Partnership for the Assessment of Risks from Chemicals

Additional Deliverable AD2.1

Status report on NGRAroute

WP 2 – T2.2

Technical reference	
Work package	WP 2 – A common science-policy agenda
Task	T 2.2 – Knowledge management and uptake into policy
Dissemination level [1]	PU
Lead Beneficiary/ Responsible AE	BfR
Contributing Participants	BfR, INSA, EAA, UBA, UoB
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Due date of deliverable	30 September 2024
Actual submission date	10 October 2024

[1] PU = Public

[2] WP/task co-leader(s) from the same WP, i.e. WP 2

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Document history

Version	Date	Reviewer name/Institutions	Short description of changes
1	12/02/2024	Matthias Herzler	First draft ToC
2	16/04/2024	Isabella Apruzzese, Matthias Herzler, Rita Paiva Pessoa	First draft of Deliverable
3	09/07/2024	Matthias Herzler	Second draft of Deliverable
4	06/09/2024	Matthias Herzler	Third draft of Deliverable for external reviewers
5	04/10/2024	Matthias Herzler	Final draft based on reviewers' comments

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Abstract

This document describes the current state of the works under the NGRAroute activity, carried out by Task 2.2 under work package (WP) 2 ("a common science-policy agenda") of the European Partnership for the Assessment of Risks from Chemicals (PARC). It builds on the first NGRAroute interim report submitted in April 2023 (PARC, 2023b).

The main intention behind this Additional Deliverable is to summarise the learnings acquired so far under NGRAroute as a helpful input into the activities of the European Commission towards a "roadmap for phasing out animal testing in chemical safety assessments" in the EU (cf. section 3), specifically:

- to demonstrate the necessity of developing a unified Next-Generation Risk Assessment (NGRA) framework,
- to propose a set of guiding principles for such a framework based on New Approach Methodologies (NAMs) as the goal and backbone of the future roadmap work and
- to describe and structure the further work towards the roadmap by proposing work streams and to characterise a number of anticipated tasks under these work streams, based on the proposed guiding principles.

Key Words

next-generation risk assessment (NGRA), next-generation environmental risk assessment (NGERA), roadmap, new approach methodologies (NAMs), regulatory acceptance, uptake into policy

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Table of contents

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Acknowledgements

The authors would like to thank the numerous stakeholders who have taken the time and effort to send comments during the development of this document. Table 1 shows a list of the institutions from which feedback was received during one or more of the multiple commenting rounds carried out in preparation of this document between July 2023 and August 2024 (for details, cf. section 3).

Table 1: Institutions, members of which provided feedback on this Deliverable

Stakeholder group	Institutions[1]
Authorities and agencies	ANSES (FR), BAuA (DE), BfR (DE), Danish EPA (DK), EAA (AT), ECHA (EU), EFSA (EU), EMA (EU), Estonian Ministry of Social Affairs (EE), European Commission (EU), FOEN (CH), FPS Public Health, Food Chain Safety and Environment of (BE), IMH (IL), INSA (PT), ISS, (IT), KEMI (SE), MAK Commission (DE), MITECO (ES), NCad (NL), NIPH (NO), RIVM (NL), SCCS (EU), UBA (DE)
Academia	Eawag (CH), Eberhard Karls University Tübingen (DE), Fraunhofer ITEM (DE), RECETOX/Masaryk University Brno (CZ), Sciensano (BE), Texas A&M University (USA), University of Aveiro (PT), UoB (UK)
Private sector	BASF (DE), CEFIC (BE), EPAA (EU), ERM (UK), EURIMA (BE), Evonik (DE), Givaudan (CH), ICCS (US), L'Oréal (FR), LVMH (FR), NETRI (FR), Syngenta (CH), Veltox Strategy Consultancy (NL),
NGOs	Comité Scientifique Pro Anima (FR), ECEAE (DE), Eurogroup for Animals (BE), HSI (US), Laboratoire Watchfrog (FR), PSCI (DE), RSPCA (UK)

Table 1 demonstrates that a broad spectrum of stakeholders has been consulted for this Deliverable. It is understood, however, that the comments received were submitted as individual expert opinions and do not by any means constitute official views of the institutions to which the commenters are affiliated.

In addition, during the roadmap workshop on December 11-12, 2023 in Brussels (cf. section 3.2), additional comments from online participants were collected anonymously.

[1] For an explanation of the abbreviations, cf. following section.

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Abbreviations and acronyms

AD	Applicability domain
ADME	Absorption, distribution, metabolism and excretion
AI	Artificial intelligence
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health & Safety)
AOP	Adverse outcome pathway
ASPA	ASPIS-initiated safety profiling algorithm
ASPIS	Animal-free safety assessment of chemicals: project cluster for implementation of novel strategies
BAuA	Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (German Federal Institute for Occupational Safety and Health)
BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
BPR	Biocidal Products Regulation (Regulation (EC) 528/2012)
CAD	Chemical Agents Directive
CLP	Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures
CMRD	Carcinogenic, Mutagenic and Reprotoxic Substances Directive
COM	European Commission
CPR	Regulation (EC) 1223/2009 on cosmetic products
CRA	Chemical risk assessment (collective term referring to one or more of the following chemical risk assessment aspects: determination of ADME properties - hazard identification (classification & labelling) - hazard characterisation (derivation of points of departure, guidance values) – exposure assessment – risk assessment/characterisation)
CRO	Contract research organisation
CSS	(EU) chemicals strategy for sustainability
DA	Defined approach
DART	Developmental and reproductive toxicity
DNA	Deoxyribonucleic acid
EA	Exposure assessment
EAA	Environment Agency Austria (Umweltbundesamt Österreich)
Eawag	Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz (Swiss Federal Institute for Environmental Science and Technology)
ECEAE	European Coalition to End Animal Experiments
ECHA	European Chemicals Agency
ED EG	Endocrine disruptor expert group
EDA	Effect-directed analysis
eDNA	Environmental DNA
EEA	European Environment Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ENV	Environment

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ERA	Environmental risk assessment
ESA	Environmental safety assessment
EU	European Union
EURL ECVAM	EU Reference Laboratory for alternatives to animal testing
FOEN	Federal Office for the Environment (Switzerland)
FPS	Federal Public Service (Belgium)
GV	Guidance value
HBGV	Health-based guidance value
HC	Hazard characterisation
HH	Human health
HI	Hazard identification
HSI	Humane Society International
IATA	Integrated approach to testing and assessment
ICCVAM	(United States) Interagency Coordinating Committee on the Validation of Alternative Methods
IMH	Israeli Ministry of Health
IPCS	International Programme on Chemical Safety
INSA	Instituto Nacional de Saúde Doutor Ricardo Jorge (Portuguese National Health Institute Dr. Ricardo Jorge)
ISS	Istituto Superiore Di Sanità
ITEM	Institute for Toxicology and Experimental Medicine (of the Fraunhofer Institute)
IVIVE	In-vitro-to-in-vivo extrapolation
JRC	(Directorate-General) Joint Research Centre (of the European Commission)
KEMI	Kemikalieinspektionen (Swedish Chemicals Agency)
MAK	Maximale Arbeitsplatz-Konzentration (maximum workplace concentration)
MITECO	Ministerio para la Transición Ecológica y el Reto Demográfico (Spanish ministry of the environment)
NAMs	New approach methodologies
NCad	National Comité advise dierproevenbeileid (Dutch national committee for the protection of animals used for scientific purposes)
NGERA	Next-generation environmental risk assessment
NGHRA	Next-generation human risk assessment
NGRA	Next-generation risk assessment
NGO	Non-governmental organisation
NIPH	Norwegian Institute of Public Health
NTS	Non-target screening
OSOA	One substance, one assessment
PARC	European partnership for the assessment of risks from chemicals

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PBK	Physiology-based kinetic (modelling)
PETA	People for the ethical treatment of animals
PNEC	Predicted no-effect concentration
PoD	Point of departure (for CRA)
PPP	Plant Protection Product Regulation (Regulation (EC) 1107/2009)
PSCI	PETA Science Consortium International
PU	Public
qAOP	Quantitative AOP
qIVIVE	Quantitative IVIVE
qST	Quantitative systems toxicology
RDT	Repeated dose toxicity
REACH	Regulation (EC) 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (Dutch National Institute for Public Health and the Environment)
RSPCA	Royal Society for the Prevention of Cruelty to Animals
SAICM	Strategic approach to international chemicals management
SCCS	Scientific Committee on Consumer Safety
T	Task
TMR	Regulation (EC) 440/2009 (Test Method Regulation)
ToC	Table of contents
Tox21	Toxicity in the 21st century
UoB	University of Birmingham
US EPA	United States Environmental Agency
US FDA	United States Food & Drug Administration
WHO	World Health Organisation
WoE	Weight of evidence
WP	Work package

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1. Introduction

1.1. New Approach Methodologies (NAMs)

It is almost impossible to provide a universal definition of the term "NAMs" that cannot be questioned in one way or another. As just one example, Sewell and co-workers have recently defined NAMs in the following way:

> *"New approach methodologies (NAMs) can be defined as any in vitro, in chemico or computational (in silico) method that when used alone, or in concert with others, enables improved chemical safety assessment through more protective and/or relevant models and as a result, contributes to the replacement of animals."* (Sewell et al., 2024)

In this definition, only methodologies would count as NAMs that offer an "improved chemical safety assessment" and "more protective and/or relevant models", whereas the basis for this comparison remains unclear. Assuming this might e.g. refer to a traditional animal test, then methodologies offering the same level of protection - or even a lower, but still acceptable one - would not count as NAMs. Moreover, methodologies pursuing new endpoints previously not covered by animal testing would also not count as NAMs as they would not "contribute to the replacement of animals".

In addition, this is a moving target, because approaches which are "new" at one point may become an established routine later. Therefore, arguably the combination of *in chemico* and *in vitro* methods (e.g. in the form of the DAs for skin sensitisation published by OECD (2023a)) officially established as the default methodology in REACH Annex VII, at some point cannot be considered a "new" approach anymore. This would be even more true for e.g. the *in vitro* test methods which have been applied for skin and eye irritation/corrosion or genotoxicity testing for a long time now.

On the other hand, it is clear (also from the definition cited above), that this term has its origin in the movement towards alternatives to animal testing. Notably, some people still prefer to use the acronym "NAM" to signify "non-animal methods". In this view, the "new approach" refers to not testing chemicals in animals. However, as noted above, developments in recent years have led to the development of "NAMs" that have no direct relationship to established animal tests. In addition, adding e.g. a transcriptomics component to a 28-d rodent test goes beyond the respective established OECD Test Guideline and may help in identifying the mechanistic mode of action of a chemical, but is still an animal experiment – NAM or not?

Against this background, the following pragmatic definition is used in the context of this Deliverable:

New Approach Methodologies (NAMs) are conceptual approaches or practically applied methods designed to provide *– on their own or in concert with other approaches or methodologies –* ***data or information*** [2] ***relevant to chemical risk assessment****, which are* ***not (yet) established as standard approaches or methodologies in the respective scientific or regulatory context****.*

This definition would appear sufficiently broad as a working definition in that it not only covers experimental methods, but also new conceptual approaches.

[2] cf. https://en.wikipedia.org/wiki/DIKW_pyramid (accessed 2024-09-01)

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1.2. Next-Generation Risk Assessment (NGRA)

Before attempting to implement an NGRA framework into EU chemicals legislation, it might seem appropriate to first define what NGRA actually means. Members of the PARC consortium have proposed the following definition for NGRA:

> *"*[…] *NGRA refers to the concept of using data from New Approach Methodologies (NAMs) for chemical risk assessment. In its ideal the concept relies on tiered combinations of in silico tools, complex in vitro systems, organ models and omics approaches in conjunction with physiologically based toxicokinetic modelling and complex exposure models. While the concept of NGRA comprises Adverse Outcome Pathways (AOPs) and quantitative AOPs as established tools for hazard assessment, it is not limited to these, nor does it exclude the use of in vivo data or histopathology. However, it puts a strong emphasis on using state-of-the-art systems and as such is predominantly mechanism-driven, and not driven by apical (toxicological) endpoints."* (Marx-Stoelting et al., 2023)

While certainly a very helpful starting point, it is noted that some aspects of this definition may again require further discussion, e.g. with respect to the generation of new *in vivo* data or the interpretation of the phrase "*mechanism-driven, and not driven by apical (toxicological) endpoints".*[3]

A simpler, more product safety-oriented definition in the context of regulating cosmetic products has been given by Dent et al.:

> *"In this context, a Next Generation Risk Assessment (NGRA) is defined as an exposure-led, hypothesis driven risk assessment approach that incorporates one or more NAMs to ensure that use of a cosmetic product does not cause harm to consumers."* (Dent et al., 2018)

This definition, too, raises some questions, as it only covers one typical risk assessment workflow (exposure-driven product safety assessment) and also regarding the question whether use of one NAM in an otherwise "classic", animal-based risk assessment approach could really be considered "Next-Generation Risk Assessment" already.

The above examples show the difficulties in coming up with a one-size-fits-all, yet concise NGRA definition. In addition, it is noted that both seem to focus strongly on Next-Generation Human Risk Assessment (NGHRA) and a definition also comprising relevant aspects of Next-Generation Environmental Risk Assessment (NGERA) remains to be developed.

One aspect to be highlighted is the fact that the terms NGRA/NGHRA/NGERA refer to assessment frameworks or workflows rather than to one or more individual approaches or methodologies. Their "Next-Generation" aspect would then mainly lie in the application of paradigms and concepts that are fundamentally different from the ones routinely in use by present-day chemical risk assessment frameworks. Examples of such "next-gen" concepts include (but are not limited to):

- toxicity testing in human-relevant test systems *in vitro* based on mechanistic knowledge vs. "black box" testing in sentient experimental animals *in vivo*,
- probabilistic vs. deterministic risk assessment,
- holistic approaches integrating human health and the environment, including new conceptual approaches to account for potential combination effects.

From this, a tentative, very broad and high-level definition of NGRA could read:

[3] In addition, other aspects have been addressed in comments to previous drafts of this document, e.g. the mention of "complex" in vitro systems, when also "simple" systems might be applicable, etc.

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Next-Generation Risk Assessment (NGRA) refers to **chemical risk assessment[4] frameworks or workflows** for human health and/or the environment that are built around **new paradigms and concepts** which do not (yet) represent an established standard in the respective scientific or regulatory risk assessment context.

Three things follow from this definition:

- Because NGRA introduces new paradigms and concepts, it will strongly rely on the use of NAMs (as defined in the previous section).

- The definition above does not exclude that an NGRA framework integrates information gathered already under previous risk assessment frameworks. However, a different weight or value may be assigned to such information (e.g. historical toxicity data in rodents) based on the paradigms/concepts at the core of the NGRA framework.

- In the context of the COM roadmap for phasing out animal testing in chemical safety assessments (cf. section 3), the paradigm change lies in removing animal testing from current legislative CRA frameworks. This leads to the conclusion that an NGRA framework to be implemented for this purpose would ultimately have to rely exclusively on animal-free (NAM) methodologies.

  Notably, this is not in contradiction with the notion that during the (year-long) transition towards such an NGRA framework animal testing will not be replaceable completely any time soon. Nevertheless, while such "present-generation risk assessment" concepts might be indispensable for now, they cannot claim to be part of the NGRA framework itself.

While the desire for a more precise or detailed definition is understandable, it might be questioned whether at this point in time more detail is really needed for constructing the roadmap for phasing out animal testing in chemical safety assessments. Notably, pivotal NGRA-related reports by US EPA (2014) and Thomas et al. (2019) did not attempt to provide such a definition and it might be asked whether it is really worthwhile to spend significant resources on trying to produce a universally accepted definition, mostly for its own sake.

In PARC Deliverable D2.3 (PARC, 2023b), a non-exhaustive list of elements for a generalised, fit-for-purpose NGRA framework was provided. In the meantime, these ideas have been further developed into ten guiding principles for a future NGRA framework (cf. section 4.4). These principles already provide clear boundaries for what NGRA stands for. Moreover, based on the comments received after the first roadmap workshop, the present document attempts to characterise in more detail aspects of NGRA that should be addressed by several different work streams (cf. section 4.5).

It is anticipated that by dealing with these aspects during the further work on the COM roadmap, gradually the full picture of what distinguishes NGRA from present-day chemical risk assessment will emerge.

[4] Note that, as explained above, "chemical risk assessment" is used here as a collective term inclusive of partial aspects of CRA like hazard or exposure assessment, and does not only refer to "full" risk assessment.

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1.3. Next-Generation Environmental Risk Assessment

Compared to environmental risk assessment (ERA), the conceptual development of NGRA for human health is currently in a more advanced stage and therefore the present document is more comprehensive regarding issues relevant to human health risk assessment. However, it is expected that more content conceptually related to NGERA will be added to NGRAroute and the COM Roadmap for phasing out animal testing in chemical safety assessments over time.

Current challenges for ERA include diverse aspects, such as the assessment of low-dose effects of chemicals, indirect and combined effects of multiple chemicals, continuously repeated exposure, chemical contributions to climate change, in particular regarding greenhouse gas emissions and ozone layer depletion, cascading effects across species, extrapolation to landscape-scale effects and habitat loss on biodiversity, the delineation from other stressors (e.g. climate change, land use) as well as fragmented regulatory assessments under the current substance-by-substance approach. Innovative and integrative approaches across regulatory sectors are needed. To this end, a cross-disciplinary project is under development aiming to strengthen collaboration within PARC and with external projects to develop and implement NGERA (Hornek-Gausterer et al., 2024). The objective of this project is to establish a collaborative platform and processes for the governance of scientific coordination in NGERA by mapping existing efforts and identifying gaps, and to develop a framework and processes for cross-project collaborations. A number of people involved in this activity are also partners in the NGRAroute activity and will represent NGRAroute in the ESA WG under the COM roadmap (cf. section 3.4).

Among other things, NGERA includes new strategies and tools for monitoring, non-target analysis and suspect screening, prioritization and modelling of exposure. In addition, tools to address combined exposures in environmental risk assessment are part of this NGERA concept. Moreover it is noted that - compared to human health risk assessment - ERA is faced with the additional complexity of having to deal with many species of different sensitivity and better tools/methods to integrate this into regulation are needed, e.g. the SeqaPASS tool from US EPA mentioned in ECHA (2024).

Extensive environmental monitoring data sets have been built up over decades and networks and strategies and platforms to foster the integration and reuse of such information to improve the scientific underpinning of current as well as future prospective risk assessment frameworks are also ongoing. In addition, new monitoring approaches and methods are needed to improve the exposure assessment.

In 2022, EFSA has laid out the concept of PERA, a European Partnership for next generation, systems-based ERA:

> *"Today's regulatory ERAs call for a systems-based approach that formulate ERA issues/problems and associated protection goals holistically; address the cumulative effects of multiple regulated substances/compounds or products and stressors; analyse upstream and downstream life-cycle implications; evaluate a range of alternative solutions; involve a broad range of stakeholders; and use interdisciplinary scientific approaches. This approach would improve the scientific basis for regulatory ERA and decision-making, create opportunities for new partnerships and enhance cooperation across regulatory silos* (Burke et al., 2017).
>
> *Considering the broad array of regulatory ERAs performed under different sectoral regulatory frameworks, there are many commonalities (e.g. hazard assessment, common parameters) for which a more coherent and harmonised approach would be beneficial when characterising environmental risks. Also, next generation ERAs should be designed in a manner that facilitate their integration in EU environmental impact and sustainability assessments, and policy*

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assessments performed by relevant partners in the context of other regulatory frameworks/policies." (EFSA, 2022).

Axelman et al. (2024) have recently reported and discussed first experiences under this partnership.

The establishment of NGRA in the legislation offers a particular opportunity to significantly improve the integration of human health and environmental risk assessment. With this in mind, the COM roadmap, and NGRAroute in its support, offer an excellent opportunity to start the development of a concept for an integrated NGRA framework with the objective to unite both "worlds" into a single, holistic NGRA framework.

1.4. Background

As part of its activities, PARC Task 2.2 ("knowledge management and uptake into policy") aims to develop and oversee the implementation of strategic roadmaps to actively support the uptake of the innovative science on New Approach Methodologies (NAMs, for a definition cf. section 1.1) and Next-Generation Risk Assessment (NGRA, cf. section 1.2) developed in PARC and elsewhere into regulatory chemical risk assessment (CRA) practice.

The first activity in this line of work is "NGRAroute", a roadmap for actively implementing the paradigm shift towards NGRA in the major EU chemical regulation programmes.

Although substantial progress has been made with the development of NAMs, all major chemical regulations in Europe requiring CRA (with the exception of the Cosmetic Products Regulation) still strongly rely on animal testing.

More than a decade ago, the United States Environment Protection Agency (US EPA) published the pivotal report "Next Generation Risk Assessment: Incorporation of Recent Advances in Molecular, Computational, and Systems Biology" (US EPA, 2014), and initiated the Tox21 programme, a joint effort of several U.S. federal agencies. This programme provided the backbone for introducing a paradigm change in science-based CRA in the U.S., with worldwide repercussions. A more detailed vision and strategy was provided in the landmark report "Toxicity Testing in the 21st Century. A Vision and a Strategy", published by the U.S. National Academy of Sciences (National Academies, 2007), and followed by the formal installation of an alliance of the involved U.S. agencies in 2008. This co-operation has provided an unprecedented dynamic for the science-based innovation of CRA.

In 2018, ICCVAM, the United States Interagency Coordinating Committee on the Validation of Alternative Methods published its "Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States" (ICCVAM, 2018). Most recently, in January 2024, the US National Institutes of Health's "Complement-ARIE" fund was approved, which will provide a budget of $ 35 - 40 M per year over the next ten years, inter alia for technology development projects/centres, a data & resource coordinating centre, a validation network for regulatory implementation, community engagement and training and strategic engagement.

In Europe, major EU projects, such as Predict-IV (Mueller et al., 2015), SEURAT-1 (Daston et al., 2015; Gocht et al., 2015) or EU-ToxRisk (Daneshian et al., 2016; Moné et al., 2020) have greatly improved our understanding of the potential of NAMs to support regulatory CRA for human health. These projects have achieved great progress on the scientific side and have successfully started addressing (and bridging) the communication gap between academic researchers and regulatory practitioners. Several currently active EU framework projects, such as the ASPIS cluster featuring the human-health-related projects RISK-HUNT3R (Pallocca et al., 2022), ONTOX (Vinken et al., 2021) and PrecisionTox (PrecisionTox Consortium, 2023), or the EURION cluster with its projects ATHENA, EDCMET, ENDpoiNTS, ERGO, FREIA, GOLIATH, OBERON and SCREENED, focusing on the assessment of endocrine disruptors, are building on and complementing this work.

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On a global scale, the Organisation for Economic Co-Operation and Development (OECD), supported by its member countries, with large contributions from EU Member States, the European Commission's Directorate-General Joint Research Centre (JRC) including the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM), and European agencies like the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA), has successfully facilitated the conceptual development of innovative chemical hazard and risk assessment methodologies, such as the Defined Approaches for Skin Sensitisation (DASS) Guideline Document 497 published in 2021 (OECD, 2023a). Via the "Integrated Approaches to Testing and Assessment" (IATA) case studies project, the OECD continues to actively explore their practical application in the regulatory context. The OECD also plays a key role in further developing concepts, e.g. the concept of Adverse Outcome Pathways (AOPs) and practical approaches to validate test as well as non-test methods regarding their fitness-for-purpose for regulatory CRA and by developing relevant guidance in the field (e.g. (OECD, 2017a; OECD, 2017b; OECD, 2017c; OECD, 2023b).

In addition to the large-scale research projects named above (and others), regulatory bodies and agencies have started initiatives to actively investigate and promote NAMs and NAM-based NGRA. In the EU, the Scientific Committee on Consumer Safety (SCCS), driven by the ban on animal use for the testing of cosmetic ingredients, has frequently adapted its Notes of Guidance with a view to NAMs and NGRA (most recently in 2023, SCCS (2023)). ECHA has for many years actively supported the development of *in silico* tools and concepts for their application under the REACH and Biocidal Products Regulation, e.g. by supporting the OECD QSAR Toolbox and by developing the Read-Across and QSAR Assessment Frameworks (ECHA, 2017b; OECD, 2023b). The agency has also hosted two workshops on the use of NAMs in CRA in 2017 and 2023 (ECHA, 2017a; ECHA, 2023a) and has recently formulated "Key Areas of Regulatory Challenge" in the field of NAMs and NGRA (ECHA, 2024). EFSA has provided a number of tenders in the area of food safety, e.g. for a "roadmap for action on new approach methodologies in risk assessment" (Escher et al., 2022) or for projects such as ADME4NGRA, TXG-MAPr and NAMS4NANO (Haase et al., 2024; Usmani et al., 2024).

As an important example on the international level, the initiative for "Accelerating the Pace of Chemical Risk Assessment" (APCRA) aims to discuss *"progress and barriers in applying new tools to prioritization, screening, and quantitative risk assessment of differing levels of complexity"* and *"opportunities to increase collaboration in order to accelerate the pace of chemical risk assessments"* by means of case studies with high regulatory relevance (Kavlock et al., 2018; Paul Friedman et al., 2019).

Outside of the Cosmetic Products Regulation (CPR) with its outright ban of animal testing, most other EU chemicals legislations already foresee the use of NAMs/NGRA on a case-by-case basis under certain circumstances (e.g. REACH Annex XI) or as part of Weight-of-Evidence (WoE).

Nevertheless, while this opportunity is frequently taken e.g. by registrants under REACH (ECHA, 2023b), the actual regulatory implementation of NAM-based NG(E)RA approaches in EU chemicals legislation has been limited to certain endpoints such as skin sensitisation, skin and eye irritation/corrosion or genotoxicity in the area of human health, or bioaccumulation or intrinsic clearance under ERA. NAM-based approaches are also used as supportive information in mechanistic identification, e.g. for endocrine active substances.

However, even these promising developments have taken several decades to unfold and for other, more complex endpoints, in particular repeated dose toxicity (RDT), developmental and reproductive toxicity (DART) or carcinogenicity, progress with implementation is still slow.

Of course, in some of these complex areas, complete scientific solutions are still missing, but part of the slow regulatory and legal uptake may also be attributable to the fact that most of the above research projects have remained on the scientific level, with limited connection (beyond the communication and discussion of scientific results) to the regulatory and policy domain.

In 2020, the European Commission has noted in its "EU Chemicals Strategy for Sustainability" (CSS):

> *"Despite a strong EU policy for the protection of animals used for scientific purposes, adopted 10 years ago, which makes full replacement of animal testing its ultimate goal, animals are still required to be used systematically for testing in the field of chemicals. Safety testing and chemical risk assessment need to innovate in order to reduce dependency on animal testing but also to improve the quality, efficiency and speed of chemical hazard and risk assessments."* (European Commission, 2020)

It is clear that to reach these policy goals and to overcome the scientific, economic and political challenges associated with the paradigm change towards NGRA, not only a clear vision for a future NGRA-based CRA framework, but also a strategic roadmap, with concrete steps from taking stock of the state of science to closing knowledge gaps and, ultimately, the installation of the new framework in EU chemicals legislation, is needed, along with a concrete plan for how to facilitate and implement the transition process in a step-by-step fashion. Such a strategic plan/roadmap would then also allow to provide the necessary resources in a more targeted matter and allow for a better integration of new tenders/projects with existing or previous ones.

2. NGRAroute – vision and scope

The original vision of NGRAroute as formulated in Deliverable D2.3 of April 2023 is given below.

> *"By 2025, NGRAroute will provide a concrete and applicable roadmap proposal for implementing animal-free NGRA as the default approach to chemical risk assessment in EU chemicals legislation.*
>
> *By that time, this roadmap will already have been discussed and harmonised as far as possible with all relevant stakeholder groups from the regulatory community (i.e., researchers, risk assessors, risk managers and policy makers, from academia, authorities and industry, in and outside of PARC)."* (PARC, 2023b)

In this vision, "default approach" meant that animal-free testing and assessment strategies should become the first tier of chemical risk assessment by default, generating any additional information that may be required beyond already existing information by using NGRA methodology.

Due to more recent developments, in particular the announcement of the EU Commission's intention to deliver a "roadmap for phasing out animal testing in chemical safety assessments" (cf. section 3), this vision had to be readjusted, both in terms of scope and timelines. This rephrased vision can be found in the box below.

NGRAroute's vision is to implement ***animal-free Next-Generation Risk Assessment (NGRA)*** *as the* ***default approach*** *to chemical risk assessment in EU chemicals legislation.*

To achieve this, NGRAroute will ***significantly contribute*** *to both the preparation (by 2025) and implementation (in later years) of the* ***EU Commission's "roadmap for phasing out animal testing in chemical safety assessments"****.*

Already during a transitional phase towards a full NGRA framework, *in vivo* testing in sentient animals (cf. section 4.8) would then only be applied in higher tiers, possibly in a targeted way based on the NGRA outcome, in cases where NGRA is not yet applicable or the conclusions from NGRA are not considered sufficiently reliable, until, finally, new animal testing will have been phased out completely from chemical safety assessments.

A similar approach has been introduced in Annex VII of the REACH Regulation for the endpoint skin sensitisation: by default, a set of *in vitro*/*in chemico* tests (e.g. in the form of an OECD "Defined

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Approach" [5]) is required as the first tier of risk assessment, but *in vivo* testing (typically in mice) still needs to be carried out if the substance to be tested falls outside of the applicability domain of the *in vitro* tests, or if for other reasons these tests do not allow for a clear conclusion on classification and labelling and/or risk assessment.

The scope of NGRAroute encompasses all relevant chemicals legislations in Europe, i.e. all legislations which contain hazard/risk assessment workflows of their own (e.g., REACH, CLP[6], the Regulations on cosmetics, food additives, or food contact materials, on Occupational Safety and Health (OSH) etc.)[7].

In addition, NGRAroute will cover both, human health and the environment.

3. The European Commission's roadmap for phasing out animal testing in chemical safety assessments

3.1. Background

On 25 July 2023, the European Commission (COM), in response to the European Citizens' Initiative (ECI) "Save Cruelty-free Cosmetics - Commit to a Europe without Animal Testing", announced their intention to

> *"immediately launch the work to develop a roadmap that will outline milestones and specific actions, to be implemented in the short to longer term, to reduce animal testing and that would be prerequisites for a transition towards an animal-free regulatory system under relevant pieces of chemical legislation (e.g. REACH, Biocidal Product Regulation, Plant Protection Products Regulation and human and veterinary medicines)."* (European Commission, 2023)

Although the primary goal of the COM's roadmap will be the phasing out of animal testing in chemical safety assessments and not necessarily the establishment of a comprehensive NGRA framework like under NGRAroute, both activities obviously overlap to a very large degree.

For this reason, it was decided that all future NGRAroute activities should best be performed under the umbrella of, and in close co-operation with, the COM roadmap activity.

3.2. First roadmap workshop (Brussels, 11/12 December 2023)

As a first milestone of joint action, the COM, the European Agencies, the European Partnership for Alternative Approaches to Animal Testing (EPAA) and PARC Task 2.2 jointly organised the "Workshop on the Commission roadmap towards phasing out animal testing for chemical safety assessments"

[5] Acc. to OECD, *"A defined approach to testing and assessment consists of a fixed data interpretation procedure (DIP) applied to data generated with a defined set of information sources to derive a result that can either be used on its own, or together with other information sources within an IATA, to satisfy a specific regulatory need." where* "Integrated Approaches to Testing and Assessment (IATA) combine multiple sources of information to conclude on the toxicity of chemicals. These approaches are developed to address a specific regulatory scenario or decision context."

[6] Regulation (EC) 1272/2008 on classification, labelling and packaging of chemical substances and mixtures

[7] Cf. also section 4.2. It is noted that the pharmaceutical sector is not well represented in PARC. It is, however, part of the work under the EU Commission's roadmap for phasing out animal testing in chemical safety assessments (cf. section 3) by which most of the further work of NGRAroute will be determined.

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which took place on 11 and 12 December 2023 in Brussels and online (European Commission and Cronin, 2024) [8].

During the afternoon of the second workshop day, Task 2.2 organised two sessions on the proposed guiding principles and work streams. Lively and controversial discussions took place among ca. 70 participants in the room, representing a broad variety of stakeholders from academia, industry, EU agencies, the COM and EU Member State authorities. Moreover, anonymous written comments from over 300 online participants were collected via the online tool Slido.

After the workshop, a document [9] summarising the guiding principles as well as the proposed work streams and related preliminary conclusions for the roadmap work was shared with workshop participants as well as additional stakeholders along with a commenting table to provide further feedback.

Comments on these documents were received from a broad range of stakeholders (cf. Acknowledgments above). Taking the Slido and the post-workshop feedback together, more than 1 500 comments were received, confirming the great interest of all involved stakeholders in the roadmap work, as well as their interest to be involved in this activity.

With respect to the latter, the PARC Task 2.2 organisers also asked workshop participants about their willingness to engage in the further roadmap work and, if so, in which work stream (cf. section 4.5) and role. A tabular overview of these (non-binding) commitments is provided in Annex I of this document and, once again, reflects the broad interest across stakeholder groups to become a part of this activity.

The guiding principles, work streams and tasks, in their revised form, also formed the basis for the first draft of the present document, which was again shared [10] with a broad range of stakeholders for comments in July 2024 (and a total of 641 comments were received and addressed by 31 August 2024).

The next workshop on the roadmap for phasing out animal testing in chemical safety assessments has been announced for 25 October 2024.

3.3. NGO-initiated roundtable discussion (Brussels, 18 June 2024)

Organised by NGOs ECEAE, HSI, Cruelty Free International, Eurogroup for Animals and PSCI, a multi-stakeholder roundtable discussion with participants from the COM, EU agencies, EU Member State authorities, academia and industry took place in Brussels on 18 June 2024 to support the COM roadmap, aiming to:

> "*(1) Explore and define key elements of the roadmap and discuss the work streams which will help to build it; (2) Establish key steps and goals for enabling the transition to a regulatory framework without testing on animals. (3) Consideration of a long-term structure for phasing out animal testing.*" [11]

PARC Task 2.2 was present with two participants from BfR and EAA, as well as a third, co-operating participant from PARC WP 6 from KEMI. Recommendations from the roundtable are available in Anonymous (2024).

[8] The presentations given at the workshop are available at https://single-market-economy.ec.europa.eu/events/commission-roadmap-phasing-out-animal-testing-chemical-safety-assessments-2023-12-11_en (accessed 2024-10-04)

[9] https://www.parcopedia.eu/wp-content/uploads/2023/12/20231218_NGRAroute_principles_and_work_streams.zip (accessed 2024-10-04)

[10] https://www.parcopedia.eu/20240709_parc_ad_2-1_files_for_commenting (accessed 2024-01-04)

[11] from the thought starter document prepared by the organising NGOs together with an advisory board featuring some of the roundtable participants, including BfR for PARC Task 2.2

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3.4. Commission roadmap working groups

Since the workshop in December 2023, the COM has held a number of interdepartmental meetings to discuss the terminology and structure of the roadmap for phasing out animal testing in chemical safety assessments.

The anticipated organisational structure obtained as a result of these discussions is shown in Figure 1. Specifically, a Commission interservice group (ISG) consisting of all relevant Commission services (DGs GROW, ENV, JRC and SANTE) and agencies active in the field (i.e. ECHA, EFSA and EMA) will supervise the roadmap development. They will be supported by three working groups (WGs), one each on human health (HH), environmental safety assessment (ESA) and change management, which will receive input from a broad range of stakeholder groups, with a strong role foreseen for the two Partnerships EPAA and PARC. Working groups may meet in closed (COM and agencies only) or open sessions.

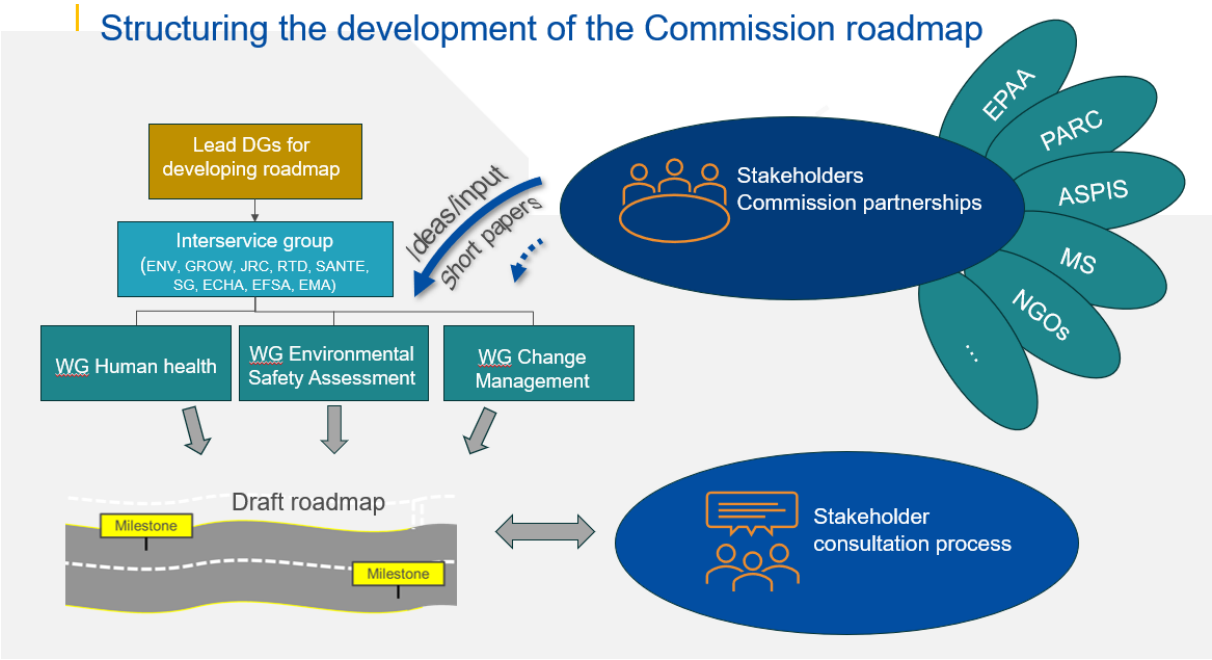

Figure 1: Organisational structure foreseen by the European Commission for preparing the roadmap for phasing out animal testing in chemical safety assessments, source: European Commission (2024b)

At the time of writing of this Deliverable, all three WGs [12] have met for the first time: ESA on 6 June 2024 (with PARC Task 2.2 represented by EAA and UBA), HH on 20 June 2024 (with PARC represented by BfR) and change management on 21 June 2024 (in a closed meeting, PARC Task 2.2 will be invited to future meetings, likely be represented by the University of Birmingham (UoB)).

During their inaugural meetings, the tasks of the HH and ESA WGs have been characterised by the COM as follows [13]:

- making proposals for specific actions and defined milestones to replace animal testing for HH and environmental assessments:
- propose short-term solutions for replacing (or reducing) animal testing
- identify longer-term solutions/methods or approaches that should be further developed:
    - recommend action to (further) develop a new method / expand applicability domain
    - recommend validation

[12] The number and scope of the WGs might be revised in the future depending on the needs.

[13] based on the inaugural presentation by the COM in the WG HH on 20 June 2024

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- propose to use several methods in integrated approaches to testing and assessment (IATAs) or defined approaches (DAs) for receiving information
- define how to use toxicokinetic & toxicodynamic properties as the basis for decision-making instead of adversity observed *in vivo*

- make proposals for switching to a regulatory system without the use of animals, considering:
  - what are we achieving with the current regulatory system: - what is the protection level now?
  - what do we want to achieve with a new regulatory system - what are the protection goals?
  - how to reach those protection goals with non-animal approaches - which information will they need to provide?

- propose criteria (in general or per area of concern), which new methods should fulfil for regulatory use (e.g. for classification)
- make proposals for validation of specific methods; prioritise methods for validation.
- collect data on methods, e.g. domain of applicability, method performance, but also on costs and laboratory capacity, and need of expertise
- consider the possibility to use information on exposure to reduce animal testing, e.g. in the sense of exposure-based waiving.

Moreover, and with particular relevance to this Deliverable, the WGs are tasked with making

> *"proposals for switching to a regulatory system without the use of animals, considering: a. what we are achieving with the current regulatory system – what is the protection level now?, b. what we want to achieve with a new regulatory system – what are the protection goals? c. how to reach those protection goals with non-animal approaches– which information will they need to provide?"* [12]

Furthermore, future action points have been subdivided into three subcategories, i.e. 1. options for short-term actions, 2. replacement of existing methods after further method development and 3. transition to an animal-free regulatory system (cf. Figure 2). In particular, the last point will be a key area of contribution for NGRAroute, as further explained in the subsequent sections.

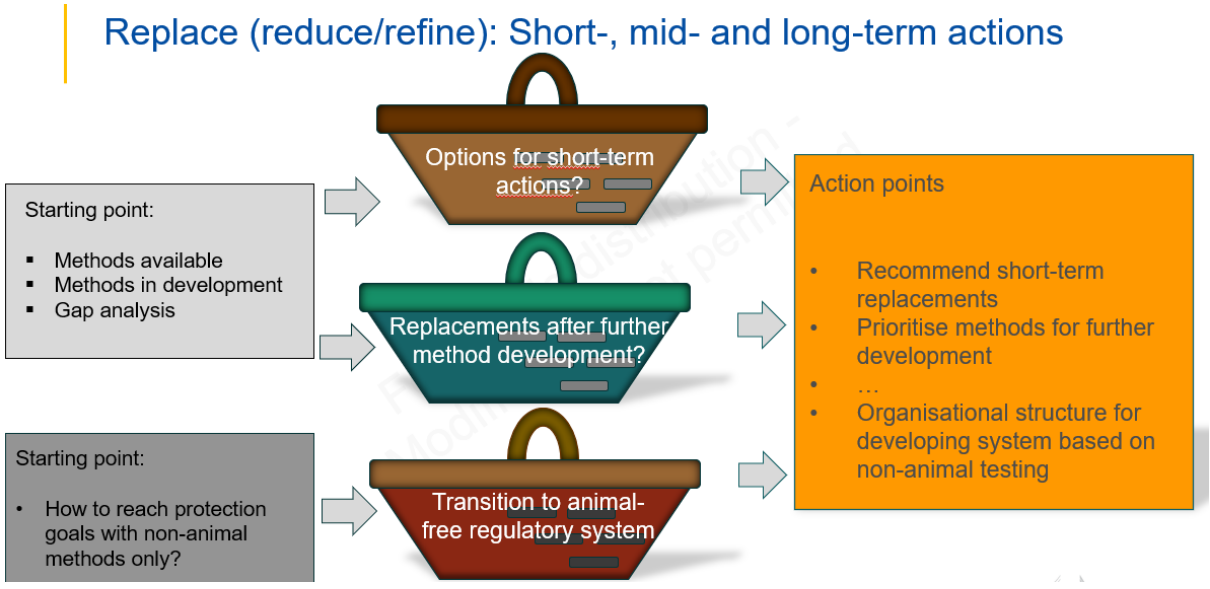

Figure 2: Action points for the Working Groups under the EU Commission's roadmap for phasing out animal testing in chemical safety assessments, source: European Commission (2024a)

3.5. European test method and validation strategy

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In response to a proposal by the Dutch and German Competent Authorities for the 52nd meeting of the Competent Authorities on REACH and CLP (CARACAL-52), asking for the installation of a CARACAL sub-group on test methods, the COM has outlined the connections between several recent activities (such as work in the One-Substance-One-Assessment working group, on the COM's roadmap for phasing out animal testing in chemical safety assessments, at the OECD, under PARC WP 2 / Task 2.2, PARC WP 5 or the ASPIS cluster) aimed at fostering the uptake of new methodologies into chemical risk assessment practice (European Commission, 2024b).

Specifically, in this document the Commission has welcomed the DE/NL initiative in general (while sceptical about the placement of such a group under CARACAL) and stated to be

> "*… ready to study all options for developing a European Test Method and Validation Strategy* […]. *This will need to tie in with other ongoing work, in particular a commitment to outline the path to expand and accelerate the development, validation and implementation of non-animal methods as part of the roadmap towards phasing out animal testing, as well as ongoing initiatives within OECD and e.g. PARC. Furthermore, in the ongoing work of developing the roadmap, the Commission services already consider developing a European Test Method and Validation Strategy, which should take into account the elements raised in the DE/NL paper and the need to adapt testing strategies, as well as test method development and validation of non-animal approaches*".

In the same document, also the NGRAroute activity is specifically highlighted, alongside the validation-related activities in PARC WP 5 (hazard assessment):

> "*A key objective of PARC is to develop next-generation chemical risk assessment to protect human health and the environment. It supports the European Union's Chemicals Strategy for Sustainability and the European Green Deal's "Zero pollution" ambition with new data, knowledge, methods and tools, expertise and networks. It aims specifically to facilitate and accelerate the acceptance of New Approach Methodologies developed in research in the regulatory practice. The PARC work package 2.2. is developing a Next Generation Risk Assessment Framework, which could play an important role in determining testing strategies based on non-animal approaches. Work package 5 is currently finalising a deliverable that describes validation-related activities within PARC to progress new methods and approaches for hazard and risk assessme*nt of chemicals."

The subsequent sections of this Deliverable will provide further background to this statement.

4. Towards a unified NGRA framework for EU chemicals legislation

4.1. Why a (unified) NGRA framework?

As explained above, the term NGRA (as interpreted in this document) refers to the conceptual/framework level rather than to individual methodologies. The current CRA frameworks feature a number of traditional concepts, e.g. substance-by-substance assessments or deterministic hazard/exposure/risk characterisation, as well as traditional test methods using experimental animals that have been challenged for various, including ethical, scientific and performance-related, reasons.

To phase out animal testing from CRA, as desired by the CON roadmap, there are in principle two strategies:

1. The CRA framework as such is basically maintained, but the (animal-based) test methodology is - one by one - replaced with animal-free NAMs.

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2. A new ("next-generation") CRA framework is introduced.

Regarding strategy 1., ECHA has recently noted that

> *"until recently, NAMs development aimed to fully replace animal testing for each specific regulatory endpoint. These developments have been successful for some relatively simple endpoints (like skin sensitisation), where the adverse effect and the mechanism(s) leading to this effect are relatively well understood. Development of NAMs for more complex endpoints has so far been less successful.*
>
> *By now, the scientific community and regulators widely accept that it would be almost impossible to develop one-to-one replacements of animal tests by NAMs for more complex endpoints such as e.g., repeated dose toxicity or reproductive/ developmental toxicity."* (ECHA, 2024)

As a consequence, and in line with strategy 2., it has become increasingly clear that not only the methods, but also the CRA frameworks themselves need to be adapted to accommodate new paradigms and concepts and to respond to regulatory challenges not well addressed by the current CRA frameworks, such as the efficient assessment and management of a huge number of chemicals on the market, possible combination effects, new types of chemicals and materials or chemical effects on biodiversity.

In addition, the development of a new, unified NGRA framework also offers the chance to better integrate human health and environmental CRA workflows across the diverse chemical sectors, resulting in a more holistic approach in the spirit of the "One Substance, One Assessment" philosophy formulated inter alia in the CSS (European Commission, 2020).

Changing the current CRA framework, however, comes at a price, as it will trigger the need for deeper changes in the current chemicals legislation and the accompanying guideline and guidance documents, compared to a one-by-one test method replacement strategy.

The necessary first step in this direction consists of elaborating the conceptual details of a new unified NGRA framework, to better understand where (and which) legislative and other fundamental conceptual changes are needed.

The rest of this section summarises the progress in this direction made so far under Task 2.2:

- Section 4.2 contains some further reflections on the potential for the harmonisation of CRA across chemical sectors and problem formulations, followed by the example of an NGRA framework currently developed under the ASPIS research cluster (in collaboration with partners in PARC task 2.2 and WPs 5 and 6) in section 4.3.

- Section 4.4 formulates ten guiding principles for the further development of a unified NGRA framework, while section 4.5 delineates four work streams that might be helpful to consider for the further work on the COM roadmap.

- Finally, sections 4.6 to 4.15 discuss the guiding principles and their ramifications in more detail, along with consequences for the proposed work streams, before the section closes with an outlook on the next steps under NGRAroute and the COM roadmap for phasing out animal testing in chemical safety assessments.

4.2. Chemical risk assessment workflows

A large variety of overarching as well as sector-specific EU chemicals legislation is in place and covers a wide range of different chemicals, uses and exposures. A list of the sectors in scope of the COM roadmap comprises:

- *"chemicals registered under the REACH Regulation;*
- *biocides;*
- *pesticides;*

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- *food improvement agents (food additives, food enzymes and food flavourings);*
- *chemicals used in food contact materials;*
- *feed additives;*
- *human medicinal products;*
- *veterinary medicinal products and MRLs for active substances in veterinary medicinal products for food- producing animals;*
- *medical devices;*
- *chemicals used in materials/products in contact with drinking water;*
- *chemicals covered by the CAD and CMRD [14];*
- *chemicals used in human nutrition;*
- *detergents;*
- *classification, labelling and packaging of chemicals; and*
- *water and waste legislation (identification of priority substances).”* [15]

Although not explicitly mentioned in the COM’s list above, further sectors, e.g. contaminants in the food chain, might in the end be affected, cf. also the report “Mapping of Data Requirements and Assessment Methodologies Linked to the Regulatory Frameworks and Remits of the Relevant EU Agencies (ECHA, EFSA and EMA) and EC Scientific Committees (SCCS and SCHEER)” by Oltmanns et al. (2023).

While CRA under some of the sectorial regulations may have to address issues specific for that sector (e.g. certain unique exposure scenarios), in general only a limited set of basic CRA workflows will be used across all sectors in the vast majority of cases:

- determination of the fate of a chemical in the human body (absorption, distribution, metabolism, excretion, bioaccumulation) or the environment (biodegradation, abiotic degradation, persistence, mobility etc.);
- hazard identification/classification and labelling;
- hazard characterisation, i.e. identification of points of departure (PoDs) for risk assessment, derivation of safe exposure levels (health-based or environmental guidance values environmental quality standards, MRLs), including extrapolation across species, duration, and routes;
- exposure assessment (estimation of migration from products, single-source or aggregate internal human exposure in serum/plasma or in target tissues, in environmental compartments etc.);
- screening/prioritisation of substance of high concern;
- full-scale risk characterisation / exposure-driven safety assessment.

The notion of these commonalities has in fact been a major driver for the inclusion of the “one substance, one assessment” (OSOA, 1S1A) principle as a prominent goal into the CSS.

Under the current CRA system, each of the above workflows is designed to provide specific, “tangible” outputs needed to address the CRA problem at hand. Examples of such outputs from typical human health CRA workflows are shown in Figure 3.

[14] Chemical Agents Directive/Carcinogens, Mutagens or Reprotoxic Substances Directive

[15] as presented by the COM during the multi-stakeholder roundtable discussion on 18 June 2024

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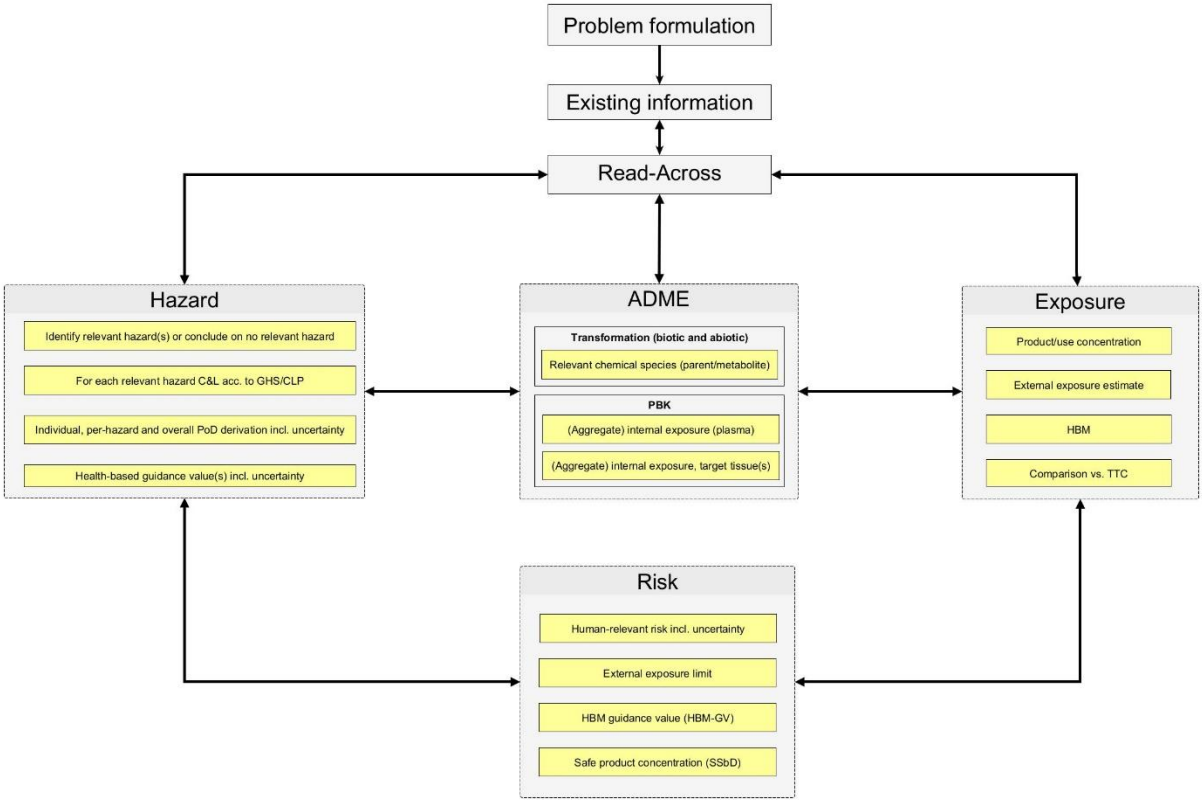

Figure 3: Overview of human health CRA workflows with typical outputs required for the further assessment

It is noted that a comparable scheme for environmental risk assessment workflows has not yet been developed under PARC Task 2.2. Moreover, Figure 3 is a simplified representation blending out additional layers of complexity, such as tiered or iterative testing/assessment schemes, probabilistic risk assessment, or cumulative/mixture risk assessments.

Still, Figure 3 covers most, if not all of the HH-related workflows listed above as well as their typical outputs. In addition, these workflows build on another. Therefore, a future unified NGRA framework should in the end be able to integrate all relevant human health HH and ENV workflows into a modular scheme in which some of the modules may or may not apply, depending on the specific CRA problem formulation at hand.

Understanding the outputs required by CRA is also an essential prerequisite for creating such a future NGRA framework; at least for the foreseeable future, these outputs, e.g. an estimate of internal exposure, a safe dose or a measure of risk for humans/the environment, will still have to be delivered, albeit with different methods/approaches/strategies. In addition, new workflows/outputs (such as the identification of safe-and-sustainable-by-design (SSbD) chemicals, the impact of chemicals on biodiversity, greenhouse effect or acidification) may eventually be added.

4.3. The ASPIS-initiated Safety Profiling Algorithm (ASPA)

The ASPIS cluster, and specifically the ASPIS cluster project RISK-HUNT3R, is currently developing the so-called "ASPIS-initiated Safety Profiling Algorithm", or ASPA. The ASPA attempts to lay out a modular NGRA framework as described in the previous section for addressing the outputs required by hazard/exposure/risk assessment, using only animal-free, NAM-based methodology. Depending on the specific problem formulation, only a subset of the boxes shown may apply.

While ASPA is still under construction, it is already clear that it can become of paramount relevance for the further development of the EU Commission's roadmap for phasing out animal testing in chemical safety assessments, because it may inter alia serve:

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- to demonstrate how a future animal-free NGRA framework can produce the same or equivalent outputs to those provided by the current, largely animal-based framework;
- to provide a concrete blueprint for an NGRA-based strategy to be incorporated into the legislation;
- to help identify, by means of dedicated case studies, knowledge or methodological gaps in the workflow, which can then be addressed in a targeted way by the COM working groups, and to support these groups in developing concrete decision criteria regarding the acceptability of NAM-generated outputs and associated uncertainties.

With this said, it needs to be noted that the ASPA's focus is currently placed on systemic toxicity to humans after repeated exposure. To become a universal NGRA framework template, further work is required regarding its expansion to other human health endpoints and on developing corresponding ERA modules that can be integrated with the human health part.

At the time of writing this Deliverable, a version 2.0 of the ASPA is under preparation. It is foreseen to be shared with a variety of stakeholders in Q4/2024, inter alia to stimulate further targeted case studies. The results from these new as well as other case studies that are currently ongoing (e.g. under RISK-HUNT*3R*) are planned to be discussed at a dedicated workshop currently foreseen for Q2/2025, with the aim to produce a third ASPA/NAMASTOX version to be rolled out in 2026.

Formulating NGRA frameworks such as the ASPA is an indispensable prerequisite for the further work on the COM roadmap: only if the details of such a framework have become sufficiently clear can the needs for changing the legislation and/or respective guideline and guidance documents be formulated.

4.4. Guiding principles for a unified NGRA framework

As mentioned above, PARC Task 2.2 has presented ten guiding principles for a future NGRA framework at the first roadmap workshop in December 2023. These principles were proposed as a suitable means to

- foster broad consensus on fundamental questions at an early developmental stage instead of getting into lengthy discussions on small details early on;
- define the political, scientific and regulatory boundaries of NGRA; thereby helping to
- structure the further roadmap work and provide a basis for focused topical discussions.

The original principles have been revised based on the comments received during and after the December 2023 workshop. This new version is provided in the box on the next page. Still, it represents a draft requiring further discussion.

It is noted that the principles describe an ideal CRA system, with some of them not well fulfilled by the current animal-based regime. In this regard, the principles may also be used to highlight possible gains to be made by switching from the current to an NGRA-based regulatory system.

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1. **PROTECTION**
   The framework allows to determine whether an *adequate*[16] *level of protection* for human health and the environment is attained.

2. **CONFIDENCE**
   The framework allows to determine whether an *adequate* [16] *level of confidence* is attained, especially when concluding on the absence of relevant hazard, exposure or risk.

3. **GENERATION OF INFORMATION**
   The framework takes into account *all relevant* [16] *existing information*. If additional information is required [16], it is generated *without in vivo testing* [17] *in sentient species* [18].

4. **SCIENCE**
   The framework uses *adequate* [16] *and reliable* [16] *modelling, testing and assessment methodology*, with *high scientific relevance* [16] to the protection of human health and the environment, representing the *current state of scientific knowledge*.

5. **EVIDENCE INTEGRATION**
   The framework can integrate *all relevant* [16] *lines of evidence* with *acceptable* [16] *reproducibility*.

6. **REGULATORY WORKFLOWS**
   The framework is applicable to *all relevant* [16] *chemical hazard, exposure and risk assessment workflows*.

7. **BIOLOGY**
   The framework covers *all relevant* [16] *(eco)toxicological pathways and endpoints*.

8. **EXPOSURE**
   The framework covers *all relevant* [16] *exposure levels, durations and routes*.

9. **CHEMISTRY**
   The framework is applicable to *substances*[19] *in their relevant* [16] *physical forms* [20]*, their transformation products, groups of substances, mixtures* [19] *and to substances in articles* [19].

10. **EFFICIENCY**
    The framework allows for assessments within an *acceptable* [16] *time- and cost-frame*. It includes *integrated testing and assessment approaches* that are *as complex as scientifically necessary*, but also *as simple and straightforward as possible*.

[16] As defined by the respective regulatory context and problem formulation

[17] Note that these principles describe the aspired final state of the framework. In line with the COM roadmap for phasing out animal testing in chemical safety assessments, an animal-free risk assessment framework marks the ultimate goal, notwithstanding that, during the transition to NGRA, *in vivo* testing might still continue to be performed.

[18] It is acknowledged that the question which species can be considered "non-sentient" e.g. fruit flies, water fleas, round worms and embryos of zebrafish and frogs as explored by the ASPIS cluster project PrecisionTox is the subject of an ongoing scientific debate beyond the scope of this report. The term is nevertheless used here to clarify that new *in vivo* testing in species that have been identified as "sentient" should not be performed under next-generation chemical risk assessment.

[19] as defined by Art. 3 of the REACH Regulation

[20] e.g. ionic speciation, nano or fibre form, also including other "advanced materials"

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4.5. Work streams and tasks

In order to conceptually structure and focus the future work for developing NGRAroute, and to allow for the effective sharing of workloads and responsibilities, the following four work streams for the further roadmap work are proposed:

- scientific development;
- regulatory implementation;
- policy implementation and
- change management.

Notably, these work streams are not meant to build an organisational structure alternative to that chosen by the COM (Figure 1). Rather, the first three (scientific development, regulatory implementation and policy implementation) can be seen as delineating "sub-streams" for the HH and ESA WGs, whereas the proposed work stream "change management" defines more closely tasks and topics for the WG of the same name.

Moreover, there is significant overlap between the individual work streams, which requires good overall co-operation and co-ordination, not least on the project management level. Despite these commonalities, and while all work streams share common elements (network building, framework development and conceptual work), there are also differences within these elements which merit a differentiated discussion as shown in more detail in Table 2 (next page) and further elaborated on in subsequent sections in relation to the guiding principles presented in section 4.4.

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Table 2: Overview of specificities of the proposed work streams

	Scientific development	**Regulatory implementation**	**Policy implementation**	**Change management**
Network building	– NG(E)RA-relevant projects/activities in Europe and worldwide – Key players in NAM/NG(E)RA research (academic and industry as well as regulatory authorities involved in research) – EU and MS authorities – End users (industry, contract research organisations, CROs) – Research-/science-oriented industry and NGOs	– EU and MS agencies, – Key regulatory players/bodies (e.g. SCCS, RAC, BPC, ED EG, EFSA panels etc.), also inter-/supranational (e.g. OECD, regulatory agencies from outside of Europe – Working group on OSOA ("One substance, One Assessment") – Regulatory industry associations, NGOs (regulatory focus) – End users (industry, CROs)	– Key relevant risk managers and policy makers and relevant bodies, – COM, EU and MS agencies/ministries – Consumer, worker/employee and environmental protection organisations, NGOs and worker/employee organisations (e.g. ETUC) and industry associations active in the field of chemicals policy, academic institutions with legal/policy focus	– Social scientists, experts on transitional governance, psychologists, opinion leaders in key institutions/bodies including industry and authorities (incl. enforcement), legal experts – Identify key regulatory and political decision-makers/-making bodies and develop targeted communication strategies, highlighting ethical, scientific, economic and other benefits – Academic institutions with knowledge in transformation management – Involve civil society multiplicators – Communication specialists
Research and conceptual work, frameworks	– Map state of the science/gaps, – Develop plan for necessary method development – NGRA framework incl. specific topics, cf. sections 4.6 - 4.15	– Define and characterise regulatory workflows and required outputs in all relevant legislations, e.g. using Oltmanns et al. (2023) as starting point – Characterise relevance and reproducibility of current i*n vivo* test designs for comparison – Regulatory implementation framework incl. specific topics, cf. sections 4.6 - 4.15 – Case studies, e.g. comparing (NG)RA for data-poor vs. -rich substances – Acceptance/validation criteria	– Characterise relevant global (e.g. SAICM), EU (e.g. CSS) and national legislation/policies with respect to NAM/NG(E)RA readiness – Identify knowledge/data/legislative gaps, develop remediation strategy – Mapping of hazard/exposure/risk assessment workflows found in all relevant EU legislations with relevance to the work stream's tasks as well as existing concepts related to the topics listed in sections 4.6 - 4.15 relevant for the roadmap. – Holistic risk management framework incl. specific topics, cf. sections 4.6 - 4.15	– Analyse social, practical and legislative barriers to overcome, required psychological/mindset changes – Training and framing/communication strategies towards key risk assessment and management bodies about limitations of current and chances of new paradigm – Identify economic incentives and compensation for those negatively affected by the required changes – Specific topics, cf. sections 4.6 - 4.15

4.6. Protection

Guiding Principle 1 demands that a future NGRA framework should allow to determine whether an "adequate level of protection" (as defined by the regulatory context and problem formulation) for human health and the environment is attained.

Before discussing specific consequences for the scientific development aspect of the further roadmap work, some general remarks on the matter seem warranted. It is noted that the following deliberations mostly apply to the area of human health risk assessment, and a deeper analysis with respect to ERA is needed.

One of the most frequently heard requirements for NGRA is that, compared to the current system, it needs to provide "the same or a higher level of protection for human health and the environment".

Risk can be defined as the product of the degree of negative impact of an unwanted event or consequence, multiplied with its likelihood of occurrence. At the same time, it is known from everyday experience that there cannot be zero risk in life.

As a consequence, all risk management, whether of chemicals or in other societal areas, can only aim to define (whether im- or explicitly) and ensure a sufficiently low likelihood that people or the environment are experiencing a negative impact of unacceptable degree, while an attempt towards total (100 %) risk aversion is not only futile, but may lead to other severe and unwanted societal consequences.

An "adequate level of protection" (as in Guiding Principle 1 above) can then be defined in quantitative terms as a risk level for which the product of severity of effect and likelihood of occurrence is acceptable, or at least tolerable, from a societal risk-benefit perspective.

Obviously, the quantitative benchmarks for "sufficiently low", "acceptable" or "tolerable" need to be defined on the policy/risk management level. Moreover, transparency about their underlying rationale may enhance societal acceptance of the resulting risk management measures.

Criteria for demonstrating that these benchmarks have been attained need to be provided on the regulatory level. It is then the task of the scientific community to develop and propose methods and workflows within an NGRA framework by means of which it can be demonstrated that these criteria can be met.

However, with respect to the current, mostly animal-based human health CRA framework, Chiu & Paoli have noted that

> *"Paradoxically, risk assessments for the majority of chemicals lack any quantitative characterization as to the likelihood, incidence, or severity of the risks involved"* (Chiu and Paoli, 2021).

As a consequence, any future NGRA framework is challenged with the "mission impossible" to prove that it lives up to a level of protection that currently is mostly not characterised explicitly for individual assessments, but rather defines safety in an indirect way, e.g. by absence of relevant effects in a set of standard tests or by ensuring, via monitoring programmes, that exposure of the human population or the environment is not exceeding "safe" levels.

With one of the world's most sophisticated chemical management systems in place in the EU, it is "safe" to say that the overall protection level ensured by the current CRA framework is in general very high by comparison to the situation in many other regions of the world. However, this generic statement is of little help for assessing the level of protection present when dealing with a specific scenario of exposure to hazardous chemicals.

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Since the current risk assessment framework does not offer a benchmark protection level to which an NGRA result could be compared, there are basically two strategies for demonstrating that an adequate protection level has been reached:

a) Comparison with existing assessments

Assuming that the protection level provided by the current CRA framework is "in general very high", a defensible scientific strategy can be to demonstrate that on average, over a large number of cases with sufficient coverage of chemical and biological space, the output from the NGRA framework coincides with that from the current one, e.g. in the form of a hazard classification result, point of departure (PoD) for further risk assessment, Predicted No-Effect Concentration (PNEC) in the environment or Health-Based Guidance Value (HBGV),

On the positive side, this approach is practically feasible, cf. (Paul Friedman et al., 2019) or (Lu et al., 2024). Nevertheless, two problems are associated with it:

i.) As in the current system, this strategy is not helpful when trying to assess the level of protection provided by a specific individual assessment (e.g. in terms of which percentage of an exposed population is protected from which severity of effect and likelihood of occurrence).

ii.) Possible flaws of the previous system, e.g. the deterministic combination of possibly overconservative worst-case assumptions or animal models with poor predictivity for the target species of the assessment, are potentially propagated.

b) Probabilistic risk assessment

The protection level provided by an individual assessment can be characterised using probabilistic assessment methodologies. Maertens and co-workers have recently described the benefits of such approaches over current "deterministic" [21] risk assessment outputs (Maertens et al., 2024; Maertens et al., 2022). It may also be argued that probabilistic methods more adequately reflect the reality of potential chemical effects on populations. A conceptual framework has been provided by Chiu and Slob (2015) and further elaborated on by WHO IPCS (2018), who also developed a tool for practical application of "Approximate Probabilistic" risk characterisation, APROBA(-Plus) [22].

However, experience with the practical application of such methodology is currently limited (cf. e.g. EFSA (2019) or BfR (2023)) and required input data may be lacking or difficult to access (however, NAMs, and in particular high-throughput methods, may be particularly suited to generate such data, since e.g. more concentrations or replicates could be tested at lower cost compared to adding further animals. In addition, NAMs can be utilised to assess e.g. intraspecies variability (Rusyn et al., 2022; Zeise et al., 2013)

Table 3 summarises specific tasks regarding Guiding Principle 1 which should be addressed by the four proposed work streams in the course of the further roadmap work.

[21] In this context, deterministic refers to the unambiguous relationship between e.g. an HBGV, given as a single number, and the protection level of an exposed individual. In contrast, a probabilistic risk assessment describes this relationship in terms of a probability distribution, bearing witness to the fact that e.g. different individuals may possess different susceptibility to a chemical's adverse effect.

[22] https://www.rivm.nl/en/aproba-plus (accessed 2024-10-04)

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Table 3: Tasks for the proposed work streams regarding Guiding Principle 1 (protection level)

Work stream	Specific tasks
Scientific development	– Assess how effect markers based on early mechanistic key events qualitatively and quantitatively relate to actual adverse effects in humans or the environment as well as to the apical adverse outcomes observed in present-day test systems. – Describe the current state of science with respect to probabilistic hazard, exposure and/or risk assessment. and analyse which knowledge gaps, if any, need to be filled by further research. Focus on both, human health and environmental risk assessment. – Assess how probabilistic risk assessment methodology can be integrated into a future NGRA framework. – Analyse which input data are required for probabilistic risk assessment and, if not already available, how such data can be generated and made publicly available. Discuss the potential use and pitfalls of Artificial Intelligence (AI) in this context. For ERA, explore novel methods to analyse available data based on AI, deep learning to cover possible effects of a broader range of species and possibly identify overlooked hazards and risks. – For the environment, synergise with relevant external initiatives and leverage on knowledge being built within PARC, other Horizon Europe projects, Biodiversa+ and beyond to benchmark protection level of current and future NG-(E)RA framework.
Regulatory implementation	– Analyse how consistency between assessments with respect to protection levels across different classes of substances within and across regulatory sectors can be improved/safeguarded (both under the current and a future NGRA paradigm), e.g. by standardisation and harmonisation of testing and assessment strategies, but also by stimulating cross-communication. – Define benchmarks for assessing the achieved protection level (as defined by the policy level), i.e. how to demonstrate that the aspired protection level has been met.
Policy implementation	– Specify the desired protection level more explicitly in the legislation or associated guidance, ideally in a goal-oriented, method-independent manner. – To that end, perform deeper risk-benefit analyses, develop, over time, a holistic scenario where protection of human health and the environment is defined in an integrated fashion and across all sectors of chemicals legislation.
Change management	– Review public communication about protection levels and goals. – Capacity building towards probabilistic understanding of risk among CRA recipients (risk managers, policy makers, media, general population). – Develop strategies to increase realistic risk perception (i.e. safety ≠ zero risk) and to manage expectations regarding protection level in the general public. – Efforts towards the "One Substance, One Assessment" (OSOA) ideal are needed to accomplish a holistic approach across all sectors of chemicals legislation, with strong engagement of the European agencies EMA, EFSA and ECHA to support this activity. This will require a huge mindset change effort in the various different regulatory silos. – Particularly address industry to get support for specific changes in legislations even if data needs to be disclosed, more work, the generation of more data or more stringent assessments are to be expected.

4.7. Confidence

Principle 2 demands that a future NGRA framework should allow to determine whether an adequate level of confidence (as defined by the problem formulation and regulatory context) is attained, especially when concluding on the absence of relevant hazard, exposure or risk.

How to ensure trust in new scientific methods and their results in general, e.g. by validation, is addressed in section 4.7. While a necessary prerequisite, this trust alone, however, does not help when trying to assess the uncertainties associated with a specific assessment outcome.

All methodology used in the context of CRA either consists of models (*in silico*, *in vitro*, *in vivo*) or measurements (subject to experimental error) with limited sample size. Both are then used to make inferences about larger collectives (of chemicals, populations or species).

Without exception, therefore, all CRA results are associated with uncertainty. The latter has been defined by WHO IPCS as

> "*imperfect knowledge concerning the present or future state of an organism, system, or (sub)population under consideration.*" (WHO IPCS, 2004)

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Acc. to the WHO IPCS guidance document on characterising uncertainty in hazard characterisations,

> "*it can be further defined as lack of knowledge regarding the "true" value of a quantity, lack of knowledge regarding which of several alternative model representations best describes a system of interest, or lack of knowledge regarding which probability distribution function and its specification should represent a quantity of interest.*" (WHO IPCS, 2018)

In contrast, acc. to the same document, variability marks the

> "*heterogeneity of values over time, space or different members of a population, including stochastic variability and controllable variability. Variability implies real differences among members of that population. For example, different individual persons have different intake and susceptibility. In relation to human exposure assessment, differences over time for a given individual are referred to as intraindividual variability; differences over members of a population at a given time are referred to as interindividual variability.*" (WHO IPCS, 2018)

Biological variability is a reality that may be measured and – e.g. in terms of experimental settings – be partly controlled, but not changed. In contrast, uncertainties (e.g. about the variability of the response of a given population towards a chemical stressor) may in principle be remediated by generation of further knowledge, e.g. by applying a refined assessment strategy or by generating additional data.

However, under the current CRA framework, assessment outcomes are mostly only reported in a deterministic way[23], without a transparent, quantitative statement regarding the associated uncertainties. This is often compensated by using what are supposed to be worst-case and/or justified generic assumptions, the combination of which, however, may result in highly (and potentially over-)conservative assessments[24]. Notably, such (over-)conservatism may not be a problem or even be desired in certain cases, but needs to be made at least transparent to avoid misinformed risk management decisions.

An additional, undesirable consequence of this practice is that different risk assessments may differ vastly with respect to their inherent uncertainties rendering their results incomparable.

A non-exhaustive list of elements in current CRA typically associated with uncertainties includes e.g.

- the outcome of dose-response analyses,
- extrapolations across species, exposure routes or durations,
- assumed use patterns for chemicals/products or
- the variability with respect to susceptibility towards a certain chemical's toxicity within the target population or between target species,
- exposure assessments, as use tonnages, uses, associated use patterns, emissions, fate & leaching potential are often no/ rarely published or known.

In NGRA, some of these aspects, e.g. the extrapolation across species in human health assessments, will become less relevant. However, additional uncertainties will have to be considered, e.g. in relation to extrapolating the results from *in vitro* experiments to the *in vivo* situation (*in-vitro*-to-*in-vivo* extrapolation, IVIVE).

[23] For example, a health-based guidance value (HBGV, single number) is compared to an exposure estimate (single number), resulting in a single number (e.g. the risk characterisation ratio, RCR = exposure/HBGV) to characterise a possible risk.

[24] For example, the likelihood that, for a given substance, the discrepancy between the sensitivity of rats vs. humans towards that chemical's toxicity is tenfold AND a person's individual susceptibility to the effect is again tenfold that of the population median AND exposure of that person is at the maximum level experienced in the population is extremely low, yet, such a combined assumption is frequently made in CRA.

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Qualitative statements to the end that uncertainties are present are of limited practical help in risk management and communication. The same holds for semi-quantitative statements that uncertainty was "low", "medium" or "high", as long as no exact boundaries between these classes are provided (but then these boundaries could also be stated directly). In addition, acceptance of an assessment outcome implies that, whatever the uncertainty, it was considered acceptable in the end.

The benefits of quantitative approaches to uncertainty assessment have been described by WHO IPCS:

> "*More transparently representing in quantitative form the confidence we can have in toxicological risk projections and estimates of the relationship between dose and health effect, thereby facilitating choices of preventive measures and/or further information gathering by risk managers. For instance, health-based guidance values* […] *may be defined based on a pre-specified and harmonized level of conservatism, or estimated health risks for a given exposure situation can be expressed in terms of an uncertainty distribution or a confidence interval.*" (WHO IPCS, 2018)

Another noteworthy aspect of uncertainty in the context of CRA is that it works in both directions, i.e. a statement on the risk posed by a certain exposure scenario may be uncertain regarding a potential under- as well as overestimation of hazard, exposure and/or risk.

The degree of uncertainty/confidence acceptable under a given problem formulation and regulatory context needs to be defined at the policy level and concrete benchmarks for acceptance need to be set at the regulatory level.

The ability to provide a transparent measure of the uncertainties inherent in a risk assessment would then also mark a superior quality of NGRA over the current CRA framework, where such a measure is often not available or provided. Where uncertainties/confidence cannot be sufficiently quantified, (semi-)qualitative methods may be applied, but this will result in additional uncertainty about the outcome of the assessment, which should be made transparent.

Table 4summarises specific tasks regarding Guiding Principle 2, which should be addressed by the four proposed work streams in the course of the further roadmap work.

Table 4: Tasks for the proposed work streams regarding Guiding Principle 2 (confidence level)

Work stream	Specific tasks
Scientific development	– Describe the uncertainties/factors determining confidence inherent in all steps of an NGRA-based assessment framework, – Provide robust and reliable, yet pragmatic, methodology to adequately determine them in a quantitative way as well as to compare them to the regulatory benchmarks, – Explore the use of probabilistic methodology in this context, including both, frequentist and Bayesian approaches.
Regulatory implementation	– Develop clear confidence benchmarks for both, the current and a future NGRA framework and implement in guidance documents.
Policy implementation	– Consider and interpret the precautionary principle in the context of required confidence levels.
Change management	– Develop communication strategy towards CRA recipients (risk managers, policy makers, media, general public) to enhance acceptance of uncertainties as a reality in chemical risk assessment. – Capacity building in the area of uncertainty/confidence/variability is strongly needed for ALL stakeholders.

4.8. Generation of information

Principle 3 requires that a future NGRA framework should take into account all relevant (as defined by the problem formulation and regulatory context) existing data. If additional information is required, as again determined by problem formulation and regulatory context, in the vision of NGRAroute (and the COM roadmap), it is generated without *in vivo* testing in sentient species.

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It was already noted above that during the time of transitioning to such a framework, it may still be necessary to use *in vivo* testing where the problem formulation cannot be adequately addressed by use of existing data or new testing using non-animal NAMs or in non-sentient species.

To ensure that the potential of any other approaches is explored before new testing in sentient animals *in vivo* is initiated as the last resort, a stepwise strategy should be followed which e.g. could look as follows [25]:

1. Assess and integrate all existing and relevant hazard and exposure data of sufficient quality, in order to identify any relevant knowledge gaps as well as any relevant regulatory concerns triggering the need for further data generation. In cases where biological mechanisms or pathways are conserved between humans and other species, it could also be justified – subject to a case-by-case analysis - to use existing data generated for environmental assessment in non-mammalian species to fill data gaps when evaluating human health (and similarly using mammalian, including human, data to support ERA).

   Already in the near future, machine-learning or other AI methods may dramatically improve the consideration of existing toxicity or exposure data (provided that the required high-quality curated datasets are generated and become publicly available).

2. Follow up on such gaps or triggers first by means of read-across from similar substances, where possible. [26]

3. Depending on the problem formulation and regulatory context, use filters based on physico-chemical, *in silico* models or ADME [27] properties to rule out relevant exposure, where possible (to be further explored), taking into account all potentially relevant chemical species (parent, metabolites, abiotic degradation products).

4. Next, if possible, perform a bioactivity screening step using *in chemico*, *in silico* or *in vitro* models to identify possible relevant pathways of biological perturbation. This bioactivity screen is meant as a gatekeeper to focus any further testing on any triggers that might have been detected during the previous steps and therefore, it

   - needs to have a high biological coverage (cf. section 4.12), to ensure that no relevant bioactivity is missed,

   - also needs to be "protective", i.e. it must have a high negative predictive value (likelihood that a negative outcome really is a negative), thereby providing high confidence when concluding that no relevant hazard/exposure/risk is present, and

   - should preferably also have a high positive predictive value to keep positive follow-up testing (see next steps) within reasonable limits (cf. also section 4.15).

[25] It is noted that the approach presented here is written from a human-health perspective. Also for ERA, new ways of tiered information generation e.g. based on monitoring data could be considered, but such work has not (yet) been undertaken under NGRAroute.

[26] Computational tools will also help to improve and streamline the assessment in the RA field. An example, already available today, is the generalised read-across framework (GenRA) as an algorithmic approach to read-across (Patlewicz and Shah, 2023).

[27] absorption, metabolism, distribution, excretion

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5. Where possible and appropriate, follow up on any triggers from the screening approach and/or remaining gaps by means of targeted *in vitro* testing or testing in non-sentient animals [28].

   In this case, the methodology used should be "predictive", i.e. it should have high predictive accuracy (i.e. a high fraction of correctly identified positive and negatives) regarding adverse outcomes for the species or population subject to the assessment. It should allow hazard classification, where required, cover all relevant mechanistical pathways and also consider the need for metabolic competence to account for relevant transformation products.

   It should also provide dose/concentration-response information to derive points of departure (PoDs) for further risk assessment, where this is required by the problem formulation and regulatory context.

6. (Only during the transition to NGRA:) Perform new *in vivo* testing in sentient animals after a case-by-case assessment concluding that the remaining knowledge gaps need to be closed because a) there is reason to assume that closing them will have a relevant impact on risk management, that b) alternative methodology is inadequate for addressing the knowledge gap and c) there is a legal requirement or other societal need to close the knowledge gap. If all of this is the case, the *in vivo* testing strategy pursued should minimise suffering and stress of the experimental animals to the maximum extent possible. It should also make best use of the animals, e.g. by adding relevant NAM components, such as omics (cf. concept of "TG+" studies, PARC (2023a))

It is noted that work on such stepwise approaches is already ongoing, e.g. in the form of the "ASPIS-initiated safety profiling algorithm" (ASPA, cf. section 4.3) and ASPIS-PrecisionTox case studies.

In the above concept, it is critical to define after each step whether the knowledge obtained is already sufficient to inform risk management (and therefore the assessment can stop). Non-animal methods should effectively be used to determine both the presence or absence of hazards, ultimately resulting in classification without testing on animals. For this, clear decision criteria are needed, in particular for reliably demonstrating "no relevant toxicity" (to be defined by the problem formulation and regulatory context).

While the knowledge required for the hazard/exposure/risk assessment as well as the potential areas of concern need to be defined *a priori*, and also a catalogue of established methods and models may be provided, the latter steps of the stepwise approach may also require case-by-case decisions which would need to be standardised as much as possible to avoid subjectivity/bias, unnecessarily lengthy assessments and delayed decisions due to the tiered approach. PARC Task 6.3 (review of risk assessment methodology) conducts projects that can provide insights on this matter.

Incorporating a stepwise approach such as the one described above would mean a major revision of legislative texts. One possible way to go about it might be to open the legislation for a stepwise assessment approach in general, and then define the details in associated soft law (guidance/guidelines which are not legally binding) over time. Since the fallback option would always be the traditional approach, the pace of the actual practical implementation of the stepwise approach would then follow scientific progress. The benefit would lie in the fact that soft law can be adapted to technical progress much faster than hard law (i.e. Regulations).

During the transition period to NGRA, *in vivo* testing will still be required for some time. In the current risk assessment framework, however, a catalogue of standard *in vivo* test requirements is often in place, while the likelihood that such tests will provide added value to risk management is not clear in an individual case. To better address this aspect already while *in vivo* testing cannot be completely

[28] see footnote 18 above

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replaced for certain areas of concern, criteria and methodology should be developed to determine the likelihood that additional *in vivo* testing in sentient animals will provide added value for risk management.

Table 4 summarises specific tasks regarding Guiding Principle 3 which should be addressed by the four proposed work streams in the course of the further roadmap work.

Table 5: Tasks for the proposed work streams regarding Guiding Principle 3 (generation of new data)

Work stream	Specific tasks
Scientific development	– Develop the stepwise assessment approach and map to all relevant regulatory workflows (cf. sections 4.1 and 4.3) one after the other, ie. develop a prioritisation scheme. – Develop methods to fulfil the requirements described in the text, in particular methods for bioactivity screening with high biological coverage and high negative predictive value (possible candidates could be transcriptomics/multiomics approaches or test batteries like ToxCast).
Regulatory implementation	– Explore whether early filters for the absence of relevant risk, such as the "Threshold of Toxicological Concern" (TTC) concept, or other criteria for "negligible" exposure, e.g. based on toxicokinetic considerations regarding internal exposure, could be accepted in certain situations. – Develop criteria for acceptance of "no relevant" or "negligible" toxicity/exposure/risk. – For each step of the stepwise approach, develop criteria for concluding that the knowledge is sufficient to terminate the assessment at this step. – Develop criteria and methodology to determine the likelihood that additional *in vivo* testing in sentient animals will provide added value for risk management.
Policy implementation	– Discuss how existing legislation could be gradually opened to the stepwise approach, taking into account all relevant legislations, regulatory silos and regulatory workflows, also keeping in mind enforceability. For this purpose, involve the OSOA WG and particularly target EU agencies EMA, EFSA and ECHA – Consider introducing an appropriate procedure and a competent committee to approve experiments on sentient animals (possibly together with the committee mentioned in Table 6), i.e. responsible for asserting confirming that these experiments are only used as the last resorts (the procedure could be similar to that for Testing Proposal Examination (TPE) under REACH, however with stronger requirements to check for alternative solutions). – Consider opening the current data protection on hazard and exposure data in chemicals law to make the data better and more easily usable for further risk assessments. – Review existing legislation and develop a work plan for how to revise it with respect to implementing the stepwise approach. – Devise work plan for implementing the stepwise approach.
Change management	– Initiate a broad and accessible training and capacity building programme over all compartments of the chemical risk assessment community. – Shifting from a highly standardised and harmonised approach to a more flexible and weight-of-evidence based workflows integrating diverse lines of evidence, potentially assisted by machine-learning and other AI tools, will be a major challenge especially for risk assessment authorities, but also in industry and contract research organisations, from the expertise and training of the scientific staff to investments into data infrastructure etc. The change management work stream should consider aiding such institutions in developing concrete transition plans, otherwise lack of structural adaptation could become a major obstacle for implementation of the paradigm change. – For industry and regulators, a change of mindset towards more flexibility is needed regarding regulatory expectation, when information requirements are updated more frequently. – Train/discuss with enforcers (e.g. ECHA, chemical inspectors?) how to enforce these new information requirements.

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4.9. Science

4.9.1. Developing new methods and building trust

Guiding Principle 4 calls on a future NGRA framework to use adequate and reliable modelling, testing and assessment methodology, with high scientific relevance to the protection of human health and the environment.

Validation [29] of individual models, test methods, DAs or IATAs is required to demonstrate their fitness for purpose. Cross-validation, reproducibility of results and comparison of performance of different methods are established building blocks to ensure scientific underpinning and confidence in risk assessments. Respective activities are ongoing e.g. at the level of the OECD, in particular in the expert group currently revising OECD Guidance Document 34 (OECD, 2005) and the future roadmap work needs to be co-ordinated with these and other international activities as much as possible.

On the other hand, traditional procedures for method validation have been criticised as slow and ineffective, potentially delaying the application of new, fit-for-purpose methods in CRA. Among other considerations, this has triggered a desire to develop qualification and method-readiness criteria (Bloch et al., 2024; Haase et al., 2024; Holzer et al., 2023), enabling the use of new methodologies in certain contexts even if they have not undergone all steps of a full validation protocol yet, e.g. as additional evidence within WoE schemes. Method qualification protocols have already been introduced in the pharmaceutical sector by authorities such as the European Medicines Agency (EMA) and the U.S. Food & Drug Administration (FDA) and their experience should factor into the further development of such protocols for other chemical sectors.

Moreover, test method validation today still relies strongly on reference substances, the bioactivity of which has been established using animal tests. Care must be taken to ensure that such substances indeed provide valid benchmarks for human-and environmental NAM development.

NGRA methodologies and procedures need to be transparently documented, to allow for reproducibility, independent review and appropriate interpretation of results, using established templates for easy access, such as the one published by Krebs and co-workers (Krebs et al., 2020; Krebs et al., 2019) or the templates developed in the context of OECD Guidance Document 34 and the OECD IATA case study [30] project. Moreover, the characterisation of new methodologies needs to include an adequate description of their domain of applicability in chemical as well as biological (both in terms of bioactivity and taxonomy) space.

Further aspects of creating trust in NAMs have been summarised e.g. by van der Zalm and co-workers in the context of presenting their "*framework for establishing scientific confidence in new approach methodologies*" (van der Zalm et al., 2022).

Mechanistic understanding will be key to relevance assessment in NGRA. For a full quantitative risk assessment, the relevance of the applied testing and assessment methods/strategies with respect to apical adverse outcomes for humans and the environment should (ideally) be established qualitatively and quantitatively. In this respect, particular weight should be placed on elucidating the predictivity of biomarkers indicative of early key events within an Adverse Outcome Pathway (AOP) for such apical adverse outcomes. Specifically, quantitative AOPs (qAOPs) (Cronin et al., 2022), quantitative Systems

[29] This term is used here in its pure sense, i.e. demonstrating that something is fit for its purpose, without prejudice to any specific procedure.

[30] https://www.oecd.org/en/topics/sub-issues/assessment-of-chemicals/integrated-approaches-to-testing-and-assessment.html (accessed 2024-09-04)

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Toxicology (qST) (Bloomingdale et al., 2017) and physiological maps [31] can become an important factor in building trust in quantitative in-vitro-to-in-vivo extrapolations (qIVIVE). In addition, the propagation of uncertainties in AOP-based assessments should be investigated in order to learn more how the uncertainties about each individual key event combine into an overall uncertainty of the KE-based prediction of the adverse outcome at the indivdual or population level.

Especially with respect to the latter, physiology-based kinetic (PBK) models play a central role in a future NGRA framework, as a means to relate nominal test concentrations to relevant human or environmental exposure concentrations and vice versa. Although great progress in the field has been made in recent years, research questions such as predicting achieved concentrations inside the cell from nominal concentrations in cell culture media or developing generic, yet robust PBK models that can be automatically parametrised with substance-specific data remain scientific challenges for the coming years. Again, robust quality criteria for acceptance and transparent documentation are important (Debad et al., 2024; Najjar et al., 2022; WHO IPCS, 2010).

Furthermore, trust in NAM models requires demonstration that the model reflects relevant aspects of the modelled organism(s)/population(s) that are subject of the assessment. *Inter alia*, this includes the presence of relevant mechanistic pathways and adequate metabolic competence. For example, interactions of different cell types are often not covered by simple (e.g. single cell-based) *in vitro* bioassays. To bridge this gap, where relevant to reflect a certain AOP, advanced cell culture systems and microphysiological systems (MPS) should be explored and developed further.

Another aspect requiring attention regards the time-dependence of toxicological effects. Exposure times in *in vitro* test systems are usually much shorter than in *in vivo* tests, which is an advantage in terms of efficiency, but may also pose problems, specifically when effects are to be predicted, which normally would require long-term exposure to develop in an intact organism *in vivo*. Here, trust needs to be built e.g. by means of case studies which compare new *in vitro* results with subchronic or chronic test results from the literature. The applicability of NGRA assessments to intermittent or infrequent exposure scenarios adds another layer of complexity. On the other hand, the current animal-based system usually covers such scenarios with subchronic or chronic studies, which may result in over-conservative assessments.

4.9.2. Assessments representing the current state of science

Guiding Principle 4 also demands that assessments under a future NGRA framework reflect the current state of science.

Under the present system, updates of e.g. the EU Test Method Regulation (Regulation (EC) 440/2009, TMR) or the UN GHS have often been slow and tedious. Therefore, information requirements in EU chemicals legislations should be revised in such a way that they can be updated more quickly in line with scientific progress. One way to achieve this could be to only lay down the general risk assessment framework (e.g. regarding the stepwise assessment and testing scheme, knowledge required in general and areas of potential concern) in hard law (legally binding legislation, such as REACH, Regulation (EC) 1107/2009 for Plant Protection Products (PPP) or Regulation (EC) (Biocidal Products Regulation, BPR); whereas specific information requirements should be moved outside of the core legislation, possibly into soft law (guidelines/guidance).

For example, information requirements in sectorial legislation could be rephrased to specify the inputs needed for risk assessment rather than specific test methods that have been approved. These methods could then be collected in a central place such as or similar to, the TMR, with a yearly update routine in place. This would have the additional benefit that acceptance of new methods could be ascertained

[31] https://ontox-project.eu/physiological-maps-2/ (accessed 2024-10-04)

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easily and more quickly across all regulatory sectors. Cf. also the SCCS Notes of Guidance under the CPR.

Furthermore, the installation of a permanent scientific committee in the EU should be considered, with the competence to decide on the acceptability of emerging NGRA concepts and methods for regulatory application and to provide corresponding recommendations to the policy level. Such a committee representing scientific experts from all relevant stakeholder groups (EU and MS authorities, industry, scientific NGOs) could be tasked with regularly updating the TMR (or whichever central place would be assigned) with approved methods, in close cooperation with other inter- and supranational bodies such as OECD, WHO or the UN level.

In addition, this committee could support registrants and regulatory agencies in dealing with topics related to the use of NAMs to avoid animal testing, such as testing proposals, review of waiver requests, and review of current practices to ensure they are in line with the last resort principle. Such a committee could provide independent advice and recommendations to foster and increase the use of non-animal methods by registrants.

Last, not least, programmes seem desirable for further enabling EU and Member State agencies to actively drive scientific progress and initiate (or even carry out) research to address open questions which they have identified (ECHA, 2024) as relevant for promoting the innovation of CRA themselves.

Table 6 summarises specific tasks regarding Guiding Principle 4 which should be addressed by the four proposed work streams in the course of the further roadmap work.

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Table 6: Tasks for the proposed work streams regarding Guiding Principle 4 (science)

Work stream	Specific tasks
Scientific development	– Develop NGRA framework (cf. ASPA) with respect to testing and assessment strategies, incl. aggregate, cumulative and mixture risk assessment, probabilistic risk assessment, for HH and ENV. – Develop methods to characterise the Applicability Domain of NAMs. – Develop methods to determine or model ADME parameters (for parents and metabolites), incl. PBK modelling. – Further develop (q)IVIVE, (q)AOP, qST methodology. – Identify knowledge/data gaps and develop a remediation strategy for closing them. – Organise the development of case studies to support the above developments.
Regulatory implementation	– Further develop, at international level (OECD), criteria and processes for method readiness, qualification and validation. – Review and, where necessary refine available guidance and templates for adequately documenting NAM-based chemical risk assessments (method documentation, data reporting and interpretation) in a standardised, ideally machine-readable manner. – Map existing regulatory workflows and associated outputs as input in the development of the stepwise approach to testing and assessment. – Enforceability
Policy implementation	– Appropriate capacities (including financial and personal infrastructure) for validation in Europe need to be provided, as lack of such capacities constitutes a major hurdle for the introduction of new methods. Experiences with public-private partnerships like PEPPER (public-private platform for the validation of endocrine disruptors characterisation methods, cf. Grignard et al. (2022)) should be taken into account. – Redefine information requirements regarding the required input for hazard/exposure/risk assessment while moving specific test methods to a central inventory which is frequently updated under supervision of a dedicated scientific committee. – Align closely with the work on 'one-substance-one-assessment' and 'Safe and Sustainable by Design' framework.
Change management	– Analyse, and develop strategies to remediate general psychological barriers towards trusting *in silico* and *in vitro* methods, especially with risk assessors trained in the traditional approach. – Develop strategies for capacity building among scientists to overcome knowledge barriers regarding regulatory requirements for test methods to be regulatory applicable. – Develop stakeholder-specific strategies for communication/dissemination, training and capacity building. – Develop plan for how to build networks within CRA sub-communities (industry/CRO labs, risk assessors in authorities, risk managers/policy makers) as “germ cells” for large scale dissemination.

4.10. Evidence integration

Guiding Principle 5 demands that a future NGRA framework should be able to integrate all relevant lines of evidence (as defined by problem formulation and regulatory context) with acceptable reproducibility.

A future NGRA framework should therefore incorporate WoE schemes to integrate the diverse lines of NAM-based evidence - as such and with existing “traditional” information - using concepts and tools established for systematic review (WHO, 2021). To produce consistent risk assessment outcomes, WoE approaches should be based on standardised workflows, integrating machine learning and other AI (Hartung, 2023; Kleinstreuer and Hartung, 2024) and *in silico* approaches, where appropriate and available, and standards for transparent documentation should be introduced to allow for a review of the assessments with a view to traceability and reproducibility. It is noted that the broad availability of relevant data in machine-readable and FAIR (Findable, Accessible, Interoperable and Reusable) formats is a prerequisite for the successful deployment of such techniques.

Guidance on WoE is already available (EFSA, 2017; OECD, 2019), but may need to be adapted to include NAMs and NGRA concepts as described in this Deliverable.

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Integration of information of different type and from different scientific domains (academic research, databases) requires a transparent process and criteria for how to weigh these different lines of evidence against each other. In addition, approaches for determining whether different lines of evidence are independent of each other (and, hence, can mutually add weight to each other) need to be developed.

EU chemicals legislations as well as associated guidance might need to be revised in concert with existing technical guidance, e.g. by ECHA, EFSA or OECD, to provide clear rules and boundaries for WoE assessments are needed to allow for consistent and efficient regulatory processes, which are in the best interest of both, regulators and the regulated. This includes rules/boundaries for evidence generated by NAMs, incorporating all types of traditional and NAM information sources and integrating uncertainty assessment (cf. section 4.7). Although WoE assesments by their very nature contain subjective/expert judgements, as much standardisation as possible is desirable in order not to delay regulatory assessments. In addition, the legal basis for information sharing between regulatory sectors should be broadened to facilitate the transition to a more integrated risk assessment in the spirit of "One Substance, One Assessment" (OSOA/1S1A).

Table **7** summarises specific tasks regarding Guiding Principle 5 which should be addressed by the four proposed work streams in the course of the further roadmap work.

Table 7: Tasks for the proposed work streams regarding Guiding Principle 5 (evidence integration)

Work stream	Specific tasks
Scientific development	– (Further) Develop tools and algorithms to automate data collection, data extraction and processing as well as integration. – Further develop standards for quality of NAM data and its documentation. – Develop standards and infrastructure for FAIR data storage and sharing.
Regulatory implementation	– Develop standardised WoE workflows. – Develop criteria for the relative weight of different evidence streams, "good evidence integration practice" [32].
Policy implementation	– Resolve issues of data sharing incl. protection of confidential business information. – Streamline Woe scehmes and workflows under the OSOA project.
Change management	– Training, but also infrastructural changes and investments might be required in industry and authorities if WoE approaches are increasingly automatised – Develop concept to overcome barriers to data sharing in industry.

[32] Cf. also the work of the Evidence-Based Toxicology Collaboration (EBTC, https://www.ebtox.org)

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4.11. Regulatory workflows

Guiding Principle 6 demands that a future NGRA framework should be applicable to all relevant (as defined by the problem formulation and regulatory context) chemical hazard, exposure and risk assessment workflows, including the assessment of the associated uncertainties. Since most of these workflows share common elements, the framework should best be built in a modular fashion.

Related issues have already been addressed above (workflows: section 4.1; uncertainty assessment including probabilistic methodology: section 4.7), including the need for accommodating probabilistic methodology.

Table 8 summarises specific tasks regarding Guiding Principle 6 which should be addressed by the four proposed work streams in the course of the further roadmap work.

Table 8: Tasks for the proposed work streams regarding Guiding Principle 6 (regulatory workflows)

Work stream	Specific tasks
Scientific development	– Ensure that the modular NGRA framework (e.g. ASPA, cf. section 4.3) covers all relevant workflows in an integrated way (cf. also Figure 3 in section 4.2).
Regulatory implementation	– Revise regulatory workflows to become NGRA-ready (while still delivering the outputs needed for hazard/exposure/risk/uncertainty assessment).
Policy implementation	– Develop timelines and work plans towards updating current legislation/guidance for their readiness to accept new NGRA framework-based workflows, also in order to create legal certainty for all stakeholders.
Change management	– No specific activities (other than training and capacity building have been identified at this point in time.

4.12. Biology

Guiding Principle 7 demands that a future NGRA framework should cover all relevant (as defined by the problem formulation and regulatory context) (eco)toxicological pathways and endpoints.

Under the current CRA framework, relevant endpoints have mostly been defined in relation to the outcome of certain standardised test designs (i.e. “repeat-dose toxicity after oral exposure” is represented by adverse effects observed on one or more of those parameters experimentally assessed by a 90-day oral rodent test acc. to OECD TG 408).

These parameters in turn can be seen as representing main hallmarks of toxicity frequently seen in a whole organism, such as effects on body weight, clinical parameters, macro- or microscopical organ tissue damage etc.

In section 4.8, the concept of a stepwise NGRA approach was introduced. Following the evaluation of existing information, opportunities for read-across and certain filters to establish “negligible” toxicity and/or exposure, step 4 in this approach consists of a broad bioactivity screening required to possess a high degree of biological coverage as well as a high negative predictive value. A high positive predictive value is also desirable, but less critical, as positive results are meant to be followed up for confirmation by further targeted testing anyway.

Since absence of toxicity in this screening step would stop the assessment, it is of the essence that it covers all relevant (as defined by problem formulation/regulatory context) pathways of biological perturbation, in order not to miss any relevant toxicity.

However, that does not necessarily mean it would have to mimic exactly the outcomes of the *in vivo* measurements. Based on a comprehensive knowledge of the (adverse outcome) pathways involved in

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e.g. liver toxicity, perhaps combined with the use of key characteristics [33] of organ-/system-specific toxicity, a new way of representing "all possible and relevant liver effects" in an NGRA-ready fashion could be developed.

Some of the comments received after the December 2023 roadmap workshop raised the point whether not also the definition of "relevant toxicity" as implemented by the current risk assessment system should be questioned.

In addition, it is pointed out that also the current array of, mostly animal-based, testing methods has difficulties to adequately detect certain emerging endpoints of concern, e.g developmental neurotoxicity or immunotoxicity.

Comprehensive biological coverage without animal testing can, in theory, be achieved in several ways (with no preference attributable to any at this stage):

- Multiple NAMs could be jointly used in a battery approach, where the applied methods in concert cover all relevant pathways and target organ/systems that need to be covered by the assessment. Critical findings in one or more of these NAMs can then be followed up on by suitable test methods with high predictive accuracy for adverse effects along the respective perturbation pathway(s). Such a battery approach is e.g. facilitated by the U.S. ToxCast [34] programme.
- *In vitro* "omics" [35] methods or omics experiments in non-sentient species (cf. a recent article by Colbourne et al. (2022) on "toxicity by descent") could be applied to assess all relevant pathways on the genome, transcriptome, proteome or metabolome level, if it can be shown that the test system used provides sufficient biological coverage to predict perturbations in all relevant organs/systems. Morphological profiling could also be explored: by quantifying phenotypic responses and using the obtained morphological profiles for comparison with known (reference) compounds, effects not covered by other assays could become visible, as kind of untargeted in vitro 'omics'. Again, biological plausibility, i.e. predictivity for apical adverse effects, should be ascertained.
- If comprehensive bioactivity databases with reliable toxicity data were available, covering both sufficient chemical and biological space, machine-learning and other AI approaches could acquire sufficient predictive power (cf. Luechtefeld et al. (2018)) as a proof-of-concept study).

In the future, a smart combination of these approaches as part of one or several IATAs or DAs, e.g. a broad, *in silico*-based screening combined with one or more transcriptomic or metabolomic experiments and complemented with a battery of *in vitro* tests assessing pathways not well covered by the two other approaches could become a successful alternative to today's repeat-dose testing scheme.

In the area of environmental safety, a future NG(E)RA framework ideally should accommodate higher-system level risk assessment such as landscape-level Environmental Risk Assessment (ERA) assessing long-term impact of chemicals on biodiversity and improved accuracy in extrapolation from laboratory tests to long-term impact in the environment. Ideally, the framework would also allow to promote an integrative workflow (e.g. biodiversity assessment, safe-and-sustainable-by-design (SSbD) assessment) in the future. In the future, existing monitoring programs on chemical exposure, hazards and biodiversity loss could be better exploited and aligned using novel methodologies such as e.g.

[33] Cf. https://www.niehs.nih.gov/research/supported/centers/srp/phi/archives/advances/keycharacteristics/index.cfm (last accessed 2024-07-09)

[34] https://www.epa.gov/comptox-tools/toxicity-forecasting-toxcast (last accessed 2024-07-08)

[35] https://www.oecd.org/chemicalsafety/testing/omics.htm (last accessed 2024-07-09)

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environmental DNA (eDNA), non-target screening (NTS) or effect-directed analysis (EDA), to better reflect real-world impacts of chemicals and chemical mixtures on ecosystems.

Table 9 summarises specific tasks regarding Guiding Principle 7 which should be addressed by the four proposed work streams in the course of the further roadmap work.

Table 9: Tasks for the proposed work streams regarding Guiding Principle 7 (biological coverage)

Work stream	Specific tasks
Scientific development	– Method development in the area of test batteries, IATAs/DAs, omics methods and AI approaches – Develop methods to characterise the biological space covered (taxonomical space, effect/bioactivity space) – Systematically evaluate the evidence for chemical-induced pathologies in humans, which are especially attributed to environmental exposure. The disease-centered systems biology approach used by Rider (NTP/NIEHS) in cumulative risk assessment is an example.
Regulatory implementation	– Development of criteria for "sufficient biological coverage" – Conceptual work on adversity and "relevant toxicity". – Conceptual work on the integrated assessment of chemicals for the protection of both human health and the environment (One Health Approach)
Policy implementation	– Review and, where necessary, revise existing legislation with respect to redefining relevant endpoints of chemical risk assessment in an NGRA-ready way without compromising protection or confidence levels.
Change management	Convincing risk assessors that a new, NGRA-ready definition of the endpoints addressed in chemical risk assessment can overall achieve an adequate protection level for humans and the environment, will require a major change management process. – analysis of required institutional/organisational changes; – analysis of required conceptual changes; – analysis of required legal changes (soft/hard law)

4.13. Exposure

Guiding principle 8 demands that a future NGRA framework should cover all relevant (as defined by problem formulation and regulatory context) exposure levels, durations and routes.

Where the framework requires new *in vitro* tests, these need to achieve test concentrations which are high enough to reflect the corresponding real-life exposure levels of the target organism/system/population that is the subject of the assessment. To account for uncertainties in both dosing and exposure assessment methodologies, in practice this means that maximum nominal test concentrations need to be higher than the corresponding (measured or modelled) real-life exposures.

Conversely, the methodology needs to be sensitive enough to assess comparatively low environmentally relevant concentrations with relevant bioactivity, also in the light of potential combination effects.

In response to these requirements, the framework needs to incorporate reliable in-vitro-to-in-vivo extrapolation (IVIVE) methods. For full quantitative risk assessment, qIVIVE methods need to be developed, especially also for non-mammalian organisms in ERA. Monitoring data (both regarding internal and external exposure) should be considered to inform about real-life exposure levels and to verify models.

As for the current CRA framework, a major scientific challenge is also posed by the question of how treatment durations in *in vitro* tests correlate with the diverse temporal exposure patterns occurring in real-life (acute, chronic, intermittent, infrequent etc.). For one, it will need to be shown that *in vitro* tests with shorter treatment durations do not miss long-term effects. Second, methodology needs to be developed to calculate risk for variable exposure scenarios, the occupational setting being one example (out of several), where risk assessment may need to address a variety of scenarios, such as

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defining a 15-min Short-Term Exposure Limit (STEL), an 8-h occupational exposure limit (OEL) or the risk over a full working life of 40 years.

On the other hand, there should be a careful reassessment of the practical relevance of testing very high substance concentrations (i.e. concentrations much higher than the actual exposure concentrations expectable for the substance(s) under assessment) *in vivo* for the purpose of hazard assessment/classification and labelling in cases, where such concentration may not be achievable by an otherwise suitable *in vitro* test system. Along these lines, one comment from industry challenged the relevance of unspecific effects secondary to high systemic toxicity at comparatively high doses:

> *"With the huge amount of animal data generated on repeated dose/repro/developmental studies within REACH over the last decade, it can be evaluated how often effects occur only at doses close to systemic toxicity. Indeed, developmental and reproductive effects are quite often seen at the dose with systemic toxicity only or at one dose below. In the frame of change management, it is important to discuss (in relation to exposure) the question whether this is 'relevant bioactivity' for real life scenarios – or whether we generate data 'relevant only to the high-dose scenario'. Mechanistic NAMs will probably never be able to mimic the unspecific secondary effects e.g. on reproduction and development due to the physiological stress of sentient animals at doses leading to systemic effects. But whether this is 'relevant bioactivity' and 'should not be missed' needs a broad discussion to facilitate the change management. Without this discussion, regulators will continue to ask for the high-dose tests as they perceive the effects observed only in high-dose tests as relevant."*

It has been argued (also in some of the comments received after the December 2023 workshop) that hazard characterisation needs to reflect all possible, including yet unknown, future use scenarios. However, the current system already uses limit test concentrations, e.g. of 1 000 mg/kg bw/d for repeat-dose testing acc. to OECD TG 408, implicitly assuming that higher real-life exposures are of negligible regulatory relevance. If such a study were to be performed in humans *in vitro*, the limit dose could already be reduced by 10-fold (compared to using rats), due to the absence of a need to consider interspecies variability, i.e. to the *in vitro* equivalent of an external dose of 100 mg/kg bw/d (still 7 g/d for a 70 kg human).

Notably, the limit dose set in OECD TG 408 does not take into consideration whether the dose is administered as a bolus by gavage or via the diet or drinking water, although these administration routes may significantly affect toxicokinetics, e.g. peak plasma concentrations.

Furthermore, the above figures relate to external exposure and – for a comparison with relevant *in vitro* test concentrations – would need to be converted to concentrations in the target tissue. With the development of suitable PBK models also accounting for the time pattern of exposure, metabolism and absorption, distribution and excretion kinetics, combined with a probabilistic understanding of human (or, in the case of ERA, non-human) intraspecies variability as well as inherent uncertainties of the assessment, NGRA might in the future be much better equipped than the present-day framework to derive meaningful limit doses linked to expectable real-life maximum exposure levels. Where sufficiently high doses cannot be reached for technical reasons, it should in addition be assessed whether the underlying reasons, e.g. low solubility, may not prevent high internal doses in the *in vivo* situation as well.

As in all other areas of chemical risk assessment, the availability of meaningful and reliable data as a benchmark as well as a thorough understanding of exposure (Cronin et al., 2023) are key for developing robust new methodology. To this end, information requirements in the EU safety legislations should include more precise information on uses and exposure patterns as input for modelling. It is noted that, depending on the outcome of the discussion on this principle, a revision of the GHS/CLP Regulation might be indicated.

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Table 10 summarises specific tasks regarding Guiding Principle 8 which should be addressed by the four proposed work streams in the course of the further roadmap work.

Table 10: Tasks for the proposed work streams regarding Guiding Principle 8 (exposure coverage)

Work stream	Specific tasks
Scientific development	– Improve methodology to assess real-life exposure, including exposure modelling, HBM and other monitoring data. – Develop suitable PBK models for qIVIVE.
Regulatory implementation	– Review limit concentrations with a view to NGRA methodology, but also to aggregate, cumulative and combined exposures. Perform comparative research identifying relevant examples where not testing at the maximum tolerable would have had detrimental regulatory consequences.
Policy implementation	– Review and, where necessary, revise existing legislation with respect to defining maximum required test levels. – Consider stepping up legal information requirements regarding use and exposure conditions of chemicals along the supply chain and over full product life-cycles to generate more and better exposure data for improving existing as well as developing new exposure models.
Change management	– Target chemical producers and downstream users (especially the latter) to sensitise them more for the need to better understand how their substances are used and what exposure of people or the environment can be expected from those uses. – Beyond that, no specific conclusions have been identified at this stage, but first responses to this principle have been controversial in nature, with the main fear of regulators that relevant bioactivity might be missed when not testing high enough. This should also be addressed by targeted discussions.

4.14. Chemistry

Guiding Principle 9 demands that a future framework should be applicable to substances in their relevant (as defined by the problem formulation and regulatory context) physical forms, their transformation products, groups of substances, mixtures and articles (the two latter as defined by REACH Art. 3). The framework should incorporate aggregate and cumulative risk assessment, as well as the assessment of unintentional (e.g. "randomly" occurring in the environment) and intentional mixtures (formulations, e.g. cosmetic or household products).

To ensure the coverage of transformation products, both modelling and testing approaches need to account for biotransformation/-degradation as well as relevant abiotic transformation processes. To achieve this, the framework should be able to integrate sources of exposure across regulatory domains (e.g. occupational, environmental, via food and drinking water, indoor and outdoor air, use of consumer products etc. for humans) and also cover diffuse releases as well as point sources, such as waste water treatment plant effluents for environmental exposure assessment.

Moreover, the framework should incorporate information on direct exposure (e.g. migration of substances from consumer products upon use) or release into the environment (e.g. for volatile substances).

The framework should be able to handle multi-constituent substances and, to the degree possible, polymers. As for the current CRA framework, substances of unknown or variable composition and/or biological origin (UVCB substances) bring additional challenges, as do "difficult-to-test" (e.g. volatile, insoluble or unstable) substances. Further research is required with respect to under which circumstances such substances can be reliably assessed by *in silico*, *in chemico* or *in vitro* models.

With respect to specific physical forms, Hristozov et al. (2024) have summarised the current status and perspectives of NGRA for nanomaterials. The EFSA-funded NAMS4NANO project has receibtly published an approach for the qualification of NAMs for the assessment of nano- and other so-called "advanced materials" (Haase et al., 2024).

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Furthermore, where not already done, EU chemicals legislation needs to be revised to incorporate aggregate and cumulative risk assessment, as well as risk assessment of intentional and unintentional mixtures.

Table 11 summarises specific tasks regarding Guiding Principle 9 which should be addressed by the four proposed work streams in the course of the further work on the COM roadmap.

Table 11: Tasks for the proposed work streams regarding Guiding Principle 9 (chemical coverage)

Work stream	Specific tasks
Scientific development	– Method development regarding PBK/IVIVE, testing of mixtures and adequate AD characterisation. – Method development regarding UVCBS, insoluble substances, formulations, nano or microparticles (different size) and other "advanced materials". – Explore and address technical challenges with the non-animal methods which set boundaries to their applicability domain and to provide a system that covers the complete chemical space.
Regulatory implementation	– Discuss criteria for relevance of difficult-to-test substances
Policy implementation	– Review and, where necessary, revise legislation to integrate aggregate, cumulative and mixture risk assessment.
Change management	– No specific tasks regarding this principle have been identified at this point in time.

4.15. Efficiency

Last, not least, Guiding Principle 10 demands that a future NGRA framework allows for assessments within an acceptable (as defined by the problem formulation and regulatory context) time- and cost-frame. It should include integrated testing and assessment approaches that are as complex as scientifically necessary, but also as simple and straightforward as possible. Therefore, approaches reducing the complexity without compromising other aims of the risk assessment (e.g. in ERA: more realistic exposure, effect-based monitoring or system- based approaches) are of prime relevance.

Phasing out animal testing for chemical safety testing based on ethical considerations is a clear political goal, as underpinned by the EU Commission's roadmap. Moreover, certain scientific advantages of an NGRA framework vs. the current system have been discussed.

In addition, NGRA also offers a promise to (at least partly) overcome the inefficiency of the current CRA framework as characterised inter alia by

- the availability of high-quality toxicity/exposure and risk data only for a small subset of the chemicals on the market;
- time-consuming animal testing (sometimes only after years of legal battle between industry and authorities);
- (legal) uncertainty regarding the use of alternative approaches;
- a substantial delay of many years between first identification of a concern and the respective regulation,
- testing of substances based on canonical test catalogues rather than in a targeted way based on triggers provided by existing knowledge on the respective chemical(s), leading to a large fraction of chemicals for which the performed tests do not lead to any change in risk management measures.

Compared to this present-day situation, with high-throughput and high-content approaches, mechanism-informed targeted testing and the use of methodology providing results in days or weeks rather than months and years, NGRA has great potential to increase the efficiency (as in cost- and time-effectiveness) of chemical risk assessment significantly in the future, while at the same time providing data on a greater number of chemicals.

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In addition, while health protection and environmental safety must remain the highest priority, chemical risk assessment nevertheless takes place within societal and economic boundaries, and therefore the aspect of cost- and time-effectiveness also needs to be considered when designing a future NGRA framework. For example, the "Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery" published by OECD [36] lists 17 different *in vitro* test systems to investigate various AOPs leading to adverse DNT outcomes and it needs to be determined which minimum subset of these tests can be combined into an IATA that is cost- and time-effective while providing sufficiently protective results.

Subject to further research (cf. section 4.12), and in line with established concepts within the current CRA framework, the stepwise approach described in section 4.8 should be further developed into a tiered scheme, starting with cost- and time-effective, highly (i.e. potentially over-)protective methodology representing "worst-case" assumptions and moving to refined schemes only in case further concerns have to be clarified. For example, the protectivity of *in vitro*-based PoDs for risk assessment has been demonstrated by the APCRA initiative.

On the regulatory side, high-throughput methods and advanced *in silico* methods (e.g. AI-based) might call for the development of additional acceptance criteria. In addition, in the context of AI-based methodology, transparency and reliability issues might need to be addressed.

Under the change management work stream, the argument of saving time and resources should be used prominently when promoting the introduction of NGRA, notwithstanding that the introduction of NGRA will likely require structural adaptations in both industry (producers/importers of chemicals, but also CROs and consultants) and the authorities, initially calling for additional extra investments. Moreover, the variety of available methods (and, in consequence, expertise required) will increase, as will the number of less schematic, case-by case risk assessments.

Among other things, it will be crucial for the success of the roadmap to demonstrate how investments in these structural changes will pay off in the end. In this context, proportionality and predictability are aspects of major importance for achieving acceptance, in particular by industry.

Future efforts towards revising existing legislation procedures to improve the efficiency of evaluation procedures and allow for stepwise, tiered approaches should take note of and build on ideas published in the recent literature e.g. by Ball et al. (2022), Pereira et al. (2022) or Berggren and Worth (2023).

Table 12 summarises specific tasks regarding Guiding Principle 10 which should be addressed by the four proposed work streams in the course of the further roadmap work.

Table 12: Tasks for the proposed work streams regarding Guiding Principle 10 (efficiency)

Work stream	Specific tasks
Scientific development	– Differentiate stepwise approach (cf. section 4.8) into tiered testing strategy "as complex as scientifically necessary, while as straightforward and cost-effective as possible".
Regulatory implementation	– Develop acceptability/decision criteria for tiered approach.
Policy implementation	– Review and, where necessary, revise existing legislation to allow for stepwise, tiered approach. – Comparatively assess the cost and time expenditure of and differences between different approaches. This is generally claimed but little data seems available to demonstrate this. Also, what is cost-effective for one industry may not be for another - SMEs also need to be considered.
Change management	– Develop concepts to communicate net efficiency gains to the respective stakeholders.

[36] OECD Series on Testing and Assessment No. 377, https://one.oecd.org/document/ENV/CBC/MONO(2023)13/en/pdf (last accessed 2024-07-09)

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4.16. Anticipated next steps

As described in section 3 of this document, work on the EU Commission's roadmap has been initiated and is expected to pick up steam after the summer break of 2024, with a second roadmap workshop foreseen to take place in Brussels on 25 October 2024.

The next meetings of the HH, ESA and Change Management working groups (cf. Figure 1, all with participation of representatives of PARC Task 2.2) under the COM roadmap will bring more clarity as to the exact role of PARC Task 2.2 in the further process.

One of these roles could be that of a hub connecting stakeholders within and outside of PARC with the roadmap development process. As a next step in this direction, the first draft of the present document will be shared for commenting with a broad range of stakeholders, including PARC activities related to NGRA development, the PARC management and governing board, members of the ASPIS cluster, participants in the multi-stakeholder roundtable in Brussels on 18 June 2024 and members of the roadmap working groups, including the COM and European agencies, as well as further colleagues at OECD and in the US. Further engagement with all stakeholder groups will be sought via PARC Task 2.2's other activity, i.e. in the NGRAroute discussion group [37] on PARCopedia, the online knowledge management and community platform created by PARC Task 2.2 for everyone working towards safer chemicals for human health and the environment.

The present Deliverable is expected to be submitted to the EU Commission in early October 2024 and will provide timely input from NGRAroute into the roadmap workshop on 25 October 2024.

In parallel, some NGRAroute activities, which have been started already in 2022/23 (cf. PARC (2023b)) and were put on hold to await further details regarding the COM roadmap work, will be picked up again over the coming months:

- providing a comprehensive literature repository for NGRA-related publications as a resource for assessing the current state of scientific research and regulatory concepts around NGRA;
- mapping of EU chemicals legislation with respect to risk assessment workflows and outputs as well as key regulatory bodies involved in executing, shaping or changing the respective legislation;
- mapping of past and current NGRA-related projects (together with PARC WP 6) to avoid duplication of work and enable coordination between these projects for further research.

In addition, plans need to be further developed for stepping up activities directed at the development of NAMs and their uptake in ERA along with all existing data and information in a systems-based analysis to tackle the broader concept of NGERA.

[37] https://www.parcopedia.eu/groups/ngraroute-group/ (last accessed 2024-07-09)

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Co-funded by the European Union

Annex I – Stakeholder indications of interest to engage in the further roadmap work

Table 13 reports the responses from Stakeholders during and after the roadmap workshop on 11/12 December 2023 with respect to their interest in engaging with the further roadmap work and, if so, in which role (lead, active participant, reviewer, observer). It is understood that the feedback was given on an individual basis and does not necessarily imply an official wish to engage of the respondents' employers.

Table 13: Declarations of interest by various stakeholders after the December 2023 roadmap workshop to engage in the further roadmap work

Role	Work stream			
	Scientific development	**Regulatory implementation**	**Policy implementation**	**Change management**
Lead	SCAHT	-	-	-
Active participant	BfR, CSPA, EFSA, EPAA, ERM, FOEN, Fraunhofer ITEM, ICCS, Laboratoire Watchfrog, MAK Commission, NETRI, RECETOX, Sciensano, UBA, University of Aveiro, VelTox	BfR, CEFIC, EFSA, EPAA, ERM., FOEN, Fraunhofer ITEM, ICCS, IMH, Laboratoire Watchfrog, NETRI, SCAHT, Sciensano, UBA	CEFIC, CSPA, EPAA, ERM, HSI, IMH, Laboratoire Watchfrog, SCAHT, UoB	CEFIC, CSPA, EPAA, ERM, HSI, Laboratoire Watchfrog, IMH, SCAHT, UoB
Reviewers	ANSES, BASF, CEFIC, COM, Danish EPA, Eawag, Evonik, Givaudan, ISS, L'Oréal, LVMH, MUI, PSCI, RECETOX, RIVM , Syngenta, UBA	ANSES, BASF, COM, Danish EPA, Eawag, EMSA, ERM, Eurogroup for Animals, Givaudan, HSI, ISS, L'Oréal, LVMH, MAK Commission, MUI, PSCI, RIVM, UBA, UoB, VelTox	ANSES, BASF, COM, Eawag, ECEAE, Eurogroup for Animals, FOEN, L'Oréal, LVMH, NETRI , PSCI, RIVM, RSPCA, UBA, VelTox	ANSES, BASF, COM, ECEAE, Eawag, EPAA, Eurogroup for Animals, FOEN, L'Oréal, LVMH, NETRI , PSCI, RIVM, RSPCA, UBA, VelTox
Observers	Danish EPA, EAA, HSI, NCad, RECETOX, RIVM, EECEAE, Eurogroup for Animals	CSPA, Danish EPA, EAA, ECEAE, EURIMA, Evonik, FOEN, IMH, NCad, RECETOX, RIVM, Syngenta	EAA, ERM, EURIMA, Fraunhofer ITEM, Givaudan, IMH, NCad, RECETOX, RIVM, UBA	EAA, ERM, EURIMA, Fraunhofer ITEM, Givaudan, IMH, NCad , RECETOX, RIVM, UBA

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