ECHA
EUROPEAN CHEMICALS AGENCY

Key Areas of Regulatory Challenge

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FOREWORD

Dr Sharon McGuinness

Executive Director

I am pleased to present the Agency's research needs related to the scope of the Partnership for the Assessment of Risks from Chemicals (PARC). Since 2007, ECHA has implemented various EU legislative tasks related to chemicals management. In an era where safeguarding human health and the environment is crucial, ECHA, as an EU agency, is playing its part, together with the Commission and Member State authorities, in delivering the EU's ambitious goals on chemical safety.

As a result of the Chemicals Strategy for Sustainability as well as the broader policy development under the EU Green Deal, ECHA has focussed its advisory role on those topics where it can provide most valuable input. Activities such as 'One Substance, One Assessment' and more recently 'One Health' demonstrate the dynamic and integrating nature of several EU policy ambitions.

While significant progress has been made, many challenges still persist. Ongoing research and innovation are needed to ensure that the regulatory frameworks remain robust and effective for the chemical safety assessment. It is through collaborative efforts and a shared dedication to advancing scientific knowledge that we will address emerging risk and protect public health and the environment.

In its Strategy 2024-2028[1], ECHA has detailed its goals and priorities over the next five years to protect health and the environment. This new strategy adopted a vision of chemical safety through science, collaboration and knowledge. One of ECHA's strategic goal is to lead on chemical knowledge and expertise.

In this context, ECHA has mapped its key areas of regulatory challenge, translating into ECHA's needs for further scientific research, under the umbrella of the Partnership for Assessment of Risk from Chemicals PARC[2].

Building strong partnership and collaboration between regulators and researchers is crucial. By connecting the latest scientific discoveries with regulatory practices, we can tap into the knowledge of academia and other experts to stimulate innovation in chemical safety. This approach will not only make the EU chemical market safer but also more competitive on a global scale.

1 ECHA Strategy Statement 2024-2028

2 Partnership for the Assessment of Risks from Chemicals | Parc (eu-parc.eu)

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LIST OF ACRONYMS

ADME	Absorption, distribution, metabolism and excretion
AOP	Adverse Outcome Pathway
AUC	Area under the curve
B	Bioaccumulation
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BMF	Biomagnification factor
BPR	Biocidal Product Regulation
CLP	Classification, Labelling and Packaging of substances and mixtures (Regulation)
CMax	Maximum concentration
Css	Steady-State Concentration
CSS	Chemicals Strategy for Sustainability
DIT	Developmental immunotoxicity
D_{MLW}	Membrane lipid-water distribution coefficient
DNT	Developmental neurotoxicity
EATS	Estrogen, Androgen, Thyroid, and Steroidogenesis
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
GHS	Globally Harmonised System of classification and labelling of chemicals
IRS	Integrated Regulatory Strategy
IVIVE	*In vitro* to *in vivo* extrapolation
KE	Key Events
K_{MLW}	Membrane lipid-water partition coefficient
Koc	Organic carbon-water partition co-efficient
Kow	N-Octanol/Water Partition coefficient
LOAEL	Lowest observed adverse effect level
LRTP	Long-range transport potential
MAD	Mutual Acceptance of Data
MW	Molecular weight
NAM	New Approach Methodologies
NBP	Non-bee pollinators
NOAEL	No-observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
OHAT	Office of Health Assessment and Translation
OMICS	Branches of science known informally as omics are various disciplines in biology whose names end in the suffix -omics, such as genomics, proteomics, metabolomics etc. In toxicology, these are used as marker to indicate a possible adverse effects
P	Persistence
PARC	Partnership for the Assessment of Risks from Chemicals

PBK	Physiologically-Based Kinetic (Modelling)
PBPK	Physiologically-based Pharmacokinetic (Modelling)
PBT	Persistent, Bioaccumulative, Toxic
PBTK	Physiologically Based Toxicokinetics
POPs	Persistent Organic Pollutants
PPPR	Plant Protection Product Regulation
QAF	OECD QSAR assessment framework
QIVIVE	Quantitative *In Vitro In Vivo* Extrapolation
RAAF	Read-Across Assessment Framework
RAC	Committee for Risk Assessment
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
T	Toxicity
TG	OECD Test Guideline
TK	Toxicokinetics
TMax	Time to maximum concentration
TMF	Trophic magnification factor
$ThCO_2$	Theoretical carbon dioxide
ThOD	Theoretical oxygen demand
UN GHS	United Nations Global Harmonisation System
UVCB	Substances of unknown or variable composition, complex reaction products or of biological materials
VMS	Volatile methyl siloxanes
vPvB	Very Persistent Very Bioaccumulative
WoE	Weight of evidence

1. EXECUTIVE SUMMARY

The Partnership for Assessment of Risk from Chemicals (PARC) provides a forum for collaboration across Europe between scientists and regulators and aims to pioneer scientific areas addressing most urgent regulatory challenges. In 2023, ECHA published a first map of its key areas of regulatory challenge with the aim to inform and inspire the PARC community developing research of most regulatory relevance.

For the 2024 update of its current regulatory needs, ECHA has further detailed its view on research needs, in particular, regarding the further development of new approach methods, and methods to identify and regulate bioaccumulative substances. Compared to 2023, all other topics received a status update.

The research needs are organised under the four chapters: Provide protection against most harmful chemicals; Addressing chemical pollution in the environment; Shift away from animal testing; and Improved availability on chemical data.

For each research area, ECHA reflects why the topic is of relevance, where it fits in the regulatory landscape and what could be short (or longer) term research impacts. These are meant as illustrations to inspire possible outcomes.

As researcher, you may be interested to follow-up on some of the topics ECHA has included. You may also have research ongoing for which you think the results may support one or more of our needs described in the different chapters of this document. When this is the case, please reach out to us for a further exchange via the functional mailbox PARC@echa.europa.eu.

The below summarises the different chapters.

Provide protection against most harmful chemicals

This chapter highlights the need for protection against harmful chemicals, focusing on gaps in identifying and understanding their effects for the immune, neurological, and endocrine system impairments. Further development of test methods, understanding of the toxicological modes of action and how to translate the outcome to risk management is essential to identify these hazards, facilitate safe use and take regulatory action where needed. This chapter provides first suggestions on areas and concrete research topics that are detrimental to the challenges ECHA is facing.

Addressing Chemical pollution in the environment

As recognised in the Chemicals Strategy for Sustainability (CSS), chemical pollution is one of the key drivers contributing to ecosystems degradation and biodiversity loss. One key element in the management of the risks posed by chemical pressure on ecosystems is the development of targeted new approach methods (NAMs) that can efficiently address the manifold interactions between chemicals and ecosystems. These include *in vitro* and *in silico* methods for hazard and fate assessment of different chemical substances. This chapter also describes the need to better understand the sensitivity of non-bee pollinators to biocidal active substances and to monitor specific substances such as linear and cyclic siloxanes.

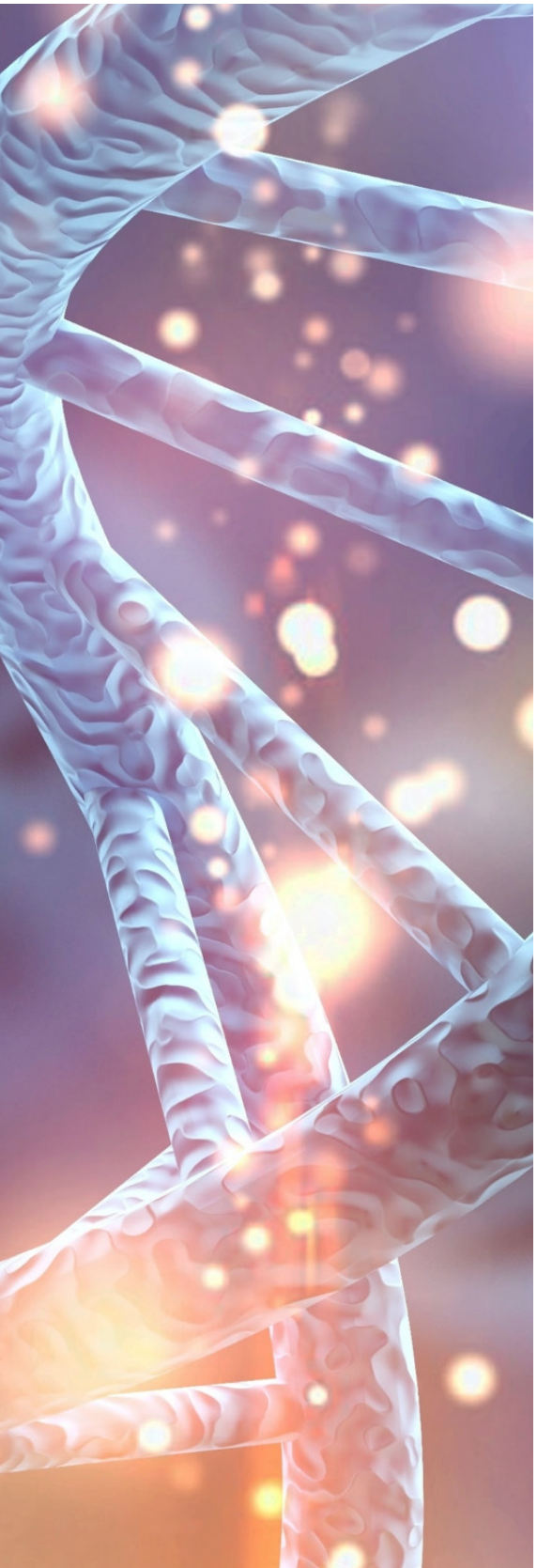
Shift away from animal testing

It is in the core of ECHA's mandate to minimise and where possible, shift away from animal testing. For chemicals management processes to shift away from animal testing, it is of utmost importance that this does not happen at the expense of nature or human health protection. To make this shift, NAM based (e.g., *in vitro* or *in silico*) methods need to be developed to substitute or reduce the use of *in vivo* test methods currently in place to support hazard identification. This chapter covers different research areas like read-across, ADME (absorption, distribution, metabolism and excretion) and Physiologically-Based Kinetic models, short-term and long-term fish toxicity and carcinogenicity.

Improved availability on chemical data

The sound management of chemicals in Europe depends on the ability to make decisions based on robust and relevant, up-to-date knowledge. For decades, the EU has generated a wealth of information for chemical management and risk assessment providing adequate protection for human health and the environment. Yet, there is still a lack of comprehensive information on many substances. Among those, polymers and nanomaterials deserve particular attention. The availability of analytical methods that ensure a proper assessment of the presence of restricted chemicals and chemicals falling under authorisation is also a critical aspect that can limit the efficiency of chemical management.

2. KEY AREAS OF REGULATORY CHALLENGE



2.1. Provide protection against most harmful chemicals

The CSS has put in the spotlight several potential hazardous effects of chemicals for which current possibilities for identification are limited, e.g., because appropriate test methods are scarce or all together lacking, or because the toxicity mechanisms underlying the effect are not yet well understood. Most notably are effects leading to the impairment of the immune or neurological system, and the endocrine system (both in humans and for environmental organisms). These add to those adverse effects that were already in focus as most harmful until now, i.e., chemicals with carcinogenic, mutagenic or reproductive toxic effects (CMR properties).

The European Commission has recently adopted new hazard classes for endocrine disruptors (ED) (human health and environment) and has set out criteria for the classification, labelling and packaging of substances and mixtures. Currently, there is no such harmonized classification for immunotoxicity and neurotoxicity, that are under the hazard endpoints 'Specific target organ toxicity' and 'reproductive toxicity'.

Under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and Biocidal Product Regulation (BPR), the current standard information requirement may inform on some aspects of neurotoxicity, developmental immunotoxicity and ED properties.

ECHA has summarized in Annex 1 the current regulatory structure of immunotoxicity and neurotoxicity under REACH, BPR and Classification, Labelling and Packaging of substances and mixtures (CLP). For ED properties, ECHA refers to ECHA/ EFSA Guidance[1] and the Guidance Document 150[2] .

1 ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

2 Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption | en | OECD

2.1.1. Neurotoxicity

Why further research is needed:

We need to better understand developmental and adult neurotoxicity to improve the identification and regulation of neurotoxicants.

Regulatory needs:

Focus research on NAMs development to avoid animal tests. E.g., validation of existing test systems, development of test batteries and adverse outcome pathways (AOPs).

Impact:

- Improved identification and regulation of neurotoxic substances.
- Moving away from animal testing.

Under REACH, data that may inform on some aspects of adult neurotoxicity (ANT) and developmental neurotoxicity (DNT) is embedded within several standard information requirements, which inform on respectively acute, sub-acute, sub-chronic and reproductive (developmental) toxicity. Information on intrinsic properties of substances may also be provided by other means than the tests above, provided that certain conditions are met (REACH, Article 13).

The current regulatory structure summarised in Annex 1 introduces several challenges to implement NAMs for ANT and DNT as standalone information in REACH, BPR and CLP. For example, the CLP criteria for STOT SE and STOT RE are based on effects in humans and/or experimental animals (CLP Annex I, Table 3.8.1 and 3.9.1, respectively). Similarly, the CLP criteria for developmental toxicity are mainly based on human and/or animal data (CLP Annex I, Table 3.7.1(a)). However, for both STOT SE/RE and developmental toxicity, *in vitro* data can be included as supplemental information in a weight of evidence approach and to support grouping and read-across. Currently, NAMs informing on ANT or DNT are in themselves unlikely to be considered equivalent for any of the REACH or BPR information requirements listed above. In addition, ANT and DNT NAMs currently face a plethora of scientific challenges, which are reflected in the research needs below.

2.1.1.1. Research on new AOPs, further development of existing AOPs and establishing their interlink with NAMs

Why the topic is relevant. Ultimately, adverse outcome pathways (AOPs) may help to predict the adverse outcomes of *in vitro* tests if can be shown that the *in vitro* test is able to depict a key event (KE) in the specific AOP. As given in ENV/JM/ MONO(2013)6[5], Key Events (KEs) in an Adverse Outcome Pathway (AOP) are causally linked, essential to the adverse outcome (AO) under consideration, and measurable. An AOP is anchored at the one end by a molecular initiating event (MIE), representing the direct interaction of a chemical with a biological target, and at the other end by an adverse outcome (AO). The AO can be at any biological level of organisation but should be relevant to a regulatory decision. DNT is a complex adverse endpoint, where timing (e.g., developmental day) and location (specific cell types/ species, tissues, organs) of the (chemical) insult are likely to play a critical role for the MIE and KE leading to a specific AO. Most ANT and DNT AOPs are currently rudimentary and/or described at such high level that many of the molecular or cellular mechanisms studied in NAMs cannot be confidently linked to their MIE or KE, and thus to their AO. A more profound mechanistic basis,

5 Organisation for Economic Co-operation and Development (2017) 'Revised Guidance Document on Developing and Assessing Adverse Outcome Pathways' pdf (oecd.org).

including sufficient spatial and temporal resolution, is beneficial for the continued development of AOPs and NAMs and for the establishment of their interlink.

Where it fits into the regulatory landscape. This concerns basic research, which is needed to gain a better understanding of the scientific possibilities regarding new NAMs for ANT and DNT and their regulatory applicability.

Short- and long-term impact. In the short-term this research may help to prioritize the development of NAMs that can be reliably linked to an AOP, and which may in the long term be able to reliably predict adverse neuro(developmental) effects (outcomes). Having a clearer view on the scientific possibilities presented by the AOP landscape for ANT and DNT may also enable a long-term shift toward pursuing the realistic development of NAMs for specifically ANT or DNT.

2.1.1.2. Identification of reliable positive and negative reference chemicals for NAM validation

Note that for the purpose of this specific research need, the term "reference chemicals" is used to identify substances that are primarily used for the validation of NAMs, both individually and as part of a battery.

To address this research need, several approaches could be considered, such as:

1. Identifying substances that have been considered ANT or DNT by at least one and ideally multiple recognised (regulatory) committees, and that may have received a related hazard classification as a result thereof. This approach is considered a priority by ECHA, as it would reflect the current regulatory landscape. ECHA intends to publish soon a reference list of neurotoxic chemicals, based on entries in CLP Annex VI, with harmonised classifications STOT SE or STOT RE (nervous system as the target organ). It is recommended that ECHA's list is combined with other objectively assembled reference lists to form a consensus list of reference chemicals to validate ANT or DNT NAMs;

2. Expanding on the above, a large-scale systematic review of literature, conducted in line with standardised principles (e.g., laid out by the Office of Health Assessment and Translation - OHAT) would need to be done. This is a desirable approach to identify in a comprehensive manner (and with minimum bias) known neurotoxicants (i.e., positive reference substances) and reliable negative reference substances. This review may investigate both human and non-human (e.g., rat) data.

Why the topic is relevant. NAMs that have been under development often lack extensive testing with systematically selected positive and negative reference chemicals (for the purpose of validating the predictive capabilities of the technique). However, identifying reliable positive and negative control substances is a challenge due to the heterogeneity of academic literature, the limited availability of reliable and comprehensive regulatory data, and the notable lack of established relationships between cellular events and specific adverse outcomes.

Where it fits into the regulatory landscape. Systematic validation of NAMs is an important consideration before ANT or DNT NAMs may be used in wider regulatory context.

Short- and long-term impact. Depending on the performance of the NAMs, and their predictive comparability to the current regulatory standards (i.e., OECD TG 443 and OECD TG 426), they may in the long term take a more central role in the regulatory field. Besides assay validation, assay developers could select suitable concurrent experimental controls for their assays from the list of positive reference chemicals.

2.1.1.3. The DNT IVB battery: further validation and refinement by increasing data density and by developing new tests to fill coverage gaps, using reference control substances identified as part of research need point 2.1.1.2

Why the topic is relevant. Further validation and development of the DNT IVB (*in vitro* battery) is needed to improve its predictability and its regulatory applicability. However, the data density and thus the level of validation is currently limited (i.e., low number reference control substances which were tested by all *in vitro* assays encompassed by the battery. Refinement of the DNT IVB may lead to the inclusion of new *in vitro* assays, for the purpose of additional mechanistic coverage, provided it improves the battery's predictivity.

Where it fits into the regulatory landscape. Increased validation and data density (i.e., more positive and negative controls tested with most or all assays included in the battery) will enable authorities to better understand the battery's true performance (e.g., specificity, sensitivity) and the types of neurotoxicants covered by the battery, potentially expanding its regulatory relevance.

Short- and long-term impact. In the short term, increasing the data density of the battery will help understand its performance and may help uncover yet unknown challenges regarding interpretation of positive and negative results. In the long term, this understanding may support the implementation of the battery in regulatory processes, e.g., hazard and risk assessment.

2.1.1.4. Early-stage development of a NAM battery dedicated to ANT

Why the topic is relevant. Recent research efforts focused primarily on the development of NAMs for DNT, with the development of NAMs for ANT (and their merger into a battery) lagging considerably. Unlike with DNT NAMs, temporal exposure considerations are less crucial when it concerns NAMs for ANT. This is because the sensitivity of adult neuronal tissue is expected to fluctuate less over time than that in a developing embryo, foetus or juvenile individual. These considerations would simplify ANT NAM development over that of DNT NAM development.

Where it fits into the regulatory landscape. As described in the introduction of this chapter, there are multiple standard information requirements under REACH and BPR which may inform on ANT. However, the standard information requirements under BPR and REACH do not include a specific study for ANT testing such as OECD TG 424. Such specific studies may be requested though, when the possibility for ANT effects are identified, e.g., in the form of mechanistic studies, but the available evidence is yet inadequate for toxicological or risk characterisation (see information requirement 8.13.2 according to BPR Annex II and REACH ANNEX VIII 8.6.1. column 2). The data triggering further ANT testing is generally stemming from *in vivo* studies but also the mechanism (such as acetylcholine esterase inhibitor) or structure of the chemical (e.g., organophosphorus compounds) may indicate ANT properties. With the further development of NAMs for ANT, the regulatory implementation of mechanistic studies could potentially improve.

Short- and long-term impact. In the short term, the identification of available AOPs and existing methods, and the early-phase development of new NAMs, could lay the foundation for designing a prototype ANT NAM battery. Such a battery could help prioritize the further development of the individual ANT NAMs, where the focus could lie on ascertaining the method's general feasibility and determining their added value to the battery. In the long term, the aforementioned efforts could help refine the prototype ANT NAM battery and support their validation for possible future regulatory application.

2.1.1.5. Addressing the known gap of current DNT and ANT NAMs informing on effects of metabolites

Why the topic is relevant. Although (PBK) modelling may in part inform on toxicokinetics and the formation of possible metabolites, it may fall short when the composition of a substance is (highly) complex, e.g., in the case

of a substance of unknown or variable composition, complex reaction products or of biological materials (UVCB). As such, it is of interest to not only explore *in silico* methods, but also the possibility of implementing the aspect of metabolism in DNT and ANT NAMs. For example, by exploring the metabolic activity of the currently used cell lines, assessing the feasibility of co-culturing the used neural (stem) cell lines with metabolically active cells, or by exposing the test system in the culture medium to an ex-vivo mimic of the metabolic system (e.g., S9 extract). In parallel to developing 'wet-lab' coverage of metabolism, the further development of *in silico* modelling to address this metabolic aspect remains encouraged.

Where it fits into the regulatory landscape. Before extensive regulatory acceptance of DNT and ANT NAMs can be considered, it is crucial to ensure the technique can be used to identify metabolically activated neurotoxicants.

Short- and long-term impact. Enabling the detection of metabolically activated neurotoxicants would enhance the scientific and regulatory relevance of the NAM.

2.1.2 Immunotoxicity

Why further research is needed:

We need to understand when the human immune system is most sensitive to chemical exposure. More specifically we need to identify and characterise the effects on the foetus, developing children and adults.

Regulatory needs:

Identify sensitive moments in the development of the human immune system. Identify available NAMs to address these effects and develop new NAMs where needed. Apply these NAMs to better regulate the adverse impact of these chemicals.

Impact:

- Improve identification and regulation of immune-toxic substances.
- Support potential future development of dedicated CLP hazard classes for immuno-toxicity.

The development of the immune system can be divided into multiple processes such as development of primary immune organs (such as bone marrow and thymus) and secondary immune organs (such as spleen and lymph nodes). However, there is currently no scientific consensus on the critical time window(s) in which the development (immune organ developments and formation of the peripheral immune homeostasis) is most sensitive to chemical perturbation that can lead to adversities in the function of the immune system. Due to the scientific uncertainties, regulation still relies on using *in vivo* developmental immunotoxicity studies to ensure that all critical windows are covered. Currently there are some initiatives by CAAT (The Johns Hopkins centre for alternatives to animal testing) to investigate this endpoint[6].

2.1.2.1. Identification of critical windows of development of the immune system

Why the topic is of relevance: Developmental immunotoxicity is of concern because of an observed increase of diseases that are linked to the immune system (e.g., allergies, autoimmune diseases)[7]. Currently, assessment of possible developmental immunotoxicity effects is a standard information requirement at the highest tonnage

6 DIT Alternatives Group - The Johns Hopkins Center for Alternatives to Animal Testing (jhsph.edu)

7 F Miller, The increasing prevalence of autoimmunity and autoimmune diseases: an urgent call to action for improved understanding, diagnosis, treatment, and prevention; Curr Opin Immunol. 2023

level under REACH (i.e., for substances brought onto the European market above 1000 tonnes per year), in case a concern for immunotoxicity is observed in previously performed studies in adult animals (EOGRTS with cohort 3). As the concern for developmental immunotoxicity (and the request for further assessment of this effect) is based on data generated in adult animals, it is possible to miss substances causing immunomodulation in developing animals.

As there is a lack of scientific consensus on the critical windows for the development of the immune system, further work is needed. Without this scientific understanding it is impossible to develop a NAM based battery to assess developmental immunotoxicity, even for screening or priority setting. Once those critical windows have been identified, a next step would be to assess what type of methods may already be available and whether those could be used (perhaps with further development and validation) for assessing developmental immunotoxicity in regulatory processes. Based on current state of science (mainly linked to academia-based research), there are multiple methods containing standard *in vitro* techniques, as well as new types of tissue cultures.

There is however only one NAM based technique[8] with international approval for assessing immunotoxicity, which hampers the inclusion of non-animal-based methods or test batteries into the regulatory system. Due to the general concern of this endpoint, it is important to have at least NAM based methods for priority setting or screening, to better decide on testing needs and to understand the potential risks of e.g., industrial chemicals for the developing immune system.

Currently, developmental immunotoxicity is included under reproduction toxicity in CLP regulation. Discussions are ongoing to develop a specific hazard class for immunotoxicity (containing both adult and development immunotoxicity). Also for this purpose, it would be beneficial to have (more) NAMs available.

Where it fits into the regulatory landscape: this concerns basic research, which is needed to gain a better understanding of the scientific possibilities and regulatory applicability of new NAMs to address immunotoxicity. Depending on their applicability domain, the NAMs could be used for priority setting (better targeting of *in vivo* testing), support of read-across and possibly even for classification and labelling purposes.

Short-term impact: To identify critical windows in the development of the immune system and to analyse the NAM related methodologies that are already available. In case promising methods are not available, further consideration of development of NAMs is needed.

Long-term impact: To develop or validate a testing battery of NAMs for the assessment of developmental immunotoxicity to implement in a regulatory context e.g., screening, priority setting, supporting evidence, hazard identification or risk assessment.

8 OECD TG 444A: *in vitro* Immunotoxicity

2.1.3 Endocrine Disruption

Why further research is needed:

ED assessment relies heavily on animal testing and test methods focus mainly on EATS modalities. We also need more knowledge and test methods for non-EATS modalities to address all ED substances and improve their risk management.

Regulatory needs:

Develop and validate NAMs to assess and interpret ED activity for EATS and non-EATS modalities.

Impact:

- Allow and speed up the identification and regulation for (non-)EATS ED substances.
- Reduce the need for animal testing.

2.1.3.1. Development of NAMs

The assessment of endocrine disruption (ED) heavily relies on vertebrate animal testing to obtain information on adversity and endocrine activity to satisfy the current criteria to identify an endocrine disruptor. In the attempt to reduce vertebrate animal testing, efforts should be made to achieve an equal level of information by using NAM approaches, for example developing non-protected embryo assays capable of predicting ED adverse effects.

Why the topic is relevant: There is currently a gap of NAMs for ED. ECHA identifies three areas where the development of NAMs is needed:

1. Develop new or improve existing assays for (non-)EATS endocrine modalities: EATS modalities (estrogen, androgen, thyroid, and steroidogenesis) are the endocrine pathways where we have a relatively good mechanistic understanding of how substance-induced perturbations may lead to adverse effects via an endocrine- disrupting Mode of Action (MoA). However, the ED criteria apply to all endocrine-disrupting MoAs, i.e., adverse effects that may be caused by any endocrine modality (e.g., insulin receptor signalling). Therefore, there is a need to develop NAMs for both EATS and non-EATS modalities. Ideally, the NAM method developed should investigate multiple modalities in one test.

2. Establish the biologically plausible link: More ED-related (quantitative) AOPs should be developed by the scientific community to facilitate the assessment and interpretation of an observed endocrine activity and the concurrent occurrence of an adverse effect. It may be promising to systematically elucidate and group AOPs starting with the same molecular initiating event (MIE) and then try to systematically identify the pathways leading to different adverse effects.

3. Develop NAMs based on invertebrates: Invertebrates are a very important class of organisms that are crucial for biodiversity and the ecosystem. Consequently, by ensuring well-functioning ecosystem services, they affect human wellbeing as captured under the 'One Health' perspective. Endocrine disruption also affects non-vertebrate organisms (endocrine disruption was first studied in invertebrate species). Currently, the ED assessment focuses on vertebrate organisms (understanding of the endocrine system and availability of test methods most advanced), i.e., mammals, fish, and amphibians. Some of the

endocrine systems are conserved though evolution and are also present in invertebrates. However, the identification of endocrine disruptors in invertebrate species is hampered by the scarce knowledge of endocrinology in these species and the difficulty to postulate the biological plausible link. Therefore, further research is needed to better understand the endocrinology of invertebrates, represent a wider range of environmental species, with a focus on developing test guidelines for the identification of EDs, including also mechanistic parameters.

Where it fits into the regulatory landscape: The development of NAMs is important because information on adversity and mechanism of action as well as the demonstration of the biologically plausible link (i.e., mode of action / AOP) is needed for ED identification. Information on the mechanism through which a substance could be considered endocrine active (e.g., by binding to and activating a receptor or interfering with hormone production) is an Information Requirement for BPR and the Plant Protection Product Regulation (PPPR) and it is the basis for classification under the newly included ED criteria under CLP[9].

Short- and long-term impact: Once developed and validated for their regulatory purpose, these methods could be introduced as information requirements in the different regulatory frameworks and replace more traditional (*in vivo*) methods. Improved screening methods and regulatory confidence is expected to reduce the need for higher tier (animal) testing for all compounds and may this need to only those cases where this cannot be avoided. Well established AOPs will in the long run speed up the CLH or other hazard identification processes and allow for greater efficiency because the existing knowledge can be used to link an adverse effect to an endocrine modality, thereby establishing the biological plausibility of the postulated mode of action. The short-term impact would be an increase of identified ED substances and, in the long-term, a reduced number of vertebrate tests. This will allow to reduce or avoid further animal testing and steer industry to "greener chemistry".

The development of NAMs based on invertebrates will probably require some time as new ED methods can only be developed after gaining basic understanding of the invertebrate endocrinology. Therefore, the horizontal time span in this case is rather long-term. Once developed, methods based on invertebrates could be introduced as information requirements into the different regulatory frameworks and allow the identification of endocrine disruptors that target invertebrates which currently are undetected due to the lack of suitable methods. In addition, in the long term, methods based on invertebrates potentially could replace vertebrate methods for ED identification, thereby allowing a (further) reduction of vertebrate animal testing.

2.1.3.2. Expansion of the OECD toolbox to other non-EATS modalities

The CLP criteria apply to all endocrine modalities, including non-EATS modalities. However, for those modalities, such as the retinoid acid pathway and the metabolism disorders with a clear known adverse effect, the existing mechanistic knowledge is limited.

Why the topic is relevant: There is a lack of methods investigating adverse effects and endocrine activity for non-EATS modalities. Therefore, there is a need to develop and validate more methods to address these. ECHA identifies the following areas:

1. Develop methods for the Retinoid system pathway: The OECD has recently developed a detailed review paper on the retinoid system (DRP Series on testing and assessment No. 343[10]) highlighting the importance of this pathway across different phyla and for many life processes.

   Retinoids are essential molecules that are needed for normal physiological functions, including neurodevelopment, growth, and cellular metabolism. The importance of retinoid signalling is reflected

9 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023R0707&from=EN

10 https://one.oecd.org/document/ENV/CBC/MONO(2021)20/en/pdf

in the conservation of genes and pathways across many phyla, including vertebrates and invertebrates. It is therefore not surprising that dysmorphogenesis of various tissues associated with altered retinoid transport, metabolism and signalling is reported in wild populations of fish, birds, amphibians and mammals. Subtle increases or decreases in concentrations of retinoic acids (the main biologically active form of Vitamin A) or some of its metabolites can directly influence the expression of genes that regulate cell differentiation and maturation with direct consequences for fundamental life processes in virtually every organ and species. Examples include sex determination, neural tube formation and formation of craniofacial structures.

Increasing evidence shows that certain environmental chemicals (including organochlorine pesticides, alkylphenols and styrene dimers) can bind to, and transactivate, the retinoic acid receptor. Considering the critical role of retinoids in key physiological processes, it is important to develop a thorough understanding of the extent of retinoid disruption in humans and wildlife, the most important mechanisms for disruption, and to initiate a systematic process to identify and develop a suite of assays to accurately test for potential retinoid system modulators.

Due to the complexity of retinoid signalling across multiple organ systems, this effort is foreseen as a multi- step process with an initial focus on efforts to identify retinoid signalling pathway test methods, markers, and endpoints for consideration.

Despite the importance of retinoid signalling in many life processes, and the potentially broad adverse effects of disrupting this signalling system, there are currently no OECD test guidelines that specifically cover retinoid system perturbation.

Due to the complexity of the retinoid system, there is a need for using an AOP framework to help understand the link between specific *in vitro* and -omics targets with non-specific downstream effects. AOPs can also help to unravel the complexity of crosstalk between pathways and understand the relationships between key events in an AOP, as well as identify gaps in biological understanding.

2. Develop methods for identifying metabolism disorders (obesity, diabetes): Recently, the risk of obesity, hypertension, and distorted lipid and glucose metabolism has been increasing, which together are also known as metabolic syndrome. Metabolic syndrome is a strong predictor of cardiovascular disease morbidity and mortality. Traditionally, metabolic syndrome has been related to unhealthy lifestyle factors, such as high calorie and ultra-processed diets, decreased physical activity, and genetic predisposition. However, epidemiological and experimental data on the close association of endocrine disruption and adverse metabolic effects are mounting. Despite the importance of metabolism in maintaining life, fat and glucose metabolism are largely overlooked in current OECD test guidelines. One of the reasons for this could be that to detect adverse effects related to metabolic disorders, additional stressors are needed such as use of high fat diet and or test systems which use transgenic animals. Therefore, current testing methods do not appropriately identify adverse effects related to metabolic syndrome.

   At the same time, there is a multitude of methods developed by academia and the pharmaceutical industry that are specifically designed to detect alterations in the metabolic system. To make these methods useful for regulatory purposes, existing methods need to be reviewed and integrated into the existing test method scheme.

   Like for the retinoid system (see above) there is a need for using an AOP framework to help understand the link between specific *in vitro* and -omics targets with specific downstream effects, unravel the complexity of crosstalk between pathways. understand the relationships between key events in an AOP and identify gaps in biological understanding.

Where it fits into the regulatory landscape: These methods will support the ED identification under PPPR, BPR and CLP.

Short- and long-term impact: Once developed and validated, these methods could be introduced as information requirements across different legislative frameworks and will allow the identification of endocrine disruptors acting via this pathway which are currently undetected.

In the interim, while knowledge is being gained, and despite challenges posed due to the interplay of retinoid signalling with other pathways/ bioregulators and spatial/ temporal signalling complexities, a retinoid AOP approach and an AOP approach for metabolic disorders may (or will) aid integrating useful AOPs and moving forward towards the goal of chemical screen development.

2.1.3.3. Endocrine disruption risk assessment

There is still no consensus in the scientific community on whether and how certain toxicological principles such as the 'safe threshold', (i.e., the dose below which no adverse effect is expected to occur) are applicable in assessing the safety of substances identified as endocrine disruptors.

Why the topic is relevant:

1. Explore current challenges with performing a risk assessment: The main issues that raise questions on whether it is possible to derive safe levels for substances with endocrine disrupting properties are related to complex phenomena such as non-monotonic dose response curves, low doses/ concentrations effects, delayed effects, multigenerational effects, critical (time) windows of exposure, and cross-species extrapolation. Therefore, there is a need for the scientific community to further investigate these phenomena to support regulators and to reduce the overall uncertainty if a risk assessment for EDs is carried out. Also, further research could be carried out to understand if probabilistic methods of prediction of thresholds would work for substances with endocrine disrupting properties. Other research needs described above, such as the consideration of additional non-EATS endocrine pathways and the development of test methods for underrepresented taxa (e.g., invertebrates) will also contribute to reduce the uncertainty in the risk characterization of ED.

2. Explore improvements of available tests to ensure critical windows of exposure are covered and all useful sensitive parameters are included. The possibility to perform a risk assessment for substances with endocrine disrupting properties is hampered by knowledge gaps and testing deficiencies in relation to issues mentioned in the previous paragraph. There is a need to further investigate how sensitivity varies with developmental stage to ensure the most critical windows of exposure are captured in ED tests, as well as assess the most sensitive endpoints and species, and based on these adapt and improve the existing ED tests.

Where it fits into the regulatory landscape: A risk assessment for ED is performed under the PPPR and BPR, and it is a possibility under the REACH processes of authorization and restriction. More clarity is needed if a scientifically underpinned safe threshold can be established for ED acting substances. That research will also support the ED identification under the PPPR, BPR and CLP.

Short- and long-term impact: Research in this area can support the regulators in taking decisions, when managing endocrine disruptors across different legislative frameworks.

2.2 Addressing chemical pollution in the environment

As recognised in the CSS, chemical pollution is one of the key drivers contributing to ecosystems degradation and biodiversity loss. Release of chemicals that are resistant to degradation will lead to increasing concentrations in the environment. Increasing concentrations of these persistent chemicals raise the probability of the adverse effects in wildlife and humans. It is acknowledged that identification, including testing, of persistent chemical substances might be often challenging[11]. Increasing pressure of chemicals on ecosystems has led to the need to improve environmental hazard and risk assessment approaches.

In practice, environmental risk assessment of chemicals is conducted by evaluating exposure pathways and the fate of a single substance (or constituent) within the environment. This includes its persistence and bioaccumulation, and its toxicity to a limited number of organisms through standardised laboratory tests. If a chemical fulfils the PBT/vPvB criteria, comparing their persistence (P), bioaccumulation (B) and toxicity (T) with hazard criteria in Annex XIII of REACH or Annex I of CLP, it is not possible to establish a 'safe' concentration in the environment for such chemicals. PBT or vPvB chemicals have potential to accumulate in the environment and stopping emissions may not lead to a reduction in their environmental concentration due to their persistence. PBT or vPvB chemicals may also have the potential to contaminate remote areas. The long-term effects of exposure to such chemicals are difficult to predict. Appropriate methods to identify PBT/vPvB chemicals are therefore of high priority to protect wildlife and humans.

One key element in the management of the risks posed by chemical pressure on ecosystems is the development of targeted NAMs that can efficiently address the manifold interactions between chemicals and ecosystems. These include *in vitro* and *in silico* methods for hazard and fate assessment of different chemicals. Advances in monitoring approaches and analytical method development are also key aspects in identifying emerging risks posed by chemicals in the environment.

The development and mapping of NAMs (e.g., *in vitro*, omics, *in silico*) is also needed to improve determination of most sensitive species per chemical, reduce animal testing, and at the same time increase the biodiversity protection by expanding our capacity to extrapolate toxicity results ideally at the ecosystem level.

This chapter also develops the research needs for the assessment

11 E.g. Technical guidance on biodegradation testing of difficult substances and mixtures in surface water. Heidi Bircha, Rikke Hammershøj, Mette Torsbjerg Møller, Philipp Mayer. MethodsX, Volume 10, 2023, 102138.

of the bioaccumulation potential of chemicals, for non-bee pollinators (NBPs) sensitivity to biocidal active substances as well as the importance to develop new approaches to monitor and analytically verify chemicals present in the environment.

2.2.1 Bioaccumulation

Why further research is needed:

Accumulation of chemicals in organisms can lead to toxic effects after long-term exposure even when external concentrations are very low. Bioaccumulation assessment should be improved and become less dependent on in vivo vertebrate studies.

Regulatory needs:

Develop NAMs to predict bioaccumulation in general, and to improve prediction for specific substances (e.g., surface-active, ionisable or super-hydrophobic) as well as for air-breathing organisms.

Impact:

- Improve bioaccumulation assessment for all substances and different organisms
- Help the identification and regulation of substances of very high concern under REACH and classification of substances as PBT/vPvB or PMT/vPvB under CLP
- Reduce the need for animal testing.

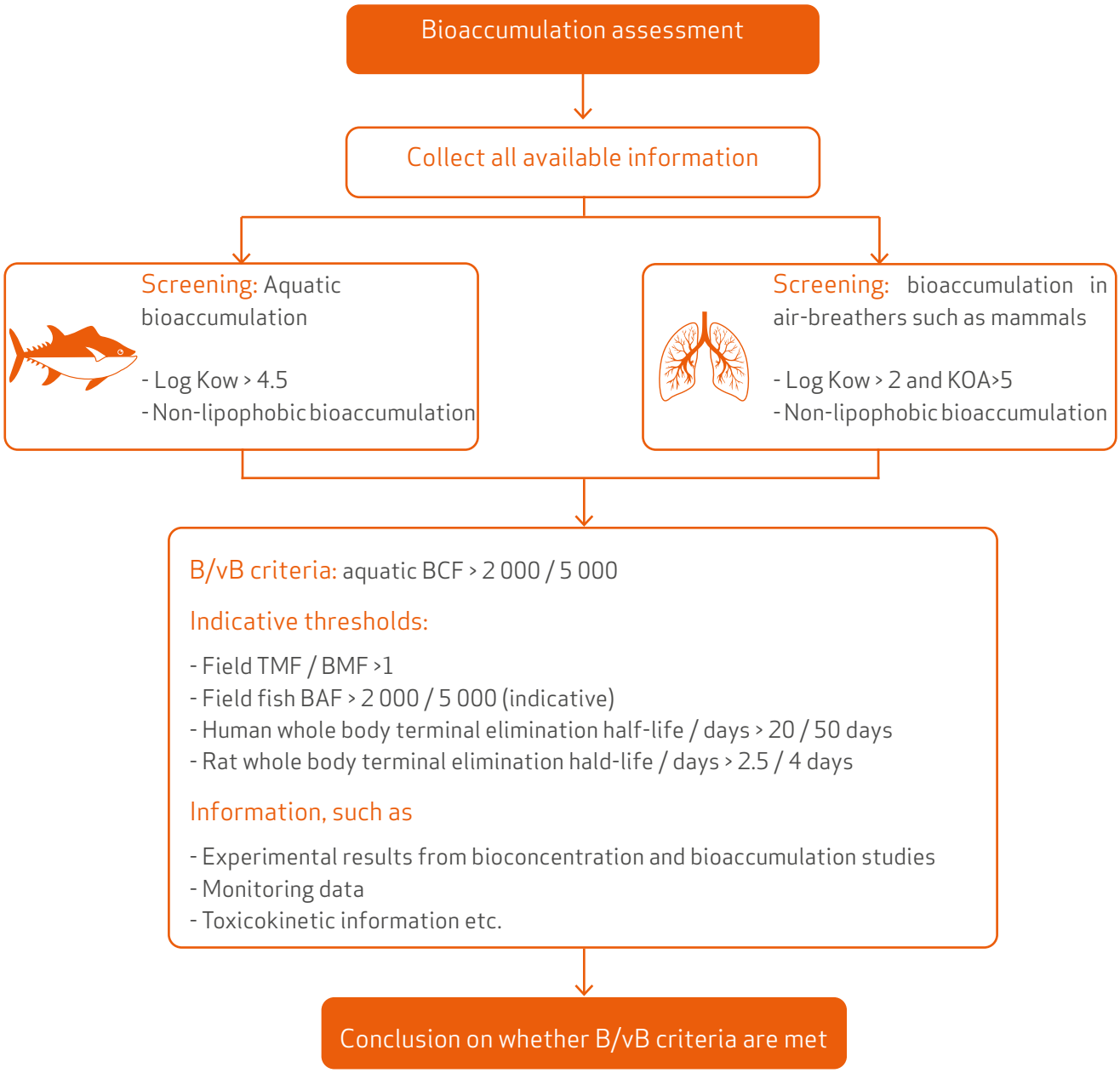
Bioaccumulation may be defined as the net result of uptake (via various routes of exposure), distribution, transformation and elimination of a substance in an organism. Bioaccumulation data is necessary for understanding the environmental behaviour of a chemical. Bioaccumulation can lead to internal concentrations of a substance in an organism that cause toxic effects over long-term exposures even when external concentrations are very low. Highly bioaccumulative substances may also transfer through the food web, which in some cases may lead to biomagnification. Biomagnification is the accumulation of a substance via the food chain, from prey to predator.

Within different Regulations (i.e., REACH, BPR, CLP) information on bioaccumulation is used in 1) PBT assessment, 2) hazard classification, and 3) chemical safety assessment (e.g., food chain exposure assessment). Bioaccumulation data is also a factor in deciding whether long-term ecotoxicity testing might be necessary.

REACH, BPR and the CLP Regulation emphasise importance to identify and regulate PBT and vPvB substances. According to REACH Annex XIII and CLP Annex I, a substance is considered to be bioaccumulative if it has a bioconcentration factor (BCF) in aquatic species higher than 2000 and very bioaccumulative if it has a BCF in aquatic species higher than 5000. Bioconcentration is the net result of uptake, transformation and elimination of a substance in an organism due to waterborne exposure only.

The most important and widely accepted indication of bioaccumulation potential for organisms with aquatic respiration such as fish, is a high value of the n-octanol/water partition coefficient, Log Kow. Log Kow is generally used as a first-tier screening indicator for substances which are expected to partition to lipids. Depending on the regulatory context, higher-tier data is generated by performing *in vivo* fish testing following the OECD 305 TG. Bioaccumulation in aquatic invertebrates such as mussels may also be evaluated and there is an OECD TG in preparation which measures BCF in the freshwater amphipod *Hyalella azteca*[12]. The bioaccumulation assessment of sediment-associated chemicals in endobenthic oligochaete worms and the bioaccumulation of chemicals in soil oligochaetes may also be relevant.

12 https://www.oecd.org/env/ehs/testing/test-guidelines-for-comments-section3-degradation-and-accumulation.htm



3. Figure 1: Steps in Regulatory Bioaccumulation Assessment for PBT/vPvB assessment under REACH, CLP, BP

Intrinsic hepatic clearance in fish can be estimated from *in vitro* clearance assays according to OECD TG 319 A and B using either cryopreserved rainbow trout hepatocytes or liver S9 subcellular fractions. Clearance rates can then be extrapolated to a (BCF) using *in vitro-in vivo* extrapolation (IVIVE) methods. Such methods may also help to support read-across and grouping approaches by allowing a comparison of the *in vitro* behaviour of different chemical substances as well as to better understand mechanisms of metabolism and support development of a mechanistic models.

Although for many substances the assessment of bioaccumulation in aquatic species is sufficient, some substances (for example, endosulfan, beta-hexachlorocyclohexane, many PFAS or highly lipophilic substances) may accumulate more than expected in air-breathing organisms such as mammals. These substances would not be recognised as highly bioaccumulative if only aquatic BCF data were used in the assessment. One reason for this different outcome may be the ability of gill-breathing organisms to eliminate non-volatile substances into the water that cannot be eliminated by air-breathing organisms by respiration. For mammals and birds,

bioaccumulation essentially occurs through uptake from food, associated with elimination via urination and the gastrointestinal tract, metabolism, exhalation and growth (dilution). In this context, air-breathing organisms also include marine mammals and humans.

ECHA works to avoid unnecessary testing on animals and promotes alternative test methods. ECHA proposes the following research needs to reduce the need for *in vivo* vertebrate studies and to improve bioaccumulation assessment for difficult substances:

- develop non-vertebrate and/or non-*in vivo* methods to predict the bioaccumulation potential of surfactants, ionisable substances and organo-metals;
- improve the bioaccumulation assessment for air-breathing organisms;
- improve the assessment for secondary poisoning and man via environment, especially for mixtures;
- develop new methods and assessment approaches to evaluate the bioaccumulation potential of super hydrophobic substances.

It should be emphasised that any alternative method or approach developed is most useful for regulatory purposes if the outcome can be compared with regulatory thresholds.

2.2.1.1. Development of non-vertebrate and/or non-*in vivo* methods to predict the bioaccumulation potential of surfactants and ionisable substances as well as of organo-metals

Why the topic is relevant: Log Kow is used as a screening tool in bioaccumulation assessment as an indicator for partitioning of the substance to lipids.

REACH Annex IX section 9.3.2 states that it is not possible to waive the bioaccumulation test in aquatic species based on low Log Kow if the substance is ionisable or surface active at environmental pH. Log Kow is not a good indicator of the bioaccumulation potential of surfactants or ionisable substances because they may have additional binding interactions (e.g., with proteins) and mechanisms for transport across cell membranes, which are not accounted for by the Log Kow which only measures partitioning to lipid.

Log Kow is also not a good indicator of the bioaccumulation potential for metallo-organic substances. Such substances may react inside organisms to form more lipophilic substances (e.g., methylation) or may bind with cell constituents[13].

Aspects of the bioaccumulation potential of ionisable substances in fish that are thought to be characterised relatively well include the pH dependence of gill uptake and elimination, uptake in the gut, and sorption to phospholipids (membrane–water partitioning).

Key challenges include the limited empirical data for biotransformation and binding in plasma where fish possess a diverse array of proteins that may transport ionised substances across cell membranes. Furthermore,the general phenomenon known as the “ion trap” effect due to the large pH gradient between lysosomes and cytoplasm, may result in the preferential concentration of the charged form in the lysosomal compartment, with differences of about 2-3 orders of magnitude, compared to the cytosol.

The fish-water partition coefficient or membrane lipid-water partition/ distribution coefficient (K_{MLW}/D_{MLW}) could play a role at a screening level to trigger a bioaccumulation concern for organo-metals, ionisable and/or surface- active substances. There is currently no standardised test guideline for the experimental determination of K_{MLW}/ D_{MLW}. The three most common experimental methods are[14]: 1) dissolved unilamellar liposomes, 2) lipid

13 Revised introduction to the OECD Guidelines for testing of chemicals, Section 3 (23 March, 2006)

14 Guidance on Information Requirements and Chemical Safety Assessment - Chapter R.7c: Endpoint specific guidance Version 4.0 December 2023, European Chemicals Agency, Helsinki.

bilayers non-covalently coated on microporous silica and 3) covalently linked phospholipid monolayers on HPLC grade silica. K_{MLW}/D_{MLW} can also be predicted.

There is a need to assess and/or develop relevant parameters and thresholds, alternative testing and assessment strategies for bioaccumulation assessment of such substances in order to minimise the need for *in vivo* testing with vertebrate animals.

Methods that avoid the use of vertebrate animals are needed to predict or assess the bioaccumulation potential of organo-metals, surface active and/or ionisable substances to avoid automatically requesting fish BCF tests on these substances. Such methods would reduce the need for vertebrate testing on fish and allow improved B assessment of these substances, feeding into the identification of substances of very high concern and for classification of substances as PBT/vPvB.

Especially cationic substances seem to present challenges for predicting their bioaccumulative properties (e.g., applicability of *in vitro* to *in vivo* extrapolations (IVIVE)), and a better understanding of parameters influencing their behaviour is needed.

Where it fits into the regulatory landscape: Substances that persist for long periods of time in the environment and have a high potential to accumulate in biota are of specific concern because their long-term effects are rarely predictable. Once they have entered the environment, exposure to these substances is very difficult to reverse, even if emissions are stopped. Identification of PBT/vPvB substances is part of the hazard assessment of substances under REACH, BPR and CLP.

Log Kow is used as a screening tool in bioaccumulation assessment, as an indicator of partitioning to lipid. For some groups of substances, such as organo-metals, ionisable substances and surface-active substances, Log Kow is not a valid descriptor for assessing the bioaccumulation potential. Information on bioaccumulation of such substances should therefore take account of descriptors or mechanisms other than hydrophobicity. There is a need to improve knowledge and develop methods which would allow to predict bioaccumulation potential of organo-metals, ionisable and surface-active substances.

Short-term impact: It is expected that understanding bioaccumulation mechanisms for organo-metals, ionisable and surface-active substances will be improved. The fish-water partition coefficient, membrane lipid- water partition/ distribution coefficient or other identified parameters could play a role at screening level to trigger or remove a bioaccumulation concern for such substances.

Long-term impact: Such methods would reduce the need for vertebrate testing on fish and allow improved B assessment of these substances, feeding into the identification of substances of very high concern and for classification of substances as PBT/vPvB.

2.2.1.2. Improve bioaccumulation assessment for air-breathing organisms (e.g., terrestrial mammals)

Why the topic is relevant: Current regulation on bioaccumulation focuses on the bioconcentration factor (BCF) for fish. However, certain substances do not bioaccumulate in aquatic food-webs, but in air-breathing animals (e.g., terrestrial mammals, birds), posing a threat to terrestrial food webs. In air-breathing organisms, bioaccumulation typically occurs via the diet. Fish are rather efficient in clearing themselves of chemical substances via the ventilated water. In contrast, air-breathing organisms cannot clear themselves effectively from chemicals via physico-chemical partitioning into exhaled air, or excreted urine and faeces because the respective sorption capacities of these media are small, and their excreted volumes are insufficient for clearance of hydrophobic chemicals.

Especially for terrestrial food-webs, certain types of substances (Log Kow > 2, log K_{OA} >5, difficult to metabolise), can pose a long-term threat to top predators (including humans), and the information sources to identify such kind of substances are limited. The numerical cut-off values are still subject to scientific review. Recently, Saunders and Wania (2023)[15] evaluated thresholds for air-breathing animals across species and found that animals with lower rates of respiration (e.g., manatees and sloths) and those ingesting high-lipid diets (e.g., polar bears and carnivorous birds) were predicted to be able to biomagnify persistent chemicals with log K_{OA} < 5. This was also observed for several temperate reptiles due to their lower respiration rates and internal temperatures. The discussion paper "Bioaccumulation assessment of air-breathing mammals[16]" (2022) outlines an approach on the use of toxicokinetic data for assessing bioaccumulation in air-breathing mammals. The paper is based on discussions from a working group with leading experts from academia, industry and government. The proposed approach (tiered strategy, including *in vitro* methods based on material from rat) is reflected in the PBT guidance R.11 (2023)[17].

Information feeds into the bioaccumulation assessment for the identification of substances of very high concern and for classification of substances as PBT/vPvB.

Where it fits into the regulatory context: Historically, bioaccumulation assessment has focused mainly on aquatic (water-breathing) species. Field measurements[18] and theoretical mathematical models[19] have indicated that some chemicals that may not be considered bioaccumulative using the aquatic-based BCF and associated criteria are bioaccumulative in air-breathing organisms, e.g., endosulfan, beta-hexachlorocyclohexane and many perfluorinated alkyl substances[20].

Under REACH and CLP, besides results from bioconcentration or bioaccumulation studies in aquatic species, other information on the bioaccumulation potential or information on the ability of the substance to biomagnify in the food chain can be used to assess bioaccumulative (B) or very bioaccumulative (vB) properties (REACH Annex XIII, 3.2.2; CLP Annex I, 4.3.2.3.2).

Short-term impact: Improved methods to assess bioaccumulation in apex organisms (e.g., development of an OECD test guideline for rat S9 and/or hepatocytes assay, verification of IVIVE approach, determination of hindered uptake for air-breathing species, use of toxicokinetic data for extrapolation to apex organisms, expanding the concept to other air-breathers such as birds).

Long-term impact: Improved bioaccumulation assessment for air-breathing organisms which feeds into the identification of substances of very high concern and for classification of substances as PBT/vPvB.

2.2.1.3. Improve the assessment for secondary poisoning and man via environment, especially for mixtures.

Why the topic is relevant: Secondary poisoning refers to toxic effects in the higher members of the food chain, either living in the marine, aquatic or terrestrial environment, which result from ingestion of organisms from lower trophic levels that contain accumulated substances. Previous cases have demonstrated that severe effects can

15 Saunders, L.J. and Wania F. (2023). Cross-Species Evaluation of Bioaccumulation Thresholds for Air-Breathing Animals. Environmental Science & Technology 2023 57 (29), 10491-10500.

16 Arnot J., et al. Bioaccumulation assessment of air-breathing mammals (2022)

17 Guidance on Information Requirements and Chemical Safety Assessment - Chapter R.11:PBT/vPvB assessment, Version 4, December 2023, European Chemicals Agency, Helsinki.

18 Kelly BC, Gobas FAPC. 2001. Bioaccumulation of persistent organic pollutants in lichen-caribou-wolf food chains of Canada's Central and Western Arctic. Environ Sci Technol 35:325–334.

19 Kelly, B.C., Gobas, F.A.P.C., An Arctic terrestrial food-chain model for persistent organic pollutants. Environ. Sci. Technol. 2003, 37, 2966-2974; Czub, G., McLachlan, M.S., Bioaccumulation potential of persistent organic chemicals in humans. Environmental Science and Technology 2004, 38, 2406-2412

20 Kelly, B.C., Ikonomou, M. G., Blair, J.D., Morin, A.E., Gobas, F.A.P.C., Food web-specific biomagnification of persistent organic pollutants. Science 2007, 317, 236-329

arise after exposure of animals via their food and that bioconcentration, bioaccumulation and biomagnification in food chains need to be considered. The pathway for secondary poisoning is referring exclusively to the uptake through the food chain.

Similar considerations apply for humans via the environment. For human exposure via the environment, the systemic hazard for long term effects is based on exposure via inhalation and via the oral route. Human behaviour related to food consumption shows appreciable variation between different EU countries but also within the countries. Equally, large variations can occur between individuals. The distribution and intensity of local sources of exposure will also be different between EU countries. Consequently, indirect exposure is likely to vary greatly within a given population. Therefore, the exposure model (with its underlying assumptions) will have a major influence on the result of the assessment. In EUSES (European Union System for Evaluation of Substances[21]), the local scale represents a worst-case situation as people do not consume 100 % of their food obtained from the immediate vicinity of a point source. Equally, the regional assessment represents a highly averaged exposure situation, which does not describe individuals who consume food products from the vicinity of point sources[22].

There is a need to give more attention to the topic of secondary poisoning and humans via the environment by integrating the concept of mixture toxicity into these assessments[23] and increasing realism of such assessments. Furthermore, monitoring data could be used to assess the potential of a chemical for secondary poisoning and approaches to do so should be developed.

Where it fits into the regulatory landscape: According to Annex I of REACH Regulation, the environmental hazard assessment shall consider the potential effects on the environment, including the potential effects that may occur via food-chain accumulation. ECHA Guidance[24] explains that in the chemical safety assessment (CSA), fish BCF and BMF values are used for the secondary poisoning assessment for wildlife, as well as for human dietary exposure. A BMF for birds and mammals may also be relevant for marine scenarios. An invertebrate BCF can be used to model a food chain based on consumption of sediment worms or shellfish. When a derived no-effect level (DNEL) is derived for long term systemic exposure via the inhalation and oral routes for the general population, risk characterisation for man via the environment based on exposure estimates for the different environmental compartments is systematically required.

Short-term impact: To further improve understanding and develop methodologies enabling adequate secondary poisoning and man via environment assessments, including for mixtures and complex substances.

Long-term impact: Improved identification and regulation of substances raising concern due to secondary poisoning or exposure of man via the environment.

2.2.1.4. Development of new methods and assessment approaches to evaluate the bioaccumulation potential of super hydrophobic substances.

Why the topic is relevant: It is a widespread opinion that super-hydrophobic substances, with a Log Kow > 8, have limited bioaccumulation potential in aquatic or air-breathing organisms because they cannot be taken up to any significant extent due to low bioavailability. However, several super-hydrophobic substances, such

21 Theo Vermeire; Tjalling Jager; B Bussian; J Devillers; K den Haan; B Hansen; I Lundberg; H Niessen; S Robertson; H Tyle; P T van der Zandt (1997) European Union System for the Evaluation of Substances (EUSES). Principles and structure. Chemosphere 34(8):1823-36.

22 Guidance on information requirements and chemical safety assessment, Chapter R.16: Environmental exposure assessment, Version 3.0, February 2016.

23 Chemicals Strategy for Sustainability, European Commission, 14 October 2020.

24 Guidance on information requirements and chemical safety assessment, Part B: Hazard assessment, Version 2.1, December 2011.

as Dechlorane Plus[25] and MCCPs[26], have been shown to bioaccumulate and super-hydrophobic substances are starting to be detected in biota. Such substances are expected to be taken up and eliminated only very slowly and it may take years to reach steady state in an organism.

Consequently, current standard bioaccumulation tests are not suitable to determine the bioaccumulation of super-hydrophobic substances. It is also very difficult to handle such lipophilic substances in the laboratory due to their tendency to stick to glassware. New testing and assessment approaches are needed to assess the potential of super-hydrophobic substances to undertake and to bioaccumulate, preferably minimising the use of vertebrate testing. This would allow improved B assessment for these substances, feeding into the identification of substances of very high concern and the classification of substances as PBT/vPvB.

Where it fits in the regulatory context: There is evidence that significant accumulation via the food chain takes place from certain highly persistent and super hydrophobic substances (e.g., chlorinated paraffins, chlorinated flame retardants). Under REACH and CLP, along with results from a bioconcentration or bioaccumulation study in aquatic species, other information on the bioaccumulation potential or information on the ability of the substance to biomagnify in the food chain can be used to assess bioaccumulative (B) or very bioaccumulative (vB) properties (REACH Annex XIII, 3.2.2 and CLP, Annex I, 4.3.2.3.2).

Short-term impact: More information needs to be gathered on mechanisms, matrices and parameters enabling assessment of bioaccumulation of super-hydrophobic substances. This will allow development of tools and methods for the bioaccumulation assessment of such substances.

Long-term impact: Improve bioaccumulation assessment of super-hydrophobic substances which feeds into the identification of substances of very high concern and for classification of substances as PBT/vPvB.

2.2.2 Expanding protection of biodiversity using NAMs

Why further research is needed:

Environmental hazard assessment relies on a limited number of species from selected trophic levels. Usually, it is based on endpoints like mortality, growth and reproduction. This approach does not protect sufficiently the high diversity of species in our ecosystems.

Regulatory needs:

Develop and map available NAMs to improve the determination of most sensitive species per chemical (group).

Impact:

- Improved protection of biodiversity by enabling use of toxicity results at ecosystem level.
- Reduce animal testing.
- Inventory of possible "bio-conserved" pathways of toxicity for different species.

Why the topic is relevant: Environmental hazard assessment is focused on the generation of data for only a few species based on acute and chronic toxicity standardised laboratory tests (e.g. OECD TGs 202, 201, 203, 211, 210). Toxicity data on algae represents the hazards to primary producers, data on *Daphnia magna* represents

25 Larisch W, Goss KU. Modelling oral up-take of hydrophobic and super-hydrophobic chemicals in fish. Environ Sci Process Impacts. 2018 Jan 24;20(1):98-104. doi: 10.1039/c7em00495h. PMID: 29235599

26 https://www.echa.europa.eu/documents/10162/98611952-49d5-b0be-d4b9-3df6579315c9

the hazards to invertebrates, and data on fish represents the hazards to vertebrates. These organisms are considered to represent different trophic levels of the ecosystem and form the basis for classification and for risk assessment to the aquatic compartment. For the latter, safety factors are applied to account for the degree of uncertainty when extrapolating from test data to the real environment.

The testing species, which are chosen based on practical aspects such as availability of test guidelines and test organisms rather than on biological grounds, are only a small surrogate of the total biological diversity. In addition, hazard assessment of chemicals focuses almost exclusively on three standardized and directly observable toxicity endpoints — survival, growth, and reproduction of individual organisms — and are selected for populational and ecological relevance. However, new methods may be available in the future to more efficiently protect a wider range of species in the ecosystems.

Increasing understanding of pathways causing toxicity holds the promise to increase our capacity for extrapolating results across different species and biological levels. New methods (e.g., *in vitro*, omics, *in silico*) could help to relate molecular changes (e.g., on proteins) to cellular, organism and population outcomes and allow the identification of the most sensitive species to a particular substance. By testing a limited number of organisms, the impact on a community or ecosystem could be better predicted.

However, for mechanistic biology to be better able to protect species and ecosystems diversity, it is necessary for research to advance in multiple areas. These include, among others, the creation of an inventory of possible "bioconserved" pathways of toxicity for different species, the development of gene expression signatures that can be used to predict toxicity through pattern recognition and probabilistic assessment, the translation of *in vitro* responses to *in vivo* effects (considering toxicokinetics), the mapping of the methods (e.g., omics, SeqAPASS) which could extrapolate any concern for a specific (sensitive) phyla as well as to population and ecosystem level effects.

Where it fits into the regulatory landscape: One of the fundamental aims of the REACH regulation is to improve the protection of human health and the environment from the risks that can be posed by industrial chemicals. To achieve sufficient level of protection across ecosystems, the regulation relies on generation of data for a limited number of species and uses the information in classification under CLP for aquatic acute and chronic hazards and PBT and PMT assessment. New methods may be developed to allow more comprehensive prediction of toxicity across different species.

Short- and long-term impact: Developing further and ultimately using NAMs for this particular challenge offer a great prospect to protect biodiversity more comprehensively in the future.

2.2.3 Non-bee pollinators (NBPs) sensitivity to biocidal active substances

Why further research is needed:

Risk assessment for arthropod pollinators may become a standard information requirement for biocides. More data is needed to conclude on sensitivity differences between bee and NBP species.

Regulatory needs:

Research to compare the sensitivity of NBPs and bees to biocides for acute contact and oral toxicity. Also, the most relevant route and life stage of exposure to NBPs should be studied.

Impact:

- Protect wild pollinator populations better and conserve the overall ecosystem structure and functioning
- Steer data generation from bees towards NBPs.
- Produce information to build the EU's data base of chemical effects on arthropod pollinators.

Why the topic is relevant: Arthropod pollinators and their decline is a growing concern globally and chemical pollution has been recognised as one of the major reasons for this phenomenon (EU_Pollinators_Initiative[27]). In February 2024, ECHA published its guidance[28] for the risk assessment of the use of biocides for bees. However, it was not possible to develop a risk assessment scheme for other arthropod pollinators than bees, the so-called non-bee pollinators (NBPs), due to a number of knowledge gaps identified.

Before we can run a full risk assessment for NBPs we need to be able to conclude on sensitivity differences between bee and NBPs species. However, information on the ecology and sensitivity to chemicals for the relevant species is scarce. To allow comparison of the sensitivity between NBPs and honeybees, we need more laboratory data to evaluate the acute contact and oral toxicity for NBPs.

Moreover, further studies are needed to find out which is the most relevant route of exposure for NBPs from the use of biocides. It is relevant to notice that the routes and pattern of exposure from the use of biocides may be considerably different compared to the exposure from plant protection products for which, in general, more information is available for the assessment of risks to terrestrial arthropods.

Another important aspect that needs further investigation is the life stage during which NBPs are most exposed to chemicals under environmental conditions. For this purpose, investigating the full life cycle of NBPs is needed. Such information could be further used in spatially explicit agent-based population models (similar to BEEHAVE[29]). These models are already used for bees and allow efficient assessment of population level effects to chemical exposure. These models also provide information on the most exposed life stages (depending on use/ exposure pattern of biocidal products). However, such models still need to be developed for NBP. Generating further data on these aspects would facilitate making reliable comparisons and elaborating the necessary conclusions to develop risk assessment methodologies that cover also NBPs.

Where it fits into the regulatory landscape: In 2019, the Commission mandated ECHA to develop a methodology and a guidance to assess the risk to bees and other non-target arthropod pollinators from the use of biocides,

27 Revision of the EU Pollinators Initiative (24.01.2023): eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52023DC0035

28 Guidance on the assessment of risks to bees from the use of biocides

29 An Evaluation of the BEEHAVE Model Using Honey Bee Field Study Data: Insights and Recommendations - Agatz - 2019 - Environmental Toxicology and Chemistry - Wiley Online Library BEEHAVE | The Model (beehave-model.net)

under Article 75(1)(g) of the Biocidal Products Regulation (BPR). During this work, ECHA and the expert group noted that currently the available information on NBP species' sensitivity and role in pollination is very limited and significant data gaps exist. This work and suggestions for future research and data generation are documented in ECHA 2022 publication[30].

The political pressure from the Commission and the public to consider NBPs in chemical risk assessment is ever-increasing. For ECHA to be able to develop guidance for the risk assessment of NBPs in the future, it is essential to gain data especially on the sensitivity of NBPs. In addition, the data generation would complement the Commission's "EU Pollinators Initiative" and its objectives to address the decline of pollinators in the EU and contribute to global conservation efforts. Furthermore, the BPR legal text already provides information requirements for NBPs, namely under section 9.5. 'Effects on arthropods' in Annex II for active substances (9.5.2. 'Other non-target terrestrial arthropods') and under section 9.3. 'Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk' in Annex III for biocidal products.

In addition, the proposed research would complement the on-going EFSA non-target arthropod project, AENEAS (*On advancing the environmental risk assessment of non-target arthropods for plant protection products by accounting for the impact on ecosystem services and on the ecological function*) as well as iPOL-ERA (*Advancing the Environmental Risk Assessment of Chemicals to Better Protect Insect Pollinators*).

Short- and long-term impact: Short-term benefit of this research is to steer data generation from bees towards NBPs, taking into account the specific aspects related to exposure from the use of biocides. In the long-term, the produced information would contribute to the EU's data base on arthropod pollinators, and in the end, hopefully also benefit the wild pollinator populations, environment and conservation of the ecosystem services provided by the pollinators.

2.2.4 Monitoring

Why further research is needed:	Regulatory needs:	Impact:
Better use of monitoring data could speed up identification and regulation of hazardous chemicals.	Research, including case studies, to better understand how to use monitoring data to identify bioaccumulation and persistence and assess long-range transport potential.	• Speed up and improve the identification and regulation of PBT/vPvB, PMT/vPvM and POP substances.

2.2.4.1. Development of approaches based on monitoring field data enabling persistence, long-range environmental transport and/or bioaccumulation assessment

Why the topic is relevant: The use of monitoring and field data generated by various authorities and academia, including for research purposes, for bioaccumulation, long-range environmental transport and/or persistence

30 European arthropods and their role in pollination: scientific report of their biodiversity, ecology and sensitivity to biocides

assessments could be improved. This could allow to speed up identification and regulation of emerging chemicals of concern. For example, samples collected for analysis in various specimen databanks could be used to establish trophic magnification factors for prioritised substances for bioaccumulation assessment. Or, monitoring data in environmental or biota matrices in remote areas far away from point sources would enable identifying new Persistent Organic Pollutants (POPs).

Field bioaccumulation or trophic magnification factors as well as monitoring data can provide relevant lines of evidence indicating that a substance has or does not have bioaccumulation properties. Bioaccumulation or biomagnification factors, dietary accumulation, trophic magnification and detection of chemicals in biota can inform bioaccumulation screening and assessment. Monitoring data in environmental matrices can support persistence assessment (including the long-range transport potential (LRTP) assessment), especially if the substance is found in remote areas far away from point sources etc. Overall, indications of an increase of the substance concentration levels in environmental or biota matrices over time is of particular interest for the persistence, bioaccumulation and LRTP assessment.

There is a need to develop further understanding on the use of field and monitoring data for bioaccumulation, persistence and LRTP assessment (e.g., via a benchmarking approach from known bioaccumulative substances), including developing better understanding of associated uncertainties. The scenarios in which such data could be used standalone or in combination with other evidence to conclude on bioaccumulation or persistence (including LRTP) should be identified. For example, 'Food web on ice' is a pragmatic approach to investigate the trophic magnification of chemicals of concern and one could further consider how such information could possibly allow to conclude on bioaccumulation.

Where it fits into the regulatory landscape: Under REACH, CLP and the Stockholm Convention on persistent organic pollutants (POPs), information from field studies (such as field bioaccumulation/ biomagnification potential) or monitoring studies can be used to assess P/vP and/or B/vB properties (REACH Annex XIII, 3.2.1 and 3.2.2) and the LRTP of substances. This information could be used in addition to information available from simulation studies in water/ soil/ sediment and from a bioconcentration or bioaccumulation study in aquatic species.

Short-term impact: Development of methodologies and scenarios enabling adequate identification of PBT/ vPvB/PMT/vPvM/ POP substances based on other data than generated by the test conducted according to the standard test guideline.

Long-term: Identification and regulation of PBT/vPvB/PMT/vPvM/POP substances is improved.

2.2.4.2. Case study: Environmental monitoring data for linear and cyclic volatile methyl siloxanes (VMS)

For a substance to have potential for long-range environmental transport according to the Stockholm Convention on Persistent Organic Pollutants (further called the "Convention"), it needs to be transported over long distances via air, water and/or migratory species, and it needs to transfer to a receiving environment.

The cyclic volatile methyl siloxanes (cVMS) such as D4, D5 and D6 are volatile substances having half-lives in air exceeding two days (which is one of the criteria in the Convention for the long-range atmospheric transport (LRTP) of substances) that have been measured in air in remote regions. However, whether these substances can deposit from the air to surface media is still under discussion. Many experts believe that the cVMS would not back-deposit from air to surface media due to their physical-chemical properties and based on certain modelling studies. Nevertheless, there have been detections or quantifications of these types of substances in environmental and biota samples from remote regions (Arctic and Antarctic) suggesting deposition did take place.

The linear VMS can be alternatives to cyclic VMS (substitutes for specific uses) and the linear VMS could share similar hazard properties to the cyclic VMS. This is why collecting monitoring data on the linear ones in addition to the cyclic ones would help in obtaining an overall understanding on the fate properties for the group of VMSs.

Monitoring of the VMS in precipitation (rain and snow), as well as in freshwater and/or marine sediments and soil far away from point sources would aid the understanding of the deposition mechanisms from air to surface media of these substances. ECHA understands that currently there is no measured data of VMS in rain or snow (only modelling-based data) and little data from water, sediment and soil. As long as that remains the case, the understanding of the deposition mechanisms will not, in our view, significantly develop.

If monitoring of VMS in snow is performed, ECHA strongly recommends that an ice core is taken instead of sampling surface snow in order to investigate the deposition potential of VMS. An ice core will better reflect a possible deposition mechanism of VMS compared to surface snow as it contains several layers of snow including trapped air (in case of a strong snow events) which are likely to contain VMS. Furthermore, ice cores enable temporal trends to be determined.

Analytical methods and techniques are currently available to monitor concentration of VMS in air. If a measurement of the VMS in rain or snow is technically challenging due to the volatile properties of these substances, an alternative approach would be to measure the concentrations of VMS in remote air (away from point sources) before and after heavy precipitation events (e.g., heavy snowfall or heavy snow rain events). A decrease in the concentrations of the VMS after precipitation compared to concentrations before precipitation would then indicate and/or support a potential for atmospheric deposition.

Increased understanding of the transfer mechanism is needed for the assessment of the LRTP of the VMS. This in turn could be used for the overall POP assessment of VMS and other similar substances. Additionally, this type of monitoring would increase our understanding of the long-range environmental transport potential of substances with similar physical-chemical properties to the VMS.

Furthermore, risk and exposure assessments related to the substances could be improved if the transfer mechanisms would be better understood. Extending environmental monitoring to other environmental compartments than air would shed light on the transfer mechanisms.

In addition, monitoring data of VMS in migratory species would further support the LRTP assessment of these substances. Measurement of VMS in migratory species (such as penguins) and their faeces (such as guano or ornithogenic soils near bird colonies) from remote areas (such as Antarctica) would provide information on the importance of migratory animals as vectors of VMS transport. For instance, ornithogenic soils contain a layer of indurated guano crust on mineral soil and they have previously been used to examine e.g., biovectors and can reflect pollutant levels[31] resulting from animal activity in Antarctica.

Most of the current (bio)monitoring data available on the cyclic VMS from remote areas are dated almost a decade ago. Considering the scientific improvements in detecting/ quantifying these substances, new monitoring data in remote areas would help better understand the current levels of these substances in the environment and wildlife.

Finally, for the monitoring data on VMS to be used in a regulatory context, it is important to follow precautionary measures to avoid contamination of the samples. This means that relevant blank samples (field, procedural) are taken in parallel during the sampling. The reference matrices used for the blanks or the method detection limits, should be exempt from VMS contamination or the contamination should be kept at a strict minimum (i.e., at trace levels) in order to avoid an underestimation of the real concentrations in the samples. Furthermore,

31 Pollutant Level - an overview | ScienceDirect Topics

loss of substances or reaction of VMS should be avoided by following appropriate sample transport, storage, preparation and instrumental methods. In view of future monitoring programmes in remote areas, ECHA highly recommends that the deployment time of air samplers is sufficiently long to enable correct detection/quantification of VMS.

The time period (winter versus summer) and the sampling locations can have a significant impact on the measured concentrations of the siloxanes. The sampling locations should be selected sufficiently away from point sources, and they should represent a site where the concentrations of the VMS are not expected to be underestimated compared to other locations. The time period and the sampling locations should be selected so that the results can be used in a regulatory context and the obtained measurements cannot be considered to be biased (i.e., underestimated concentrations).

Why the topic is relevant: Monitoring of the volatile linear and cyclic methyl siloxanes in precipitations (rain and snow), as well as in freshwater and/or marine sediments and soil in remote regions (far away from point sources) would aid to understand the deposition mechanisms from air to surface media of these substances. This information helps to evaluate the environmental long-range transport potential of these substances. VMS are high volume chemicals with consumer uses and have been identified as chemicals of emerging concern by the Arctic Monitoring and Assessment Programme (AMAP).

Where it fits into the regulatory landscape: The cyclic VMS D4, D5 and D6 have been identified as SVHCs[32] under REACH due to their PBT/vPvB properties and RAC and SEAC opinions have been adopted for the proposed restriction under REACH Annex XVII[33]. Norway plans to submit further SVHC proposals for the linear VMS L2, L3, L4, and L5 due to their PBT or vPvB concern[34].

Global regulatory action under the Stockholm Convention can be warranted only for substances that lead to significant adverse human health and/or environmental effects as a result of their long-range environmental transport.

Short-term impact: Improved scientific understanding of the deposition mechanisms from air to surface media of VMS substances, that will allow a better understanding of the current environmental and biota concentration levels.

Long-term impact: Ensuring high level of protection for the environment and the human health from substances that could potentially meet the criteria for persistent organic pollutants.

32 Substance of Very High Concern

33 https://echa.europa.eu/documents/10162/a3e8195a-23d3-5859-6fdc-7805a3148b46

34 https://echa.europa.eu/registry-of-svhc-intentions accessed on 18 March 2024

2.3. Shift away from Animal Testing

Why further research is needed:

We need to move away from animal testing and find alternative methods to safeguard human health and the environment. The aim is to stop unnecessary animal tests and to speed up identification and management of hazardous substances.

Regulatory needs:

Develop new methodologies and invest in regulatory acceptance of already existing NAMs, in particular, on toxicokinetics and toxicodynamics. Case studies should assess the applicability of NAMs in regulatory purposes, build regulators' confidence and demonstrate their use in read-across.

Impact:

- Move away from animal testing.
- Speed up identification and regulation of hazardous chemicals.
- Reduce the costs involved in overall hazard assessment.

The CSS aims to regulate chemicals at a faster pace by improving the current regulatory framework to ensure appropriate hazard and risk characterisation. The anticipated changes to the regulatory landscape (such as the introduction of new hazard classes to CLP) may lead to additional animal testing. At the same time, the CSS emphasises the need to become less reliant on animal testing. NAMs development is closely linked with this ambition to move towards replacement of animal testing.

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. To identify and characterise the adverse effects underlying these complex endpoints, NAMs derived information should:

- allow a conclusive outcome on the (lack of) hazardous properties for given regulatory endpoint: the conclusion should be scientifically sound;
- reliably identify hazard and derive reference values to set safety levels, to communicate the hazard and assess the risks; and
- reliably inform on the severity of the effect.

Several roadmaps and initiatives[35] are addressing these critical needs. Besides developing new assays, models and technologies to address these, there is a particular need for research investments to focus on the application of already "mature" methods to specific regulatory areas, e.g.,

- Support the development and use of QSAR models for lower tier endpoints, by better exploiting regulatory data (e.g., submitted under REACH) and promoting the use of transparent and clear validity

35 US EPA. Interim science policy: Use of alternative approaches for skin sensitization as a replacement for laboratory animal testing. EPA-740-R1-8004. 2018. US EPA. New approach methods work plan (v2). EPA/600/X-21/209. 2021. Escher et al. Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment. 2022.

criteria (reliability and relevance) for the predictions developed under the OECD QSAR assessment framework (QAF).
- Development of case studies that investigate and demonstrate the practical use of omics for grouping and read-across for hazard assessment or regulatory risk management purposes.
- Generation of omics datasets within current TG studies to close the gap between the current *in vivo* studies used for decision-making (OECD test guidelines) and emerging methods such as omics.
- Demonstration of the utility of NAM-based approaches to inform on key parameters (i.e., NOAEL, LOAEL, classification, use NAM based indication for hazard as a trigger for further testing) used in the current risk management framework for the challenging systemic toxicity endpoints or generate similar insight.
- Continued investment in data dissemination and exchange, and format harmonisation for the development of new NAMs.

Some of these needs are further exemplified in the following subsections. Also, the need for NAM development for specific endpoints like bioaccumulation, neurotoxicity, immunotoxicity, endocrine disruption and mutagenicity are reflected separately under those respective sections.

2.3.1 Read-across and NAMs – Development of case studies

Why further research is needed:

Read-across can possibly substitute the need for (in vivo) data generation under REACH. NAMs may be used in the read-across justification to strengthen predictions regarding similarity of structural, toxicokinetic and -dynamic, and toxicological properties.

Regulatory needs:

Explore and demonstrate the possible use of NAMs in supporting read-across and build regulators' confidence with case studies.

Impact:

- Reduce the need for animal testing.
- Speed up identification and regulation of hazardous chemicals
- Reduce the costs.

Why the topic is relevant: Read-across is considered one of the main possible adaptations for more complex toxicological endpoints such as repeated dose toxicity, developmental and reproductive toxicity. This is presuming that a scientifically plausible hypothesis can be justified and used to derive a quantitative prediction for the targeted substances. Read-across is the most used adaptation to the standard information requirements in REACH and accounts for circa 23 % of all information requirements (all other adaptations: 14 %, experimental data: 31 %)[36].

The read-across approach starts with identifying a structural/ physicochemical similarity between target (the substance for which one would like to better understand in hazard properties) and source (the substance for which information on a specific hazard property is available) substance, provided that similar structural characteristics lead to similar hazards. In addition, similarity should be demonstrated for the toxicokinetic and toxicodynamic properties of the target and source substance. Many read-across cases fail to demonstrate toxicokinetic and toxicodynamic similarities. Reasons for this include deficiencies in the quality of the source studies and lacking data to support predictions based on toxicokinetics. Also, there are often shortcomings in the hypothesis and justification of the toxicological prediction. And on top of that, the variation in the severity and type of the adverse outcome makes it often difficult to conclude on a "similar" toxicological hazard.

36 ECHAs summary report on alternatives to animal testing, 2023

The deficiencies related to the supporting evidence are particularly relevant for more complex human health and environmental endpoints. To increase the robustness and regulatory acceptance of those adaptations, additional data is needed. Particularly, further data is needed related to toxicological mechanisms and absorption, distribution, metabolism and excretion (ADME) properties.

NAMs, *in vitro* and *in silico* tools, can support read-across by generating data on the toxicokinetic and toxicodynamic profile of the substances which are candidates for read-across and defining category boundaries of similar substances. This will facilitate a conclusion on toxicological similarity between the source and the target substance strengthening and validating the read- across hypothesis. A major challenge is how to use molecular data with no direct link with toxicity to group substances for similar adverse effects and how to cover wide range of possible toxicological pathways. The application of omics approaches could be beneficial in this context. Still, further development is needed of methodologies and objective criteria for regulatory acceptance. These further developments should consider at least the following elements:

- the relevance of the biological model (NAM) used to generate NAM information to 'bridge' the information from the source to the target substance and vice versa;
- the threshold of similarity for the target and source substance, in particular when aiming at grouping multiple substances (conditional to hazard mechanism);
- the toxicological relevance of the NAM information in the context of regulatory endpoint of interest.

Through PARC, ECHA can facilitate and support the development of case studies for using NAMs (i.e., OMICs, PBTK, etc) to consolidate grouping and read-across.

Where it fits in the Regulatory Landscape: Grouping of substances and read-across is one of the most used alternative approaches for filling data gaps in registrations submitted under REACH. Applying read-across correctly speeds up risk management and reduces the need for experimental testing on animals. The clear acceptance criteria for incorporation of NAMs into read-across will make read-across hypotheses more robust and helps to address deficiencies found for supporting (experimental) evidence for adverse effects.

Short- and long-term impact: If grouping and read-across are applied correctly, experimental testing can be reduced, as there is no need to test every substance in a group for all required endpoints. New approach methodologies have the potential to further substantiate the hypotheses of read-across approaches helping to define substance category boundaries and characterise similarities/ dissimilarities between source and target. The development of case studies will facilitate the incorporation and understanding of NAMs for read-across for regulatory purposes.

Associated Detailed Research Needs: As described above, the major challenge is how to use molecular (mechanistic) data with no direct link with apical/ adverse effects, for grouping substances with similar adverse effects. Research needs associated to this challenge include the following.

- How to describe confidence and consistency in NAM-based grouping hypothesis? To what extent does the level of significance of the NAM-based bioactivity (e.g., 'omics bioactivity signature) or ADME properties affect both the confidence and consistency of deriving a grouping hypothesis? A critical element of this includes the metabolization of a non-hazardous substance to a hazardous metabolite, because current in-vitro methods incorporate only a limited set of mammalian metabolization conditions (oxidate phase-I; not reductive, not phase-II) (see next sub-section 2.1.12).
- What factors are critical for defining the relevance of the biological model used to generate NAM-based bridging evidence for grouping? Are these factors dependent on the specific endpoint that is being read across?

- How to enhance our knowledge and confidence of molecular biomarker/ bioactivity versus adverse effect associations (e.g., relevance of the biomarker panels) to facilitate the use of molecular and bioactivity data to support grouping?
- What factors are critical for defining reliability of the NAM evidence for grouping hypothesis?
- Development of relatively standardised operating protocols (best practices) for generation, processing and interpretation of NAM data (to support read-across), including the standardised reporting of a NAM-based grouping study such as 'omics-based grouping.

2.3.2. *In vitro/ in silico* ADME and Physiologically-Based Kinetic models

Why further research is needed:

We need to understand the adsorption, distribution, metabolism and excretion of chemicals (ADME), as well as their toxicokinetic (TK) and -dynamic behaviour to move from in vivo to in vitro/ in silico testing for regulatory purposes. ADME/ TK have been proposed as future information requirement under REACH.

Regulatory needs:

Research to evaluate the generation of TK information for industrial chemicals and its use in physiologically based kinetic models to explain TK properties of substances.

Impact:

- Support the introduction of ADME/ TK under REACH.
- Move towards an animal free chemical hazard and risk assessment system relying on in vitro and in silico approaches.

Why the topic is relevant: An animal free chemical hazard assessment system will rely on *in vitro* and *in silico* approaches. Therefore, models such as physiologically-based kinetic modelling (TK) will be needed for hazard assessment. Furthermore, current standards for *in vitro* metabolic activation need to be reviewed and updated. This is because so far, only oxidative phase-I metabolism is covered and consequently, certain groups of hazardous substances are falsely identified as negative (i.e., not hazardous). This is relevant for all *in vitro*-based NAMs that are meant for a regulatory system which covers human health assessment.

In vitro to *in vivo* extrapolation (IVIVE), covers the process of converting an *in vitro* concentration associated with bioactivity to an external dose level associated with a potential hazard. Characterisation and quantification of this process is a pre-requisite to allow *in vitro* test methods to be more accepted in toxicity testing, regardless of the regulatory approach or the type of hazard. For this, data on absorption, distribution, metabolism, and excretion (ADME) of a chemical is needed. The ADME characteristics of a chemical within an organism can be collectively described by a set of mathematical equations within a PBK model. A PBK model considers physiological, anatomical and chemical specific parameters and to simulate a chemical's movement and transformation throughout the body following exposure from one or from multiple routes. Many parameters can be derived from such model, but the most common ones are AUC, CMax, TMax, Css[37], elimination rate, elimination half-life(s). These parameters can be used to inform about levels of chemicals in the organism and relate the chemical concentration/ dose to the observed toxicity.

Furthermore, IVIVE models are also needed for environmental endpoints, e.g., to extrapolate results derived from *in vitro* clearance assays with material from fish (e.g., OECD TG 319 A/B) to estimate a bioconcentration factor (BCF).

37 AUC: Area under the curve. CMax: maximum concentration, TMax:Time to maximum concentration. Css: Steady-State Concentration

There are various areas that need further development in current IVIVE-PBK models. The applicability domain of these models needs to be better characterised in terms of chemical and biological/ physiological properties. Furthermore, some ADME areas are not fully explored. Metabolism is generally considered in the liver, while the metabolism in other organs is often not known in detail. Another limitation when considering metabolism relates to quantitative measures or estimates of the metabolites of the metabolised (parent) substance. In fact, while qualitative metabolic information is easier to obtain, especially for the first levels of metabolism, quantitative information is more difficult to obtain and is associated with higher uncertainty. It is also challenging to properly reflect *in vivo* metabolism with *in vitro* methods in terms of coverage of organs, cell types, and enzymes. These limitations should be understood, described, and considered when developing pharmacokinetic models. It would be beneficial to assess the performance of IVIVE-PBK models in comparison to *in vivo* ADME studies for relevant substances or substance classes to characterise the variability and uncertainty of IVIVE-PBK models and for different substances.

Where it fits into the regulatory landscape: *In vitro* ADME/TK has been proposed by the Commission as an information requirement for REACH. In this context, we consider that it will be beneficial to gather information about the generation and use of TK data for industrial chemicals using a comparatively simple paradigm (i.e., oral for solids & liquids; inhalation for gas). This will allow us to consider the information generated, and its use in physiologically-based kinetic models to explain TK properties. Also, *in vitro* clearance assays with fish material are addressed in the updated ECHA PBT guidance[38] and support the assessment of the bioaccumulation potential, thus can contribute to avoid *in vivo* fish bioaccumulation testing.

Information on ADME/TK properties have useful regulatory applications and are already widely used in the following applications:

- estimation of the half-life (used for bioaccumulation assessment);
- REACH information requirement waivers[39] or triggers[40];
- building read-across hypothesis and justification (by demonstrating similarity in the TK profile between source and target substances).
- improved risk assessment, including (exposure) route to route extrapolation, and interspecies and intraspecies extrapolation of toxicokinetic;.
- reliable PBK modelling is a prerequisite for Quantitative *In Vitro In Vivo* Extrapolation (QIVIVE). QIVIVE is necessary for development and implementation of reliable alternative methods for systemic toxicity endpoints.

Short-term impact: In the short term, the work will support the inclusion of *in vitro* ADME/TK as a standard information under REACH through identification of what methods are available in Europe and what are their performance for different type of substances. This allows setting up realistic expectations and/or standards for the methods. The work will also improve optimisation of methods to increase their reliability and relevance.

Biotransformation can be an important mechanism of elimination for a given hydrophobic substance in an organism. Therefore, *in vitro* clearance assays such as OECD TG 319 A and B have the potential to support the bioaccumulation assessment in a Weight of Evidence approach.

Long-term impact: The *in vitro* ADME/TK is critical to potentially cover any systemic toxicity endpoint because the metabolic (de)activation must be considered. In practice it means that the biological models used to generate

38 Guidance on Information Requirements and Chemical Safety Assessment - Chapter R.11:PBT/vPvB assessment, Version 4, December 2023, European Chemicals Agency, Helsinki

39 e.g. a study might not need to be conducted if the substance (and its metabolites) do not show indications for a long biological half-life (based on e.g., toxicokinetic information, including *in vitro* tests, and physico-chemical parameters)

40 e.g. there are indications that the internal dose for the substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure

information on toxicity need to be metabolically competent or complemented with a reliable simulation of metabolism. In the long term, the introduction of *in vitro* ADME/TK as standard information requirement might have a major impact on hazard assessment practice. Also, it may increase the quality and robustness of the adaptations used to address standard information requirements under REACH. The *in vitro* ADME/TK information and related IVIVE is critical for defining safety levels for regulatory use and a pre-requisite for an animal free chemical risk assessment system relying on *in vitro* and *in silico* approaches.

2.3.3. Short-term fish toxicity

Why further research is needed:	Regulatory needs:	Impact:
Certain in vitro studies could be used to predict acute toxicity in fish. However, it is uncertain whether this prediction is correct for all substance types and chemical families.	A systematic assessment of these in vitro studies to better understand the applicability domain, e.g., for bulky, very poorly soluble, or volatile substances.	• Substitute in vivo tests with in vitro tests

One of the fundamental aims of the REACH regulation is to improve the protection of human health and the environment from the risks that can be posed by industrial chemicals. To achieve sufficient level of protection across ecosystems, the regulation relies on generation of data for only few species and extrapolates the effects to other non-tested species. One of these is fish (acute and chronic toxicity testing), which is needed to extrapolate the effect estimation for vertebrates. They are used in classification for aquatic acute and chronic hazards under CLP regulation. While it is important to cover the effect assessment for vertebrates, it is also acknowledged that vertebrate testing could be reduced for animal welfare reasons.

Why the topic is relevant: NAMs and *in vitro* testing has potential to reduce testing on living vertebrate animals such as fish. Certain *in vitro* studies could be used to predict whether a substance could be likely toxic to fish. By catching early key events taking place at cellular level which allow predicting acute fish toxicity directly for some substances. For example, responses at cellular level (of rainbow trout cells) may be captured by OECD TG 249 (Fish Gill cell line toxicity assay) or by OECD TG 236 (Fish Embryo toxicity test) to predict the effects to occur in an acute fish toxicity study (e.g., OECD TG 203).

However, currently it is not clear to what extent the gill cell line study can be applied and correctly predict fish acute toxicity of all substance types, including difficult substances such as bulky, very poorly soluble, adsorptive, or volatile substances. It is already highlighted by the OECD TG 249 that this *in vitro* test is not applicable for neurotoxic chemicals acting through specific ion channels or receptors typical of brain tissue. Similar limitation is highlighted for biotransformed substances, but it is not yet clear e.g., if an addition of enzymes into the system is possible and could mitigate this limitation.

To allow more intense use of these *in vitro* methods in regulatory context, their limitations need to be well understood to ensure safe use of all registered substances. For this purpose, a systematic assessment of the applicability of these methods should be conducted. The assessment should include comparison of *in vitro*

results to the existing high quality *in vivo* studies and report a detailed assessment of the predictivity against different modes of actions and substance characteristics (including physicochemical properties available for REACH registered substances).

Furthermore, it would be of additional value to the current risk assessment scheme to develop cell lines/ test systems for different organs and species. This would further foster protection of the whole ecosystem with much less uncertainty (see also on the topic of protecting biodiversity, section).

Short- and long-term impact: NAMs offer a great prospect to reduce vertebrate testing while still providing a same level of protection of the environment from industrial chemicals. Eventually, introduction of the *in vitro* systems as regulatory information requirements can be considered, provided that there is a clear applicability domain identified for these methods.

2.3.4. Long-term fish toxicity

Why further research is needed:	Regulatory needs:	Impact:
Certain in vitro studies could be used to predict chronic toxicity in fish and may be used to reduce and steer vertebrate testing while still providing same (or even higher) level of protection of the environment from industrial chemicals.	Assess systematically the use of available alternative methods to predict chronic fish toxicity in a regulatory context.	• Substitute in vivo tests with in vitro tests.

For the same reasons as mentioned under 'Short-term fish toxicity' more specific research is needed to cover the long-term effects on fish. Overall, the generated chronic toxicity data on fish represents chronic hazards to vertebrates but this data generation approach may not be protective enough for all vertebrate species. Test species are chosen by practical aspects such as availability of test guidelines and test organisms rather than for biological grounds such as sensitivity of the species.

Why the topic is relevant: NAMs and *in vitro* testing has potential to reduce testing on living vertebrate animals such as fish. For example, *in vitro* studies could be used to predict when a substance is likely toxic to fish or other vertebrates by catching early key events taking place at cellular/ tissue level, triggering a need to perform an *in vivo* study on a sensitive species because it would be of high importance in further risk management (e.g., classification of substances according to CLH). However, in turn the *in vivo* study(s) may not be needed for substances which do not produce a strong response in cellular levels/ tissues. The use of omics data and NAMs can steer the data generation to a species that is predicted to be sensitive.

Efforts to develop AOPs, *in vitro* systems and embryonic assays with fish, amphibians and birds to predict chronic toxicity to fish/ vertebrates have been made. For example, the EcoToxChip Test System may have the potential to prioritize chemicals for management and further testing the effects on growth, survival, reproduction of fish, amphibians and birds. A validation exercise has been launched recently in Environment Canada to investigate its

use in regulatory context[41]. Similar exercises could be done for REACH substances using different tools which are available to predict chronic toxicity to vertebrates. Furthermore, considering that the *in vitro* systems are limited by representative species/ cell lines, some methods to extrapolate further the effects across a wide range of species could be to use the similarity between the protein target in a model organism (such as rat) to other species (e.g., Sequence Alignment to Predict Across Species Susceptibility [SeqAPASS]). Such tools can be useful to predict when adversity can be expected in different species and thus can further steer the generation of *in vivo* data based on e.g., mammalian data.

However, the potential of such tools in terms of their usefulness to prioritise chemicals for chronic toxicity testing (or to predict the effects directly) under REACH is yet unknown. To allow more intelligent *in vitro* / Adverse Outcome Pathways (AOPs) to be applied in regulatory context, assessment of the predictivity of the methods should be conducted for REACH relevant substances. The existing tools should be mapped in terms of the adverse effects which they are able to predict and whether they are able to predict the outcome of e.g., OECD TG 210 or OECD 234 studies (in terms of prioritisation or prediction of effect levels). Assessment of such new methods to predict chronic toxicity should include comparison to existing high quality *in vivo* studies (for substances registered under REACH) and report a detailed assessment of the predictivity for different substance characteristics (including e.g., highly lipophilic substances) and modes of action.

Short- and long-term impact: NAMs offer a great prospect to reduce and steer vertebrate testing while still providing same (or even higher) level of protection of the environment from industrial chemicals. Eventually, introduction of the *in vitro* systems as the regulatory information requirements can be considered, provided that there is a clear applicability domain identified for these methods.

2.3.5. Carcinogenicity

Why further research is needed:	Regulatory needs:	Impact:
We need to explore the possible regulatory use of available NAMs to speed up identification and regulation of genotoxic and non-genotoxic carcinogens. For this, we also need to better understand how different types of mutagenic substances act in vivo.	Assess systematically the use of available alternative methods to predict (non-)genotoxic carcinogens in a regulatory context.	• Speed up and improve identification and regulation of carcinogens.

Under REACH, the current strategy for identifying carcinogens relies on the two-year rodent bioassay (OECD TG 451 or 453). The information requirement is conditional to triggering by risk via two conditions that must be fulfilled by demonstrating:

a) Exposure:

   a. "the substance has widespread dispersive use or
   b. there is evidence of frequent or long-term human exposure, and"

41 Validation of the use of the EcoToxChip test system for regulatory decision-making (genomequebec.com)

b) Hazard:

a. "the substance is classified as germ cell mutagen category 2 or
b. there is evidence from the repeated dose study(s) that the substance is able to induce hyperplasia and/ or pre-neoplastic lesions".

Until now, less than ten carcinogenicity tests could be performed under REACH. At this rate, testing will continue for decades or centuries before currently unknown, but likely numbers of carcinogens are identified. Therefore, the following proposals focus on the use of alternative and new approach methods to speed up this process.

2.3.5.1. Improve the detection of carcinogens including those that act through a non-genotoxic mode of action

Why the topic is relevant: Cancer is the leading cause of death in rich countries[42] despite improvements in therapies and (early) diagnostics. ECHA estimates that 1-3 times as many carcinogens are yet unidentified, compared to those that have been identified in the last decades of carcinogenicity testing (vom Brocke et al, in preparation/2023). The current methodology selects for genotoxic carcinogens and has not led to a measurable increase in identifying novel carcinogens among industrial chemicals during the last 15 years[43].

Where it fits into the regulatory landscape: NAMs suitable to be included in future regulations could be identified by testing known human carcinogens in several available robust NAMs. This benchmarking would then identify which NAMs are relevant for identifying human carcinogens, with high sensitivity. Benchmarking against "known human non-carcinogens" would then provide the necessary high specificity and result in an overall top-down approach. The approach is expected to take several iterations, since not all promising NAMs will withstand the scrutiny of being validated against substances for which the effects are known to be relevant to humans. Also, it is likely that not all tests will be relevant for all classes of substances and therefore, combinations of (a large number of) tests are inevitable.

Improvements in the methodology for identifying carcinogens will likely affect time, economic costs and (pathology) know-how, because the currently available rodent bioassay takes two-years of in-life study duration and again at least as much time for analysing and interpreting the results, while requiring numerous mammals to ensure sufficient statistical power. Its outcome has frequently been challenged as being too unspecific, and thus, not relevant enough for humans[44].

An expert group organised by the OECD is currently identifying a (non-exhaustive) list of NAMs that are evaluated for their inclusion in testing regimes according to several robustness criteria[45]. Key events (hallmarks of cancer) for which NAMs have been identified include genotoxicity, metabolic activation, oxidative stress, immunosuppression/ evasion, gene expression and signalling pathways, increased resistance to apoptosis.

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42 Dagenais, G.R. and et.al. (2020) 'Variations in common diseases, hospital admissions, and deaths in middle-aged adults in 21 countries from five continents (PURE): a prospective cohort study', The Lancet, 395(10226), pp. 785–794. doi: https://doi.org/10.1016/S0140-6736(19)32007-0.

43 Karamertzani, P.G. and et.al. (2019) 'The impact on classifications for carcinogenicity, mutagenicity, reproductive and specific target organ toxicity after repeated exposure in the first ten years of the REACH regulation', Regulatory Toxicology and Pharmacology, 106(August 2019), pp. 303–315. doi: https://doi.org/10.1016/j.yrtph.2019.05.003.

44 Suarez-Torres, J.D., Orozco, C.A. and Ciangherotti, C.E. (2021) 'The 2-year rodent bioassay in drug and chemical carcinogenicity testing: Performance, utility, and configuration for cancer hazard identification', Journal of Pharmacological and Toxicological Methods, 110, p. 107070. doi:10.1016/j.vascn.2021.107070. / Marone, P.A., Hall, W.C. and Hayes, A.W. (2014) 'Reassessing the two-year rodent carcinogenicity bioassay: A review of the applicability to human risk and current perspectives', Regulatory Toxicology and Pharmacology, 68(1), pp. 108–118. doi:10.1016/j.yrtph.2013.11.011

45 Jacobs, M. et.al. (2016) 'International regulatory needs for development OFAN IATA for non-genotoxic carcinogenic chemical substances', ALTEX, 33(4). doi:10.14573/altex.1601201. / Jacobs, M.N., Colacci, A., Corvi, R. et al. Chemical carcinogen safety testing: OECD expert group international consensus on the development of an integrated approach for the testing and assessment of chemical non-genotoxic carcinogens. Arch Toxicol 94, 2899–2923 (2020). https://doi.org/10.1007/s00204-020-02784-5.

Key hallmarks for which further development is needed are e.g., pathogenic neo-/angiogenesis and genetic instability, as well as the critical gap from inflammation and hyperplasia to tumour formation.

An assessment framework for weighing the different pieces of evidence is being developed. It will be flexible enough to incorporate any new methods as they become available.

Short- and long-term impact: The approach above can only be realised through top-down research as in PARC and will lead to a completely novel approach for identifying carcinogens that are relevant to humans, instead of other (test) species. This is based on the uniquely available information from testing known human carcinogens with NAMs for benchmarking these methods for their sensitivity and specificity. It will be possible to also identify those carcinogens whose toxicity is primarily driven by non-genotoxic mechanisms, including epigenetic events, as long as reliable NAMs for that mechanism are included in the process.

2.3.5.2. Development of Adverse Outcome Pathways (AOPs) for specific modes of genotoxic or mutagenic action

Why the topic is relevant: Further research is needed to understand how different types of mutagenic substances act *in vivo* and identify the key steps leading to their genotoxic or mutagenic effects. This information could then be used to develop Adverse Outcome Pathways (AOPs) for specific modes of genotoxic or mutagenic action.

For instance, AOP 296 on "Oxidative DNA damage leading to chromosomal aberrations and mutations" has recently been developed by OECD and may be relevant to mutagenicity hazard assessment as indirect genotoxic effects caused by oxidative damage are assumed to be threshold effects, contrary to direct genotoxic effects. Therefore, safe levels of exposure could in principle be derived for substances causing indirect genotoxic effects after oxidative damage only, and specific risk management measures put in place. This AOP could be used to develop non-animal test methods specific for each of the AOP key events and possibly develop testing strategies or defined approaches under the OECD TG programme in the future.

Another potential AOP could be targeted at germ cell mutagenicity. Specifically, some research is needed to identify key factors or key events that determine whether a substance that is mutagenic/genotoxic in somatic cells *in vivo* will also be mutagenic/ genotoxic in germ cells. Further understanding of the key steps leading to germ cell mutagenicity *in vivo* would be valuable to develop non-animal test methods that could eventually replace animal testing and potentially lead to a revision of the GHS/CLP criteria.

Where it fits into the regulatory landscape: Although AOPs are not covered by the Mutual Acceptance of Data (MAD) principle, which allows the data generated under MAD to be accepted by authorities in any OECD member countries, they could be used to develop non-animal test methods specific for each of the AOP key events and possibly develop test guidelines, testing strategies or defined approaches under the OECD TG programme, which would be covered by MAD.

Short-term impact:

- further characterisation of the mode(s) of genotoxic or mutagenic action of a substance;
- better selection of the most appropriate *in vivo* follow-up test(s) based on the identified modes of genotoxic or mutagenic action.

Long-term impact:

- development of non-animal test methods specific for each of the AOP key events;

- development of testing strategies or defined approaches under the OECD TG programme based on validated AOPs;
- development of specific risk management measures based on the identified modes of genotoxic or mutagenic action.

Potential for partial of complete replacement of animal testing for the identification of genotoxic or mutagenic substances, provided that AOP coverage of the different types of genotoxic or mutagenic modes of action is exhaustive and validated non-animal test methods are available for all key events.

2.4 Improved availability on chemical data

2.4.1. Polymers

Why further research is needed:

High molecular weight polymers may no longer be considered innocuous by default. We need research to understand their bioavailability and better support hazard and risk assessment for regulatory purposes.

Regulatory needs:

Focus research on interpreting a polymer's bioavailability, stability to degradation, and overall toxicity.

Impact:

- Understanding of hazard properties of polymers.
- Identification and regulation of hazardous polymers.
- Possible future development of a protective environmental and human health regulatory framework for polymers.

Historically, regulatory frameworks have considered polymers of lower hazard than the monomers they are synthesised from. It has been assumed that the higher molecular weight of a polymer compared to its monomer units would lead to lower bioavailability and hence lower toxicity. This has been supported by the 'rule of five' (Ro5) which posits that substances with a MW > 500 Dalton have poor absorption and permeation, thus their (systemic) bioavailability will be limited. However, 20 years from the introduction of the Ro5, scientific research demonstrates that the 500 Dalton cut-off is questionable.

The literature reports molecules with MW > 1200 Dalton (e.g., cyclosporine) that are not hindered in their (cell) membrane permeability. Also, the example of chlorinated paraffins (CPs) proves that concerns for bioaccumulation and aquatic toxicity should not be neglected for high MW polymers. Despite their large molecular size, the experimental studies on Daphnia show the uptake, bioaccumulation and chronic toxicity of CPs. Based on these findings, high MW polymers can no longer be regarded as innocuous by default. To prepare for a possible future extension of the REACH registration to polymers, more research is needed to understand their bioavailability and better support the future hazard and risk assessment for the regulatory purpose.

Several practical challenges are highlighted. Most importantly, polymers are most often not homogeneous in composition. One specific polymer may in fact consist of a distribution of polymer chain-lengths of monomer units with different corresponding molecular weight (MW). Depending on the (polymeric) material's desired properties, a polymer may actually be designed to include different MW fractions. The fact that different MW ranges may have different bioavailability and hazard properties complicates the interpretation of bioavailability and hazard assessment of polymers for regulatory purpose. This also complicates identifying meaningful testing material, e.g., the whole polymer, certain chain lengths or certain MW fractions of the polymer. Moreover, polymers may contain low-MW oligomers or additives that may be released upon degradation and may drive the hazard profile of the bulk polymer. Polymers are thereby similar to UVCB substances for which their unknown, complex or highly variable composition brings some level of uncertainty on the hazard properties, and research needs are similar too. Moving away from animal testing forms an additional challenge.

2.4.1.1. Interpretation of polymer's bioavailability

How to demonstrate lack of bioaccumulation/ intrinsic toxicity of high-MW polymers without unnecessary vertebrate tests?

Ongoing discussions on possible future regulatory scheme's that could be proposed as part of a future REACH update, considers reduced information requirements depending on the polymer's type (defined by its MW and tonnage band). Within such scheme could be proposed e.g., for high-MW polymers (> 1000 Da) to require less information based on the assumption of limited bioavailability. However, to minimise the uncertainty regarding bioaccumulation and aquatic toxicity, a screening methodology would be needed to either confirm the reduced bioavailability, or to spot the exceptions to the assumption. For example, if the screening allows to conclude on polymer's negligible bioavailability, no aquatic toxicity studies would be required. On the other hand, if negligible uptake cannot be confirmed, aquatic toxicity and fate studies could be triggered, depending on the tonnage, similarly to non-polymer substances.

Contrary to non-polymer substances, hardly any public, experimental bioaccumulation, aquatic toxicity or toxicokinetic data exist for bulk polymers. Hence, the evidence that could support the physico-chemical indicators of hindered uptake (as described in ECHA guidance R.11)[46] for high MW polymers is missing. Similarly, there is the need for the bioaccumulation assessment of superhydrophobic substances (see page 25, section 2.2.1.4). With the intention to minimise the generation of animal data wherever possible, there is a risk that the screening method will not be protective enough and potentially hazardous polymers may "slip" through the regulatory "safety net".

To support the ongoing regulatory developments, it would be helpful to explore NAMs for the (screening) assessment of bioavailability for polymers in the absence of experimental (eco)toxicological studies. Screening should also consider that polymers may contain low-MW oligomers or additives that may be released upon degradation, may become bioavailable and may drive the hazard profile of the bulk polymer.

2.4.1.2. Assessment of polymer's stability to degradation under environmental conditions

Degradation of polymers in the environment and release of substances of concern is another exception to the assumption that high-MW polymers are less hazardous. In the envisaged information requirements for polymers under REACH, there is a need for screening methodology and triggering criteria to establish whether high-MW polymers are either (a) adequately 'stable' under environmental conditions to biotic and abiotic degradation, or if in contrast (b) they are 'completely degraded' (i.e., fully/rapidly mineralised), or (c) if any 'substances of concern' are released upon degradation.

"Failing" such assessment would trigger further environmental fate studies (simulation tests, identification of degradation/transformation products). The technical complication in using 'ready' or 'inherent' biodegradability test data is that even if a polymer is not 'readily' or 'inherently' biodegradable according to the test method criteria it does not follow that it is 'inert' which complicates the environmental 'stability' assessment. In addition, interpretation of biodegradability studies of polymers in general should be linked to real-life factors (light, extreme temperatures, physical damage, etc.) that may change the size and properties of the polymer and increase its bioavailability in the environment. In addition, there are challenges in applicability of standard screening and simulation tests for polymers (difficulties in quantifying ThOD/$ThCO_2$ of polymers, limited bioavailability, test duration, application of test substance to test compartment, high number of transformation products, radiolabelling often not possible, lack of calibration standards, etc.). To overcome these issues, alternative test systems and/or approaches dedicated for bulk polymers need to be developed.

46 38 Average maximum diameter (Dmax) > 1.7 nm, Log Kow > 10 or octanol solubility [mg/L] < 0.002 [mM] x MW [g/mol]. Guidance on Information Requirements and Chemical Safety Assessment - Chapter R.11:PBT/vPvB assessment, Version 4, December 2023, ECHA, Helsinki.

2.4.1.3. Polymer bioavailability; assessment and relevance for human health hazard assessment

It is unclear whether the hypothesis that higher molecular mass is associated with reduced absorption, and consequently lower levels of toxicity, holds for all routes of exposure (oral, dermal, inhalation). Also, a possible quantitative relationship between molecular mass, absorption and toxicity for polymer-type molecules is not characterised. Further it would be desirable to have rapid methods available for characterisation of polymer bioavailability.

2.4.1.3.1. Screening methods for assessing polymer toxicity

Repeated-dose toxicity can (inter alia) affect a variety of organs, result in cancer, or affect reproduction or development. However, performing REACH Annex IX and X tests according to OECD Test Guidelines on all polymers would be costly, in terms of time, animal use and financial costs. It would be desirable to develop screening methods/ strategies that are capable of targeting definitive tests (i.e., REACH Annex IX and X tests performed according to OECD Test Guidelines) to polymers that are most likely to be hazardous.

2.4.1.3.2. Characterisation of polymer toxicity

There is a scarcity of data on the repeated-dose toxicity of polymers. It is important to understand if polymers have specific characteristics or common toxicity as a result of being polymers. Such information is important for hazard assessment and protection of human health as well as for the development of methodologies to assess toxicity of polymers. Such analysis of the toxicity of polymers should have regard to the route of exposure and the chemical structure of the polymers.

Why are the topics relevant: Understanding polymer's bioavailability (both for environment and human health) and stability to degradation in environment is critical for developing and implementing rational and hazard-proportionate information requirements for polymers under revised REACH Regulation. Efficient screening methodologies will help to spot the potential polymers of concern and reduce the excessive experimental testing and tests on vertebrate animals.

Short-term impact:

- understanding hazard properties of polymers;
- development of the protective environmental and human health regulatory framework for registration of polymers under REACH.

Long-term impact:

- ensuring high level of protection for environment and human health based on science-based assumptions on polymer's bioavailability and (hazard) properties.

2.4.2. Micro- and nano-sized materials

Why further research is needed:

Critical test methods to address the human and environmental hazard and risk assessment of nanomaterials are still missing. Furthermore, for most NAMs, validation is missing to allow regulatory acceptance and uptake.

Regulatory needs:

Test method development for nanomaterials as well as suitable NAMs covering regulatory relevant endpoints. These should consider the analytical characterisation of the materials to shed light on toxicokinetics and -dynamics under different exposure scenarios. Further research should focus on the (bio)degradation potential, long-term effects in e.g., sediments and soils and their bioaccumulation potential.

Impact:

- Standard test methods for the hazard and risk assessment of nanomaterials.
- A framework for regulatory accepted NAMs to help the assessment of single nanoforms or sets of nanoforms.
- Moving away from animal testing.

In December 2018 the Commission Regulation (EU)2018/1881 was adopted to modify REACH Annexes I, III and VI-XII, introducing nano-specific clarifications and new provisions in the chemical safety assessment (Annex I), registration information requirements (Annex III and VI – XI) as well as downstream user obligations (Annex XII) which came into force on 1st January 2020. To comply with the amended REACH Annexes, all nanoforms that are manufactured or imported must be reported in the registration dossier of the substance. This can be done individually for each single nanoform, or, by derogation, several individual nanoforms can be grouped into sets of similar nanoforms.

During the last decade good progress has been made in terms of adapting some of the standard OECD test protocols for characterising as well as testing the (eco)toxicological hazard of nano-sized materials to address the specific challenges brought in by nanoforms. But fate and toxicity are not only driven by intrinsic properties (core composition, size, particle size distribution, surface functionalisation/coating/capping, crystallinity, dissolution, shape) but also by extrinsic properties (chemical transformation, physical transformation (agglomeration/ aggregation), biological transformation and interactions with macromolecules) complicating a realistic human health and environmental hazard and risk assessment. Despite the good progress it is therefore not surprising that there are still substantial gaps in terms of test system adaptation or development for (eco) toxicological endpoints. Therefore, ECHA highlights the critical need to urgently finalise the ongoing OECD test methods and guidelines revisions under the Malta Initiative[47] as well as the Malta initiative priority list[48]. These test methods are essential for the implementation of the REACH provisions for nanoforms. Without such test methods, the generation of specific information on intrinsic properties of nanoforms is delayed, hampering their safety assessment, as well as impacting innovation in the 'key enabling technology' linked to nanomaterials and advanced materials.

The continuously increasing number, complexity, and diversity of micro-and nanosized materials are making a case-by-case assessment of each of them undesirable and impossible from a practical perspective but also and specifically in the light of the increasing pressure to reduce vertebrate testing for hazard and risk assessment purposes.

47 https://web-archive.oecd.org/2022-10-25/644037-status-report-test-guidelines-guidance-documents-nanomaterials.pdf

48 https://malta-initiative.org/MaltaInitiative_UPLOADS/20240301_The_Malta_Initiative_Priority_List.pdf

All this clearly shows the need to break down this unsurmountable number of candidates by reducing the complexity brought in by nano specific characteristics. This reduction can be done by generating an understanding on how nanomaterial properties link to functional behaviour and to simplify where possible through functional and behavioural groupings of nanoforms.

However, it is vital that this reduction is not leading to an increased uncertainty in terms of potential adverse effects on human health or the environment. To be still able to provide effective and reliable hazard and risk assessment for these highly diverse materials the area of NAMs is promising in terms of developing suitable screening tools for single nanoforms and to support the building of set of nanoforms through reliable grouping and read across. Progress has been made in the development of NAMs for nanomaterial safety testing (e.g., the development of a 3D tissue models for the assessment of genotoxicity of nanomaterials in parallel to other endpoints such as cytotoxicity or inflammatory responses; a screening test to analyse the biodegradability of nanomaterial coatings, the development of computational models to predict hazard, fate and exposure). However, these are efforts originating from international research projects and for most cases sufficient validation is still missing and consequently preventing regulatory acceptance.

To progress the field, suitable NAM approaches covering regulatory relevant endpoints are needed. These should specifically target the area of analytical characterisation of the materials – both pristine as well as in the respective exposure situation while specifically addressing the characterisation of materials in complex matrices (e.g., organ tissue, environmental samples such as soils, biofilms, sewage sludge) to shed light on the toxico-kinetics and -dynamics of the materials under different exposure scenarios. Other areas of high interest are the (bio)degradation potential, long-term effects in e.g., in sediments and soils taking into consideration (multiple) transformation processes and the bioaccumulation potential in humans and the environment. All these endpoints targeting fate, (eco)toxicity and bioavailability should be combined for a NAM framework, combining experimental set ups with *in silico* methods where appropriate, to help the assessment of single nanoforms or sets of nanoforms.

The development of such a framework should go hand in hand with the validation against testing outcomes from 'conventional' standard OECD TGs to be able to progress towards regulatory acceptance in the future.

During this development phase the gained experience will help to generate and to refine a robust set of key criteria which will have to be considered in the building of the NAM framework.

Short-term impact: to gain experience in the use of NAMs and available science and technology for the hazard and risk assessment of micro-and nanosized materials under the current regulatory system. This will help to refine the available tools as well as developing suitable NAMs to cover identified knowledge gaps.

Long-term impact: the application of NAMs in a regulatory context for the hazard and risk assessment of micro- and nanosized particles. In the long term, this will contribute to the reduction of vertebrate testing while simultaneously contributing to a more realistic hazard and risk assessment of nanoforms by considering intrinsic (particle specific characteristics) as well as extrinsic properties (transformation, fate).

2.4.3. Analytical methods for enforcement

Why further research is needed:

Effective and efficient analytical methods are needed to enforce regulatory measures. They should be able to detect specific substances in possibly a wide variety of different matrices (e.g., textiles, different types of plastics, metals and ceramics).

Regulatory needs:

Develop sensitive but affordable analytical methods for compliance controls to allow inspectors to apply (high throughput) methods for inspection campaigns and help SMEs to self-control their products.

Impact:

- Validated analytical methods to monitor compliance of e.g., REACH restrictions, and to make future restrictions enforceable.
- Development of fast and affordable analytical methods and laboratory capacity to protect human health and the environment from the exposure to hazardous chemicals

One of the important aspects of the enforceability of regulatory measures restricting the use of certain hazardous chemicals, e.g., under the process of REACH Restriction and authorisation, is the availability of analytical methods that ensure a proper assessment of the presence of restricted substances and substances falling under authorisation. The absence of such methods hampers a harmonised control of conformity of substances, mixtures, and articles in the EU market subject to restrictions and authorisations. In the absence of suitable methods, problems or even risks for human health and/or the environment may prevail, and the competitiveness of EU companies may be negatively impacted. Seeing that many substances may be present in different material matrices, sample preparation methods need to be validated to the different materials as well. Since millions of products are entering the EU, growing attention is needed for the development of screening techniques that can assess and prove non-compliance with EU law in a high-throughput manner.

Why the topic is relevant: there is a need for sensitive but affordable analytical methods for compliance controls. Such methods not only allow inspectorates to apply methods that they can use for their inspection campaigns but also help SMEs to self-control the products they place on the market.

Where it fits into the regulatory landscape: Having adequate analytical methods also allows ECHA and MS authorities to better deal with incoming restriction and authorisation proposals. For example, information on sampling protocols for the different ranges of substances in articles, indication of normalised methods for determining concentration values and correct calculation and interpretation of results is often key to judge on the enforceability of a REACH restriction under development. Furthermore, for a restriction to be enforceable, it is important that analytical methods are available for which the limit of quantification (LOQ) is lower than the threshold values established in the restriction. It is important that development of analytical methods is stimulated as new substances are added to the restriction.

Short- and long-term impact: In the short term, the development of international validated analytical methods will be used to monitor the compliance of e.g., REACH restrictions and will support the enforceability of the future restriction proposals. In the long term, it will support the development of laboratory capacities and networking and will protect human health and the environment from the exposure of hazardous chemicals, for example in relation to the revised Water Framework Directive and Groundwater Directives where analytical methods are a prerequisite for including substances on the watch lists.

Examples of areas of application

Characterisation of nanomaterials, including advanced materials

One emerging area of significance is that of innovative products and equipment arising from applications of nanotechnology. While having commercial and economic benefits, there is growing concern that some nanomaterials have potential human and environmental health risks. It is therefore crucial that customs laboratories are maintained at the very edge of these rapidly evolving scientific developments and use suitable techniques for screening and for characterisation of nanomaterials, including advanced materials. Specific research needs are, for example:

- developing and validating measurement techniques that can cover the entire nano range (1–100 nm) effectively. The microplastic restriction is already confronted with this problem;
- enhancing the comparability and interoperability of different nanomaterial measurement techniques to reduce variability and uncertainty;
- innovating sample preparation methods that are adaptable to a variety of nanomaterials and measurement techniques;
- establishing standardized methodologies that can be widely adopted for the characterization of nanomaterials.

Identification of CMR in leather, textiles and childcare articles

CMR screening in leather, textiles and childcare articles is important as it helps to identify and assess the presence of substances that are classified as carcinogenic, mutagenic, or toxic to reproduction (CMR). These substances pose significant health risks to both consumers and workers involved in the textile industry. The screening for these substances is crucial for implementing e.g., REACH restrictions and other risk management strategies to protect human health and the environment. Both targeted and non-targeted screening methods are needed to better understand the chemical composition of textiles, leather and childcare articles and may help to identify priority substances that require further investigation and quantification. Specific research needs are, for example, as below.

- Enhanced Analytical Techniques: Development of more sensitive and comprehensive analytical methods, such as advanced HPLC/High Resolution Mass Spectrometry, to detect a broader spectrum of chemicals in textiles and other imported goods.
- Improved Screening Methods: Implementation of target, suspect, and non-target screening methods to better identify known and unknown substances in imports. So far, the number of cheap screening methods that result in a high probability of positive testing with more advanced and more expensive techniques is limited. X-ray fluorescence is widely used to get a first indication, even at custom entrance whether certain metals in cheap toys are present. Fourier transformed infra-red spectroscopy clearly indicates the presence of for instance phthalates without having the possibility to identify the real substance identity and whether they fall under a restriction or authorisation duty. Raman spectroscopy is also used, but for the majority of restricted and substances falling under authorisation, no cheap and simply applicable screening methods are available.
- Database Expansion: Creation and maintenance of extensive compound libraries to aid in the identification of emerging contaminants.

ANNEX I

Endponts	Information Requirement under REACH and BPR	Classification under CLP
Neurotoxicity	Under REACH, Adult neurotoxicity may be indicated from: • 8.5.1 Acute toxicity (Annex VII, column 1),); • 8.5.2 or 8.5.3 Acute toxicity (Annex VIII, column 1),); • 8.6.1. Short-term repeated dose toxicity study (28 days) (Annex VIII, column 1),); • 8.6.2. Sub-chronic toxicity study (90-day) (Annex IX, column 1).); • Data on the P0 generation available under: » 8.7.1. Screening for reproductive/developmental toxicity (OECD TG 421 or TG 422) (Annex VIII, column 1); » 8.7.2. Pre-natal developmental toxicity study (OECD TG 414) on a first species (Annex IX, column 1); » 8.7.3. Extended one-Generation generation reproductive Toxicity toxicity Study study (EOGRTS, OECD TG 443) (potentially triggered at Annex IX, and standard requirement at Annex X); » 8.7.2. Pre-natal developmental toxicity study (OECD TG 414) in a second species (Annex X, column 1).	Adult neurotoxicity: Chemicals may be classified as specific target organ toxicity single exposure (STOT-SE) or specific target organ toxicity repeat exposure (STOT-RE) if they fulfil the respective CLP criteria.
Neurotoxicity	Under REACH, Developmental neurotoxicity may be indicated from: • 8.7.1. Screening for reproductive/developmental toxicity (OECD TG 421 or TG 422) (Annex VIII, column 1); • 8.7.2. Pre-natal developmental toxicity study (OECD TG 414) on a first species (Annex IX, column 1); • 8.7.2. Pre-natal developmental toxicity study (OECD TG 414) in a second species (Annex X, column 1).); • 8.7.3. Extended One-Generation Reproductive Toxicity Study EOGRTS (OECD TG 443) (potentially triggered at Annex IX, and standard requirement at Annex X). Cohorts 2A/2B (developmental neurotoxicity) shall be proposed by the registrant or may be required by the Agency in case of particular concerns on (developmental) neurotoxicity are justified. For active substances under the biocidal products regulation (BPR), in addition to the pre-natal development toxicity study (OECD TG 414) on two species and extended One-Generation Reproductive Toxicity Study EOGRTS (OECD TG 443), the OECD TG 426 must be performed as a standalone study or DNT shall be investigated as part of OECD TG 443 with cohorts 2A and 2B with additional investigation for cognitive functions. Alternatively, or DNT must be investigated by any relevant study (set) providing equivalent information. Such specific investigations on DNT provide additional information e.g. on motor and sensory functions and associative learning and memory (cognitive functions) in the offspring exposed during the developmental period.	Developmental neurotoxicity: Chemicals may be classified as developmental toxicity (Reproductive toxicity) if they fulfil the respective CLP criteria. Details on how the information listed above are used for the purpose of classification and labelling are set out in 'RAC Guidance" Note: Addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes[1]

1 Microsoft Word - RAC_CLH_Hazard_classes_to_address_DNT_and_neurotoxicity.docx (europa.eu)

Endponts	Information Requirement under REACH and BPR	Classification under CLP
Developmental Immunotoxicity	Under REACH, Developmental immunotoxicity may be indicated from: • 8.7.3. Extended One-Generation Reproductive Toxicity Study EOGRTS (OECD TG 443) (potentially triggered at Annex IX, and standard requirement at Annex X). Cohort 3 (developmental immunotoxicity) shall be proposed by the registrant or may be required by the Agency in case of particular concerns on (developmental) immunotoxicity are justified Under BPR, the nature and/or severity of the identified concern may provide guidance to select between a separate study or inclusion of parameters to other studies or a Cohort 3 in an OECD TG 443. It should be considered whether the parameters/Cohort 3 or a separate study best address the particular concern identified.	Chemicals may be classified as developmental toxicity (Reproductive toxicity) if they fulfil the respective CLP criteria.
Endocrine Disruption	Under REACH, Endocrine disrupting modes of action (Human Health) may be indicated from: • 8.6.1. Short-term repeated dose toxicity study (28 days) (Annex VIII, column 1), ; • 8.6.2. Sub-chronic toxicity study (90-day) (Annex IX, column 1); • 8.7.1. Screening for reproductive/developmental toxicity (OECD TG 421 or TG 422) (Annex VIII, column 1); • 8.7.2. Pre-natal developmental toxicity study (OECD TG 414) on a first species (Annex IX, column 1); • 8.7.2. Pre-natal developmental toxicity study (OECD TG 414) in a second species (Annex X, column 1).) • 8.7.3. Extended one-Generation generation reproductive Toxicity Study (EOGRTS, OECD TG 443) (potentially triggered at Annex IX, and standard requirement at Annex X); 8.7.2. Pre-natal developmental toxicity study (OECD TG 414) in a second species (Annex X, column 1).); Under REACH, there are no specific information requirements for Endocrine disruption for the environment, but some of the information highlighted above for mammals may also be of relevance for the endocrine disrupting properties for the environment. Under BPR, for each biocidal active substance, a conclusion on the ED properties is required. According to Annex II, the information requirement 8.13.3 for Endocrine disruption (Human Health) shall comprise: (a) An assessment of the available information from the following studies and any other relevant information, including *in vitro* and *in silico* methods: (i) 8.9.1 A 28-day oral toxicity study in rodents (OECD TG 407); (ii) 8.9.2 A 90-day oral toxicity study in rodents (OECD TG 408); (iii) 8.9.4 A repeated dose oral toxicity study in non-rodents (OECD TG 409); (iv) 8.10.1 A prenatal developmental toxicity study (OECD TG 414); (v) 8.10.2 An extended one-generation reproductive toxicity study (OECD TG 443) or two-generation reproductive toxicity study (OECD TG 416); (vi) 8.10.3 A developmental neurotoxicity study (OECD TG 426); (vii) 8.11.1 A combined carcinogenicity study and long-term repeated dose toxicity study (OECD TG 451-3); (viii) A systematic review of the literature including studies on mammals and non-mammalian organisms;	Since 20 April 2023, the new hazard classes are: Endocrine disruption for human health: ED HH Category 1 (EUH380: May cause endocrine disruption in humans) and Category 2 (EUH381: Suspected of causing endocrine disruption in humans) Endocrine disruption for the environment: ED ENV Category 1 EUH430: May cause endocrine disruption in the environment and Category 2 (EUH431: Suspected of causing endocrine disruption in the environment)

Endponts	Information Requirement under REACH and BPR	Classification under CLP
Endocrine Disruption	(b) If there is any information suggesting that the active substance may have endocrine disrupting properties, or if there is incomplete information on key parameters relevant for concluding on endocrine disruption, then additional information or specific studies shall be required to elucidate: (1) the mode or the mechanism of action; and/or (2) potentially relevant adverse effects in humans or animals Section (b) is further described in point 8.13.3.1 of Annex II to BPR specifying which additional studies to consider. This guidance on the Biocical Products Regulation[2] provides advice on the tests that an applicant can or should perform to address the ED properties of the active substance and to conclude whether the ED criteria are met or not. This guidance should be read in conjunction with OECD Guidance No. 150 (OECD 2012)[3] and the ECHA/EFSA Guidance[4] where the testing strategy is further elaborated. According to Annex II, the information requirement 9.10 for Endocrine disruption (environment) shall comprise the following tiers: (a) An assessment of the mammalian data set in accordance with 8.13.3 to assess whether the substance has endocrine disrupting properties based on data in relation to mammals; (b) If it cannot be concluded based on the mammalian data in accordance with 8.13.3 or 9.1.6.1 that the substance has endocrine disrupting properties, then studies set out in 9.10.1 or 9.10.2 shall be considered taking account of any other available relevant information, including a systematic review of the literature' The Annex II, section 9.1.6.1., describes the information to be provided from long-term toxicity testing on fish in which early life-stages (eggs, larvae or juveniles) are exposed. The Annex II, section 9.10.1, specifies the studies to investigate potential endocrine disrupting properties that may include, but are not limited to the following data requirements: (a) Medaka extended one-generation test (MEOGRT, OECD TG 240); (b) Fish life cycle toxicity test (FLCTT, OPPTS 850.1500) covering all the 'estrogen-, androgen- and steroidogenic-mediated' (EAS) parameters foreseen to be measured in the MEOGRT study The Annex II, section 9.10.2, specifies the additional studies to investigate potential endocrine disrupting properties that may include, but are not limited to Larval amphibian growth and development assay (LAGDA; OECD TG 241) The Annex II, section 9.10.3, indicates that if there is information suggesting that the active substance may have endocrine disrupting properties, or if there is incomplete information on key parameters relevant for concluding on endocrine disruption, additional information or specific studies, as necessary, shall be required to elucidate: (a) the mode or the mechanism of action; and/or (b) potentially relevant adverse effects in humans or animals.	

2 Guidance on Biocical Products Regulation

3 Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption | en | OECD

4 ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

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