Q)SARs), read-across and PBPK should be applied to evaluate the model and, separately, the robustness of the prediction. *In silico* models can be assessed against, amongst other frameworks, the OECD validation principles for (Q)SARs, ECHA's Read-Across Assessment Framework (RAAF) and the SCCS NoG. Separate to this is the assessment of a prediction for a model, for instance recently the OECD has published the "QSAR Assessment Framework" (OECD, 2023c).

There is an acknowledged need to define the limitations and characteristics of NAM approaches. The proper and full definition of the applicability domain of a NAM should be provided. Case Study presentations were able to state the applicability domain of the tested chemicals, but an overall applicability domain for most NAMs is still lacking. It is implicit that applicability domains are unique for individual NAMs. It was concluded that cross-sector knowledge will assist in understanding when NAMs could be adapted to other chemistries to broaden their domain. Skin sensitisation is a prime example of where knowledge from other industries, e.g., the cosmetics, biocide, pharmaceutical and fragrance industries, can be shared to gain a better understanding of the applicability domain.

ii) Improving Confidence and Uncertainty Assessment

There is still a need to increase confidence in NAMs and the data derived from them. The characterisation and, where possible, quantification of uncertainties is a key process in the definition of confidence in a NAM. Uncertainties can be defined for particular elements of the risk assessment workflow; for instance, uncertainties can be defined for the toxicodynamics and toxicokinetics elements. The modelling of human relevant exposure assisted in the case studies to refine risk assessment. NAM data are also able to reduce uncertainty with regard to toxicokinetics and toxicodynamics in read-across similarity.

With regard to *in silico* and other models, the confidence in a prediction from QSAR and read-across can be improved by using more than one model. In addition, NAM data have value to support SAR. This also includes modelling of receptor-binding with docking studies. Thus, a variety of *in silico* data can inform and support read-across. When a molecular initiating event is binding to a receptor, docking studies may help to interpret data. Further, the use of PBK assessments helps to refine the SAR and thus support the read-across. The value of the exploratory case studies in uncertainty characterisation should be emphasised and act as a stimulus for future work.

In addition to the current procedures for assessing the validity of a NAM, there was also a recognition of the value of shared, high quality, data sets from traditional methods for benchmarking NAM performance against. For instance, development of NAMs for skin sensitisation has benefitted from their evaluation against existing *in vivo* and, in some circumstances, human data.

iii) Metabolites

The current limitations of some NAMs to assess metabolites from the parent chemical was seen by the workshop participants as an obstacle in their application. Although the metabolic capability of NAMs was not discussed in detail, it was noted within individual case studies the effect of metabolism may not have been addressed adequately by an *in vitro*

NAM assay. A possible solution identified is the use of metabolically competent assays, although few are currently available. Other possibilities included the computational modelling of metabolism and possible metabolites, with significant metabolites being assessed individually.

iv) Other Areas of Development

The User Forum focused on the stakeholders' experiences and existing applications of NAMs with further needs and areas for development identified in addition to those stated here. It is currently accepted that validated NAMs are generally better developed and applied for local effects, as opposed to systemic effects. As such, more effort is required to address systemic, as opposed to local, adverse effects using NAM data. There is also a need for greater understanding of the technical challenges in using NAMs for chemicals that are seen as being "difficult" to test, e.g., low water solubility, volatile chemicals, etc.

There is also a need to better understand and measure the free intracellular concentration for NAM data. This is especially true for compounds that may sorb to vials and plastic culture dishes/wells, or are volatile. *In vitro* biokinetic models assist in the comprehension of large differences in NAM data for potentially similar compounds. Such models (e.g., Armitage et al., 2021; Fisher et al., 2019) correct for the loss of a compound due to the *in vitro* study design as well as the ability of the compound to cross cellular membranes. As such, the use of *in vitro* biokinetic models is recommended to correctly interpret and use *in vitro* NAM data.

3.7. Use of -omics technologies

The User Forum was provided with various illustrations of the use of data derived from various -omics technologies for use as NAMs to support chemical risk assessment. Case studies utilised a variety of methods to analyse -omics data with the CMap methodology being a key approach. When CMap is utilised within the AOP framework, it represents information of the molecular initiating events and early cellular responses. A number of experimental issues should be considered within the CMap approach. Some chemicals were found to be highly promiscuous and activated multiple cell lines, giving responses reducing the clarity of the data. In addition, responses were found to be dependent on the dose tested and the time of exposure. Other chemicals did not produce a response and this was assumed to be an experimental artefact, possibly related to sorption to the plastic of the apparatus. Finally, there should be an assessment of the biological coverage of the cell lines to ensure it is appropriate for the mode(s) of action being assessed. There are other practical issues to overcome, e.g., there is currently no certainty in what makes a significant response. The User Forum focused on two methods to analyse -omics data (CMap and TGX-Mapper); it is acknowledged that other valid methods are available and a greater understanding of their strengths and weaknesses, as well as the relevance of particular analyses, is required.

3.8. Opportunities for regulatory use of NAMs

There was discussion in the User Forum regarding the use of NAM data to make regulatory decisions. For instance, several case studies illustrated how to provide data for hazard characterisation that contributes to the weight of evidence assessment in regulatory decisions. In order to gain a better understanding of the issues, case study presenters were invited to report on how the NAMs could address regulatory needs at the current time and in the future.

With regard to the current potential use of NAMs for regulatory purposes, several examples of NAMs within, or outside of, an NGR framework were presented. It was demonstrated that NAMs can be included in the risk assessment of cosmetics ingredients under Regulation (EC) N° 1223/2009. The current possibility to further inform hazard

characterisation, and support read-across and weight of evidence assessment, through the determination of differences in relative potency and mode of action assessment was also provided. The BER approach could also be applied in prioritisation to identify substances of greater potential concern and, as such, require further information or data to support risk assessment. The use of NAM data including *in vitro*, -omics and *in silico* models (docking and PBK) was shown to support the assessment of read-across and could be included, for example, in the ECHA RAAF.

Several potential uses of NAMs following minor changes to the current regulatory framework were also identified. Examples of these included NAMs being used in the risk assessment of cosmetics, prioritisation of substances of concern, supporting a weight-of-evidence and read-across for industrial chemicals. The need for greater experience and development of acceptable practises for reporting and interpretation of NAM data was also noted – this could build upon the current development of frameworks and guidance in areas such -omics (Harrill et al., 2021; OECD, 2023b). In addition, further work is required to better understand the refinements required to make NAMs acceptable; this may include (quantitative) uncertainty analysis. There also needs to be a continued generation of data to assist in the demonstration of the robustness, reliability and reproducibility across different exposure routes and also for biological and chemical space coverage. This may include a greater diversity in cell lines and *in vitro* models, for instance greater exploitation of spheroids, microphysiological systems, etc.

It was noted that, especially for regulatory use, there is considerable value to a NAM having an OECD Test Guideline. However, NAMs are still considered to be useful without OECD endorsement. Specifically, scientifically robust and valid non-guideline assays could allow for rapid uptake of emerging NAM approaches and potentially a broader coverage of endpoints and applicability domains. There was a call that greater trust should be placed, where appropriate, into data from assays without OECD Test Guidelines.

In order to gain a better understanding of the use of NAMs within a regulatory framework, the “safe harbour” approach of parallel submissions using NAMs/NGRA and traditional data is proposed. The aim of such an activity is to grow confidence in the new approaches for defined uses.

3.9. Capacity building: Sharing learning from experience and training next generation safety assessors

NGRA requires a change in mindset of toxicologists and risk assessors in both industry and regulatory authorities. To implement NGRA there will be a need for well-trained multidisciplinary teams. The User Forum identified a clear need for knowledge and understanding in the use of NAMs for chemical safety assessment. As part of this, the training challenges need to be defined so that the real needs and solutions to training can be identified. Much has been learned by sharing of information within groups such as the EPAA User Forums; these events allow for the sharing of experience from different industrial sectors.

In addition to training, there is a fundamental need to provide further guidance on how to interpret and use NAM data. This may come from case studies which are data rich – such as those presented in the User Forum. For instance, some case studies were supported by *in vivo* data which aided understanding and could be extrapolated using NAM approaches. Overall, there is a clear benefit to disseminating examples and exemplar case studies. Sharing information is an excellent means of initiating training and understanding.

4. Conclusions

The EPAA’s “New Approach Methodologies (NAMs) User Forum Kick-Off Workshop” allowed participants to gain insight of, and share experiences into, the use of NAMs in chemical safety assessment, with a particular reference to NGRA. Recommendations for the use of NAMs in

NGRA were made for the opportunities for the regulatory use of NAMs in exposure assessment; hazard identification; using tiered and targeted testing strategies; performing risk assessment using NAM data; the practical implementation of NAMs; the use of -omics technologies; and the needs for capacity building and training.

CRedit authorship contribution statement

Mark T.D. Cronin: Writing – original draft. **Maria T. Baltazar:** Writing – review & editing. **Tara S. Barton-Maclaren:** Writing – review & editing. **Ofelia Bercau:** Writing – review & editing. **K. Nadira De Abrew:** Writing – review & editing. **Christian Desaintes:** Conceptualization. **Sylvia E. Escher:** Writing – review & editing. **Petra Kern:** Writing – review & editing. **Gavin Maxwell:** Writing – review & editing. **Conceptualization.** **Vera Rogiers:** Writing – review & editing. **Katrin Schutte:** Writing – review & editing. **Tomasz Sobanski:** Writing – review & editing.

Disclaimer

The views and opinions expressed in this manuscript are those of the authors and contributors to the workshop, they do not represent those of the European Commission and the European Chemicals Agency.

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.

Acknowledgements

EPAA would like to thank ECHA for their help organising this event and for use of their meeting facilities for the NAM User Forum. SEE acknowledges the support by the European Union’s Horizon 2020 Research and Innovation Programme under Grant agreement no. 681002 (EU-ToxRisk).

Data availability

No data was used for the research described in the article.

References

- Armitage, J.M., Sangion, A., Parmar, R., Looky, A.B., Arnot, J.A., 2021. Update and evaluation of a high-throughput *in vitro* mass balance distribution model: IV-MBM EQP v2.0. *Toxicity* 9, 315. <https://doi.org/10.3390/toxics9110315>.
- Basketter, D., Alépée, N., Casati, S., Crozier, J., Eigler, D., Griem, P., Hubesch, B., de Knecht, J., Landsiedel, R., Louekari, K., Manou, I., Maxwell, G., Mehling, A., Netzeva, T., Petry, T., Rossi, L.H., 2013. Skin sensitisation – moving forward with non-animal testing strategies for regulatory purposes in the EU. *Regul. Toxicol. Pharmacol.* 67, 531–535. <https://doi.org/10.1016/j.yrtph.2013.10.002>.
- Basketter, D., Ashikaga, T., Casati, S., Hubesch, B., Jaworska, J., de Knecht, J., Landsiedel, R., Manou, I., Mehling, A., Petersohn, D., Rorije, E., Rossi, L.H., Steiling, W., Teissier, S., Worth, A., 2015. Alternatives for skin sensitisation: hazard identification and potency categorisation: report from an EPAA/CEFIC LRI/ Cosmetics Europe cross sector workshop, ECHA Helsinki, April 23rd and 24th 2015. *Regul. Toxicol. Pharmacol.* 73, 660–666. <https://doi.org/10.1016/j.yrtph.2015.10.005>.
- Basketter, D., Azam, P., Casati, S., Corvaro, M., Ezendam, J., Griem, P., Hubesch, B., Irizar, A., Kern, P., Manou, I., Mehling, A., Rossi, L.H., 2019. Applying non-animal strategies for assessing skin sensitisation report from an EPAA/cefic-LRI/IFRA Europe cross sector workshop, ECHA Helsinki, February 7th and 8th 2019. *Regul. Toxicol. Pharmacol.* 109, 104477. <https://doi.org/10.1016/j.yrtph.2019.104477>.
- Basketter, D., Beken, S., Bender, H., Bridges, J., Casati, S., Corvaro, M., Cuvellier, S., Hubesch, B., Irizar, A., Jacobs, M.N., Kern, P., Lamplmair, F., Manou, I., Müller, B.P., Roggeband, R., Rossi, L.H., 2020. Building confidence in skin sensitisation potency assessment using new approach methodologies: report of the 3rd EPAA Partners Forum, Brussels, 28th October 2019. *Regul. Toxicol. Pharmacol.* 117, 104767. <https://doi.org/10.1016/j.yrtph.2020.104767>.

- Basketter, D., Crozier, J., Hubesch, B., Manou, I., Mehling, A., Scheel, J., 2012. Optimised testing strategies for skin sensitization – the LLNA and beyond. *Regul. Toxicol. Pharmacol.* 64, 9–16. <https://doi.org/10.1016/j.yrtph.2012.06.003>.
- Collins, S.P., Barton-Maclaren, T.S., 2022. Novel machine learning models to predict endocrine disruption activity for high-throughput chemical screening. *Front. Toxicol.* 4, 981928. <https://doi.org/10.3389/ftox.2022.981928>.
- Collins, S.P., Mailloux, B., Kulkarni, S., Gagné, M., Long, A.S., Barton-Maclaren, T.S., 2024. Development and application of consensus *in silico* models for advancing high-throughput toxicological predictions. *Front. Pharmacol.* 15, 1307905. <https://doi.org/10.3389/fphar.2024.1307905>.
- Corton, J.C., Liu, J., Kleinstreuer, N., Gwinn, M.R., Ryan, N., 2022. Towards replacement of animal tests with *in vitro* assays: a gene expression biomarker predicts *in vitro* and *in vivo* estrogen receptor activity. *Chem. Biol. Interact.* 363, 109995. <https://doi.org/10.1016/j.cbi.2022.109995>.
- Cronin, M.T.D., Ball, N., Beken, S., Bender, H., Bercaru, O., Caneva, L., Corvaro, M., Currie, R.A., Dawson, J.L., Desert, P., Escher, S.E., Franco, A., Irizar, A., Mehta, J.M., Rogiers, V., Tremblay, R.T., Westmoreland, C., Maxwell, G., 2023. Exposure considerations in human safety assessment: report from an EPAA Partners' Forum. *Regul. Toxicol. Pharmacol.* 144, 105483. <https://doi.org/10.1016/j.yrtph.2023.105483>.
- De Abrew, K.N., Natoli, T., Lester, C.C., Wang, X., Shobair, M., Subramanian, A., Daston, G.P., 2022. A New Approach Methodology (NAM) based assessment of butylated hydroxytoluene (BHT) for endocrine disruption potential. *Toxicol. Sci.* 190, 227–241. <https://doi.org/10.1093/toxsci/kfac099>.
- De Abrew, K.N., Shan, Y.K., Wang, X., Krailler, J.M., Kainkaryam, R.M., Lester, C.C., Settivari, R.S., LeBaron, M.J., Naciff, J.M., Daston, G.P., 2019. Use of connectivity mapping to support read across: a deeper dive using data from 186 chemicals, 19 cell lines and 2 case studies. *Toxicology* 423, 84–94. <https://doi.org/10.1016/j.tox.2019.05.008>.
- Environment and Climate Change Canada, 2020. Technical consultation: proposed subgrouping of bisphenol A (BPA) structural analogues and functional alternatives. <https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/technical-consultation-proposed-subgrouping-bpa-structural-analogues-functional-alternatives.html#toc20>. (Accessed 2 January 2024).
- Escher, S.E., Aguayo-Orozco, A., Benfenati, E., Bitsch, A., Braunbeck, T., Brotzmann, K., Bois, F., van der Burg, B., Castel, J., Exner, T., Gadaleta, D., Gardner, I., Goldmann, D., Hatley, O., Golbamaki, N., Graepel, R., Jennings, P., Limonciel, A., Long, A., MacLennan, R., Mombelli, E., Norinder, U., Jain, S., Capinha, L.S., Taboureau, O.T., Tolosa, L., Vrijenhoek, N.G., van Vugt-Lussenburg, B.M.A., Walker, P., van de Water, B., Wehr, M., White, A., Zdravil, B., Fisher, C., 2022a. Integrate mechanistic evidence from new approach methodologies (NAMs) into a read-across assessment to characterise trends in shared mode of action. *Toxicol. Vitro* 79, 105269. <https://doi.org/10.1016/j.tiv.2021.105269>.
- Escher, S.E., Kamp, H., Bennekou, S.H., Bitsch, A., Fisher, C., Graepel, R., Hengstler, J.G., Herzler, M., Knight, D., Leist, M., Norinder, U., Ouédraogo, G., Pastor, M., Stuard, S., White, A., Zdravil, B., van de Water, B., Kroese, D., 2019. Towards grouping concepts based on new approach methodologies in chemical hazard assessment: the read-across approach of the EU-ToxRisk project. *Arch. Toxicol.* 93, 3643–3667. <https://doi.org/10.1007/s00204-019-02591-7>.
- Escher, S.E., Partosch, F., Konzok, S., Jennings, P., Luijten, M., Kienhuis, A., de Leeuw, V., Reuss, R., Lindemann, K.-M., Hougaard Bennekou, S., 2022b. Development of a roadmap for action on new approach methodologies in risk assessment. *EFSA Supporting Publ* 19 (6), 153. <https://doi.org/10.2903/sp.efsa.2022.EN-7341>.
- Fisher, C., Siméon, S., Jamei, M., Gardner, I., Bois, Y.F., 2019. VIVD: virtual *in vitro* distribution model for the mechanistic prediction of intracellular concentrations of chemicals in *in vitro* toxicity assays. *Toxicol. Vitro* 58, 42–50. <https://doi.org/10.1016/j.tiv.2018.12.017>.
- Gilmour, N., Alépée, N., Hoffmann, S., Kern, P., Van Vliet, E., Bury, D., Miyazawa, M., Nishida, H., Cosmetics, Europe, 2023. Applying a next generation risk assessment framework for skin sensitisation to inconsistent new approach methodology information. *ALTEX - Altern Anim Exp* 40, 439–451. <https://doi.org/10.14573/altex.2211161>.
- Gilmour, N., Kern, P.S., Alépée, N., Boislève, F., Bury, D., Clouet, E., Hirota, M., Hoffmann, S., Kühnl, J., Lalko, J.F., Mewes, K., Miyazawa, M., Nishida, H., Osmani, A., Petersohn, D., Sekine, S., van Vliet, E., Klaric, M., 2020. Development of a next generation risk assessment framework for the evaluation of skin sensitisation of cosmetic ingredients. *Regul. Toxicol. Pharmacol.* 116, 104721. <https://doi.org/10.1016/j.yrtph.2020.104721>.
- Harrill, J.A., Viant, M.R., Yauk, C.L., Sachana, M., Gant, T.W., Auerbach, S.S., Beger, R. D., Bouhifd, M., O'Brien, J., Burgoon, L., Caiment, F., Carpi, D., Chen, T., Chorley, B. N., Colbourne, J., Corvi, R., Debrauwer, L., O'Donovan, C., Ebbels, T.M.D., Ekman, D.R., Faulhammer, F., Gribaldo, L., Hilton, G.M., Jones, S.P., Kende, A., Lawson, T.N., Leite, S.B., Leonards, P.E.G., Luijten, M., Martin, A., Moussa, L., Rudaz, S., Schmitz, O., Sobanski, T., Strauss, V., Vaccari, M., Vijay, V., Weber, R.J. M., Williams, A.J., Williams, A., Thomas, R.S., Whelan, M., 2021. Progress towards an OECD reporting framework for transcriptomics and metabolomics in regulatory toxicology. *Regul. Toxicol. Pharmacol.* 125, 105020. <https://doi.org/10.1016/j.yrtph.2021.105020>.
- Matteo, G., Leingartner, K., Rowan-Carroll, A., Meier, M., Williams, A., Beal, M.A., Gagné, M., Farmahin, R., Wickramasuriya, S., Reardon, A.J.F., Barton-Maclaren, T., Corton, J.C., Yauk, C.L., Atlas, E., 2023. *In vitro* transcriptomic analyses reveal pathway perturbations, estrogenic activities, and potencies of data-poor BPA alternative chemicals. *Toxicol. Sci.* 191, 266–275. <https://doi.org/10.1093/toxsci/kfac127>.
- Middleton, A.M., Reynolds, J., Cable, S., Baltazar, M.T., Li, H., Beven, S., Carmichael, P. L., Dent, M.P., Hatherell, S., Houghton, J., Kukic, P., Liddell, M., Malcomber, S., Nicol, B., Park, B., Patel, H., Scott, S., Sparham, C., Walker, P., White, A., 2022. Are non-animal systemic safety assessments protective? A toolbox and workflow. *Toxicol. Sci.* 189, 124–147. <https://doi.org/10.1093/toxsci/kfac068>.
- Moxon, T.E., Li, H., Lee, M.-Y., Piechota, P., Nicol, B., Pickles, J., Pendlington, R., Sorrell, I., Baltazar, M.T., 2020. Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. *Toxicol. Vitro* 63, 104746. <https://doi.org/10.1016/j.tiv.2019.104746>.
- OECD, 2021. Guidance document on the characterisation, validation and reporting of physiologically based kinetic (PBK) models for regulatory purposes. OECD Series on Testing and Assessment, No. 331, Environment, Health and Safety, Environment Directorate. OECD, Paris. <https://www.oecd.org/chemicalsafety/risk-assessment/guidance-document-on-the-characterisation-validation-and-reporting-of-physiologically-based-kinetic-models-for-regulatory-purposes.pdf>.
- OECD, 2022. Case Study on the use of Integrated Approaches to Testing and Assessment for potential Systemic Toxicity and Estrogen Receptor Activation of a Group of Bisphenols and Select Alternatives Series on Testing and Assessment No. 373, p. 63. [https://one.oecd.org/document/ENV/CBC/MONO\(2022\)43/en/pdf](https://one.oecd.org/document/ENV/CBC/MONO(2022)43/en/pdf).
- OECD, 2023a. Guideline No. 497: Defined Approaches on Skin Sensitisation. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. <https://doi.org/10.1787/b92879a4-en>.
- OECD, 2023b. OECD Omics Reporting Framework (OORF): Guidance on reporting elements for the regulatory use of omics data from laboratory-based toxicology studies, OECD Series on Testing and Assessment, No. 390. OECD Publishing, Paris, p. 124. <https://doi.org/10.1787/6bb2e6ce-en>.
- OECD, 2023c. (Q)SAR assessment framework: guidance for the regulatory assessment of (quantitative) structure - activity relationship models, predictions, and results based on multiple predictions OECD series on testing and assessment, No. 386, Environment, Health and Safety. Environment Directorate. OECD.
- SCCS (Scientific Committee on Consumer Safety), 2023. SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation 12th revision. corrigendum 1 on 26 October 2023, corrigendum 2 on 21 December 2023, SCCS/1647/22. https://health.ec.europa.eu/publications/sccs-notes-guidance-testing-cosmetic-ingredients-and-their-safety-evaluation-12th-revision_en. (Accessed 29 December 2023).
- Vrijenhoek, N.G., Wehr, M.M., Kunnen, S.J., Wijaya, L.S., Callegaro, G., Moné, M.J., Escher, S.E., Van de Water, B., 2022. Application of high-throughput transcriptomics for mechanism-based biological read-across of short-chain carboxylic acid analogues of valproic acid. *ALTEX - Altern Anim Exp* 39, 207–220. <https://doi.org/10.14573/altex.2107261>.
- Westmoreland, C., Bender, H.J., Doe, J.E., Jacobs, M.N., Kass, G.E.N., Madia, F., Mahony, C., Manou, I., Maxwell, G., Prieto, P., Roggeband, R., Sobanski, T., Schütte, K., Worth, A.P., Zvonar, Z., Cronin, M.T.D., 2022. Use of new approach methodologies (NAMs) in regulatory decisions for chemical safety: report from an EPAA deep dive workshop. *Regul. Toxicol. Pharmacol.* 135, 105261. <https://doi.org/10.1016/j.yrtph.2022.105261>.
- Wu, S., Blackburn, K., Amburgey, J., Jaworska, J., Federle, T., 2010. A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogs for SAR-based toxicological assessments. *Regul. Toxicol. Pharmacol.* 56, 67–81. <https://doi.org/10.1016/j.yrtph.2009.09.006>.
- Wu, S., Ellison, C., Naciff, J., Karb, M., Obringer, C., Yan, G., Shan, Y., Smith, A., Wang, X., Daston, G.P., 2023. Structure-activity relationship read-across and transcriptomics for branched carboxylic acids. *Toxicol. Sci.* 191, 343–356. <https://doi.org/10.1093/toxsci/kfac139>.