ECHA
EUROPEAN CHEMICALS AGENCY

Key Areas of Regulatory Challenge

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ECHA's Key Areas of Regulatory Challenge (KARCs) are formulated as an 'evolving research and development agenda' aiming to support and inspire the Partnership for the Assessment of Risks from Chemicals (PARC) research community. The overview areas presented herein are not exhaustive. Other areas of relevance are currently under development. ECHA's KARC will be updated and refined as the scientific areas evolve and key challenges develop.

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1. Setting the scene

ECHA's role as independent agency in implementing various EU legislations has generated a 15 years' wealth of scientific and technical insights and competences. With the Chemicals Strategy for Sustainability[1] (CSS), a renewed policy focus has emerged that invited ECHA to revise and sharpen its advisory role on those topics where it can provide most valuable input. As a result, ECHA has started to map its key areas of regulatory challenge (KARC) under the umbrella of the PARC Project[2] that provides a forum for collaboration across Europe and aims to pioneer those scientific areas addressing most urgent regulatory challenges.

ECHA's KARCs are formulated as an 'evolving research and development agenda' aiming to support and inspire the PARC research community. The overview presented here is not an exclusive list. Other areas of relevance are currently under development. ECHA's KARC will be updated and refined as the scientific areas evolve and key challenges develop.

The CSS is currently leading to major reviews and new initiatives for EU (chemicals) legislation, and its rationale will undoubtedly live through the next European Commission. Seen through an ECHA lens, KARC can be summarized under the following CSS areas:

- *Provide protection against most harmful chemicals*
- *Address Chemical pollution in the natural environment*
- *Shift away from animal testing*
- *Improve availability on chemical data*

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.

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. ECHA's KARC provide first suggestions on areas and concrete research topics that are detrimental to the challenges ECHA is facing. In formulating these KARCs ECHA wants to draw the attention to these selected areas for possible further research within PARC. ECHA has prioritized the following KARCs:

Neurotoxicity: Good quality data for Developmental Neurotoxicity (DNT) and Adult Neurotoxicity (ANT) from animal studies is available for a very limited number of chemicals. Project research could support the development of New Approach Methods for DNT and ANT hazard assessment (e.g. new or development of existing Adverse Outcome Pathways (AOP), identification of reliable positive and negative controls for NAM reliability testing and validation, further development of Developmental Neurotoxicity In Vitro Battery (DNT IVB) battery)

Immunotoxicity: The developmental immunotoxicity is of concern due to the increase of

[1] Chemicals Strategy for Sustainability - ECHA (europa.eu)
[2] Partnership for the Assessment of Risks from Chemicals | Parc (eu-parc.eu)

diseases linked to the immune system (e.g. allergies, autoimmune diseases). The identification of critical windows of exposure of the immune system would help to assess NAM based methods available and, in the future, to use the validated NAMs methods for regulatory context (priority setting, screening, hazard identification, …)

Endocrine disruption: Following the adoption of the new ED (human health and environment) hazard classes under the CLP Regulation (regulation for classification, labelling and packaging of substances and mixtures), to develop NAMs for both EATS and non-EATS modalities (e.g. the Retinoid system pathway) needs to be improved and AOPs should be developed to facilitate the assessment and interpretation of observed endocrine activity and adverse effects (e.g. metabolic disorders).

Addressing Chemical pollution in the natural environment

As recognised in the CSS, chemical pollution is one of the key drivers contributing to ecosystems degradation and biodiversity loss. In practise, environmental risk assessment of chemicals is done by evaluating exposure pathways and the fate of a single substance within the environment, including its persistence and bioaccumulation, and its toxicity to a limited number of organisms through standardised laboratory tests. However, the increasing pressure of chemicals on ecosystems has led to the conclusion that the current approach might be insufficiently protective with the environment, and hence needs to be improved. Key to this 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, including polymers and nanomaterials. The identified key areas of research include:

Bioaccumulation: Current regulatory assessment of bioaccumulation mainly focuses on the bioconcentration factor (BCF) for fish. Generally, for assessing the bioaccumulation potential of a substance a first screening approach based on the logKow of a substance is followed. Depending on the regulatory context, higher tier data is generated by performing *in vivo* test following the OECD 305 TG Guideline. To make bioaccumulation assessment less dependent of *in vivo* studies and to improve bioaccumulation assessment for difficult substances ECHA proposes e.g. to Develop non-vertebrate methods to predict bioaccumulation potential of selected substances and improve bioaccumulation assessment for air-breathing organisms,

Expanding biodiversity protection in Ecotoxicity: Environmental Hazard assessment is currently performed with a limited number of species from selected trophic levels and usually based on directly observable endpoints (mortality, growth and reproduction). It is recognised that this approach might not be protective enough for the multitude of species present in the environment. It might therefore opportune to develop and map NAMs (e.g. *in vitro*, omics, *in silico*) to improve determination of most sensitive species per chemical, reduce in vivo animal testing, and at the same time increase the biodiversity protection by expanding our capacity to extrapolate toxicity results ideally at the ecosystem level. To reach this, it would be helpful to e.g. create an inventory of possible "bioconserved" pathways of toxicity for different species and develop gene expression signatures that can be used to predict toxicity through pattern recognition and probabilistic assessment.

Exposure assessment: New approaches to monitor and analytically verify chemicals present in the environment is critical to inform regulatory action. This will lead to improved use of monitoring and field data for bioaccumulation, long-range environmental transport and/or persistence assessment by authorities, causing more swift reactions to emerging chemicals of concern and reduce need for further laboratory testing. More environmental exposure is welcome for various chemical classes (for example for the linear and cyclic siloxanes).

Data generation for assessing the sensitivity of non-bee pollinators (NBP) to biocidal active substances: The risk assessment for arthropod pollinators may become a standard information requirement for biocides. However, more data is needed to conclude on sensitivity

differences between bee and NBP species. In this context, it would be highly valuable to have more laboratory studies to evaluate the acute contact and oral toxicity and compare the sensitivity between NBPs and honeybees. Further studies are also needed to find out which is the most relevant route of exposure of NBPs from the use of biocides. Finally, another important aspect that needs further investigation is the life stage during which NBPs are most exposed to chemicals in environmental conditions.

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 *in vivo* test methods that are currently in place to support hazard identification. Other approaches such as read across already contribute to the reduction of animal testing within REACH. Yet, this could be further enhanced by a more extensive use of mechanistic as well as toxicodynamic and toxicokinetic data. NAMs incorporation into read across could provide more scientific certainty in extrapolating hazard properties within similarly structured chemicals.

A prerequisite for NAMs incorporation into regulatory decisions is that they should at least guarantee a similar protection level for humans and the environment that is in place at the moment. Under REACH and CLP, only for hazard identification and classification of skin sensitisers NAMs are sufficiently developed to substitute *in vivo* methods. ECHA identifies below key NAM developments that need to happen to facilitate a reduction in animal testing for the following hazards in the current regulatory framework, e.g.:

Read across under REACH To date, many read-across cases fail to demonstrate toxicokinetic and toxicodynamic similarities. NAMs (*in vitro*, *in silico*, OMICs) can help to better characterise hazard and ADME of chemicals in an organism. Case studies demonstrating the context of use of NAMs for read across under different decision-making scenarios are needed. In addition, NAMs can help in defining category boundaries. This will facilitate a conclusion on toxicological similarity between the source and the target substance strengthening and validating the read across hypothesis.

Ecotoxicology - Short term fish toxicity: Responses at cellular level of rainbow trout captured by OECD TG 249 (Fish Gill cell line toxicity assay) or by OECD TG 236 (Fish Embryo toxicity test) can predict acute toxicity for fish. To allow more intense use of these *in vitro* methods in regulatory context, a systematic validation of the predictivity of the methods should be conducted. Validation 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. Furthermore, additional value to the current risk assessment scheme would be to develop cell lines/test systems for different organs and species. This would further foster protection of the whole ecosystem with much higher certainty.

Ecotoxicology - Long term fish toxicity: *In vitro* studies could be used to predict when a substance would be likely toxic to fish by catching early key events taking place at cellular/tissue level, triggering a need to perform an *in vivo* test. Some efforts to develop AOPs and identify early key events (from *in vitro* systems, or an optimised fish embryo study) leading to chronic toxicity to early life stages of fish have been made. To apply an *in vitro*/Adverse Outcome Pathways (AOPs) approach for chronic fish toxicity in the regulatory context, a systematic development and validation of the predictivity of the methods should be conducted. If these methods show potential to predict a specific mechanism causing adverse effects, possibility to translate the mechanistic information to in vivo effect levels could be explored, e.g., utilising in vitro to in vivo extrapolation (IVIVE) methods.

Toxicology: In general, ECHA expects that NAM applications hold many benefits in pushing the boundaries of toxicology. For example, NAM results generated on top of traditional toxicology

studies, can greatly improve our understanding of the mechanisms causing adverse effects in laboratory animals. This holds true for endpoints such as Carcinogenicity, where NAMs could create the basis for detecting carcinogens with non-genotoxic mode of action.

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. During 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 deserve particular attention.

Polymers: Polymers are the fundamental building blocks of plastics and due to the intrinsic chemical nature associated with different molecular mass and material's desired properties, one manufactured polymer may include different molecular weight (MW) fractions. This complicates the interpretation of bioavailability and hazard assessment for regulatory purpose. Development of knowledge and methodologies to support hazard and risk assessment of polymers including their environmental fate is urgently needed.

Micro-and nano-sized materials: Following the adoption of the Commission Regulation (EU)2018/1881 introducing nano-specific clarifications and new provisions in REACH Annexes, all nanoforms that are manufactured or imported must be reported in the registration dossier of the substance. There is a need to better understand the link between the nanomaterial properties and the functional behaviour. Suitable NAM approaches covering regulatory relevant endpoints targeting fate, (eco)toxicity and bioavailability are needed. All those endpoints 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.

Analytical methods for enforcement: One of the important aspects of the enforceability of regulatory measures restricting the use of certain hazardous chemicals is the availability of analytical methods that ensure a proper assessment of the presence of restricted substances and substances falling under authorisation. 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.

2. Key Areas of Regulatory Challenge

2.1. Provide protection against most harmful chemicals

2.1.1. Neurotoxicity

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 toxicity and reproductive (developmental) toxicity.

Under REACH, standard information requirements that may inform on some aspects of adult neurotoxicity include:

- 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 Reproductive Toxicity Study (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)

Substances with which effects indicative of ANT are observed, are subject to classification and labelling 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.

Under REACH, standard information requirements that may inform on some aspects of developmental neurotoxicity include:

- 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 Reproductive Toxicity Study (OECD TG 443) (potentially triggered at Annex IX, and standard requirement at Annex X), and
- 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 (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 BPR, in addition to the pre-natal development toxicity study (OECD TG 414) on two species and extended One-Generation Reproductive Toxicity Study (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 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.

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*' (ECHA, 2022, https://echa.europa.eu/documents/10162/17090/rac_clh_guidance_note_neurotoxicity_en.pdf/96717ed9-55d3-10e0-785b-093d07e267f3?t=1665034511575).

Under REACH, information on intrinsic properties of substances may be also generated by means other than tests above, provided that certain conditions are met (REACH Article 13). These conditions include that the data shall be considered to be equivalent to data generated by the corresponding test methods referred to in REACH Article 13(3) and must be adequate for the purpose of classification and labelling and/or risk assessment; must fulfil adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in REACH Article 13(3); exposure duration must be comparable to or longer than the corresponding test methods referred to in Article 13(3) if exposure duration is a relevant parameter; and adequate and reliable documentation of the study must be provided.

The current regulatory structure summarised above introduces several challenges to the implementation of ANT and DNT NAMs in the REACH, BPR and CLP modalities as standalone information. These include but are not limited to the fact that 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, if available, can be included as supplemental information in a weight of evidence approach and to support grouping and read-across. As such, currently ANT or DNT NAMs in themselves are 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. It is also noteworthy that currently the possibilities for ECHA to request the registrants to conduct any other tests outside standard information requirements are limited.

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

Why the topic is of relevance: ultimately AOPs may help to predict the adverse outcomes of in vitro tests if it 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, Key Events (KEs) in AOP are causally linked and essential to the adverse outcome (AO) under consideration, and they are measurable. The AOP is anchored at one end by a Molecular Initiating Event (MIE), which represents the direct interaction of a chemical with a biological target, and at the other end by an AO, which can be at any biological level of organisation that is relevant to a regulatory decision. DNT is a complex field, where timing (developmental day) and location (specific cell types/species, tissues, organs) of the 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 a MIE or KE in an AOP and thus an AO. A more profound mechanistic basis 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 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 interlinked with AOPs, 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 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 reliability testing and validation

Note that for the purpose of this specific research need, the terms "reference chemicals" is used to identify substances that are used for the validation of NAMs, both individually and as part of a battery (such substances are also commonly referred to as reference chemicals). From this consensus list of reference controls, the assay developers could then select the suitable concurrent experimental controls for their assays.

To fulfil this research need several approaches could be considered:

1. Identify 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.
2. To expand 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), is a desirable approach to identify in a comprehensive manner (and with minimum bias) known neurotoxicants (i.e. positive controls) and reliable negative controls. This review may investigate both human and non-human (e.g. rat) data.

Why the topic is of relevance: 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 in itself 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 currently an important consideration before ANT/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 fulfil a more central role in the regulatory field.

2.1.1.3. The DNT IVB battery: further validation and refinement by increasing data density and by developing new tests to fill data gaps, using reference control substances identified as part of research need point 1.1.1.2

Why the topic is of relevance: Further development of the Developmental Neurotoxicity In Vitro Battery (DNT IVB) as regards their validation including that for predictability may improve 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 is an added value to 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 (specificity, sensitivity, ...) and the types of neurotoxicants that are 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 help understand if and how the battery may be implemented from a regulatory perspective.

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

Why the topic is of relevance: 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 ANT NAMs. 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 above (1.1.1.), 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, but such specific studies may be requested when the concern has been identified, e.g in the form of mechanistic studies, but the available evidence is yet inadequate for toxicological and/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 prototype ANT NAM 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 open the frontier for their formal validation, in turn shedding light on their potential regulatory applications.

2.1.1.5. Addressing the known data gap presented by current DNT/ANT NAMs regarding toxicological information on metabolites

Why the topic is of relevance: Although (PBK) modelling may in part inform on toxicokinetics and the formation of possible metabolites, it may fall short when the substance is e.g. a UVCB. As such, it is of interest to not only explore *in silico* methods, but also the possibility of practically implementing the aspect of metabolism in DNT/ANT NAMs, e.g. 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 item 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/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

Currently the developing immune system is investigated in a regulatory setting by using *in vivo* methods (OECD TG 443, cohort 3), where the exposure to the substance starts in utero and continues until the immune system has developed (e.g. in rats around post-natal day 56 and in humans between years 12 to 18). 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 what are the critical windows in those particular immune organ developments and formation of the peripheral immune homeostasis 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 (Johns Hopkins) to investigate this endpoint.

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

Why the topic is of relevance: The developmental immunotoxicity is of concern, as there has been an increase in relation to deceases that are linked to the immune system (e.g. allergies, autoimmune diseases). Currently the assessment developmental immunotoxicity is a standard information requirement at the highest tonnage level under REACH, in case a concern for immunotoxicity has been noted in previously performed studies in adult animals (EOGRTS with cohort 3). As the concern for requesting developmental immunotoxicity studies is based on data generated in adult animals, it is possible to miss substances causing immunomodulation.

As currently there is a lack of scientific consensus on those critical windows for the development of the immune system, further work is needed, as without the 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, as a next step would be to assess what type of methods are out there and whether those could be used (perhaps with further development and validation) for assessing developmental immunotoxicity. Based on current state of science, which is mainly linked to academia-based research, there are multiple methods containing standard *in vitro* techniques, as well as new types of tissue cultures.

As there is no NAM based techniques with international approval for assessing immunotoxicity, this hampers the inclusion of non-animal-based methods/test batteries into the regulatory system. Due to the general concern of this endpoint, it would be important to have at least NAM based methods for priority setting/screening, in order to better decide on testing needs and to understand the potential risks that e.g. industrial chemicals have towards developing immune system.

Currently developmental immunotoxicity is included under reproduction toxicity in CLP regulation, however discussions are ongoing to generate a specific hazard class for immunotoxicity (containing both adult and development). Therefore, it would be beneficial for classification and labelling purposes to have NAM to assess the potential hazards of substances.

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 and their regulatory applicability. Depending on the outcome, the use of NAMs could lead into priority setting (better targeting of in vivo testing), support of read-across and possibly even for

classification and labelling purposes (depending of the suitability and availability of NAMs).

Short term: To identify critical windows in the development of 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: To develop/validate a testing battery of NAMs for the assessment of developmental immunotoxicity and to assess how this testing battery can be used in regulatory context e.g., screening, priority setting, supporting evidence, or hazard identification.

2.1.3. Endocrine Disruption

2.1.3.1. Development of NAMs

2.1.3.1.1. Develop new/improved assays for (non-)EATS endocrine modalities

Why the topic is of relevance: Currently, the ED assessment heavily relies on vertebrate animal testing to obtain information on adversity and endocrine activity, which are part of the 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, using NAM approaches, for example developing non-protected embryo assays capable of predicting adverse effects. There is currently a gap of NAMs for EDs.

EATS modalities are currently the pathways for which there is a relatively good mechanistic understanding of how substance-induced perturbations may lead to adverse effects via an endocrine-disrupting MoA. However, ED criteria cover all endocrine-disrupting MoAs, i.e. adverse effects which may be caused by any endocrine modality (e.g. insulin receptor signaling). 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 the same test.

Where it fits into the regulatory landscape: These methods are important because information on adversity and mechanism of action is needed for ED identification. Mechanistic information that informs 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 PPPR, BPR and proposed for REACH, and it is the basis for classification under the new ED criteria for CLP.

Short- and long-term impact: Once developed, these methods could be introduced as information requirements and replace more traditional methods, in the different regulatory frameworks. The short-term impact would be increased number of identified EDs and long term reduced number of vertebrate tests. Improved screening methods and confidence in them will avoid performing higher tier testing for all compounds and concentrate animal testing only where absolutely needed thereby reducing the use of animals.

2.1.3.1.2. Establish links to higher tier test systems

Why the topic is of relevance: Currently, the ED assessment heavily relies on vertebrate animal testing to obtain information on adversity and endocrine activity, which are part of the criteria to identify an endocrine disruptor. More ED-related (quantitative) AOPs should be developed by the scientific community to facilitate the assessment and interpretation of both observed endocrine activity and adverse effects. 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.

Where it fits into the regulatory landscape: Biological plausibility (i.e. mode of action /AOP)

information is required for ED identification for PPPs, BPs and CLP.

Short- and long-term impact: Well established AOPs will in the long run speed up the CLH process and allow 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. This will allow to reduce or avoid further testing and steer industry to "greener chemistry".

2.1.3.1.3. Develop NAMs based on invertebrates

Why the topic is of relevance: Invertebrates are a very important class of organisms that are crucial for biodiversity and the ecosystem, and consequently, by ensuring well-functioning ecosystem services, contribute to human wellbeing. As such invertebrates deserve more attention also in the field of endocrine disruption. Currently, the ED assessment heavily focuses on vertebrate organisms, for which the current understanding of the endocrine system and availability of test methods is most advanced, i.e. mammals, fish, and amphibians. However, some endocrine systems are conserved though evolution and are also present in invertebrates. Endocrine disruption also affects non-vertebrate organisms, and in fact, endocrine disruption was first studied in invertebrate species, but the identification of endocrine disruptors in non-vertebrate 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 for a better understanding of the endocrinology of invertebrates, more widely representing environmental species of a range of different phyla, with a focus on developing test guidelines for the identification of EDs, including also mechanistic parameters.

Where it fits into the regulatory landscape: ED identification for PPPs, BPs and CLP

Short- and long-term impact: This research need would probably need some time for development due to the need to first have a basic understanding of the invertebrate endocrinology and only based on this new acquired knowledge new ED methods for invertebrates can be developed. Therefore, the horizontal time span to look at 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 for 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 to reduce vertebrate animal testing.

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

The current test methods mainly focus on EATS modalities, which are the pathways for which there is a relatively good mechanistic understanding of how substance-induced perturbations may lead to adverse effects via an endocrine-disrupting MoA. 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. There is a lack of methods investigating adverse effects and endocrine activity for these modalities. Therefore, there is a need to develop and validate more methods to address non-EATS modalities.

2.1.3.2.1. Develop methods for the Retinoid system pathway

Why the topic is of relevance: OECD has recently developed a Detailed review paper on the retinoid system (DRP Series on testing and assessment No 343) 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 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.

There is increasing evidence 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 modulation.

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.

Where it fits into the regulatory landscape: ED identification for PPPs, BPs and CLP.

Short- and long-term impact: Once developed, 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 may (or will) aid integrating useful AOPs and moving forward towards the goal of chemical screen development.

2.1.3.2.2. Develop methods for identifying metabolism disorders (obesity, diabetes)

Why the topic is of relevance: Recently, there has been an increasing risk of obesity, hypertension, and distorted lipid and glucose metabolism, which together are also known as metabolic syndrome, 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 which are specifically designed to detect alterations in the metabolic system. In order to make these methods useful for regulation, existing methods need to be reviewed and integrated into the existing test method scheme.

Due to the complexity of the metabolic system, 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. 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.

Where it fits into the regulatory landscape: ED identification for PPPs, BPs and CLP.

Short- and long-term impact: Once developed, 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, 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

2.1.3.3.1. Explore current challenges with performing a risk assessment for endocrine disruptors

Why the topic is of relevance: there is still currently 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. 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, low dose effects and critical windows of exposure, and species to species extrapolation. Therefore, there is a need for the scientific community to further investigate these factors 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 EDs.

Where it fits into the regulatory landscape: A risk assessment for EDs is performed under Biocides and pesticides, 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.

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

2.1.3.3.2. Explore improvements to current tests to ensure critical windows of exposure are covered, all useful sensitive parameters are included

Why the topic is of relevance: 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 such as non-monotonic dose response curves, low doses/concentrations effects, delayed effects, multigenerational effects, low dose effects and critical windows of exposure. 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: ED identification for PPPs, BPs and CLP

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

2.2. Addressing chemical pollution in the environment

2.2.1. Bioaccumulation

Bioaccumulation data is necessary for understanding the environmental behaviour of a substance. Within different Regulations (i.e.: REACH, BPR) information on bioaccumulation is used in 1) PBT assessment, 2) hazard classification, and 3) chemical safety assessment (food chain exposure modelling). Bioaccumulation data is also a factor in deciding whether long-term ecotoxicity testing might be necessary. Highly bioaccumulative substances may also transfer through the food web, which in some cases may lead to biomagnification.

The most important and widely accepted indication of bioaccumulation potential is a high value of the *n*-octanol/water partition coefficient. Log Kow is generally used as a first screening approach. Depending on the regulatory context, higher tier data needs to be generated by performing in vivo fish testing following the OECD 305 TG Guideline. Less often, bioaccumulation in mussels, other aquatic invertebrates or the bioaccumulation of sediment-associated chemicals in endobenthic oligochaetes worms and the bioaccumulation of chemicals in soil oligochaetes is also evaluated.

Nowadays, a number of alternative methods have been developed, such as the freshwater amphipod Hyalella azteca bioconcentration test (HYBIT) (OECD draft TG under revision) which delivers an aquatic BCF value, or estimation of intrinsic hepatic clearance from *in vitro* assays according to OECD 319 A and B, which can be extrapolated to a BCF using *in vitro-in vivo* extrapolation (IVIVE) methods.

To make bioaccumulation assessment less dependent on in vivo vertebrate studies and to improve bioaccumulation assessment for difficult substances ECHA proposes the following Research Needs:

- Development of non-vertebrate methods to predict the bioaccumulation potential of surfactants, ionisable substances and organometals
- Improved bioaccumulation assessment for air-breathing organisms,
- Improve the assessment for secondary poisoning and man via environment specially for mixtures
- Development of new methods and assessment approaches to evaluate the bioaccumulation potential of super hydrophobic substances

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

Why the topic is of relevance: Log Kow is used as a screening tool in bioaccumulation

assessment, as an indicator of partitioning to lipid.

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 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 Log Kow which only measures partitioning to lipid.

Furthermore, revised introduction to the OECD Guidelines for testing of chemicals, section 3 (23 March, 2006) notes that also for metallo-organic substances the bioaccumulation potential cannot unequivocally be established by the n-octanol/water partitioning test. In vivo bioaccumulation testing in fish is proposed for such substances.

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.

Fish-water partition coefficient, membrane lipid-water partition/distribution coefficient (K_{MLW}/D_{MLW}) or other identified parameters could play a role at 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 commonly employed experimental methods are: 1) dissolved unilamellar liposomes, 2) lipid 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. For further information see ECHA Guidance R.7.c Appendix 7.10-3 (2023).

There is a need to assess 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 which avoid the use of vertebrate animals are needed to predict 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 still 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 and Biocidal Products Regulations.

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 other descriptors or mechanisms than hydrophobicity. There is a need to improve knowledge and develop methods which would allow to predict bioaccumulation potential of organo-metals, ionisable and/or surface/active substances.

Short-term impact: It is expected that understanding of bioaccumulation mechanisms for ionisable and/or surface active substances will be improved. Fish-water partition coefficient, membrane lipid-water partition/distribution coefficient or other identified parameters could play a role at screening level to trigger a bioaccumulation concern for organo-metals, ionisable and/or surface active substances. Such information can furthermore support their bioaccumulation assessment together with other data.

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. Bioaccumulation potential in air-breathers (e.g. terrestrial mammals)

Why the topic is of relevance: Current regulation on bioaccumulation focuses on the bioconcentration factor (BCF) for fish. Certain substances do however not bioaccumulate in aquatic food-webs, but biomagnify 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 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, logKoa >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 discussion paper "Bioaccumulation assessment of air-breathing mammals" (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) will be reflected in the PBT guidance R.11 (2023).

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 (Kelly and Gobas, 2001) and theoretical mathematical models (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.) 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 (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.).

Under REACH, besides 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).

Short term impact: Methods to assess bioaccumulation in apex organisms further being improved (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. How to improve assessment of secondary poisoning and man via environment

Why the topic is of relevance: Secondary poisoning is concerned with 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 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 man via the environment. For human exposure via the environment, the systemic hazard for long term effect is based on exposure via inhalation and via the oral route.

There is a need to give more attention to the topic of secondary poisoning and man via the environment and integrate the concept of mixture toxicity into these assessments. Furthermore, analysis of monitoring data could be used to assess the potential for secondary poisoning.

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 Part B: Hazard Assessment explains that in the 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 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: Substances which raise a concern due to secondary poisoning and/or exposure of men via environment are identified and regulated.

2.2.1.4. Bioaccumulation potential of super-hydrophobic substances

Why the topic is of relevance: 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 (low bioavailability). However, several super-hydrophobic substances, such as Dechlorane Plus and MCCPs, 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. It is very difficult to handle such lipophilic substances in the laboratory due to their tendency to stick to glassware.

Consequently, current standard bioaccumulation tests are not suitable to determine the bioaccumulation of super-hydrophobic substances. A new testing and assessment approach is needed to assess the potential of super-hydrophobic substances to be taken up and to bioaccumulate, preferably minimising the use of vertebrate testing. This would 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.

Where it fits in the regulatory context: There is evidence for certain highly persistent and super hydrophobic substances, that significant accumulation via the food chain takes place (e.g. chlorinated paraffins, chlorinated flame retardants). Under REACH, besides 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).

Short term impact: Gaining of information 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: Improved 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 by use of NAMs

Why the topic is of relevance: As recognised in the CSS chemical pollution is one of the key drivers contributing to ecosystems degradation and biodiversity loss. Current environmental hazard assessment is focused on the generation of data for only 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 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.

Testing species, which are chosen by practical aspects such as availability of test guidelines and test organisms rather than for biological grounds, are only a small surrogate of biological diversity. In addition, hazard assessment of chemicals focusses almost exclusively on three standardized and directly observable toxicity endpoints—survival, growth, and reproduction of individual organisms—selected for being population and ecologically relevant. However, new methods may be available in the future to protect more efficiently 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 levels of biological. New methods (e.g. in vitro, omics, in silico) could help to relate molecular changes (i.e 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 able to better protect species and ecosystems diversity it is necessary for research to advance in multiple areas. These include among others i.e.: 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 (Registration, Evaluation, Authorisation, and Restriction of Chemicals, no. 1907/2006) 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 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. Assessing the sensitivity of non-bee pollinators (NBP) to biocidal active substances

Why the topic is of relevance: Arthropod pollinators and their decline is a growing concern globally. It is commonly known, or at least suspected, that chemicals play a significant part in the demise of pollinator populations. The risk assessment for arthropod pollinators may become a standard information requirement for biocides (depending on the biocide product type and pattern of exposure).

However, before we can run a full risk assessment for non-bee pollinators (NBPs) we need to have more data available, as it is still not possible to conclude on sensitivity differences between bee and NBP species, as information on the ecology and sensitivity to chemicals is scarce for relevant species. In this context, it would be highly valuable to have more laboratory studies to evaluate the acute contact and oral toxicity and compare the sensitivity between NBPs and honey bees.

Moreover, further studies are needed to find out which is the most relevant route of exposure of NBPs from the use of biocides.

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

where it fits into the regulatory landscape: 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, under Article 75(1)(g) of the Biocidal Products Regulation. During this on-going 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 "European arthropods and their role in pollination: scientific report of their biodiversity, ecology and sensitivity to biocides".

However, the political pressure from the Commission and general public to consider NBPs in chemical risk assessment is ever-increasing. For ECHA to be able to fulfil the mandate for 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.

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*.

short and long term impact: Short-term benefit of this project is to steer data generation for bees versus NBPs, and whether to leave NBPs out of the first version of the pollinator guidance.

In the long-term, the produced information would build into the EU's data base on arthropod pollinators, and in the end, hopefully also benefit the pollinator populations, environment and conservation of the ecosystem services provided by the pollinators.

2.2.4. Monitoring

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 of relevance: 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 assessments could be improved. This could allow to speed up identification and regulation of emerging chemicals of concern as well as reduce a need for laborious and time consuming standard laboratory testing. E.g. samples collected and available for analysis in various specimen databanks could be used to establish trophic magnification factor for prioritised substances of concern for bioaccumulation assessment.

Field bioaccumulation or trophic magnification factors as well as monitoring data can provide relevant lines of evidence indicating that the substance has or has not bioaccumulation properties. Bioaccumulation factors, dietary accumulation, trophic magnification and detection of chemicals in biota, wildlife can generally be considered in the context of bioaccumulation screening and assessment. This information can also 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.

There is a need for development of further understanding on use, including consideration of associated uncertainties, of field and monitoring data for bioaccumulation, and persistence and LRTP assessment, e.g. via use of benchmarking approach from known bioaccumulative substances. The scenarios where such data standalone or in combination with other evidence could be used to conclude on bioaccumulation and/or persistence (including LRTP) should be identified. E.g. 'Food web on ice' is a pragmatic approach to investigate the trophic magnification of chemicals of concern and it could be further considered how such information would allow to conclude on bioaccumulation.

Where it fits into the regulatory landscape: Under REACH, besides results from simulation studies in water/soil/sediment and from a bioconcentration or bioaccumulation study in aquatic species, other information from field studies or monitoring studies and information on the

bioaccumulation/biomagnification potential can be used to assess P/vP and B/vB properties respectively (REACH Annex XIII, 3.2.1 and 3.2.2) but also POPs

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

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

2.2.4.2. Case study 1: Environmental monitoring data for linear and cyclic siloxanes

For a substance to have potential for long-range environmental transport according to the Stockholm Convention on persistent organic pollutants (”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 volatile methyl siloxanes (VMS) are volatile substances having half-lives in air exceeding two days (which is one of the criteria in the Convention) that have been measured in air in remote regions. However, whether these substances can deposit from the air to surface media is unclear and something that has been discussed among experts on many occasions. Many experts believe that the VMS would not back-deposit from air to surface media due to their physical-chemical properties and there are modelling studies supporting this. Nevertheless, there have been some detections of the substances in biota (including in Antarctica) and in deep-sea sediments away from point sources, which would suggest deposition has taken place.

Monitoring of the volatile methyl siloxanes 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. To our knowledge there is currently no measured data of VMS in rain or snow, only modelling, 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, we strongly recommend 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 siloxanes. Furthermore, ice cores enable temporal trend to be determined.

Analytical methods and techniques are currently available to monitor concentration of VMS in air. If a measurement of the siloxanes 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 (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/support a potential for atmospheric deposition.

Increasing the 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.

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. Furthermore, loss of substances or reaction of siloxanes should be avoided by following appropriate sample transport, storage, preparation and instrumental methods.

Why the topic is of relevance: 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 understanding the deposition mechanisms from air to surface media of these substances. This information is needed 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[3] due to their PBT/vPvB properties and RAC and SEAC opinions have been adopted for the proposed restriction under REACH Annex XVII[4]. Norway plans to submit SVHC proposals for the linear VMS L2, L3, L4, and L5 due to their PBT/vPvB concern[5].

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

Short term impact: Improve the scientific understanding of the deposition mechanisms from air to surface media of these substances.

Long term impact: Ensuring high level of protection for the environment and the human health from substances that could potentially meet the criteria of Persistent Organic Pollutants.

2.3. Shift away from Animal Testing

2.3.1. Read across and NAMs: Development of case studies

Read-across is considered one of the main possible adaptations for higher tier human health endpoints such as repeated dose toxicity, developmental and reproductive toxicity, presuming that a scientifically plausible hypothesis can be justified and used to derive a quantitative result for targeted substances.

The read-across approach starts with structural/ physicochemical similarity between target and source compounds, assuming that similar structural characteristics lead to similar human hazards. In addition, similarity also has to be shown for the toxicokinetic and toxicodynamic properties of the grouped compounds. However, many read across cases fail to demonstrate toxicokinetic and toxicodynamic similarities. Reasons for this include deficiencies in the quality of the source studies, lack data to support predictions based on toxicokinetics, shortcomings in the hypothesis and justification of the toxicological prediction and variation in the severity and type of the adverse outcome which make it difficult to conclude on a "similar" toxicological hazard.

The deficiencies related to the supporting evidence are particularly relevant for high-tier human health and high-tier environmental endpoints. To increase the robustness and regulatory

[3] Substance of Very High Concern
[4] https://echa.europa.eu/documents/10162/a3e8195a-23d3-5859-6fdc-7805a3148b46
[5] https://echa.europa.eu/registry-of-svhc-intentions accessed on 23 May 2023

acceptance of those adaptations for high-tier human health endpoints, additional data is needed, particularly related to toxicological mechanisms and absorption, distribution, metabolism and excretion (ADME) properties.

NAMs, in vitro and silico tools, can help to support read across by generating data on the kinetics and dynamics of different compounds and defining category boundaries. This will facilitate a conclusion on toxicological similarity between the source and the target substance strengthening and validating the read across hypothesis. The major challenge is how to use molecular data with no direct link with toxicity to group substances for similar adverse effects. This application requires further development of the methodology and objective criteria for regulatory acceptance considering the following elements:

- relevance of the biological model used to generate NAM bridging evidence (both for the source and target substances);
- threshold of similarity for substance grouping
- toxicological relevance of the NAM evidence 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 commonly used alternative approaches for filling data gaps in registrations submitted under REACH. Applying read-across correctly reduces the need for experimental testing and tests on animals. The incorporation of NAMs into read across will make read-across hypothesis more robust and help to address deficiencies found for supporting evidence.

Short term and long term: 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 category boundaries and characterise the similarities/dissimilarities between source and target substances. The development of case studies will facilitate the incorporation and understanding of NAMs for read across.

Associated Detailed Research Needs: As described above, the major challenge is how to use molecular data with no direct link with toxicity to group substances for similar adverse effects. Research needs associated to this challenge include

A. 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?
B. What factors are critical for defining 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?
C. Enhance our knowledge and confidence of molecular biomarker/bioactivity - adverse effect associations (e.g. relevance of the biomarker panels) to facilitate the use of molecular and bioactivity data to support grouping?
D. What factors are critical for defining reliability of the NAM evidence for grouping hypothesis?
E. 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 an NAM-based grouping study such as 'omics-based grouping.

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

Why the topic is of relevance: An animal free chemical hazard assessment system will rely on in vitro and in silico approaches. Therefore, models such as pharmacokinetic modelling will be needed to derive a point of departure for hazard 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 tissue distribution, metabolism, and excretion of a chemical is needed. 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 319A,B) to estimate a bioconcentration factor (BCF).

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 (e.g. some models may perform better for fast metabolising substances). Further, 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 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 are associated with higher uncertainty. It is also a challenge to properly reflect *in vivo* metabolisms with *in vitro* methods in terms of coverage of organs, cell types, and enzymes. These limitations should be understood, described, and taken into account when developing such models. A measure of the performance of IVIVE-PBK models in comparison of in vivo ADME studies to characterise the variability and uncertainty of IVIVE-PBK models for relevant compounds or compound classes would be beneficial.

Where it fits into the regulatory landscape: *In vitro* ADME/TK has been proposed by The Commission as an information requirement for REACH. In vitro clearance assays with fish material are addressed in the updated ECHA PBT guidance and support the assessment of the bioaccumulation potential, thus can contribute to avoid in vivo fish bioaccumulation testing.

Short- and long-term impact: In 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/standards for the methods. The work will also improve optimisation of methods to increase their reliability and relevance.

In long term, the introduction of ADME/TK as standard information requirement might have a major impact on hazard assessment practice and potentially to increase the quality and robustness of the use adaptations under REACH. ADME/TK information and related IVIVE is critical for defining safety levels for regulatory use and pre-requisite for an animal free chemical risk assessment system relying on in vitro and in silico approaches.

Associated Detailed Research Needs: To develop a workflow that, considering the characteristic of the target substance, will identify an integrated (defined) approach to characterise the ADME/TK properties of the substance.

To identify the key characteristics of the substances that are important for defining the applicability domain of the methods.

To generate integrated approaches that potentially combine in silico, in vitro, and or in vivo

methods, taking into account the applicability and the limitations of each ADME/TK method to make sure that the methods used are suitable for the substance of interest.

In addition, there is a need to

- catalogue what methods are currently available (and can be run by CROs) and which can be used to predict ADME/TK properties?
- characterise the performance of these methods and IVIVe modelling and how the performance is affected by given chemical space?
- Identify the current testability limitations and what are the uncertainties related to ADME/TK methods?

The findings from the steps above can be used to identify further research needs.

2.3.3. Acute fish toxicity

One of the fundamental aims of the REACH regulation (Registration, Evaluation, Authorisation, and Restriction of Chemicals, no. 1907/2006) 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. 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 by e.g., in vitro screening of substances which are unlikely to be toxic to fish.

Why the topic is of relevance: 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 when a substance would be likely toxic to fish by catching early key events taking place at cellular level, and allow predicting acute fish toxicity directly for some substances. For example, responses at cellular level of rainbow trout 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, it is not clear to what extent the gill cell line study can be applied and is predicting well the fish acute toxicity of all substance types, including difficult substances (bulky, very poorly soluble, adsorptive, volatile). 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 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, the limitations of them need to be well understood in order to ensure safe use of all registered substances. For this purpose, a systematic validation of the predictivity of the methods should be conducted. Validation 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 which exist for REACH registered substances).

Furthermore, additional value to the current risk assessment scheme would be to develop cell lines / test systems for different organs and species. This would further foster protection of the whole ecosystem with much less uncertainty.

short and long term impact: NAMs offer a great prospect to reduce vertebrate testing while

still providing same 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 the methods.

2.3.4. Validation of a systematic in vitro/silico battery to steer generation of chronic toxicity data for vertebrate species

One of the fundamental aims of the REACH regulation (Registration, Evaluation, Authorisation, and Restriction of Chemicals, no. 1907/2006) 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 and PBT assessment. 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. Furthermore, the generated toxicity data on fish represents the hazards to vertebrates but this data generation approach may not be protective enough for all vertebrate species. Test species, which 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 of relevance: NAMs and *in vitro* testing has potential to reduce testing on living vertebrate animals such as fish. For example, i*n 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(ies) 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, ambhibians and birds to predict chronic toxicity to fish/vertebrates has been made. For example, 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 ENV Canada to investigate its use in regulatory context (Validation of the use of the EcoToxChip test system for regulatory decision-making (genomequebec.com)). Similar exercise could be done for REACH substances. Furthermore, considering that the *in vitro* systems are limited by representative species/cell lines, some methods to extrapolate further the effects across wide range of species is 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 by PODs, as in e.g. DOI: 10.1002/etc.5395) under REACH is yet unknown. To allow more intelligent *in vitro* / Adverse Outcome Pathways (AOPs) to be applied in regulatory context, validation 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 PODs). Validation 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 the methods.

2.3.5. Carcinogenicity

To quantify the effect of (non-/genotoxic) 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"
b) Hazard:
    a. "the substance is classified as germ cell mutagen category 2 or
    b. there is evidence from the repeated dose study(ies) 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 to 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 of relevance: Cancer is the leading cause of death in rich countries (Dagenais et al 2019) despite improvements therapies and (early) diagnostics. ECHA estimates that 1-3x 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 (Karamertzanis et al, 2019).

Where it fits into the regulatory landscape: NAMs suitable to be included in future regulations could be identified by testing known human carcinogens in a large number of 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 effect

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 a large number of mammals to ensure sufficient statistical power. Its outcome has frequently been challenged as being too unspecific, and thus, not relevant enough for humans (Torres et al 2021, Marone et al 2013).

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 (Jacobs et al 2016, 2020; special issue in preparation/2023). 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. 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 (vom Brocke et al, in preparation/2023). 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 identify also 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 of relevance: 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 thresholded, 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. In particular, 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: Development of knowledge and methodologies to support hazard assessment

Due to the intrinsic chemical nature associated with different molecular mass and material's desired properties, one manufactured polymer may include different molecular weight (MW) fractions. This complicates the interpretation of bioavailability and hazard assessment for regulatory purpose, as one polymer can include many MW ranges, characterised by different bioavailability and hazard properties. This additionally complicates establishing of the meaningful testing material/fraction for testing purposes. In this aspect, polymers are similar to UVCB substances for which complexity brings some level of uncertainty on the hazard properties. Therefore, the research needs and challenges are similar to those identified for UVCBs. Lack of experimental (eco)toxicological studies on bulk polymers is additional obstacle in understanding their bioavailability and hazard properties. Furthermore, in the upcoming REACH revision for polymers there is an intention to minimise the *in vivo* testing with vertebrate animals.

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?

Historically, regulatory frameworks have considered polymers of lower hazard than non-polymers, as a result of their high molecular weight (MW). The assumption is that high MW is associated with poor bioavailability and hence low toxicity. This is supported by the 'rule of five' (Ro5) which posits that substances with a MW > 500 Da 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 500 Da cut-off is questionable. The literature reports molecules with MW > 1200 Da (e.g., cyclosporine) that are not hindered in their membrane permeability. The example of chlorinated paraffins (CPs) proves that the concerns on 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. On that basis, high MW polymers can no longer be regarded as innocuous by default. As the REACH registration will be extended to polymers, there is a need to highlight the areas where more research is needed to understand their bioavailability and better support the future hazard and risk assessment for the regulatory purpose.

The future proposed regulatory scheme under REACH considers reduced information requirements depending on the polymer's type (defined by its MW and tonnage band). Within

this scheme, for high-MW polymers (> 1000 Da) less information would be required based on the assumption of their limited bioavailability. However, to minimise the uncertainty on the bioaccumulation and aquatic toxicity, a screening methodology is needed to either confirm the reduced bioavailability, or to spot the exceptions to the assumption that high-MW polymers are less hazardous. If the screening allows to conclude on polymer's negligible bioavailability, no aquatic toxicity studies would be required However, if insignificant uptake cannot be confirmed, and there are concerns that polymer may reach potentially hazardous concentrations in aquatic organisms, aquatic toxicity and fate studies are 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[6] for high MW polymers is missing. With the intention to minimise whenever possible the generation of animal data, there is a risk that the screening method will not be protective enough and potentially hazardous polymers may "slip" through the regulatory "safety net".

The above-mentioned data and knowledge gaps open the opportunity to explore NAMs that could possibly be used for polymers in the absence of experimental (eco)toxicological studies on the bulk polymers. Screening should also consider that polymers may contain low-MW oligomers or additives that may be released upon degradation and 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 polymer 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 on 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, the alternative test systems/approaches dedicated for bulk polymers need to be developed.

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

There is an hypothesis that higher molecular mass of a molecule is associated with reduced

[6] Average maximum diameter (Dmax) > 1.7 nm, logKow > 10 or octanol solubility [mg/L] < 0.002 [mM] x MW [g/mol].

absorption, and consequently lower levels of toxicity. This has potential relevance for polymers, many of which have a molecular mass in excess of 1000 Da. However, it is unclear if this hypothesis holds for all routes of exposure (oral, dermal, inhalation), and the 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 paucity of data on the repeated-dose toxicity of polymers. It is important to understand if polymers have characteristic or common toxicity as a result of being polymers. Such information is important for hazard assessment and protection of human health, and also 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 of relevance: 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 scientifically-grounded assumptions on polymer's bioavailability and (hazard) properties.

2.4.2. Micro- and nano-sized materials

2.4.2.1. Improve toxicity assessment for micro- and nano-sized materials (e.g., nanomaterials or microplastics) and investigate the long-range transport, uptake and toxicity for humans and other organisms.

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 unsurprising that there are still substantial gaps in terms of test system adaptation or development for (eco)toxicological endpoints.

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.

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 (see Doak et al 2022); 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: is the application of NAMs in a regulatory context for the hazard and risk assessment of micro- and nanosized particles and therefore contributing 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

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. Seen the fact that many substances may be present in different matrices also adapted sample preparation methods is a necessity. 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 of relevance: 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 level of quantification (LOQ) is lower than the limit 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 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.

Examples of areas of application

Characterisation of nanomaterials, including advance 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:

- 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.

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