Report of the European Commission Workshop on "The Roadmap Towards Phasing Out Animal Testing for Chemical Safety Assessments"

Brussels, 11-12 December 2023

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June – 2024

EUROPEAN COMMISSION

Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs

Directorate F — Chemicals, Food, Retail

Unit F1 — REACH

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PDF ISBN 978-92-68-18602-2 doi:10.2873/34576 ET-02-24-681-EN-N

Manuscript completed in June 2024

First edition

Luxembourg: Publications Office of the European Union, 2024

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1. Executive Summary

This report summarises the main findings and discussion from the European Commission (EC) workshop on "The Roadmap Towards Phasing Out Animal Testing for Chemical Safety Assessments" (Brussels, 11-12 December 2023). The aim of the workshop was to identify the major challenges in moving towards animal-free chemical safety assessment and to inform the roadmap to achieve this goal. Over 500 delegates attended the workshop, either in person or on-line, representing relevant stakeholders. All slides, videos of the presentations and discussion from the workshop were recorded and are available at https://single-market-economy.ec.europa.eu/events/commission-roadmap-phasing-out-animal-testing-chemical-safety-assessments-2023-12-11_en.

The commitment to develop a roadmap towards animal-free chemical safety testing is part of the response from the EC to the European Citizens' Initiative (ECI) "*Save cruelty-free cosmetics – Commit to a Europe without animal testing*". The roadmap is intended to be a policy document from the EC which will outline milestones and specific actions, addressing all relevant pieces of chemical legislation relating to safety assessment. The roadmap intends to analyse and to describe the necessary steps to replace animal testing in pieces of legislation that currently require animal testing for chemical safety assessments. The roadmap will outline the path to expand and accelerate the development, validation and implementation of non-animal methods as well as means to facilitate their uptake across legislations. The roadmap is planned to be finalised in the first quarter of the term of the next Commission i.e., end of 2025 / beginning of 2026.

The workshop included presentations and discussion from a wide variety of stakeholders including Commission services, government agencies, industry, academia and non-governmental organisations (NGOs). The contributors provided examples of where, and how, Non-Animal Methods could be implemented within the context of the replacement, reduction and refinement (3Rs) of animal testing relating to human health and environmental effects. In addition, there was consideration of ongoing activities within regulatory agencies and projects to support the roadmap. The workshop received contributions and opinions from a broad range of stakeholders relating to the content and timeline of the roadmap. The purpose of the workshop was not to reach agreement or consensus on any issue, rather to record the range of opinions from individuals, organisations or institutions. The workshop should provide the basis for further discussion on these topics.

A final session of the workshop was devoted to the EU Partnership for the Assessment of Risks from Chemicals' (PARC's) Next Generation Risk Assessment (NGRA) NGRAroute. NGRAroute aims to develop a roadmap for implementing NGRA as the default approach to chemical risk assessment in EU chemicals legislation. PARC offers a platform for facilitating, as well as moderating, the in-depth and potentially controversial discussions which will be needed not only to develop a sound and realistic roadmap, but also to secure broad support across the whole chemical risk assessment community. Collaboration between the EC and PARC is being investigated to make an efficient use of resources to develop the roadmap. Discussion and feedback were received around the ten draft guiding principles for NGRAroute, which are based on policy implementation, scientific development and regulatory acceptance. In addition to change management, these areas formed the basis of four work streams to achieve NGRAroute. Many participants expressed support for the guiding principles of NGRAroute. Specific comments were captured by means of on-line and in person discussions, as well as after the workshop.

2. Abbreviations

3Rs	Replacement, Reduction and Refinement
3RsWP	Working Party on the 3Rs
ACR	Acute-to-Chronic Ratio
AFSA	Animal-Free Safety Assessment
AI	Artificial Intelligence
AOP	Adverse Outcome Pathway
APCRA	Accelerating the Pace of Chemical Risk Assessment
ASPA	ASPIS-initiated alternative Safety Profiling Approach
ASPIS	Animal-free Safety assessment of chemicals: Project cluster for Implementation of novel Strategies
BfR	Bundesinstitut für Risikobewertung
C&L	Classification and Labelling
CLP	Classification, Labelling and Packaging
DA	Defined Approach
EBW	Exposure-Based Waiving
EC	European Commission
ECHA	European Chemicals Agency
ECI	European Citizens' Initiative
ecoNAM	ecological Network for Alternative Methods
ecoTTC	Ecological Threshold of Toxicological Concern
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPAA	European Partnership for Alternative Approaches to Animal Testing
ESA	Environmental Safety Assessment
ESEC	European Specialised Expert Community
EU	European Union
FET	Fish Embryo Test
GD	Guidance Document
GHS	Globally Harmonised System
GLP	Good Laboratory Practice
HSI	Humane Society International
IATA	Integrated Approaches to Testing and Assessment
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICH	International Council on Harmonisation
IVIVE	*In vitro*-*In vivo* Extrapolation

JRC	Joint Research Centre
KE	Key Event
MAD	Mutual Acceptance of Data
ML	Machine Learning
MoA	Mode of Action
NAM	New Approach Methodology
NGO	Non-Governmental Organisation
NGRA	Next-Generation Risk Assessment
NIVA	Norwegian Institute for Water Research
NoG	Notes of Guidance
OECD	Organisation for Economic Cooperation and Development
OoC	Organ-on-Chip
OSOA	One Substance, One Assessment
PARC	Partnership for the Assessment of Risks from Chemicals
PBK	Physiologically-Based Kinetic
PEC	Predicted Environmental Concentration
PoD	Point of Departure
PNEC	Predicted No Effect Concentration
qAOP	quantitative AOP
QIVIVE	Quantitative *In Vitro In Vivo* Extrapolation
QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCCS	Scientific Committee on Consumer Safety
SIR	Standard Information Requirement
SSbD	Safe and Sustainable by Design
SSD	Species Sensitivity Distribution
TG	Test Guideline
TK	Toxicokinetics
TTC	Threshold of Toxicological Concern
UN	United Nations
US	United States
WHO	World Health Organisation
WoE	Weight-of-Evidence

3. Introduction to, and Purpose of, the Workshop

3.1. Introduction to the Workshop

This report presents the main findings from the European Commission (EC) workshop on "*The Roadmap Towards Phasing Out Animal Testing for Chemical Safety Assessments*". The workshop was a hybrid event held in Brussels and on-line over two days (11-12 December 2023). It was attended by over 500 participants representing regulatory agencies, industry, non-governmental organisations (NGOs), the Partnership for the Assessment of Risks from Chemicals (PARC), projects and academia, as well as EU competent authorities and the European Commission.

The aim of the workshop was to identify the major challenges in moving towards animal-free chemical safety assessment and to inform the roadmap to achieve this goal. Specifically, the workshop provided an opportunity for broad stakeholder input and allowed the exchange of ideas, which contribute to the roadmap. There was also an opportunity to establish closer links with external activities that can feed into the roadmap, e.g., European Union (EU) funded projects, stakeholder representations etc. As part of this process, the workshop also intended to allow critical reflection on the process of bringing Non-Animal Methods into regulatory frameworks and the changes required.

The workshop was opened by Ms Kristin Schreiber (Director, EC DG GROW) and Mrs Tilly Metz (Member of the European Parliament (MEP)). Ms Schreiber welcomed participants to the workshop and emphasised the importance of the roadmap to both the EC and citizens of the EU. Progress towards the goal of animal-free chemical safety assessment was acknowledged, with reference to funding from the EC in this area. Ms Schreiber recognised the need for further efforts to achieve the objective, whilst ensuring the safety of humans and the environment. Both Ms Schreiber and Mrs Metz acknowledged the commitment and desire to conduct animal-free chemical safety assessment. Mrs Metz also recognised the effort needed to implement the roadmap and the requirement for better coordination and cross-sector approaches to achieve this goal. Further, chemicals legislation should increasingly reflect the value of non-animal methods.

The purpose of this workshop report is not to provide detailed minutes of the workshop, but rather to bring together the main themes from the presentations and discussion. For a detailed account of the workshop, the EC has published all slides and videos of the presentations from the workshop, which can be found at https://single-market-economy.ec.europa.eu/events/commission-roadmap-phasing-out-animal-testing-chemical-safety-assessments-2023-12-11_en.

3.2. Definitions

Within the workshop, there was a divergence of opinions with regard to the exact definition of the terms "Non-Animal Methods" or animal-free methods, as well as a distinction between that and "New Approach Methodologies" (NAMs) as some participants considered the latter term may include animal testing. The goal of the roadmap discussed at the workshop is the transition to a regulatory system based on non-animal methods. Non-animal methods may include NAMs in the short- to medium-term to reduce or refine animal testing. There was no agreement in the workshop on the definition of NAMs or their implementation as non-animal testing methods. The following summarises many of the proposed methods: non-animal methods were considered in a broad sense to include *in silico*, *in chemico* and *in vitro* approaches, Integrated Approaches to Testing and Assessment (IATAs) and Defined Approaches (DAs), omics approaches or omic-enhanced studies.

3.3. Context of the Workshop

The workshop was held as a direct response from the EC to the European Citizens' Initiative (ECI) "*Save cruelty-free cosmetics – Commit to a Europe without animal testing*" which gained over one million statements of support (ECI, 2023). The response from the European Commission of 25 July 2023 to the ECI states the commitment to transform and modernise chemicals' legislation through the implementation of a roadmap towards animal-free chemical safety assessment (EC, 2023a). To support this, the European Commission committed to create the roadmap towards animal-free chemical safety assessment and to hold two workshops to present progress towards the roadmap, in the second halves of 2023 and 2024. The first of these workshops in December 2023, of which this document is the report, was intended to lay the foundations for the roadmap in terms of gaining knowledge on the potential milestones and steps to achieve them.

The workshop was attended by multiple stakeholders who contributed by podium presentations, panel discussions or via questions and comments to the plenary sessions (both in person and on-line). Several organisations were represented, including the European Commission, agencies such as the European Chemicals Agency (ECHA), the European Food Safety Authority (EFSA), and the European Medicines Agency (EMA), along with animal welfare NGOs, the European Partnership for Alternative Approaches to Animal Testing (EPAA), industry groups, academia, and others. PARC organised the afternoon session on the second day of the workshop.

3.4. Presentations Made at the Workshop

The workshop was organised into five sessions over the first one and a half days and a further half day session organised by PARC to present and discuss their views on Next Generation Risk Assessment (NGRA) and included discussion of the "Guiding principles for NGRAroute - a roadmap proposal for implementing Next-Generation Risk Assessment (NGRA) in EU chemicals legislation". There was a focus in the workshop on reflection and exchange stimulated by panel discussions in each session, with the aim to encourage active audience participation and input.[1] The workshop sessions are summarised in Table 1 with the full agenda provided in Appendix 1.

Table 1. Summary of presentation topics and presenters at the workshop

Session 1. Introduction and setting the scene
• Welcome, housekeeping and opening, DG GROW
• Welcome and ambition for the roadmap and workshop, Ms Kristin Schreiber (Director), DG GROW
• Opening Keynote, Mrs Tilly Metz MEP, European Parliament
• Introductory presentation 1: The roadmap towards phasing out animal testing for chemical safety assessments Commission Communication, commitments, timeline, DG GROW

[1] https://single-market-economy.ec.europa.eu/presentations-workshop-commission-roadmap-towards-phasing-out-animal-testing-chemical-safety_en

- Introductory presentation 2: Workshop on the roadmap towards phasing out animal testing for chemical safety assessments – Scope, aim and concept of the workshop, DG GROW
- Main outcomes from ECHA New Approach Methodology workshop and Key Areas of Regulatory Challenge report, ECHA
- Towards phasing out regulatory animal testing, a perspective from European Food Safety Authority and the European Chemicals Agency, EFSA and ECHA
- Implementation of 3Rs at the EMA: current activities and future perspectives, EMA
- PrecisionTox/ASPIS: Socio-technical barriers to the uptake of NAMs, ASPIS
- Towards chemical safety assessments using solely non-animal methods: the PARC contribution, PARC
- Destination Animal Free, Humane Society International (HSI)
- Industry perspective on the roadmap, CEFIC

Question and Answer Session with speakers

Session 2. How to replace animal testing for the concern of systemic human health effects

- Introductory presentation: How to address systemic health effects with non-animal methods? - Gaps, overlaps and research needs, EU Commission, JRC
- A national risk assessor's perspective to move towards the assessment of systemic health effects using non-animal methods, ANSES, France
- How are human health systemic effects covered when animal testing is not allowed? SCCS
- What does it take to start reducing systemic animal testing now, and to phase it out soon? CEFIC LRI
- Paving the way towards a One-Health approach to chemical risk assessment, People for the Ethical Treatment of Animals (PETA)
- An initiative towards a future solution: the EPAA Designathon, EPAA

Panel discussion on Session 2, Moderator: EC JRC, Panellists: CEFIC, SCCS, CEFIC LRi, PETA, EPAA, ECHA, PARC

Session 3. How to replace animal testing for the concern of long-term aquatic toxicity?

- Introductory presentation: Long-term aquatic toxicity as area of concern – current regulatory status - differences between legislative areas, DG GROW
- How to address fish aquatic toxicity with alternative approaches? – Possibilities, gaps and challenges to be addressed, Norwegian Institute for Water Research (NIVA)
- NGRA for the aquatic environment, EC JRC

- Presentation by a MS authority on their view how to replace fish long-term toxicity testing, UBA
- Feedback from the EPAA Partner Forum: Possibilities to address the area of long-term aquatic toxicity, EPAA

Panel discussion on Session 3, Moderator: DG GROW, Panellists: DG GROW, NIVA, UBA, CEFIC, PETA Science Consortium International e.V.

Session 4. Enhancing the translation of non-animal methods into regulation

Part 1: Setting the scene

- Introductory presentation on Validation and Regulatory Acceptance, DG ENV
- Different needs of legislative areas, DG ENV

Part 2: Validation – how can it evolve

- Validation needs to evolve: Update of OECD GD 34, EC JRC
- OECD stakeholders' survey and workshop on operational and financial aspects of validation, OECD

Part 3: Acceptance for regulatory use

- PARC NGRAroute – a roadmap for making EU chemicals legislation NGRA-ready, German Federal Institute for Risk Assessment (BfR)
- Pathways to regulatory acceptance - Looking beyond validation, EC JRC
- Experiences from the US-Roadmap to regulatory acceptance of non-animal methods, NICEATM/ICCVAM

Panel discussion on Session 4, Moderator: UK Health Security Agency, Panellists: OECD, NICEATM/ICCVAM, BfR, EC JRC, Pepper, RIVM

Session 5. Next steps and closing remarks

Panel discussion, Moderator: EPAA, Panellists: DG ENV, EC JRC, DR GROW, HIS, ECHA, CEFIC, EPAA**:**

- Take home messages from the sessions
- What are the next steps to develop the roadmap?
- Which topics necessary for the development of the roadmap require follow-up events?

4. Purpose of a Roadmap Towards Animal-Free Chemical Safety Testing

The planned roadmap is intended to be a policy document from the EC that will "outline milestones and specific actions" and address all "relevant pieces of chemical legislation (e.g., Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation, Biocidal Product Regulation, Plant Protection Products Regulation and human

and veterinary medicines)". The roadmap will not cover animal use for research e.g., bio-medical research.

The roadmap intends to analyse and to describe the necessary steps to replace animal testing in pieces of legislation that currently require animal testing for chemical safety assessments. The roadmap will outline the path to expand and accelerate the development, validation and implementation of non-animal methods as well as means to facilitate their uptake across legislations. EC (2023a) defined elements for the roadmap:

- Replacing animal testing: to analyse for each (eco-)toxicological endpoint the options to replace animal testing, identify gaps that have to be closed and development needs.
- Joining forces - stakeholder involvement: to communicate e.g., with workshops, the report being based on the first such workshop with a second planned in the second half of 2024.
- Strengthen collaboration of agencies and expert committees: an EC Proposal "Streamlining EU scientific and technical work on chemicals through the EU agencies" (EC, 2023b) to strengthen collaboration of agencies and expert committees to accelerate the transfer of available scientific expertise to legislation. An analysis of the strengths and weaknesses of the current landscape of agencies, committees and working groups that provide advice on non-animal methods. Exploration of opportunities for a stronger collaboration and analyse possibilities to accelerate the transfer of available scientific expertise to legislation.
- Advisory scientific committee on non-animal methods: analysis of the need/feasibility of an expert scientific committee to provide advice on the development of non-animal approaches and their use in the regulatory context.
- Acceptance of methods: Analysis of the ways to accelerate the acceptance of new non-animal methods, while taking into account the importance of mutual acceptance of data across different jurisdictions This includes the need to increase validation but also the regulatory uptake of non-animal methods.
- International dimension: Outreach to non-EU partner countries and multilateral organisations to foster the development and acceptance of non-animal testing methods for regulatory purposes. Consideration of the UN Globally Harmonised System of Classification and Labelling of Chemicals.
- Agencies involvement in international forums: Analysis of how to increase the agencies' visibility and impact in international forums, such as Organisation for Economic Cooperation and Development (OECD), World Health Organisation (WHO), Accelerating the Pace of Chemical Risk Assessment (APCRA) etc.
- Improve availability and accessibility of information: A proposal on a regulation on chemicals data that will improve accessibility to information on chemicals. Analysis of how to facilitate access to information such as upcoming events, calls, guidance.
- Outreach to scientific community and stakeholders: Increase outreach to stakeholders and the scientific community, with support of its agencies, to receive the necessary input on how to replace animal testing with non-animal approaches, e.g., via the organisation of workshops, the annual conference under the umbrella of EPAA or contributions to conferences.

Feedback was sought on these elements and is summarised in Section 4. Further, the workshop considered advances in scientific approaches and methodologies with regard to chemical safety assessment in order to help prepare a roadmap for this specific area. These included developments in *in vitro* and other non-animal methods as well as tiered strategies for their implementation. Existing initiatives towards the general aim of animal-free chemical safety assessment were presented. These include those from the United States (US) to improve the regulatory acceptance of non-animal methods (ICCVAM, 2018) and from EFSA for the use of new approach methodologies (Escher et al., 2022).

5. Stakeholders' Perspectives on Requirements for the Roadmap

The workshop heard a range of opinions relating to the need for a roadmap and the ultimate aim of achieving animal-free chemical safety assessment. The workshop aimed to gather comments, feedback, and suggestions from a wide range of stakeholders rather than to reach agreement or consensus. The first session of the workshop received contributions from regulatory agencies, ECHA, EFSA and EMA, as well as from representatives from the chemical industry (CEFIC), a non-governmental organisation (Humane Society International (HSI)) and EU collaborative projects (Animal-free Safety assessment of chemicals: Project cluster for Implementation of novel Strategies (ASPIS) and PARC). These contributions are summarised below which act as support for, and a basis for part of, the roadmap.

5.1. Past and On-Going Activities that May Inform the Roadmap

The development of the roadmap was seen as a collective effort and requires buy-in by all stakeholders. Stakeholders reported activities that could inform and support the development of the roadmap and, more specifically, the implementation of non-animal methods for animal-free chemical safety assessment. Some of these activities are summarised in this section or referred to in subsequent sections.

ECHA is proactively promoting an increased use of non-animal methods and recognises the need to agree on critical elements to be addressed to enable a transition to an animal-free regulatory system. ECHA reported on the main outcomes from a Workshop on new approach methodologies organised (31 May – 1 June 2023) to discuss the critical needs to move towards an animal free regulatory system for industrial chemicals. The workshop brought together perspectives from different stakeholders and explored opportunities to increase the use of new approach methodologies in the short- and long-term. The ECHA workshop demonstrated a strong commitment from all stakeholders but with different expectations on how to progress and how rapidly progress can be made. The full report on the workshop and recommendations is available (ECHA, 2023b). ECHA also published in June 2023 a document on the "Key Areas of Regulatory Challenge" (ECHA, 2023a), with the document to be updated annually. Four key areas are foreseen, namely to provide protection against the most harmful chemicals, to address chemical pollution in the environment, the shift away from animal testing and to improve availability of chemical data. Three steps were identified to move away from animal testing using new approach methodologies: the identification of critical needs, application of existing new approach methodologies and re-design of the overall the overall safety assessment process (use of information about chemical activity and exposure to manage risk). Key areas for new

approach methodologies development are identified including supporting read-across, better detection of non-genotoxic carcinogens and better use of toxicokinetic modelling.

EFSA also supports the use of NAMs in risk assessment. EFSA provided several examples of the use of NAMs to fill data gaps where traditional data are not available, to complement existing data as part of a Weight-of-Evidence (WoE) approach and to support the phasing out of testing on animals. However, validated and accepted NAMs are not currently available for key elements of the existing regulatory requirements (in common with other regulations), namely providing standardised quality data to allow classification into hazard classes defined in Classification, Labelling and Packaging (CLP) which use classification criteria harmonised under GHS. In particular, there is also a lack of validated NAMs for systemic toxicity. It was observed that the paradigm of one-to-one replacement of *in vivo* tests with NAMs is very challenging, especially for the complex endpoints. Thus, the formation of batteries of NAMs within tiered testing strategies will be essential. Use of NAMs is considered by EFSA (EFSA strategy 2027, Roadmap https://www.efsa.europa.eu/en/supporting/pub/en-7341).

EMA prioritises the 3Rs (Replacement, Reduction, Refinement) in evaluating pharmaceuticals, biologically-based medicines, and vaccines. EMA considers endorsement and acceptance as the most important criteria for NAMs. A Working Party on the 3Rs (3RsWP) has also been instigated by EMA.[2] This has high level strategic goals to implement 3Rs into the authorisation of medicinal products. In addition, EMA has a platform for information sharing and facilitating interactions between experts in the non-clinical field, including NAMs; this is termed the Non-Clinical and New Approach Methodologies European Specialised Expert Community (ESEC). More information is available from: https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/non-clinical-new-approach-methodologies-european-specialised-expert-community. Further, there are a number of Drafting Groups, which are established to comment on reflection papers on 3Rs opportunities (human and veterinary medicinal products) and on alternatives to the use of non-human primates.

CEFIC, representing the European Chemical Industry is committed to the development and regulatory acceptance of animal-free methodologies for the advancement of chemical safety. In alignment with change management approaches, CEFIC proposed to consider three core elements: (1) the planning, discussion and agreement on objectives, deliverables and timelines; (2) the design and implementation at global level of high quality standards; and (3) the continuous verification and calibration of expectations throughout the change management process. Important points for each of these core elements were flagged:

- More flexibility in chemical regulation and the use of New Approach Methods where already possible, e.g. for simple endpoints or read-across was identified as a measure that could be implemented short term. The long-term vision on the other hand would require a paradigm shift, mainly for EU CLP/ Union Nations Globally Harmonised System (UN GHS) classification rules away from today apical endpoints towards a holistic approach to chemical safety.

- Internationally harmonised standards for methods becoming Standard Information Requirements (SIRs) under REACH and the respect of OECD Mutual Acceptance of Data (MAD) rules were identified as key success factors for the implementation of new approaches. Due care should be taken during the standardisation and validation processes to the needs and limitations for difficult to test substances.

[2] https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/3rs-working-party.

- These tasks will require adequate resourcing and hence only enabled by appropriate partnerships and resource sharing. The creation of safe spaces and knowledge hubs should further improve mutual confidence in how NAMs are applied (e.g. on case studies). Coherent NAM approaches between sectors (food, cosmetics, chemicals in general, etc) will only be possible if the use and exposure information of REACH registered substances is refined.

CEFIC proposed that there should be an agreement first on the ambition level. Differentiating between what is required for extending or adapting SIRs (e.g. in terms of global standardisation, confidence and trust required, what is in the evaluation pipeline or to come) and what is required from NAMs for use other than SIRs would then further allow speeding up the uptake of robust and reliable NAMs that are fit for weight -of-evidence and read-across already, and a reduction of animal testing achieved by using exposure to inform testing strategies.

HSI promoted the open, transparent and inclusive building of the roadmap. In particular, the inclusion of defined milestones within a roadmap, particularly focussing on the removal of the requirement in chemicals legislation for redundant and / or duplicative tests, the full phasing out of animal testing in chemical safety assessment and the need to address intersectional inconsistencies in regulation. HSI emphasised the need to establish mechanisms to monitor progress, a commitment to transforming validation and alignment on common language on non-animal methods and health protection goals.

Contributions were also received from two EU-funded projects. In addition to the NGRAroute roadmap activity already mentioned (cf. also Section 5 below), activities in PARC include work packages on hazard assessment and innovation in regulatory risk assessment. It can provide toxicity data, innovative NAMs – including those with a high level of regulatory readiness, IATAs and understanding of criteria for NAMs for regulatory use. ASPIS has the ASPIS-initiated Alternative Safety Profiling Approach (ASPA) framework which will provide a tiered strategy for the implementation of NAMs. In addition, there will be considerable learnings available from EPAA's "NAM Designathon 2023" challenge for human systemic toxicity. The Designathon seeks to identify classification systems capable of categorising chemicals based on the intrinsic toxicodynamic and toxicokinetic properties. The results are to be released in a workshop in March 2023. More details on the Designathon, including updates on results, are available from https://single-market-economy.ec.europa.eu/calls-expression-interest/epaa-launches-designathon-human-systemic-toxicity_en.

6. Recommendations for the roadmap

This section compiles and summarises the discussion in the workshop that were considered by participants as relevant for the roadmap. Many participants of the workshop stated that the roadmap needs well-defined milestones with a robust plan to achieve them. Consistent with the purpose of the workshop, participants expressed a broad range of opinions. This summary attempts to provide some insight into the range of opinions, noting that consensus was not sought or achieved and there was no common agreement on any recommendation. As appropriate, the summary in this section is organised with regard to human health and environmental effects representing individual contributions to the workshop, although it is noted that some topics may be common to both.

Where possible and appropriate, contributions have been attributed to the individuals, institutions or represented in square parentheses i.e. **[Including contributions from…]**. This is not exhaustive of the contributions received and it is recognised that many participants contributed to the discussion, representing many opinions, not all of whom are

acknowledged directly. The purpose of the citations to institutions within Section 4 is to emphasise that a number of opinions on many topics, representing considerable diversity, was received in the workshop. Association as being a contributor to a section does not imply agreement with the content in the report, or that that section represents the opinion of the institution, it merely implies one or more opinion is represented.

6.1. Mapping and Gap Analysis of Needs and Available NAMs [Including contributions from ANSES, JRC]

Several participants considered that there is a need for a co-ordinated approach to identify the existing alternatives to animal testing. As part of this, a fundamental requirement is to understand and document the state of the science with regard to alternatives to *in vivo* animal tests. As such, it is crucial to map the current information requirements for chemical safety assessment in different product-specific regulatory frameworks, i.e., to map the endpoints provided by the current animal-based studies on the basis of information and lists that could be provided, for instance, from the EC and Agencies. To assist in this, a defined list of protection goals and regulatory needs will be a good starting point. The information should cover hazard identification and hazard characterisation along with the need for exposure information and for risk assessment. The research and development needs for animal-free chemical safety assessment should be defined including a gap analysis to determine what is missing and how to prioritise further method development.

The consideration of needs can be supplemented by mapping which NAMs and other approaches are currently available. This will enable the identification of where NAMs are suitably developed for use, as well as the limitations, gaps and needs for development or acceptance of new methodologies. NAMs should be mapped to understand the information they provide and, if necessary, matched with the endpoints from animal-based studies. The non-animal approaches need to be assessed against the protection goals established through current and upcoming regulation. The certainty of current and future non-animal approaches should be assessed.

Considering the mapping process, it is important to create a global perspective on the needs and possibilities of animal-free chemical safety assessment. The purpose here is to facilitate exchange and harmonisation amongst jurisdictions and industrial sectors. The possibility to foster cooperation with ongoing activities on research projects, as well as ensuring the crossover between human health and environment, should be encouraged.

The workshop identified some existing approaches and NAMs that could be applied in the short-term with the current level of data requirements and safety assessment paradigms (in addition to the mapping process described above). It was noted that there is a difference between what is implementable in the short term (i.e., within the current paradigms) and what is required in the longer term (i.e., for allowing a transition of the paradigm). The examples provided in this report are not exhaustive, (which would be a result of the mapping process), rather it presents a selection of examples presented at the workshop. As noted previously, a broad range of opinions were received and no consensus was achieved on any of the measures that could be implemented in the short to longer term.

In order to make NAMs usable for chemical safety assessment, it is possible that a set of NAM information could be defined in the short-term which could support health protection goals. This could provide common information basis for assessments across regulations that would be in line with One Substance, One Assessment (OSOA), and can extend to use in the application of Safe and Sustainable by Design (SSbD). For legislation such as REACH, a set of additional “common information requirements”, dependent on tonnage and exposure, could be considered. Adapting regulation to increase exposure control and

allowing for exposure-driven decisions on higher tier testing is faster than developing and validating new experimental methods. For other industrial sectors such as plant protection products, biocides, medicines etc., further "specific information requirements", where possible based on NAM data, could be introduced in replacement of existing requirements. The aim is to build confidence in the use of the non-animal data to support the phasing out of animal testing. Some of the specific examples presented at the workshop are summarised below and represent a broad vision for the possible solutions, to phase out animal testing, but were agreed as a final solution.

6.2. Regulatory Decisions on New Types of Data [Including contributions from ECHA, EFSA, PETA UK, SCCS]

There is an opportunity and need to progressively adapt legislative frameworks to account for the progress and possibilities of NAMs. This can take account of scientific principles as well as societal needs and expectations expressed in the ECI. The modernisation of legislation should take advantage of the opportunity to allow for international harmonisation to assist with the global replacement of animals in chemical safety assessment. The adaptation of legislation should allow for the phasing out of redundant tests i.e. those that do not provide any new information.

To understand how changes could be achieved, there was discussion of various pieces of legislation. Classification and Labelling (C&L) relates to all chemicals on the market. It crosses all industrial sectors (e.g. plant protection products, biocides, cosmetics, human and veterinary medicines, industrial chemicals etc). Data to make C&L decisions are obtained from the information required under various pieces of legislation e.g., REACH. The GHS for C&L is implemented in the EU through the CLP regulation. This requires knowledge of local and systemic health effects across a range of toxicities and will include endocrine disruption. It is important to assess systemic health effects, addressing C&L as well as risk assessment, to ensure comprehensive understanding. The possibility of developing NAMs to be used for classification of systemic toxicity is explored in initiatives such as RISK-HUNT3R[3] and PARC WP5. Within the workshop, there were different understandings amongst the stakeholders (e.g. EC, Agencies, member states, industry, NGOs) about whether or not sufficient reliable NAMs are available for hazard classification (CLP) and/ or for risk assessment and the degree of confidence in them is needed. There were also differences of opinion with regard to new approach methodologies such as read-across, grouping, omics etc which may reduce testing as compared to NAMs which replace testing fully.

The use of NAMs has the possibility to reduce animal testing anticipated in REACH registrations and reduce uncertainty in human protection, this could be achieved by their support for exposure-driven decisions on waiving for higher tier testing. As uncertainty in human and environmental protection is reduced through the application of NAM and reliable information on exposure, exposure driven decisions on higher tier testing may also be employed to reduce animal testing. An illustration of an area where regulatory focus and emphasis could be enhanced is the understanding of exposure before resorting to animal testing (e.g., with regard to REACH tonnage requirements). As an example, the principles of NGRA (Berggren et al., 2017) could be applied in this situation.

The Cosmetics Regulation (EC) No 1223/2009 is the first EU regulatory framework to have completely banned animal testing and marketing of cosmetic products tested on animals since March 2013. This has meant that the use of NAMs became vital and much

[3] https://www.risk-hunt3r.eu/

has been learned. Various *in silico* and *in vitro* NAMs are utilised for the assessment of cosmetic ingredients NAMs are considered to be of greater value and reliability for local endpoints e.g., skin corrosion, irritation and sensitisation, genotoxic mutagenicity, but further work is required regarding systemic effects following prolonged exposure.

6.3. Validation of NAMs [Including contributions from DG ENV, DG GROW, HSI, JRC]

Several prerequisites for the use of NAM data to replace animal tests were identified in the workshop. These include the validation of methods, models and tools. Following from this is the need for improved understanding of the criteria for regulatory acceptance of NAMs. In addition, terms such as “valid” and “validated” may be interpreted differently depending on the context, use and sector to which they are applied.

The workshop dedicated a session to the consideration of validation of NAMs. The process of validation is currently seen as a precondition, but in practice also a challenge and a bottleneck, to the regulatory acceptance of NAMs. There was discussion regarding the need to update the current paradigm for validating/ demonstrating scientific and relevance of NAMs. The following summarises a range of opinions on the current situation, needs and some proposals to update the validation paradigm.

6.3.1. Current State-of-the-Art of Validation of NAMs [Including contributions from DG GROW, DG ENV, ANSES, JRC, NICEATM/ICCVAM, OECD, SCCS]

Validation, in the context of making NAMs acceptable for regulatory use, is a process that is anchored in scientific principle and serves to demonstrate the reliability and relevance of a new method, such as a NAM, for a particular purpose. It also serves to build trust and confidence within a regulatory context of use. Validation paves the way for regulatory acceptance which is needed to assure that the data from the validated method can be used in regulatory decision making.

NAMs must be shown to be ready for regulatory use, which may include having an OECD Test Guideline (TG). However, tests can be considered valid without undergoing formal validation. The Scientific Committee on Consumer Safety (SCCS) Notes of Guidance (NoG) provide guidance on the use of NAMs including those being developed. A guiding principle is that NAMs will be expected to be fit for purpose, for instance to be able to inform on hazard and risk. In terms of meeting legislative requirements, such as REACH, there is a clear requirement to demonstrate scientific validity, which implies, amongst other requirements, adequate documentation including statements on the reproducibility, sensitivity, specificity and applicability domain of the NAM. In addition to these considerations, it is acknowledged that NAMs adopted in OECD TGs is the prerequisite for MAD.

At the current time OECD-accepted methods, carried out under Good Laboratory Practice (GLP), are the accepted norm. These are considered to be reliable and reusable, allowing for MAD between legislations and geographic regions. Data from OECD-accepted methods provide regulatory predictability and demonstration of compliance.

The current system for validation and acceptance of alternative methods for hazard assessment is based on OECD Guidance Document 34 (GD 34) (OECD, 2005). GD 34 is perceived as being out of date with regard to progress in toxicology and the technology and science on which many of the NAMs are now based, in addition the process is too slow to be fit for purpose. The principles of validation as defined by GD 34 are universal,

however the overall context has changed. There is an ongoing effort to update GD 34 as described in Section 4.3.3.

Key concepts in validation of NAMs are described in van der Zalm et al., (2022) as well as in a more recent proposal from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM, 2023). These documents describe the key concepts to consider during the development and implementation of flexible, fit-for-purpose validation processes of NAMs. These are that the purpose and context of use must be stated and appropriate, there is human or environmental biological relevance, data integrity, appropriate technical characterisation and transparency of all information associated with the NAM. Examples of endpoints (skin sensitisation, endocrine disruption, developmental toxicity, inhalation toxicity, acute toxicity) were presented where biological and mechanistic relevance of NAMs has been demonstrated to support regulatory applications.

A further aspect relating to validation presented was the credibility. Much can be learned from other areas, for instance concepts from *in silico* medicine which has derived procedures to assess the credibility of computational modelling through verification and validation (ASME, 2018). As such, this could help determine the influence of the NAM to the decision relative to other available evidence in any defined scenario as well as the decision consequence, specifically the significance of an adverse outcome resulting from an incorrect decision.

6.3.2. Needs for Updated Validation Procedures [Including contributions from ANSES, CEFIC, EFSA, ECHA, JRC, OECD, PETA UK, SCCS]

A need for a paradigm shift in how validation is performed was recognised within the workshop by several participants. It was discussed that such a shift requires an understanding of the needs for validation as well as an evolution of thinking (Hilton et al., 2023). The principle path from validation of a new method to its acceptance at the regulatory level was highlighted. There are four steps:

i) Demonstration that the method is reliable i.e., sufficiently robust and reproducible.
ii) The method has to demonstrate toxicological validation in terms of scientific relevance for a particular purpose.
iii) The method must be fit for purpose by which the context of use is evaluated, in terms of regulatory relevance this process will build trust and confidence.
iv) There will be acceptance amongst regulators for the use of the new method for a particular purpose.

Validation must be adaptable for different needs depending on the use of the NAMs, which could range from initial screening and priority setting up to full hazard assessment and C&L. The full set of use case scenarios will need to be defined for the validation process.

Key questions can be defined to accelerate the validation process. These were aligned to the full steps of validation to acceptance defined above and include some open questions:

- What is the degree of reproducibility (intra- and inter-laboratory) that is required for the NAM?
- What are the key requirements in terms of toxicological validation, how and which reference data should comparison be made with and how many reference substances are needed?
- How is the NAM to be used in decision making and what additional evidence is required to make such a decision?

- What are the key drivers for regulators to be able to accept a decision based on hazard data?

A new approach to validation must meet the levels of validation expected under the legislation which will apply it. These can be defined, for instance for REACH the provisions of Article 13 must be met. For plant protection products test methods must be validated either through the OECD or an equivalent international organisation. For biocidal products, information requirements on active substances are described in Annexes II and III of the Biocidal Product Regulation. With regard to cosmetics, the SCCS provides guidance in the NoG for the acceptance of new alternative methods. This includes the use of officially validated replacements, a definition of valid replacement methods and they use, mechanistic methods and physiologically-based kinetic (PBK) models that meet specified criteria. For pharmaceuticals, there is specific International Council on Harmonisation (ICH) guidance and EMA advice.

The OECD conducted a Stakeholder Survey in 2023 of validation practitioners on practical and financial aspects of validation. This brought together various opinions, notably on the financial aspects of validation which are estimated to cost between 200,000€ and 500,000€ for a single NAM. The survey concluded that funding validation should not be left to the method developer alone. With regard to the organisational aspects, there was support that validation continues to follow (updated) OECD guidance but should be flexible to accommodate different approaches and situations. Survey correspondents reported that the process should be streamlined to accelerate overall acceptance of methods. There was a call to separate technical as well as specific suggestions including optimising protocols before validation, consider adequate numbers of reference chemicals and limitation of laboratories needed for reproducibility checking, early engagement of regulators, and timely data sharing considerations. In addition, a call was made to equally consider difficult to test chemicals.

It was highlighted that validation does not equal regulatory acceptance. To go beyond validation to regulatory acceptance, a number of criteria should be met including an impact-based credibility assessment, ensuring the provision of multiple pathways to acceptance and clarity on who will be responsible for accepting information for a given context of use.

6.3.3. Discussion of the Possibility to Update the Validation Process [Including contributions from HSI, JRC, NICEATM/ICCVAM, OECD]

As stated above, the principles and process of validation is presented in OECD GD 34. As part of the activity of updating GD 34, the following have been identified as the top priorities:

i) Validation of DAs and their building blocks.

ii) Inclusion of more practical guidance on validation, such as chemicals selection, data integrity, quality assurance, study design.

iii) Defining the concept of technical validation.

iv) Assessment of relevance beyond accuracy to predict animal data.

v) Validation of new technologies such as Organ-on-Chip (OoC) and Artificial Intelligence (AI) assisted methods.

vi) Revision of the process to assess reproducibility and transferability.

vii) Evolution of performance standards.

A need was identified to develop the validation process further for DAs which involve fixed information sources (e.g., *in silico* predictions, NAM data) used in a specific combination where the resulting data are interpreted using a fixed data interpretation procedure. It was suggested that the DA process can be validated and is amenable to MAD, which involves a defined approach providing instructions on how to use specific combinations of non-animal methods and interpret resulting data with a fixed procedure. IATA are a more flexible approach applying a WoE or expert judgement. As such for IATA, there is a need to build a confidence framework for the validation.

With regard to NAMs based on a specific mechanism of action, for instance as part of a DA, the need was recognised to expedite test guideline development and approval as part of the DA approval process. Acceptance of mechanistic NAMs can be facilitated through technical characterisation including assessment of reproducibility, biological relevance and regulatory usefulness. This may be independent of having to establish a regulatory application.

Future work on validation should consider the relevance of the NAM, with a particular emphasis on how to benchmark new methods if *in vivo* animal data are not available or not appropriate. In such cases, relevance may focus on mechanistic relevance to the endpoint of interest, and concordance with other NAMs for the same property. The validation of certain new technologies is not addressed in the current OECD GD 34. For instance, OoC technologies far exceed current *in vitro* methods in terms of complexity. The emphasis in the validation efforts should be placed on physiological and biological relevance and to address the specific elements of the OoC. Machine learning (ML) and AI methods will be crucial both as NAMs themselves or in the interpretation of NAM data (for instance the OECD GARD TG 442E). Thus, there is little experience of validating ML and AI approaches for these methods. However, the OECD quantitative structure-activity relationship (QSAR) validation principles have been found useful. In addition, the model must be demonstrated to be robust and for ML in particular, a description of feature importance and avoidance of overfitting should be stated.

It has also been proposed to re-consider the conduct of multi-laboratory ring trials. These may not be required in the same manner as has been historically performed i.e., with large-scale laboratory ring trials. Of greater importance will be well-designed studies that are able to demonstrate the reproducibility of the NAM between laboratories. There will be a requirement for well-designed transfer studies between labs to demonstrate portability as well as appropriate training in the new method.

Traditionally, performance standards have focused on the components of the test method. Looking forward, there will be a need to evolve and apply performance standards that can be used to demonstrate equivalent information to other methods for the same endpoint. These will characterise the components of the test method, consider outputs to reference chemicals, and aim to demonstrate reproducibility and predictive capacity. There will be a need to apply standards that can be used as benchmarks to demonstrate equivalent information to other methods for the same endpoint can be obtained.

6.4. Regulatory Acceptance of NAMs [Including contributions from DG GROW, JRC, NICEATM/ICCVAM, PARC]

A number of opinions on regulatory acceptance of NAMs were received at the workshop, with validation seen as a key step. To achieve regulatory acceptance of NAMs, in addition to a validated method, there is a need to gain an understanding of the acceptable level of uncertainty for a particular purpose. Acceptable uncertainty may be put in the context of what is currently acceptable e.g., as part of the mapping of NAMs and endpoints.

Therefore, it is important to define the milestone(s) for achieving faster regulatory acceptance in the roadmap.

There are multiple pathways to the acceptance of NAMs for use in chemical safety assessment. These include “Rebuilding” – the design of a new regulatory framework with NAM-based criteria (which may be informed by the EPAA Designathon); “Replacing” - NAMs to replace an animal test in a current regulatory framework; “Repurposing” – adaptation of established NAMs for a different application; or “Augmenting” - using a NAM to address an otherwise neglected adverse event.

There was an appreciation that validation and regulatory acceptance need harmonisation between different initiatives, regulations and geographic sectors. The experiences in the US from developing a roadmap to improve the regulatory acceptance of NAMs were also described. The ICCVAM strategic roadmap is a resource to help guide the development of new methods applying flexible approaches to establish confidence in new methods (ICCVAM, 2018). It aims to encourage the adoption of new methods by U.S. Federal Agencies and regulated industries and recognises that advances in science and technology have not yet been properly leveraged to predict adverse human health effects. The PARC Project is developing “NGRAroute” which is a roadmap for making EU chemicals legislation NGRA-ready. The vision for NGRAroute is to provide a concrete and applicable roadmap proposal by April 2025 for implementing NGRA as the default approach to chemical risk assessment in EU chemicals legislation. This will build on the “ASPA”, the “ASPIS cluster safety profiling approach” an NGRA framework currently developed under the ASPIS cluster. [4]The scope is intended to include all European chemicals legislation with a risk assessment component (i.e., hazard and/or exposure and/or risk), for human health and environmental risk assessment. Ten guiding principles for an NGRA framework to be established in EU chemicals legislation have been drafted and are discussed in more detail in Section 5. Collaboration on the roadmap will be closely coordinated with partners from other work packages in PARC, as well as other ongoing EU projects.

6.5. Consideration of Exposure and Role of Toxicokinetics (TK) [Including contributions from CEFIC, ECHA, EFSA, EPAA, JRC, NIVA]

Understanding of exposure is a significant part of the determination of chemical safety assessment for human health for many substances. A number of opinions on how exposure could be integrated into non-animal chemical safety assessment were presented at the workshop, some of which are presented below, although no agreement or consensus was reached on any particular approach.

Assessment of exposure is fundamental to the application of NGRA and new approach methodologies therein. It will require consideration of both internal and external exposure estimates. Approaches such as the Threshold of Toxicological Concern (TTC) are applied used for impurities (e.g. in food) and low concentrations of ingredients (e.g. in cosmetics). The concept could be extended to the development of an internal TTC i.e., a threshold set on extrapolating internal concentrations for the derivation of a Point of Departure (PoD) in human health assessment.

With regard to understanding and utilising exposure, there is a need to learn from best practices, with considerable experience in some sectors e.g., pharmaceuticals, cosmetics. In general, there is a necessity to reassess exposure estimates and models for workplace

[4] https://aspis-cluster.eu

and occupational exposure for chemicals. Several participants pointed towards short-term measures that could lead to an imminent reduction of animal testing and uncertainty in human and environmental protection. It is based on the adoption of exposure-driven decisions on higher tier testing requirements and the improvement of exposure knowledge in case confidence in this knowledge is lacking. Policy changes towards such an increased exposure management would enable resources to be re-invested in faster development and validation of new experimental methods.

It is crucial to understand real exposure patterns in the environment, for instance to better use monitoring and modelling to improve exposure assessment. Reduced uncertainty could facilitate use of exposure-based waiving (EBW) that cannot be seen as NAM by itself but could reduce the need to consider animal tests before full replacement is available. A number of approaches to waiving were described which could be improved and developed further for EBW. A key approach is the ecological threshold of toxicological concern (ecoTTC) which could be applied in combination with exposure triggers. Another fundamental method is the inclusion of acute-to-chronic ratios (ACRs). ACRs have been found to work well for inert and / or narcotic chemicals when a mode of action can be established (Kienzler et al., 2017). Exposure-driven evidence for a low likelihood of exposure can be used as the basis of EBW.

It's important to gain a better understanding of when sufficient confidence is attained for EBW opportunities. This could be achieved by combining different lines of evidence, but still requires guidance on the overall WoE and stakeholder confidence and agreement on protection goals.

In common with human health, a better understanding of internal exposure of environmental species is needed. Helpful tools to consider internal exposure and interpret *in vitro* data are the use of toxicokinetics (TK). In addition, biomonitoring data can help with internal exposure, such data are available e.g. via IPCHEM.[5]

Due to the lack of viable alternatives for *in vivo* chronic fish testing, a strategy to understand the requirements for the application of NAMs in this area, as well as a plan to develop them, are required. It is anticipated that this information could be compiled in the medium term and will assist with the ultimate replacement of *in vivo* testing. The action plan should consider other methods to reduce the need for chronic fish toxicity testing. These include the use of ecoTTC and EBW (see above), grouping and read-across, with the possibility of integrating omics technologies with read-across approaches.

6.6. Mode and Mechanism of Action [Including contributions from CEFIC, EPAA, JRC]

With regard to systemic human health effects, there will be no one-to-one replacement of a complex *in vivo* test with a single NAM. The use of Adverse Outcome Pathways (AOPs) should also be included in the roadmap. Mechanistic knowledge will create trust in the use of NAM data, this will increase confidence in their use. It is unlikely that there will be complete coverage of all (human-relevant) AOPs, so the focus should be on covering intermediate effects shown to be of concern for adversity avoiding unnecessary overlap, to minimise the number of predicted effects. For such effects quantitative NAM readouts are preferred. One way in which this could be achieved is through an improved linkage of biomarkers for tissue injury with mechanistic key events.

With regard to the application of AOPs there is a need to identify intermediate key effects (KEs) that are responsible for adversity, through the use of NAMs, which lead to the adverse outcome, rather than assessing or predicting the adverse outcome itself.

[5] https://ipchem.jrc.ec.europa.eu

Realistically, a prediction of all effects detected in animal models and in humans to fit the current CLP-based chemical management framework will not be possible. To achieve a phase out of animal testing, a shift in mindset and change in regulations is hence required towards improved but targeted mechanistic knowledge. Additionally, there is a requirement to establish consensus on the extent of coverage needed for various uses, along with the endpoints and exposure categories to be included. Regardless of the NAM methods utilised, demonstrating the ability to maintain current protection levels is essential.

Knowledge and understanding of mechanism of action is important for the replacement of *in vivo* fish toxicity testing. For instance, appreciation of whether there is a non-specific (e.g., narcosis) or specific mechanism of action is important for the accurate extrapolation from Fish Embryo Test (FET) outcomes to *in vivo* fish effects. The extrapolation is frequently observed to be more robust for non-specific mechanisms, thus greater uncertainty may be associated for specific mechanisms. Other issues arise with chemicals with multiple modes of action or those with specific, but unknown, modes of action.

Whilst progress has been made, NAMs are currently not adequate to identify substances with specific modes of action. Therefore, so-called mechanistic “eco-drivers”, e.g., NAMs or biomarkers that are highly indicative of mechanisms, will be crucial in the long-term ambition of replacing the chronic fish test. There is an opportunity to incorporate omics-based endpoints to support mechanistic understanding and integrate with endocrine assessment – albeit with the caveat that these would incorporate -omics in traditional *in vivo* tests.

In addition, AOPs and AOP networks are helpful in structuring information. Case studies with data-rich chemicals will assist in understanding and implementation of NAMs, especially if the effects can be extrapolated to the population, and ultimately ecosystem, level. It is acknowledged that approaches such as the development of quantitative AOPs (qAOPs) and other AOP-derived models have been shown to be useful to implement NAM data (Villeneuve et al., 2023), however, in the short term there will be very few available and these will need to be developed further to assist in the use of the outputs from NAM assays.

6.7. Development of Frameworks for Data Integration and Decision Making [Including contributions from ANSES, NIVA, PARC, SCCS]

The following section is a collection of statements from different participants at the workshop. Single NAMs in isolation are unlikely to provide sufficient information for hazard characterisation, especially for systemic toxicity. Therefore, there will be a need to utilise more than a single NAM, which implies the need for a battery of tests or framework to integrate the data to create an appropriate WoE. There is also a need to develop frameworks to apply NAM data to make a decision. Frameworks are likely to be tiered allowing for efficient decision making incorporating various lines of evidence. The integration of data should allow for the creation of links across methods, endpoints, and species / taxa.

Several such structured frameworks for implementing NAM data to make safety assessment decisions exist. Examples include DAs for skin sensitisation. Most DAs are limited to where a mode of action (MoA) and key molecular events are known – with these providing the basis for the NAMs to be utilised. Other approaches include exposure-led frameworks, such as NGRA applied within the Cosmetics Sector, which allows for decisions to be made based on tiers of information, starting with exposure and leading through to NAM-based evaluation of hazard. Other existing or proposed frameworks described in the workshop included those from ECETOC (Ball et al., 2022) and EC Joint

Research Centre's (JRC's) vision for "Chemicals 2.0" (Berggren and Worth, 2023). There is also a need for intelligent testing to support the development of a WoE from *in vitro* (and other) lines of evidence (Hall et al., 2017). Bayesian Approaches have already been shown to be useful as part of a WoE and can be applied in this context (Moe et al., 2020). Other approaches presented included the ASPIS ASPA, which defines a tiered approach and the relevant methods and approaches to use, along with uncertainty assessment. Some of these approaches will be investigated in the EPAA Designathon.

Relevant AOPs will be key to the development of tiered testing strategies in the future. There is a short-term opportunity (as part of the mapping process) to identify usable frameworks and to structure existing NAMs into similar frameworks. The medium-term is likely to benefit from new *in vitro* methods to replace existing *in vivo* tests as well as reduction in testing through more efficient screening. For environmental species and effects, the long term-aim is seen as the development of NAM-based Environmental Safety Assessment (ESA), specifically applying an IATA-based design, e.g. using PoDs from other taxa or *in silico* predictions. It is important to integrate different assays such as combining toxicity, bioaccumulation and toxicokinetics and gaining a better understanding of internal concentrations in toxicity tests. There is also a need to design the ESA requirements as part of chemical legislations to support wide environmental protection policies e.g. Water Framework Directive, biodiversity protection etc.

6.8. Data Sharing and Access – Ensuring Transparency [Including contributions from ANSES, PETA UK]

Some participants opined that there is a requirement to make data from NAMs and regarding the validation process available for inspection by regulatory risk assessors. Aligned to this is an opportunity to enrich available databases of hazard information, for instance ECHA's database on chemicals. This will allow for better understanding of the capability of a NAM and avoid the possibility of having black boxes which are untransparent, thus facilitating their regulatory acceptance. One possibility is to utilise data for pharmaceuticals as standards. To assist in this goal, a common platform to share data and test guidelines is needed.

6.9. Safe Spaces for Exchange of Data Ensuring Mutual Confidence and Trust [Including contributions from CEFIC, HSI, PETA UK]

The concept of safe spaces was proposed. Safe spaces could facilitate free exchange of ideas, methodologies and data. They would allow an open, back-and-forth, dialogue to be conducted between industry and regulatory agencies. One aspect of the safe space concept could allow data on NAMs to be submitted simultaneously with data currently required for regulatory purposes, thus allowing for an assessment of the non-animal data without prejudice and / or regulatory consequence. It is recognised that this requires sufficient expertise within both industry and regulatory stakeholders to interpret and evaluate the data, as well as sufficient resources from regulators, hence would be difficult for all substances falling under REACH. An option proposed to facilitate the use of safe space could be to form interagency committees staffed by experts on NAMs.

6.10. Replacements for *In vivo* Chronic Fish Toxicity and Bioaccumulation Testing [Including contributions from BfR, DG GROW, EPAA, NIVA]

The workshop discussed as a case study the possibilities for the replacement of animals in the assessment of long-term aquatic toxicity. Predicted No Effect Concentrations (PNECs) are derived from a variety of tests for toxicity and are then compared to the Predicted Environmental Concentration (PEC). Risk assessment is performed using the PEC/PNEC ratio. The discussion focussed on how to replace animal tests in bioaccumulation and long-term toxicity assessment.

It may be possible to replace testing for bioaccumulation in the short term with a number of tests available e.g., bioaccumulation in invertebrates (the HYBIT study); OECD TG 319a and 319b *in vitro* intrinsic clearance tests (combined with *in vitro-in vivo* extrapolation (IVIVE) bioaccumulation models). Other possibilities include read-across and grouping approaches.

For bioaccumulation there is a need to harmonise replacements across different pieces of legislation after initiating legislative changes and guidance updates. There is also a need for case studies and research on domains of applicability and IVIVE models for bioaccumulation.

Some alternatives that exist could be used as partial replacement for fish chronic toxicity testing. An example provided was the OECD TG 210 Fish Early Life Stage Test, although it remains an *in vivo* assay. For acute testing, the OECD TG 236 Fish Embryo Acute Toxicity Test is already applied. Despite this progress, there are currently no alternatives foreseen for *in vivo* chronic fish toxicity testing in the short term.

6.11. Outreach and Involvement of Stakeholders [Including contributions from HSI]

The crucial aspect of the full involvement of stakeholders in the implementation of NAMs was emphasised within the workshop. The aim is to identify and integrate best practice, to highlight international partnerships and precedents from other international regulatory frameworks and to secure the involvement of stakeholders across sectors throughout the entire change management processes. There are many routes to achieve outreach and stakeholder involvement, these are summarised in this section drawing on evidence from all sessions and presentations.

6.11.1. Importance of Partnerships, Networks and Case Studies to Facilitate Dissemination and Communication [Including contributions from EMA, EPPA, NIVA, PARC, PETA UK]

It is crucial for transparency in the use of NAMs between all stakeholders and the value of sharing knowledge, experience, data and methodologies. International partnerships and networks are seen as valuable to share information and knowledge. Examples of partnerships include PARC, ASPIS Cluster and APCRA – all of which are on-going. Case studies were seen as a very valuable tool to demonstrate and increase confidence in the use of NAMs in a regulatory context. Such communication tools, along with sharing of common guidance on the application of NAMs will allow for their greater understanding and uptake.

Another, different type of approach described was the ecological Network for Alternative Methods (ecoNAM: econam.org). This is a platform to facilitate international information exchange about non-animal alternatives for ecological safety assessment of chemicals. The aim is to foster cooperation between all stakeholders involved in the research,

development, and application of alternative ecological hazard and risk assessment tools and methods.

Dissemination events and organisation of workshops on how to progress on the roadmap and the proposed measures will be required, for instance with the collaboration of the EPAA.

6.11.2. Managing Change: Dealing With Socio-Technical Barriers [Including contributions from PrecisionTox / ASPIS]

It's important to consider socio-technical barriers to the uptake of new methodologies and safety assessment. This is from the point of view not only of industry but also the regulators. For instance, a survey (University of Birmingham, UK, as part of the PrecisionTox Project[6], found a predominance of social, rather than technical, barriers. These were categorised, with the aim of identifying possible solutions to assist in the change in mindset. Rules and standards for chemical safety assessment require incremental change in the law and guidance. There is a need for NAMs to be shown as being relevant, reliable and fit-for-purpose to make decisions. There should be appropriate communication to and between all stakeholders, showing leadership and clear direction, thus coordination will facilitate dialogue and collaboration. The clear value of NAMs must be demonstrated with regard to ethics, time and cost comparison, the use of better science and legal considerations. Transition theory is one method that may be applied to clear these socio-technical barriers (Abarkan et al., 2022).

6.12. Education and Training [Including contributions from ANSES, BfR, HSI, OECD, PETA UK]

Some participants emphasised the need for capacity building across all stakeholders. Training and education in all aspects of NAMs, from university courses to professional qualification, are essential. Participants mentioned that training is required specifically for the regulators in Governmental Agencies and a need to provide training to the trainers themselves. This process would require appropriate funding, as well as an appreciation of which topics to include in the training. There also needs to be training provided to industry, with a common understanding of the goals and acceptability that is provided to regulators. Further suggestions for training and mutual education were to consider NAM workshops, to use the existing expert groups of ECHA (e.g., PBT Expert Group, ED Expert Group) and to set up problem dedicated safe spaces for regulators and applicants (e.g., REACH registrants). Examples of current training provided included the Animal-Free Safety Assessment (AFSA) Collaboration Master Class.[7]

6.13. Other Suggestions of Measures Made During the Workshop for Implementation in the Short and Medium Term [Including contributions from BfR, ECHA, EFSA, JRC, PETA UK, SCCS]

A variety of non-test approaches and *in silico* models were referred to in the workshop. These ranged from read-across, to the use of (quantitative) structure-activity relationships

[6] https://precisiontox.org/policy-brief-on-socio-technical-barriers-to-the-uptake-of-nams/
[7] https://www.afsacollaboration.org/masterclass/

((Q)SARs), machine learning, qAOPs, PBK exposure modelling. Usable *in silico* approaches and their applications should be identified. For complex endpoints such as systemic toxicity, it is highly probable that individual *in silico* models on their own will not be acceptable (in the short- or medium- term), but may form a consensus with other modelling approaches and will support the overall WoE for a decision. Read-across is seen as a plausible technique for complex endpoints, specifically where a WoE may be formed using NAM data.

Related, in part at least, to the consideration of the relevance of exposure consideration is to confirm the higher sensitivity of other non-animal taxa (based on *in silico* predictions and possibly mechanism of action). This may allow for the better application of EBW, which could focus on the most sensitive species alone.

In light of the need for comprehensive evaluation, it is important to include data from all species and assess their relative sensitivities, as well as Species Sensitivity Distributions (SSD). This will establish the sensitivity of species, e.g., if fish are not the most sensitive species, there is no relevance in testing them. A recent study demonstrated that fish testing could be excluded for approximately one third of active pharmaceutical ingredients based on species sensitivity alone (Coors et al., 2023). Indeed, with regard to the environmental effects of pharmaceuticals, their well-established and specific modes of action are likely to increase the likelihood of significant species sensitivity. Similar thinking for chemicals with well-known specific modes of action could be applied to other chemicals.

6.14. Other Needs [Including multiple contributions from the presenters and other workshop participants]

Other opinions relating to the roadmap were made, which include:

- Comparison of *in vitro* data with human data is essential to increase confidence in NAMs.
- Funding is required for development and validation of NAMs, in addition to education.
- More work on is needed complex mixtures and substances that are difficult to test. Examples stated include UVCBs such as hydrocarbons, fragrances; surfactants, ionisable chemicals and other substances.
- Regulatory predictability for the use of NAMs with regard to compliance with regulatory requirements should be assessed.
- In addition to the current expert groups, there may be a need to create more expert groups to facilitate the use of NAMs.

6.15. Specific Suggestions for Milestones in the First Five Years of the Roadmap

At the conclusion of the fifth session of the workshop (before the PARC NGRAroute session), a discussion session discussed suggestions for milestones to be achieved in the first five years after publication of the roadmap. These are listed below in the order in which they were discussed and relate to the broader topics described in Section 4.1 of this report. These suggestions were proposed from a variety of participants (as noted below), but no agreements or conclusions were sought or made at the workshop.

- Further use of NAMs in toxicokinetics [ECHA]
- Addressing validation to increase confidence [ECHA, DG GROW]
- Maintain and develop international aspects of NAM development [ECHA, DG ENV]
- Declaration of ambition and commitment to phasing out of animal testing [HSI]
- Commitment to phase out redundant test, to be structured around a short, medium, long-term timeline [HSI]
- Ensure harmonisation e.g. of validation and best use of data [HSI]
- Clear definitions of terminologies e.g. validation, NAM, safe spaces, protection goals [HSI, DG GROW, CEFIC]
- Education, training and support mechanisms to implement NAMs, change management [HSI, DG ENV, CEFIC]
- Action required on the timeframe, reporting and possibility to redress [DG ENV]
- Define who contributes to the roadmap [DG ENV]
- Ensure small steps are taken towards the goal of animal-free testing to allow for reflection [DG ENV]
- Clear vision and goal required [CEFIC, JRC]
- Clear projects with deliverables and measurable milestones allowing targeted progress [CEFIC]
- Resources need to be identified [CEFIC, DG GROW]
- Start with a project uncertainty analysis and assessment of current status [CEFIC]
- The roadmap will be multidimensional and structural elements [DG GROW]
- Consider whether animal tests can be replaced or if there is a need to repurpose and consider protection level [DG GROW]
- Consider how to change process and procedures e.g. to bring in animal-free methods [DG GROW]
- Requirement for an Expert Working Group on Non-Animal Methods [DG GROW]
- How to define good and achievable milestones within the roadmap in or plan activities to meet them [JRC]
- Development of workflows e.g., IATAs [EPAA]
- Identify actions that can be implemented at the current time to implement animal-free testing [EPAA]

Further suggestions were made from the Panel regarding possible topics necessary for the development of the roadmap that require follow-up events. There was a broad range of opinions, some of which are summarised below in the order in which they were stated. No agreement or overall consensus was sought or achieved with respect to these suggestions.

- Working groups should be established to consider standardisation and validation of NAMs [JRC, HSI]
- Phasing out of redundant tests [JRC, HSI]
- Focus on the change of the paradigm, considering regulatory needs, but also to identify and protect more susceptible groups [EPAA, HSI]
- Work and effort should be shared between agencies and international partnerships such as PARC, ASPSIS etc. [DG GROW]
- The organisation and coordination of activities of the roadmap to be undertaken by the EC, as a first step in the roadmap [DG GROW]
- Need to use regulatory momentum e.g., revision to REACH, update of UN GHS. Identify where NAMs can be used at the current time [CEFIC]
- Involve CRO and SME businesses to make NAMs available [CEFIC]
- Classification on the basis of upstream mechanistic intermediate effects rather than adverse effects [DG ENV]
- Consideration of how far regulatory systems can be changed and how to be realistic on changes to legislation [DG ENV]
- Timelines and key performance indicators of NAMs should be defined [HSI].
- Focus on problem formulation [HSI]
- Educational needs and training [HSI]
- Consensus from the scientific community should be sought, particularly what can be achieved by NAMs (ECHA]
- Need for concrete next steps for the roadmap [Many contributions / from Slido]
- There should be concerted action on validation, particularly with regard to funding and resources in the regulatory context. [Many contributions / from Slido]

7. Discussion on Guiding Principles and Workstreams for NGRAroute – a PARC initiative

7.1. Introduction to the Workshop

The final session of the workshop was organised by the PARC WP2[8] and chaired by Dr Matthias Herzler (Bundesinstitut für Risikobewertung (BfR), Berlin, Germany) who co-leads the activity "NGRAroute". PARC offers a platform for facilitating, as well as moderating, the in-depth and potentially controversial discussions which will be needed not only to develop a sound and realistic roadmap, but also to secure broad support across the whole chemical risk assessment community. The form of collaboration

[8] https://www.eu-parc.eu/

between EC and PARC is being investigated to make an efficient use of resources to develop the roadmap.

The agenda for the PARC workshop is provided in Appendix A2. Following an introduction to the themes of the workshop covering PARC and NGRAroute, the workshop aimed to gather input from as many stakeholders as possible for the further work on the roadmap proposal for NGRAroute. The overall vision of NGRAroute is to develop, by the end of April 2025, a concrete and applicable roadmap proposal for implementing NGRA as the default approach to chemical risk assessment in EU chemicals legislation. The scope of NGRAroute includes all major chemicals legislation with a hazard, exposure or risk assessment component of their own. In addition, it pertains to both human health and environmental risk assessment.[9]

NGRAroute has ten guiding principles, which are summarised in Table 1. These principles are intended to guide the development of NGRA frameworks by fostering broad consensus of fundamental questions. They should define political, scientific and regulatory boundaries of NGRA and the further roadmap work. In addition, the guiding principles are intended to help structure further work and focus discussions. Four workstreams are proposed to further develop a roadmap for the uptake of NGRA into policy. The workstreams are scientific development, regulatory acceptance, policy implementation and change management. Overall, the workstreams have a number of tasks including the building of networks between, for instance, research projects, regulatory authorities, policy makers and social scientists/ communication experts. The networks will involve EU regulatory bodies, experts from PARC, supranational organisations (OECD, WHO, UN), EU regulatory and policy-making bodies, identifying key players to implement the necessary changes. The workstreams will analyse the state of the art and identify concrete research questions to be answered in the short, medium and long term. Further, the workstreams will define specific and concrete goals and steps to be taken and develop a detailed work plan.

Table 1. Draft guiding principles for an NGRA framework to be established in EU chemicals legislation (taken from PARC, 2024)

Policy Implementation
1. The framework ensures a high and transparent level of protection for human and environmental health that meets the overarching policy targets.
2. The framework relies on new *in vivo* testing in sentient animals only as a last resort and only until a full replacement is possible.
3. The framework allows for a resource-efficient assessment of a large number of chemicals within an appropriate time-frame.
Scientific Development
4. The framework uses state-of-the-art methodology for modelling, testing and assessment with high scientific relevance to the protection targets, i.e. human health and the environment.
5. The framework provides a high and transparent level of confidence, in particular when concluding on the absence of relevant hazard, exposure and/or risk.
6. The framework is capable of integrating multiple lines of evidence from a wide range of data, information and knowledge sources in a highly reproducible way.

[9] More information on NGRAroute is available from: https://www.eu-parc.eu/sites/default/files/2023-10/PARC_D2.3.pdf (PARC, 2024).

Regulatory Acceptance
7. The framework is applicable to all chemical hazard, exposure and risk assessment workflows required by legislation.
8. The framework covers all relevant pathways and endpoints of regulatory interest and ideally is able to address also new and emerging areas of chemical risk assessment.
9. The framework allows for the assessment of single substances and their transformation products, groups of substances, intentional and unintentional mixtures and articles across all relevant routes of exposure.
10. The framework allows for risk assessment of real-life exposure levels and durations across all relevant routes of exposure.

The aim of the PARC session was to collect a wide range of opinions and feedback firstly on the ten proposed guiding principles for an NGRA framework to be established in European chemicals legislation (see section 5.2) and secondly the four proposed work streams to further develop a roadmap for the uptake of NGRA into policy (see section 5.3). Feedback was collected in the face-to-face meeting and on-line via a Slido poll. A very significant number of comments were received, representing a broad range of opinions. As well as comments received in the face-to-face session, over 750 comments were received electronically via Slido. The report below and comments detailed Appendix 3 attempt to summarise the main themes of these opinions. Comments may be taken from one person, or a summary of similar comments. Since many comments were received via Slido (and were recorded anonymously), comments have not been attributed to individuals or institutions. No agreement on any comments was sought or achieved in the PARC session, they are considered to represent a broad range of opinion throughout the session.

7.2. PARC Sub-Session 1: Guiding Principles for a Next-Generation Risk Assessment (NGRA) Framework to be Established in EU Chemicals Legislation

Session 1 of the PARC workshop introduced the overall concept of NGRAroute and, specifically, ten guiding principles for an NGRA framework that could be compliant with EU chemicals legislation. The ten principles are summarised in Table 1. The principles are organised into three areas i) policy implementation, ii) scientific development and iii) regulatory acceptance, with regard to the utilisation of NAMs within NGRA.

The feedback on the guiding principles is summarised below. The comments include those from the face-to-face workshop as well as the main themes submitted electronically via Slido.

7.2.1. Comments on Policy Implementation

There was generally good agreement with, and support for, principles 1 – 3. Comments are reported in Appendix A2.1.1 as the responses to individual principles. The comments (for all principles and workstreams) represented a broad range of opinions ranging from the need for explicit definitions of the principles themselves, as well as terminology, through to the need for defining protection withing the context of current chemicals legislation.

7.2.2. Comments on Scientific Development

There was general agreement and support for the principles of scientific development of NAMs. One suggestion was that all methods, including existing *in vivo* methods, should be evaluated (or reviewed) with the opportunity to remove redundant [taken to imply providing no new information] tests. The need to have one or more expert group(s) to advise on the uptake and validation of NAMs was confirmed. Currently there are many international activities and there could be greater harmonisation and integration of these, the OECD is seen as an ideal place for such an activity. Comments are reported in Appendix A2.1.2 as the responses to individual principles.

7.2.3. Comments on Regulatory Acceptance

There was general agreement and support for principles 7 – 10 relating to the regulatory acceptance of NGRA. For instance there was discussion as to whether the goal of addressing all endpoints and substances was too high, however, it was stated that the aim should be to cover all eventualities. A further principle to be considered for regulatory acceptance was proposed namely “to aim for a certain level of accuracy of risk assessment conclusions, both less and more conservative, to provide confidence as well as practical applicability”. Comments are reported in Appendix A2.1.3 as the responses to individual principles.

7.2.4. Additional Comments on the Guiding Principles

A number of additional comments, more general in nature, were provided on the guiding principles. These covered aspects such as structuring dialogues between stakeholders, as well as the consideration of other legislation and frameworks. General comments on the guiding principles are reported in Appendix A2.1.4.

7.3. PARC Sub-Session 2: Feedback on the Work Streams to Develop a Roadmap for NGRA Uptake into Policy

Feedback was gathered on the four work streams to develop NGRAroute: scientific development, regulatory acceptance, policy implementation and change management. There are a number of generic tasks to each work stream including the requirement to build networks, to analyse the state-of-the-art and to identify relevant research questions, and to define specific goals and steps to be taken as part of a detailed work plan. Each work stream was discussed to obtain relevant feedback from delegates (in the face-to-face meeting and electronically via Slido), which is summarised below. As above, a broad range of opinions was obtained. The comments below may be for a single person or institution or a summary of similar comments. No agreement or consensus was sought or achieved in the workshop.

7.3.1. Comments on the Scientific Development Work Stream

The scientific development work stream includes a number of activities. These are the building of networks of stakeholders to connect projects and activities. Method development and validation as well as defining the readiness criteria. The development of a NGRA framework to implement NAMs (with reference being made to ASPA). Finally to ensure appropriate documentation standards to allow review of NAM methods, demonstration of their reproducibility etc. There was general agreement and support for the scientific development work stream. Specific comments relating to scientific development are reported in Appendix A2.2.1.

7.3.2. Comments on the Regulatory Acceptance Work Stream

The specific tasks for the regulatory acceptance work stream include developing acceptance criteria for NAMs building on uncertainty, method readiness and relevance. There is also a need for network building to connect risk assessors and managers as well as ensuring engagement of key stakeholders. Regulatory frameworks will be reviewed to ensure NGRA readiness, along with the adaptation of risk assessment workflows. The was general support for the regulatory acceptance work stream. Specific comments relating to scientific development are reported in Appendix A2.2.2.

7.3.3. Comments on the Policy Implementation Work Stream

The tasks for the policy implementation work stream include the specification of protection level and confidence benchmarks that are required in the new legislation. To allow for this, there will be preparation and revision of legal texts to allow for the use of NGRA (make these texts “NGRA-ready”). Crucial to the new implementation of policy is that it enables rapid uptake of new methodologies including central repositories for accepted methods. Part of these activities will be the increased capacity for validation of NAMs and a framework for the use of Artificial Intelligence (AI) methods. This work stream will also create a network of policy makers and risk managers. There was general support for the policy implementation work stream. Specific comments relating to scientific development are reported in Appendix A2.2.3.

7.3.4. Comments on the Change Management Work Stream

The tasks of the change management work stream relate to engaging external stakeholders. The work stream includes better communication on the protection levels and goals that can be achieved by NGRA, highlighting the benefits of these approaches going beyond ethical reasons. This is anticipated that better communication will support changes in institutional organisation and the mindset of individual scientists. Strategies to overcome psychological barriers in trusting NAMs will be developed. Part of this process will be training and capacity building for the implementation of NAMs within NGRA. There was broad support for the change management workstream and associated tasks. Specific comments relating to scientific development are reported in Appendix A2.2.4.

8. Conclusions

A "*Roadmap Towards Phasing Out Animal Testing for Chemical Safety Assessments*" is being prepared by the European Commission, as a response to the ECI "*Save cruelty-free cosmetics – Commit to a Europe without animal testing*". The workshop introduced the purpose of the roadmap as being a policy document that will outline milestones and specific actions and address all relevant pieces of chemical legislation towards achieving animal-free chemical safety assessments. The possible content of, a roadmap was discussed by the participants of the workshop, who provided extensive input into its requirements. The contributions (in terms of presentations) the workshop participants covered many topics, including expectations from an animal-free assessment approach, mode and mechanism of action, consideration of exposure and toxicokinetics, validation and regulatory acceptance, socio-technical barriers to change. There was a broad range of opinions on many topics, especially related, but not limited to timelines, requirements to make a safety assessment, terminology and validation of NAMs as well as the priorities and milestones for the roadmap. There was no overall consensus or agreement from the range of opinions. Further to discussion of the EC roadmap, the PARC project presented "NGRAroute", i.e., work ongoing on a roadmap for implementing NGRA as the default approach to chemical risk assessment in EU chemicals legislation. The workshop participants provided input into the ten scientific principles that underpin NGRAroute and the workstreams proposed to develop a roadmap for the uptake of NGRA into policy.

9. Acknowledgements

Financial support from the European Partnership for Alternative Approaches to Animal Testing (EPAA) to prepare the report is gratefully acknowledged. The organisers also express gratitude to all speakers at the workshop, members of the workshop Steering Committee and all others, including participants, who provided input at the workshop and into this report.

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11. Appendix 1. Agenda of the Workshop on the Commission roadmap towards phasing out animal testing for chemical safety assessments.

Workshop on the Commission roadmap towards phasing out animal testing for chemical safety assessments

Brussels, Breydel 1, Auditorium (hybrid)

11 - 12 December 2023

Draft agenda

Session 1 – Introduction and setting the scene			
11 December, 9:00	5 min	Welcome and housekeeping	Georg Streck (EU Commission, DG GROW)
9:05	10 min	Welcome and opening	Kristin Schreiber (EU Commission, DG GROW)
9:15	10 min	Opening keynote speech	Tilly Metz (European Parliament)
9:25	10 min	Introductory presentation: The roadmap towards phasing out animal testing for chemical safety assessments – Commission Communication, commitments, timeline	Georg Streck/Marco Fabbri (EU Commission, DG GROW)
9:35	10 min	Introductory presentation: Workshop on the roadmap towards phasing out animal testing for chemical safety assessments – Scope, aim and concept of the workshop	Katrin Schutte (EU Commission, DG ENV)
9:45	10 min	Main outcomes from ECHA NAM workshop and Key Areas of Regulatory Challenge report	Ofelia Bercaru (ECHA)
9:55	20 min	Towards phasing out regulatory animal testing, a perspective from European Food Safety Authority and the European Chemicals Agency	Andrea Terron (EFSA)(online) and Tomasz Sobanski (ECHA)
10:15	15 min	Implementation of 3Rs at the EMA: current activities and future perspectives	Sonja Beken (EMA)
10:30	20 min	Coffee break	

10:50	10 min	PrecisionTox/ASPIS: Socio-technical barriers to the uptake of NAMs	Laura Holden (ASPIS)
11:00	10 min	Towards chemical safety assessments using solely non-animal methods: the PARC contribution	Mirjam Luijten (PARC)
11:10	15 min	NGO (animal welfare) perspective on the roadmap	Jay Ingram (Humane Society International)
11:25	15 min	Industry perspective on the roadmap	Katia Lacasse (CEFIC)
11:40	20 min	Q&A	
12:00	1 hour	Lunch break	
Session 2: How to replace animal testing for the concern of systemic human health effects?			
13:00	15 min	Introductory presentation: How to address systemic health effects with non-animal methods? - Gaps, overlaps and research needs	Elisabet Berggren (EU Commission, JRC)
13:15	10 min	A national risk assessor's perspective to move towards the assessment of systemic health effects using non-animal methods	Christophe Rousselle (ANSES, France)
13:25	10 min	How are human health systemic effects covered when animal testing is not allowed?	Qasim Chaudhry (SCCS) (online)
13:35	10 min	What does it take to start reducing systemic animal testing now, and to phase it out soon?	Heli Hollnagel (CEFIC LRI)
13:45	10 min	Paving the way towards a One-Health approach to chemical risk assessment	Julia Baines (PETA)
13:55	10 min	An initiative towards a future solution: the EPAA Designathon	Carl Westmoreland (EPAA)
14:05	55 min	Panel discussion	Moderator: Elisabet Berggren Panellists: • Christophe Rousselle • Quasim Chaudhry • Heli Hollnagel • Julia Baines • Carl Westmoreland

			• Thomasz Sobanski • Mirjam Luijten
15:00	20 min	Coffee break	
Session 3: How to replace animal testing for the concern of long-term aquatic toxicity?			
15:20	15 min	Introductory presentation: Long-term aquatic toxicity as area of concern – current regulatory status - differences between legislative areas	Georg Streck (EU Commission, DG GROW)
15:35	15 min	How to address fish aquatic toxicity with alternative approaches? – Possibilities, gaps and challenges to be addressed	Adam Lillicrap (Norwegian Institute for Water Research) (online)
15:50	15 min	NGRA for the aquatic environment	Stephanie Bopp (EU Commission, JRC)(online)
16:05	15 min	Presentation by a MS authority on their view how to replace fish long-term toxicity testing	Gerd Maack (Environment Agency, UBA)
16:20	15 min	Feedback from the EPAA Partner Forum: Possibilities to address the area of long-term aquatic toxicity	José Vicente Tarazona Lafarga (Instituto de Salud Carlos III)
16:35	55 min	Panel discussion	Moderator: Marco Fabbri Panellists: • Georg Streck • Adam Lillicrap • Gerd Maack • Björn Hidding (Basf) • Christopher Faßbender (PETA Science Consortium International e.V.)
17:30		Closing day 1	

Session 4: Enhancing the translation of non-animal methods into regulation			
Part 1 Setting the scene			
12 December,	10 min	Introductory presentation on	Katrin Schutte (EU

9:00		Validation and Regulatory Acceptance	Commission, DG ENV)
9:10	15 min	Different needs of legislative areas	N.N. (EU Commission, DG ENV, DG GROW, DG SANTE)
Part 2 Validation – how can it evolve			
9:25	10 min	Validation needs to evolve: Update of OECD GD 34	Joao Barroso (EU Commission, JRC) (online)
9:35	10 min	OECD stakeholders' survey and workshop on operational and financial aspects of validation	Anne Gourmelon (OECD)
Part 3 Acceptance for regulatory use			
9:45	15 min	PARC's NGRAroute activity – towards a roadmap for implementing "Next-Generation Risk Assessment" (NGRA) as the default risk assessment approach in EU chemicals legislation	Matthias Herzler (German Federal Institute for Risk Assessment, BfR)
10:00	20 min	Coffee break	
10:20	10 min	Pathways to regulatory acceptance - Looking beyond validation	Andrew Worth (EU Commission, JRC) (online)
10:30	10 min	Experiences from the US-Roadmap to regulatory acceptance of non-animal methods	Nicole Kleinstreuer (NICEATM/ICCVAM) (online)
10:40	55 min	Panel discussion	Moderator: Miriam Jacobs (UK Health Security Agency) Panellists: • Anne Gourmelon • Nicole Kleinstreuer • Matthias Herzler • Joao Barroso • Andrew Worth • Philippe Hubert (Pepper) • Betty Hakkert (RIVM)
Session 5: Next steps and closing remarks			
11:35	50 min	Panel discussion:	Moderator: Gavin

		• Take home messages from the sessions • What are the next steps to develop the roadmap? • Which topics necessary for the development of the roadmap require follow-up events?	Maxwell (EPAA) Panellists: • Cristina de Avila • Elisabet Berggren • Georg Streck • Ofelia Bercaru • Jay Ingram • Chantal Smulders (Shell) • José Vicente Tarazona Lafarga
12:25	5 min	Wrapping up/next steps	Georg Streck (EU Commission, DG GROW)
12:30	5 min	Closing remarks	Cristina de Avila (EU Commission, DG ENV)
12 December, 12:35		End of the first part of the workshop	
12:35	55 min	Lunch	

Second Part

"Guiding principles for NGRAroute - a roadmap proposal for implementing Next-Generation Risk Assessment (NGRA) in EU chemicals legislation"

Workshop session organised by the Partnership for the Assessment of Risks from Chemicals (PARC), Work Package 2 ("A common science-policy agenda")

13:30	15 min	Introduction and housekeeping announcements
Session #1: Guiding principles for an NGRA framework to be established in EU chemicals legislation		
13:45	105 min	In each of the thematic blocks in this session, the draft guiding principles and first preliminary conclusions pertinent to that theme will be briefly introduced. The participants will then be asked to provide their feedback to a number of generic as well as theme-specific questions.

		ca. 13:45: Guiding principles related to policy implementation ca. 14:15: Guiding principles related to scientific development ca. 14:45: Guiding principles related to regulatory acceptance ca. 15:15: Additional aspects
15:30	15 min	Coffee break
Session #2: Work streams to develop a roadmap for NGRA uptake into policy		
15:45	1 hour	The purpose and scope of session #2 will be briefly introduced along with generic tasks relevant to all work streams. In each of the ensuing thematic sessions, the work streams and their specific tasks will be briefly introduced. The participants will then be asked to provide their feedback to the following generic questions: ca. 15:45 Scientific development ca. 16:00 Regulatory acceptance ca. 16:15 Policy implementation ca. 16:30 Change management
16:45	15 min	Wrap-up of the second part of the workshop and outlook
17:00		Closing day 2

12. Appendix 2. Summary of Comments Received in the PARC Session (Relating to Section 5 of the Workshop Report)

A2.1 PARC Sub-Session 1: Summary of Comments on the Guiding Principles for a Next-Generation Risk Assessment (NGRA) Framework to be Established in EU Chemicals Legislation

The feedback on the guiding principles is summarised below. The comments represent a broad range of opinions from the face-to-face workshop as well as the main themes submitted electronically via Slido. Comments may be from a single person or a summary of several similar comments. No agreement or consensus on the comments was obtained in this session.

A2.1.1 Comments on Policy Implementation

Summary of responses relevant to all of principles 1 – 3.

- All principles will need explicit definition and explanation to be usable.
- The legal framework to promote elimination of *in vivo* testing needs to be stated and defined.
- The REACH Regulation, Annex I, already provides a flexible tool for chemical safety assessment, and Annexes VII to XI provide plenty of options for adaptation of the standard information requirements; thus, development of NGRA could take inspiration from this.
- The use of NGRA for the safety assessment of cosmetic ingredients could be used to demonstrate what does and does not work successfully.
- A further principle was proposed "To allow for a new understanding of adversity and classification based on molecular/cellular burden reducing the capacity of the organism and populations to compensate for additional real world stress."

Summary of responses relevant to principle 1 (ensuring a high and transparent level of protection):

- A contributor stated that it should be the science behind the NAMs that defines and informs the protection level, as opposed to legislative needs. Although it is noted that the desired protection level is, in fact, a policy decision.
- The protection level could be made transparent through the use of systematic reviews.

Summary of responses relevant to principle 2 (new *in vivo* testing in sentient animals only as a last resort):

- There was a range of opinions on the requirement of *in vivo* testing as a last resort which ranged from support (providing it is accompanied by explicit scientific justification) to disagreement that *in vivo* testing should be included in any form in NGRA.
- The definition of "last resort", as well as requirements to reach *in vivo* testing as a last resort will need to be carefully stated.
- The terminology of "new" should be defined, i.e., existing test protocols are to be used and that this is not a newly created assay.

- There will always be a requirement to have a last resort as there will always be substances that cannot be tested in NGRA.
- The wording of what constitutes *in vivo* testing [it is considered that this refers to the definitions such as for zebra fish embryos] will need to be precise considering that not all endpoints currently have *in vivo* tests. In this case there should not be a requirement for an *in vivo* test where it is not necessary.
- It should be emphasised that *in vivo* testing would be part of a tiered testing strategy with opportunities to make a decision before reaching *in vivo* testing.
- Should *in vivo* testing be required then consider adding full omics and toxicokinetics to obtain maximum information from the tests.
- Further consideration could be made regarding using epidemiology clinical and other human data.
- There is a need to be proactive, should an *in vivo* test be requested, there should be full documentation and learning from the NAMs data.
- Consideration should be given to what has been learned from the REACH submissions where *in vivo* testing is considered as a last resort, but testing is frequently seen without the use of NAMs data.
- There was a suggestion to remove the term "and only until a full replacement is possible".

Summary of responses relevant to <u>principle 3</u> (resource-efficient assessment):

- It is important to maintain the principles of REACH, i.e., that industry is responsible for safety assessment and the burden of proof is on industry.
- Consideration should be given to prioritisation of chemicals for testing.

A2.1.2 Comments on Scientific Development

Summary of responses relevant to principle 4 (use of state-of-the-art methodology):

- There will be a need to share new methodologies and to accommodate progress in NAMs more rapidly than currently achieved.
- The state-of-the-art methodology should move towards mechanistic understanding as its basis.
- Greater consideration should be given towards whether human cell lines are predictive and protective. It is acknowledged that further evaluation is needed.
- Quantitative *in vitro in vivo* extrapolation (QIVIVE) methodology was seen as being essential to apply NAMs, with a need to extend existing and develop new methods.
- PBK modelling will play a role in translating likely exposures in human reference doses.
- NGRA needs a new way of thinking, a new Committee may be required and a common platform to share methodology and data.

Summary of responses relevant to <u>principle 5</u> (high and transparent level of confidence):

- There needs to be a clear definition of terms "high" and "transparent".
- Relating to terminology, the word "absence" with regard to relative hazard, exposure and/ or risk, should be avoided. There is always the possibility that hazard may be found at high dose or in different exposures.
- With regard to confidence in data, it would be beneficial to look at the uncertainty and variability in the currently used *in vivo* data. This would allow for an understanding of the currently acceptable level of uncertainty in risk assessment. New methods should not be more uncertain than existing methods.

- To achieve confidence in NGRA there is a need for transparency in the data and methodology.
- Exposure needs to be carefully considered.
- MAD is crucial to the use of NAMs.
- Validation of NAMs is very important and adaptation of the current system is required. The aim should be to demonstrate trust in a NAM and that it is fit for purpose i.e. through repeatability, transferability and reliability.
- Funding of validation will be required.

Summary of responses relevant to principle 6 (integrating multiple lines of evidence):

- More work is needed on defining WoE. In addition there will be a great need for training, capacity building and understanding of the meaning of WoE and integration.
- There should be an emphasis to utilise existing NAMs technologies, with an evaluation of what is missing so that it can be developed.
- It would be beneficial to have a centralised resource with information and availability on non-standard and validated methods.
- Data will need to be digitalised and FAIR to allow for their integration.

A2.1.3 Comments on Regulatory Acceptance

There was general agreement and support for principles 7 – 10 relating to the regulatory acceptance of NGRA. For instance there was discussion as to whether the goal of addressing all endpoints and substances was too high, however, it was stated that the aim should be to cover all eventualities.

A further principle to be considered for regulatory acceptance was proposed namely “to aim for a certain level of accuracy of risk assessment conclusions, both less and more conservative, to provide confidence as well as practical applicability”.

Summary of responses relevant to principle 7 (global applicability):

- It will be difficult for NGRA to be applied across all legislations, therefore there may be a need to provide flexibility to accommodate different needs and requirements.
- There was concern that an NGRA based on exposure considerations will not be able to provide sufficient information for C&L.
- Terms such as “chemical hazard, exposure and risk-assessment” are not used in the pharmaceutical industry, therefore this will restrict global applicability.

Summary of responses relevant to principle 8 (all relevant pathways and endpoints):

- This principle could be rephrased to address “all protection goals”.
- The suggestion was to delete the word “ideally” from this principle.

The suggestion was to delete the word “all” from this principle.

- Demonstration of the absence of toxicity is very challenging.

Summary of responses relevant to principle 9 (assessment of single substances cross all relevant routes of exposure):

- There was a suggestion to delete the word “all” from this principle.
- It is crucial to included terminology to indicate exposure should be aggregated and across the entire life-cycle of a substance.

Summary of responses relevant to principle 10 (risk assessment of real-life exposure levels and durations). It was explained by the moderator that this principle did not state that an NGRA framework should be limited to real-life exposure, but that the framework should be capable of dealing with both, very low and high exposure doses/concentrations, if applicable in real life:

- Definition and understanding on what "real-life exposure level" is required and indeed a suggestion to remove the term "real-life" from this principle.
- There should be greater definition of exposure and its meaning, for instance whether this is worst case exposure scenarios, combined exposure etc.
- The suggestion was to delete the word "all" from this principle.
- Exposure is one area that requires much consideration due to its complex nature.
- The dose used in *in vitro* NAMs should take into account bioavailability and *in vivo* tests and humans.
- There is an opportunity to use more human biomonitoring data to understand real-life exposure levels. There was a further recommendation to use blood levels and not concentrations in fat.
- Exposure from chemical incidents and accidents could be considered.
- There may be a difficulty to test non-toxic doses in NAMs.
- The use of PBK and IVIVE is the key utilising NAMs to gain an insight into real-life exposure.

A2.1.4 Additional Comments on the Guiding Principles

A number of additional comments, more general in nature, were provided:

- There is a need for a structured dialogue between regulators, policy makers and scientists. None of these groups can drive the change on their own.
- Use of an exposure-based system should still allow for the careful distinction of hazard and risk.
- It should not be assumed that REACH is the gold standard of risk assessment. There should be consideration of other frameworks which have found solutions to problematic questions.
- Encourage the creation of a non-formal exchange opportunity where non-standard approaches (using NGRAs) can be discussed with regulators on a case-by-case basis.
- Identify what we can be done currently and formulate what we would like to achieve in the future.
- The SSbD framework could be used for the transition to NGRA. It would serve as a training ground and help building experience and acceptance of NAMs - not only for individual endpoints but as an entire assessment system.

A2.2 PARC Sub-Session 2: Feedback on the Work Streams to Develop a Roadmap for NGRA Uptake into Policy

The feedback on the work streams is summarised below. The comments represent a broad range of opinions from the face-to-face workshop as well as the main themes submitted electronically via Slido. Comments may be from a single person or a summary of several similar comments. No agreement or consensus on the comments was obtained in this session.

A2.2.1 Comments on the Scientific Development Work Stream

- It is important to develop proper problem formulation and the questions for NAMs to address.
- NGRA should not overextend the limitations of NAMs.
- Mechanism of action approaches are preferred for NAMs, however they should not be restricted by whether there is an existing AOP or whether there is any quantification of it, i.e. a qAOP.
- There needs to be further consideration of the protection level that is required.
- Validation is an essential topic which requires a draft concept paper to illustrate how the new paradigms could be applied.
- Frameworks such as APSA need to be developed further. There should be further mention of IATA.
- NGRAroute should focus on phasing out animal tests in addition to promoting NAMs.
- Collaboration and dialogue between stakeholders is crucial – scientific and regulatory work streams should work in parallel.
- Understanding of uncertainties is required.

A2.2.2 Comments on the Regulatory Acceptance Work Stream

The specific tasks for the regulatory acceptance work stream include developing acceptance criteria for NAMs building on uncertainty, method readiness and relevance. There is also a need for network building to connect risk assessors and managers as well as ensuring engagement of key stakeholders. Regulatory frameworks will be reviewed to ensure NGRA readiness, along with the adaptation of risk assessment workflows.

The was general support for the regulatory acceptance work stream. Specific comments and feedback on the work stream included the following:

- The work stream could build on the potential to increase dialogue between stakeholders and allow for the building of consensus.
- There was concern that regulations will have difficulty in assessing individual NAMs.
- There is an opportunity to consolidate many regulatory activities, for instance within PARC and ECHA. Commonalities between activities could be sought.
- There is a need for global regulatory acceptance of NAM and NGRA.
- There is a requirement for regulators to have increased funding and resources to effectively utilise NAM data.
- Regulations needs to be more agile and flexible.
- Create NAM “Champion” regulatory expert groups with experts for all regulations.

A2.2.3 Comments on the Policy Implementation Work Stream

The tasks for the policy implementation work stream include the specification of protection level and confidence benchmarks that are required in the new legislation. To allow for this, there will be preparation and revision of legal texts to allow for the use of NGRA (make these texts “NGRA-ready”). Crucial to the new implementation of policy is that it enables rapid uptake of new methodologies including central repositories for accepted methods. Part of these activities will be the increased capacity for validation of NAMs and a framework for the use of Artificial Intelligence (AI) methods. This work stream will also create a network of policy makers and risk managers.

There was general support for the policy implementation work stream. Specific comments relating to the work stream are summarised below:

- There is a demand for a more adaptable interpretation of legal texts concerning chemicals legislation.

- There is a need for increased support for the validation of NAMs in terms of funding on the capacity to undertake validation studies.
- This is a long-term and ambitious work stream.
- Amendments to the considerations for GHS could be considered i.e. a new fully NAM-based GHS class, that can evolve with science over time.
- NAMs must have legal certainty.
- AI-based methods will need to be carefully defined and clarified.

A2.2.4 Comments on the Change Management Work Stream

The tasks of the change management work stream relate to engaging external stakeholders. The work stream includes better communication on the protection levels and goals that can be achieved by NGRA, highlighting the benefits of these approaches going beyond ethical reasons. This is anticipated that better communication will support changes in institutional organisation and the mindset of individual scientists. Strategies to overcome psychological barriers in trusting NAMs will be developed. Part of this process will be training and capacity building for the implementation of NAMs within NGRA.

There was broad support for the change management workstream and associated tasks. Specific comments relating to the work stream are summarised below:

- The process of change management is key to the implementation of NAMs within NGRA. Whilst the support for their implementation at the workshop was strong, the reluctance of other scientists across all stakeholders to implement and utilise NAMs should not be underestimated.
- There is a great need for training and capacity building. This is true for all stakeholders and a mention was made of the needs of regulators to increase awareness on the new approaches.
- Universities should be encouraged to incorporate training on NAMs within higher educational programmes. An example given was the ONTOX Hackathon (April, 2024; Utrecht, The Netherlands) which provided an opportunity for young researchers to make presentations relating to the intersection of AI and ethical toxicology.

A2.2.4 Additional Comments on the Work Streams

Relevant further comments on the work streams were also received and are summarised below:

- Worker exposure should be addressed and an interface with OSH workplace assessments provided.
- Greater efforts should be made to include the pharmaceutical sector.
- Duplication of work should be avoided. An inventory of best practices that are already developed and ready to be used should be developed that crosses all work streams.
- There is a need to build confidence and trust in NGRA before discussion on implementing NAMs and NGRAroute.
- Many data have been delivered by industry and transparently disseminated by ECHA. This lead to more information on how chemicals can be used safely (i.e. without undue risk) for the general public, workers and the environment.

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ET-02-24-681-EN-N

Publications Office
of the European Union

ISBN 978-92-68-18602-2