LIFE APEX

Systematic use of contaminant data from apex predators and their prey in chemicals management

Deliverable B5.5

A draft guideline for the assessment of PBT properties of pollutants in AP&P

Lead Beneficiary:

German Environment Agency (UBA), Germany

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Deliverable Title	B5: A draft guideline for assessment of bioaccumulation of relevant pollutants in AP&P samples
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Objective	To aid scientific and regulatory discourse the present report provides practical recommendations regarding the specific use and interpretation of chemical data from wildlife in screening and assessment of bioaccumulation under REACH. It was used to support the revision process of the field data sections of *ECHA Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment and R.7c: Endpoint specific guidance.*

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Summary and recommendations

This guidance document reports on the screening and assessment exercises as regard to bioaccumulation, which was applied under LIFE APEX using occurrence and concentration data of various chemicals detected in apex predators and fish. To aid scientific and regulatory discourse on the application of field and biomonitoring data, the present report provides practical recommendations regarding the interpretation of monitoring data (MD) from biota with regard to screening and assessment of chemicals. While the present report builds upon the existing ECHA Guidance document R.11 and R. 7b (ECHA 2017a;b) it aims to update the recommendations on current and future practices and to address issues concerning the lack of standardisation of wildlife MD for (1) PBT screening and (2) B-assessment. Based on experiences from the LIFE Apex project and other research initiatives, guidance is given at different levels, i.e. sampling, chemical analysis, screening, prioritization, data interpretation and quality assurance. A clear focus is set on the use of occurrence and concentration data from apex species from all environmental compartments (freshwater, marine and terrestrial). This report was used to support the revision process of the ECHA *Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment (ECHA, 2017a) and R.7c: Endpoint specific guidance (ECHA, 2017b)* as well as to promote the harmonisation of regulatory criteria for PBT/vPvB assessment at European level.

The detection of chemicals in wild biota provides a clear indication that a substance entered the environment and that has been taken up by that organism. However, a detection does not by itself indicate that significant bioconcentration or bioaccumulation has occurred. A qqualitative or quantitative screening for chemicals as well as time trend analysis in wildlife from various compartments can considerably help to (1) identify and prioritize potentially hazardous contaminants, in particular PBTs; (2) determine presence of environmentally relevant chemical mixtures and metabolites, and (3) provide information on real-world biota levels, which might be useful during exposure and toxicity assessment. Such data is particularly useful when it is easily accessible in online databases, as e.g. the NORMAN database. Novel *in silio* methods, like the JANUS tool have shown to reliably support the prioritization for detected PBT candidates in biota samples for further in-depth assessment.

Due to their trophic position and well-known ecology, top predators are valuable indicators of spatio-temporal contaminant trends and have been shown to serve as early warnings for the uptake and accumulation of hazardous chemicals. Apex predators are protected species and invasive sampling should be avoided on ethical grounds, especially since samples are already available in European sample collection. Generally, using opportunistic samples from natural history museums, environmental specimen banks or scientific collections substantially challenges the bioaccumulation assessment, since often predator-prey relationship cannot be established for archived specimens and exposure levels of sampled individuals are unknown. Another opportunity would be to apply non-lethal sampling methods (e.g. collection of animals that are found dead, droppings, infertile birds' eggs or biopsies of mammalian skin or blubber). However, such studies require are careful design that has to be appropriate for the respective assessment.

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Still many factors such as tissue sampled, biology of the focus species or variation in diet can confound the interpretation of results. In many cased it remains also challenging to identify emerging chemicals of concern in environmental media as analytical standards are frequently not available for such contaminants. Thus, analytical methods should be combined, e.g. target, suspected and non-target analysis to capture all potentially hazardous substances. Nonetheless, the valuable samples of various predator species are becoming more and more available in European sample collections and should be better exploited in the future. Field monitoring data, particularly from apex predators might be an alternative or supplementary to laboratory testing in certain cases, in particular for more hydrophobic substances that may take a long time to reach steady state in the laboratory or chemicals accumulating in air breathers.

An assessment based solely on field monitoring data or on routine monitoring programs to conclude upon the B-status of a compound will not be feasible in the foreseeable future due to the various cofounding factors such as variability in species ecology, unknown life-history or difficulty in determining exposure levels. Therefore, using field monitoring data as an alternative to assesses bioaccumulation or supplementary course of action to laboratory testing remains challenging. However, at such field biota data, in particular from apex species, have been shown to considerably support the B screening and to improve the confidence to decide upon the B classification in a weight-of-evidence approach. A more systematic sampling regime, including analysis of water concentrations to screen for bioconcentration in fish e.g. within the routine sampling campaigns under the Water Framework Directive would increase the value of such samples for regulatory purposes. This would increase the use of available biota field monitoring data as a more commonly applied part of the PBT- screening and assessment under REACH e.g. a better guidance on the application and use of the different monitoring database systems. A more systematic monitoring at a European scale would considerably support the screening and prioritization of hazardous chemicals, identify environmental relevant chemical mixtures as well as metabolites that are currently not considered in common risk assessment.

In conclusion, field studies with apex predators can give valuable ‘real-world’ data in the context of bioaccumulation and exposure assessments and are useful to identify chemicals of emerging concern within prioritization exercises but they are resource intensive, retrospective and have many interpretation problems. Further elaboration is needed to enshrine the knowledge summarised in the present document and other Life Apex guidance documents into the current regulatory framework und REACH and other chemical regulations. This could for instance be the work for a dedicated expert group.

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1. Introduction

New and existing substances are screened by regulatory agencies at national and international levels according to persistent (P), bioaccumulative (B), and toxic (T) cut-off, such as those from the Stockholm Convention (UNEP 2001), Environment Canada (Environment Canada 2003), the US Environmental Protection Agency (USEPA 1976), and the European Chemicals Agency (ECHA 2008). Substances exceeding these values are referred to as PBTs and are potentially subject to regulatory assessment or further scrutiny to determine their environmental and human health impacts.

Under REACH, the assessment of a chemicals PBT properties is usually conducted in a tiered approach. First, P, B, and T properties of chemicals are considered in a chemical screening phase to identify potentially hazardous substances (hereafter called screening). In a second step, an in-depth assessment of a chemical hazard is usually based on laboratory data (hereafter called assessment). Currently, the hazard and risk assessment under REACH is often challenged by large data gaps and uncertainty. For instance, ecotoxicological data and information on exposure scenarios of registered substances are often missing (EEA, 2020). This is also emphasized in a study that demonstrated that 58% of the registration dossiers for REACH substances with tonnages above 1000 tons (t) per year were non-compliant (Springer, 2015). Measured fish bioconcentration factors (BCFs) exist for only approximately 5% of the organic chemicals that require B assessment (Arnot and Gobas, 2006). In this context, field and monitoring data (MD) in particular from apex species are highly valuable to bridge the gap between laboratory data and field scenarios and to ultimately improve the screening and assessment of hazardous compounds. However, MD from wildlife species are often not available for most chemicals that are under assessment, in part due to a lack of analytical methods including certified reference standards.

Top predators, including raptors and mammals, have proven to be reliable sentinel species for persistent bioaccumulative contaminants because (i) they integrate chemical signatures across space and time, including entire biological communities, (ii) of their high trophic position within food webs (iii) provide insights in the wider ecological health including potential human exposures (Burger and Gochfeld, 2004; Elliott and Elliott, 2013). Moreover, population declines of top predators have been among the most tangible impacts of chemical pollution and have driven public pressure to enact treaties aimed at reducing such pollution (Bierregaard et al., 2014; Blus et al., 1971). Novel analytical techniques such as suspect and non-target screening now allow for the identification of thousands of chemicals in each sample, which ultimately helps to overcome the analytical gap for prioritization chemicals of yet unknow concern and potentially hazardous chemicals that under current assessment (Badry, 2022a-c; Treu, 2022). As a consequence, the Life Apex project (www.lifeapex.eu) was initiated in 2018 (2018-2022) and is aiming to screen for presence of more than 65,000 substances in marine, freshwater and terrestrial mammals, raptors and fish (as prey species) from all over Europe using state-of-the-art analytical methods for wide-scope target and suspect screening from all over Europe. The overall goal was to demonstrate how the data on occurrence and concentration of pollutants in apex species measured at an EU-wide scale can support risk and hazard assessment (Badry et al., 2022a,b; Treu et al., 2022).

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Nowadays, an increasing number of high-quality monitoring data from biota are generated under different European and global initiative such as the NORMAN network. Within the latest chemical's strategy, the EC (European Commission) calls for strengthening monitoring approaches in humans and ecosystems as key to improve the understanding of their impact and to act as EU early warning. Such initiatives will further increase the number of detected chemicals in European environmental compartments in the future (EC, 2020). Accordingly, the European Commission (EC) has made significant efforts to make (biota) monitoring data better accessible and comparable, e.g. via the platform IPCHeM (https://ipchem.jrc.ec.europa.eu/), which are now ready to use by regulators and industry. Thus, new approaches and guidelines are needed to ensure an efficient application and promote regulatory uptake of such field data including an enshrinement into the regulatory and early warning assessment schemes. Given the growing number of field bioaccumulation data, a particular need exists for a better understanding of the magnitude and causes of the variability of bioaccumulation potential between laboratory and field measurements (Burkhard et al., 2012b).

REACH and the Stockholm Convention explicitly require that all available information in registration dossiers and open literature, including monitoring data (MD), shall be considered in a weight of evidence approach (WoE) to draw a conclusion on hazard endpoints of a substance. However, there is no clear implementation strategy for accomplishing this regulatory objective in Europe or elsewhere (Arnot et al., 2022). In frame of the Stockholm Convention, the use field data in the evaluation of bioaccumulation is explicitly mentioned. In contrast to the REACH regulation, field data are integral part of the bioaccumulation criteria set out in Annex D: "Monitoring data in biota indicating that the bioaccumulation potential of the chemical is sufficient to justify its consideration within the scope of this Convention". At present, there is a lack of specific guidance on how to apply information coming from the detection of compounds in wildlife within the hazard and risk assessment under REACH and other legislations. Recognizing that field bioaccumulation data make an important contribution to WoE approach for chemical assessments, several regulatory agencies called for better guidance to develop consistent methods for collecting and interpreting biota monitoring data. Thus, to aid scientific and regulatory discourse on the application of field and biomonitoring data, the present report provides practical recommendations regarding the interpretation of MD from biota with regard to screening and assessment of PBT chemicals. While the present report builds upon the existing ECHA Guidance document R.11 and R. 7b ECHA (2017a; ECHA, 2017b) it aims to update the recommendations on current and future practices and to address issues concerning the lack of standardisation of wildlife MD for (1) PBT screening and (2) B-assessment. Based on experiences from the LIFE Apex project and other research initiatives, guidance is given at different levels, i.e. sampling, chemical analysis, screening, prioritization, data interpretation and quality assurance. A clear focus is set on the use of occurrence and concentration data from apex species from all environmental compartments (freshwater, marine and terrestrial). This report was used to support the revision process of the ECHA *Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment (ECHA, 2017a) and R.7c: Endpoint specific guidance {ECHA, 2017 a)4* as well as to promote the harmonisation of regulatory criteria for PBT/vPvB assessment at European level.

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2. Use of biota monitoring data to screen for chemicals of concern

2.1 Current practices to screen for bioaccumulative properties

REACH Annex XIII of the REACH regulation defines two levels of assessment within the PBT/vPvB assessment ("screening" and "assessment") and two sets of information ("screening information" and "assessment information"). The screening information can be understood as one subtype of assessment information. However, it should be noted that screening information cannot be directly (numerically) compared with the PBT/vPvB criteria as the screening information does not contain degradation half-life values for the assessment of persistence or BCF values for the bioaccumulation assessment, which could be directly compared with the criteria set out in REACH. Screening information indicates whether a substance fulfils the PBT or the PBT/vPvB criteria of REACH Annex XIII. This tiered process allows to identify potentially hazardous chemicals in a pool of more than 22.000 currently registered chemicals under the REACH regulation. The screening stage is essential to identifying the need for a follow-up assessment regarding the different hazard endpoints. Therefore, strong efforts should be made at the screening level to ensure that a worst-case scenario is considered and that no hazardous substance is overlooked.

At the screening level the bioaccumulation potential is screened via i) the water-octanol- partitioning coefficient (log Kow) and ii) other suitable and reliable information (ECHA, 2017a). For the latter, MD from wildlife species such as apex predators e.g. a high frequency of occurrence, increasing concentrations or occurrence of chemicals over time, or increased levels at over at an EU-wide scale. For many neutral organic chemicals, the Kow has historically provided a simple parameter to estimate organism–water partitioning (bioconcentration) in aquatic organisms in which octanol is a surrogate for organic phases, primarily lipids (Arnot et al., 2022). Since the Kow is a measure of the equilibrium partitioning of organic compounds between water and octanol, it only allows for an estimation of the aquatic bioaccumulation and is only valid as a surrogate for bioconcentration of neutral chemicals and when biotransformation rates are negligible (Mackay, 1982). For organic substances with a log Kow value below 4.5 it is assumed that the B criterion, i.e. a BCF value of 2000 Kg/L (based on wet weight of the organism, which refers to fish in most cases), is not exceeded. At very high log Kow values (> 6), a decreasing relationship between log BCF and logKow is observed. Apart from known experimental bias during the analytical determination of BCF values for these very hydrophobic substances, reduced uptake due to the increasing molecular size, described as bioaccumulation cut-off effect, is suggested to be an additional source of error (ECHA, 2017a). Research on this topic, however, suggests that there is no robust evidence for cut-offs in bioconcentration related to molecular size (Arnot et al., 2010; Nendza and Müller, 2010). According to Jonker and Van der Heijden (2007, observed cut-off effects can be ascribed to artefacts, and thus the incorporation a BCF cut-off values for very hydrophobic chemicals should be revised.
For ionisable substances, the log Kow also does not seem to be a valid descriptor for assessing the bioaccumulation potential as these substances predominantly accumulate in membrane lipids which is not reflected by the log Kow (Bittermann et al., 2014). Information on the bioaccumulation potential of such substances should therefore also consider other descriptors or mechanisms than just hydrophobicity, e.g. by the membrane-water partition coefficient (DMW) which is suitable for estimating the general uptake capacity of ionic substances in organisms (Armitage et al., 2013). This

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indicates that the use of the Kow as single screening criterion might result in many false negative cases, overlooking potentially bioaccumulative chemicals as shown e.g. for polyfluorinated alkyl substances (PFAS; (De Silva et al., 2021)).

2.2 Potential use of biota monitoring to screen for bioaccumulative properties

Due to the above-mentioned limited validity of log Kow for the prediction of bioaccumulative properties, it is important to use alternative information at the screening level, such as biota MD. Under REACH, other suitable and reliable data can be used as PBT screening information besides the log Kow. The detection of elevated levels of substance in biota, compared to levels in their surrounding environment are explicitly mentioned in frame of assessment of bioaccumulation under REACH (ECHA, 2017a).

Depending on the type of analysis performed within the monitoring program, field biota monitoring data have to be separated into i) chemical occurrence and ii) concentration data. Qualitative analysis of biota samples normally results in occurrence data for a chemical, just reporting if the chemical is present or absent in the analysed sample, e.g. in a selected organism at a selected time. In combination with quantification efforts, concentration data of a chemical can be generated. Based on this type of analysis, information on a chemicals' presence and concentration in the organisms in relation to the surrounding environment can be provided. This information can then be included within a line of evidence in the bioaccumulation assessment in line with the provisions of ECHA R.11 guideline. However, monitoring data delivering concentrations of chemicals in an organism and the corresponding media are usually not commonly available. Therefore, a stronger connection between scientific and regulatory bodies is necessary to promote the regulatory uptake of monitoring data (Wang et al 2020). As a consequence, the use of occurrence and concentrations data from biological samples should be strengthened within the PBT screening. High occurrence of chemicals in biota samples without the necessity of defined concentrations should be used as screening information on bioaccumulative properties, despite potential missing information on the occurrence and level of the chemical in potential prey species or abiotic environmental compartments. Especially for substances for which the common endpoints on bioaccumulation on screening level fails, e.g. log Kow in case of ionic and very hydrophobic substances, monitoring data can close this evaluation gap, allowing to add further proof within a line of evidence on potentially PBT properties to further contribute to an in-depth assessment of the hazard posed by the chemical.

2.3 Approaches to prioritize PBTs detected in apex species

Thousands of substances have been detected in biota samples globally, not all of them can be screened manually. To draw useful regulatory information from these huge datasets of wildlife MD, it is necessary to use a prioritization methodology that focuses and prioritizes the potential PBT/vPvB candidates. With the prioritization approach used in the LIFE APEX project, we demonstrated that this approach is able to prioritize potential emerging PBT/vPvB candidates from large biomonitoring datasets. The scheme, based on interlinked filtering steps and in silico methods, focuses on potentially interesting PBT/vPvB candidates for regulatory purposes and saves time and effort when dealing with large datasets (figure 1). The aim of a prioritization list is to provide an enumeration of chemicals of emerging concern that may be identified and regulated as by regulators

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in further assessment steps. In the first step, already identified chemicals (e.g. SVHC substances, POPs) were filtered out from our data set using ECHAs public activities coordination tool (PACT, https://echa.europa.eu/de/pact). In the second step, the list was further narrowed down in terms of its chemical class. Since substances registered under REACH are of primary interest to us, the dataset was further reduced using the ECHA list for substances registered under REACH. For this purpose, our data set was compared with the ECHA registration list using the CAS numbers (https://echa.europa.eu/de/information-on-chemicals/registered-substances). Only substances with an active registration were still considered in the further prioritization process. In a further step, the list of detected non-regulated REACH substances was filtered according to their frequency of appearance (FoA) in biota. In the LIFE APEX project, the limit for the inclusion of detected substances was set to greater than or equal to 10%. The limit of 10% were set to exclude substances that have led to accumulation in biota due to increased emissions at point sources. In the next step of prioritization, this filtered list was supplemented with information on PBT/vPvB probabilities of the individual substances generated by in-silico methods. The JANUS tool was used to calculate the persistence, bioaccumulation and toxicity probabilities. The JANUS software (https://www.vegahub.eu/portfolio-item/janus/) is based on a battery of (quantitative) structure-activity relationship ((Q)SAR) models, integrated with a specific workflow for each endpoint (UBA, 2016). The final predictions are combined in a PB or PBT score that allows to rank and prioritize the list of compounds. The PB score includes the calculated probabilities for persistence and bioaccumulation, while the PBT score additionally considers the probability of toxicity. A score of 0–0.3 means that the compound is predicted not to meet the PBT/vPvB criteria set by REACH Annex XIII, a score of 0.3-0.7 means no conclusion can be drawn, while a score above 0.7 indicates that PBT/vPvB properties are likely met. Results of validation studies demonstrated that the integrated model for PBT prioritization can reliably discriminate PBT and non-PBT compounds (Pizzo et al., 2016; UBA, 2016). The strongly reduced and ranked list can now be screened manually for their PB(T) properties based on information given in the REACH registration dossiers, similar to the procedures applied by REACH competent authorities. Suspected PBT or vPvB candidates will be listed for a further in depth PBT/vPvB assessment. Furthermore, this methodology can highlight potential PBT/vPvB substances that were previously considered non-bioaccumulative on screening level due to conservative screening methodology. The presence of substances that appear in this prioritization list despite a Log Kow of <4 or >8 indicates a possible bioaccumulative property. In addition, substances with a classical risk and exposure mismatches can be highlighted. If substances with high FoA are found in biota that are only registered as intermediates, the question arises as to how they enter the environment. Using the prioritization scheme and the JANUS tool, it is also possible to screen for other properties of concern such as CMR and ED, as JANUS also calculates a probability for these hazard classes.

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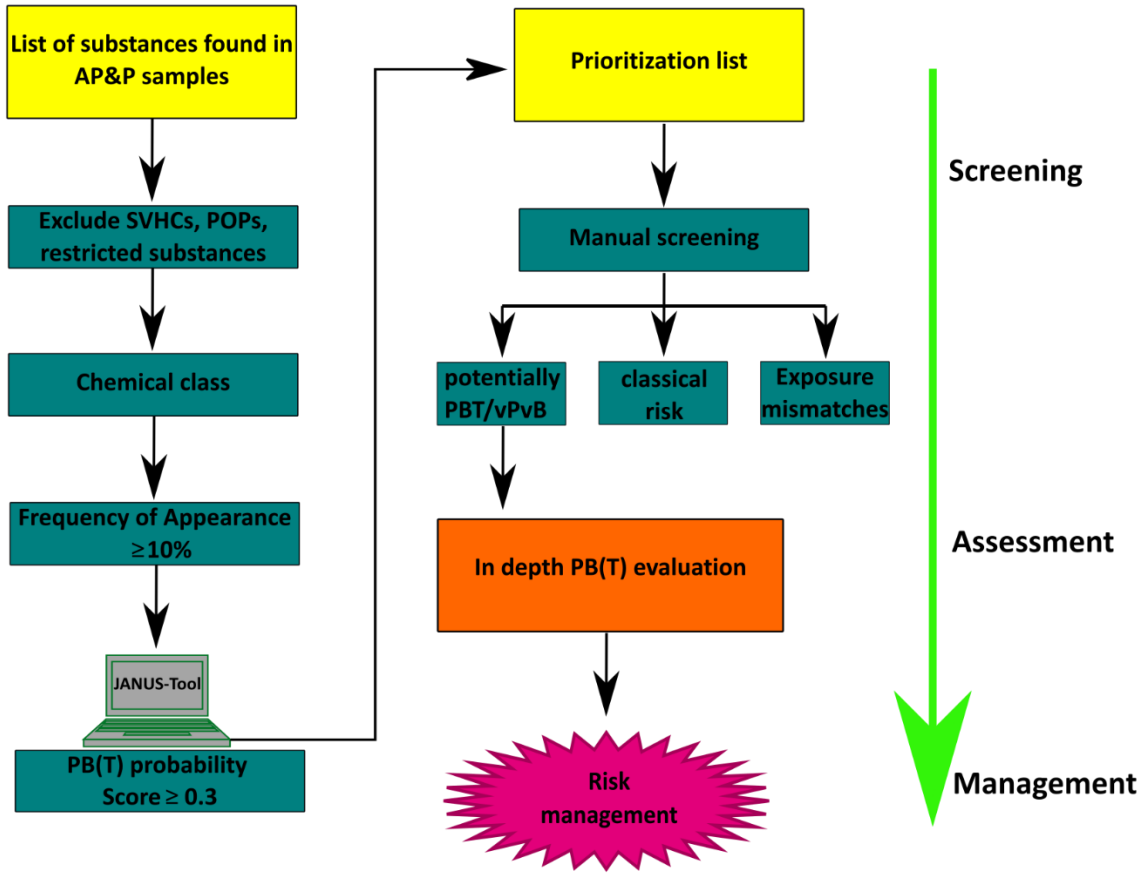

Figure 1: Approach for a prioritisation scheme to filter potential PB(T) compounds from huge datasets and highlight them for manual screening and assessment.

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3. Use of biota monitoring data to support bioaccumulation assessment

3.1 Bioaccumulation terminology and endpoint metrics

In this report, bioaccumulation is defined as net accumulation of a chemical by an organism as a result of uptake from all routes of exposure (e.g., water, sediment, air, and food). Thus, bioaccumulation refers to the net uptake rates of competing a chemical, and elimination from, an organism. Bioconcentration is defined as the net accumulation of a chemical by an organism as a result of uptake directly from water through respiratory or dermal surfaces. Biomagnification is defined as the increase in concentration of a chemical in the tissue of organisms along a series of predator-prey associations, primarily through the mechanism of dietary accumulation which can be either measured in the laboratory (lab BMF) or in the field (field BMF).

The internal concentration level reached in (aquatic or terrestrial) organisms over long-term exposures may cause adverse effects once a critical threshold is exceeded. This is why the capacity of chemicals to bioaccumulate in biota is recognized as a critical property that contributes to a chemical's risk. The degree to which bioaccumulation occurs can be expressed by different bioaccumulation or biomagnification metrics obtained from laboratory tests *in vivo* (usually fish exposed either via the aqueous or dietary path), *in vitro* (e.g. rainbow trout liver cells) or by applying mechanistic mass balance models or predicting quantitative structure-activity relationship (QSAR).

The specific metrics are defined as follows:

- Lab BCF
- BAF
- Lab BMF
- Field BMF
- TMF

The bioaccumulation factor (BAF) represents environmental exposure in the field to an aquatic organism from all routes and is referenced to the chemical concentration in water (Arnot and Gobas, 2004; Burkhard et al., 2012b). Relationships between dietary exposures and bioaccumulation can be quantified using laboratory biomagnification factors (BMFs; {OECD, 2012), field BMFs (Burkhard et al., 2012a), and trophic magnification factors (Borgå et al., 2012). One of the current difficulties in comparing BCF and BAF data to other bioaccumulation metrics is the difference in numerical scale and reference media to which chemical concentrations in organisms are compared (Burkhard et al., 2012a). BCFs and BAFs express ratios of chemical concentrations in biota to water, while BMFs and TMFs reflect ratios of chemical concentrations in predator–prey relationships (Burkhard et al., 2012a).

Chemical concentrations in biota that are orders of magnitude larger than those in water and air are important for several reasons. Such large concentrations may adversely affect organisms across food webs, especially if internal concentrations reach toxic levels (Mackay et al., 2018). Studies of bioaccumulation fall generally into one of the following categories: ecosystem monitoring using

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various biota species, laboratory tests under controlled conditions, mass balance modelling, and in vivo and in vitro ADME studies (Mackay et al., 2018).

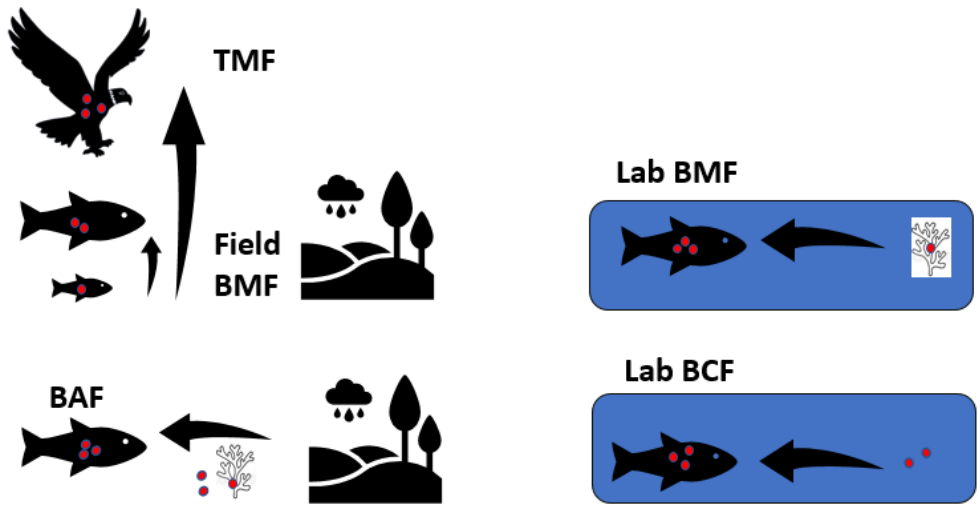

Figure 2: Simplified schematic overview on bioaccumulation metrics: laboratory bioconcentration factor (lab BCF), laboratory biomagnification factor (lab BMF), field biomagnification factors (field BMF), bioaccumulation factor (BAF), biota sediment accumulation factor (BSAF), and trophic magnification factor (TMF).

3.2 Current practice in bioaccumulation assessment under REACH

The potential of compounds to bioaccumulate in organisms and to transfer and biomagnify in food webs is a key consideration in chemical regulations (Weisbrod et al., 2009a). Generally, bioaccumulation assessment is required to i) identify persistent, bioaccumulative and toxic (PBT) and/or very persistent very bioaccumulative (vPvB) substances or persistent organic pollutants (POPs). POPs refer to PBT/vPvB substances that are also characterised by i) long-range transport potential, or by ii) posing a more generic hazard to the environment (Gottardo 2014).

If a substance shows bioaccumulation potential based at the screening level, additional data and testing, usually in fish, needs to be considered and eventually before deriving a final conclusion.

Under the European REACH Regulation (EC, 2006) a framework for PBT and vPvB assessment including both quantitative and qualitative criteria is provided in REACH Annex XIII.

The information that is required for a proper bioaccumulation assessment includes:

i) bioaccumulation in aquatic species usually under laboratory conditions

ii) other information on the bioaccumulation potential such as bioaccumulation study in terrestrial species, human body fluids or tissues, detected high levels in biota in particular in endangered and apex species populations, chronic toxicity study on animals, toxicokinetic behaviour; and

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iii) information on the ability of the substance to biomagnify possibly based on the biomagnification factor (BMF) or trophic magnification factor (TMF).

At the assessment level, usually controlled bioconcentration studies determining a laboratory bioconcentration factor (BCF) in fish are measured under controlled conditions (OECD, 2012). The numerical criteria given in Annex XIII concern bioaccumulation in aquatic species only and correspond to two cut-off values: if the BCF/BAF value is higher than 2000 L/Kg, then the substance is classified as bioaccumulative (B); if the value is higher than 5000 L/Kg, then the substance is classified as very bioaccumulative (vB). No threshold values are given for the remaining criteria mentioned in Annex XIII, e.g. TMF and BMF. ECHA developed a Guidance Document for implementation of PBT/vPvB assessment under REACH (ECHA 2017), where additional numerical criteria are suggested.

3.3 Potential use biota monitoring data as indication of bioaccumulation

As discussed by Arnot et al. (2022) the B assessment process is in practice challenging because of large data gaps and uncertainty. For example, many of the REACH registration dossiers were shown to fail quality standards (Springer, 2015) and often experimental studies are not available. For instance, measured fish BCFs exist for only approximately 5% of the organic chemicals that require B assessment (Arnot and Gobas, 2006) and the RAECH registration dossiers often lack essential information and studies which hampers a sound risk and hazard assessment of chemicals.

These requirements point out that a comprehensive assessment is necessary and bioaccumulation in other species, in addition to the aquatic ones, as well as magnification through the food chains need to be investigated before drawing a conclusion on the bioaccumulation potential of a substance (Gottardo et al., 2014). To this end, REACH Annex XIII explicitly requires that all available information in registration dossiers and others, and open literature, including MD, shall be considered in a weight of evidence approach to draw a conclusion on the P, B and T properties of a substance. These provisions give some flexibility to the classification schemes and allow for consideration of additional evidences such as high (eco)toxicity and monitoring data (Gottardo et al., 2014). Accordingly, in a more detailed bioaccumulation assessment that is usually performed at higher tiers, there is general agreement in the scientific community that all available bioaccumulation metrics, including field data, is considered as complementary information that needs to be considered in a weight of evidence approach (Gottardo et al., 2014). However, threshold values are given only for laboratory lab BCF and lab BMF values in aquatic species and there is currently no clear implementation strategy or guidance for accomplishing this regulatory objective. For instance, the respective ECHA guidance documents (R.11, R.7c) only cursorily describe how to apply the various information coming from the detection of compounds in wild biota in the PBT/vPvB assessment but mainly focus on trophic magnification studies.

Laboratory versus real world data

Uncertainty and variability in experimental fish BCF data are well recognized (Arnot and Gobas, 2006; Müller et al., 2011; Parkerton et al., 2008; Wassenaar et al., 2020), and approximately 45% of the measured BCF data have at least one significant source of uncertainty, for example, exposure concentrations exceeding solubility limits or analytical bias (Arnot and Gobas, 2006). As outlined in

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ECHA Guidance R.7C (ECHA, 2017b), the results of field measurements can be used to support the PBT assessment and to make the assessment more realistic but the interpretation of MD in wildlife currently remains difficult. Elevated concentration of a chemical in biota in comparison to prey species or surrounding media may indicate that the Field BCF or Field BMF of the substance is approximately equal to or greater than the Lab BCF estimated from laboratory experiments. This is because in the laboratory tests the fishes are exposed either via water or via the food and often no steady- state is reached, while under field conditions organisms are exposed to chemicals via all routes of exposures depending where they live (terrestrial or aquatic) and which taxa they belong to (air-breathers or water-breathers like fish). Furthermore, apex predators accumulate high concentrations of biomagnifying substances, whereas laboratory tests only use species of lower trophic level. This will ultimately lead to higher bioaccumulation in wild organism compared to the laboratory experiments for substances that are not rapidly metabolized and eliminated. Furthermore, the duration of exposure is expected to be substantially longer in wild animals as compare to the laboratory test (usually 28 days), which can play a substantial role in long-lived species such as many apex predators accumulating hydrophilic chemicals over life time. Bioaccumulation measurements of very hydrophobic, persistent chemicals that have not approached steady-state are considered to be underestimates of the true values (Burkhard et al., 2012a). This might also be one reason why certain superhydrophobic chemicals (e.g. some benzotrialzoles) tested via dietary route according to OECD 305 (OECD, 2012) are not recognized as bioaccumulative or very bioaccumulative, but have shown to magnify in wild organisms (Goss at al. under preparation).

Thus, from both a regulatory and industry perspective, there is concern about whether the existing PBT criteria and assessment schemes, may lead to either false positive or false negative conclusions on the bioaccumulation potential of individual chemicals (Burkhard et al., 2012b). False positive conclusions may lead to the unnecessary allocation of resources to further characterize a chemical, whereas false negative conclusions may lead to decisions that are not protective of environmental organisms (Burkhard et al., 2012b). For instance, a review resulting from an international Pellston workshop (Klečka et al., 2009) reported that the B-criteria used under the Stockholm Convention (UNEP, 2011) and many national risk assessment programs were unable to identify or to predict the actual bioaccumulation of several substances in organisms in the environment (Gobas et al., 2009b; Van Wijk et al., 2009; Weisbrod et al., 2009b). Thus, field and monitoring data represent a valuable source of information that can improve the screening and in part also the assessment of bioaccumulative compounds to ensure a safe use of chemicals under field conditions. Such a weight-of-evidence approach, in essence, should evaluate the extent to which the available bioaccumulation endpoint data support the hypothesis that a chemical will or will not biomagnify (Burkhard et al., 2012a). However, it needs to be considered that field and monitoring data are also subject to uncertainty (Arnot and Gobas, 2006; Burkhard, 2003; Burkhard et al., 2012a) in particular due to highly variable local exposure levels, lack of harmonization, standardisation, and guidance of sampling, chemical and statistical analysis, regulatory interpretation of the data, and others issues, which should be better addressed in future guidance documents (e.g. in ECHA Guidance R.11, R.7c).

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Identification of chemicals accumulating in air-breathing vertebrates

Previous bioaccumulation studies and regulatory assessments have predominantly focused on chemical distribution in aquatic organisms and food webs. Recent studies gave new insights into the bioaccumulation process in nonaquatic food webs. One of the key observations was that less hydrophobic (i.e., more hydrophilic) chemicals such as chlorobenzenes and lindane, which have log K_{OW} and BCF in fish experiments far below the regulatory criteria of 3 (for log K_{OW}) and 5000 L/kg ww (for BCF), were found to exhibit a high degree of biomagnification in lichen–caribou–wolf (Kelly and Gobas, 2001; Kelly and Gobas, 2003) and marine mammalian food chains (Kelly et al., 2007) in northern Canada. Also, perfluorinated sulfonic acids such as perfluorooctane sulfonate (PFOS) dos not biomagnify in laboratory fish tests (Martin et al., 2003a; Martin et al., 2003b) but show a high degree of biomagnification in birds and marine mammals (Chen et al., 2021; De Silva et al., 2021). These findings demonstrate that for certain non-hydrophobic and protein-binding chemicals the bioaccumulation behaviour in fish and aquatic piscivorous food chains is not necessarily protective for airbreathing wildlife and humans beings (Gobas et al., 2009a). Therefore, current regulatory frameworks may fail to identify a number of substances that are bioaccumulative and/or toxic in non-aquatic organisms and related food webs (exposed through soil and food). This is particularly due to fundamental differences between aquatic and terrestrial organisms with regards to metabolism such as uptake and elimination mechanisms, diet, energy requirements, and feeding rates. For instance, birds, mammals and humans are homeotherms, which are shown to have higher energy requirements, feeding rates, trophic positions, longer life time and different biotransformation abilities than poikilotherms including fish, (Fisk et al., 1998; Hop et al., 2002). Therefore, extrapolation from fish-related bioaccumulation data to other organisms should not be made (Martin et al., 2003a). In particular, (Kelly et al., 2007) explain that higher biomagnification of certain organic compounds in air-breathing organisms is due to the greater ability to absorb and digest their diet, which is related to differences in digestive tract physiology and body temperature. In this context, field data on bioaccumulation and magnification in terrestrial biota again can provide valuable information for identifying substances that accumulating in airbreathing wildlife and in human food webs (Czub and McLachlan, 2004) that were previously not flagged in the aquatic B-assessment. Field monitoring might be an alternative or supplementary course of action to laboratory testing in special cases, in particular for more hydrophobic substances that may take a long time to reach steady state in laboratory test systems.

3.4 Types of biota monitoring and interpretation with regard to bioaccumulation

Biota monitoring data used for regulatory purposes needs to be quality approved and interpreted correctly. Following types of biota field data can generally be considered in the context of bioaccumulation screening and assessment:

Detection of chemicals in organisms

- The detection of chemicals in wild biota, in particular in apex species, provides a clear indication that it has been taken up by that organism. This is especially true for chemicals with a low risk for potential cross contamination during sampling, storage and chemical analysis. The detection of chemicals in apex predators is suggested to mainly reflect the level of the chemical in the diet and surrounding media of these species at the time of sampling. However, a detection in biota

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does not necessarily mean that significant bioconcentration or bioaccumulation has occurred since exposure level from the surrounding media and/or diet would be needed for such an assessment. Data on prey species and in the surrounding media can be helpful to identify cases where chemical uptake seems unlikely (e.g. due to large molecular weight and extreme high lipophilicity) but obviously appears in wild organisms. However, for that, the sources and contemporary exposure levels (through water as well as food) must be known or reasonably estimated, which is often not the case. Thus, depending on the concern and research question, future monitoring programs and field studies should additionally sample and analyse souring media and/ or prey items of the organism studied. The regulatory question should be clearly defined a priori and frame the sampling and study design. This is often not the case within routine monitoring programs which for instance do not collect sample sets useful for bioaccumulation assessment such as water-fish or predator-prey samples.

- In case where no data is available on sources and contemporary exposure levels, a high frequency of appearance (FoA) and occurrence of chemicals in several biota species across different compartments may indicate indeed an increased bioaccumulation potential of the respective compound. It might however just reflect a continuous exposure of the focal organism e.g. due to continuous and high emissions volumes of the chemical. In cases of high production volumes or indications on continues exposures, further confirmatory B-assessment on bioaccumulation and on exposure levels would be needed to draw a final conclusion.
- A high frequency of appearance or elevated levels of a chemical in terrestrial biota, particularly in terrestrial apex species, may indicate an increased concern of terrestrial bioaccumulation, and a further in-depth B- assessment should applied.
- A screening by means of occurrence and/or measured concentrations of chemicals in biota from large data sets, in particular the NORMAN and Life Apex database can help to prioritize chemicals for further B or other assessment and should be regularly applied by authorities. To further prioritize detected chemicals in wild biota for a follow-up PBT assessment *in silico* methods such as the JANUS tool as integral part of the different NORMAN database systems are useful (see section on screening above).

Field study showing bioaccumulation in aquatic organisms (BAF)

Field studies can be used to derive bioaccumulation factors (BAFs) mainly fish, and have been used to develop water quality standards (ECHA, 2017b). Water concentrations are necessary to calculate BAFs from water to fish. Since in Life Apex did not include water samples this endpoint is not further discussed.

Field study showing biomagnification from prey to predator (field BMF)

There is a current lack of systematic data comparing field and lab data This approach also provides a basis for direct comparison of all laboratory and field bioaccumulation measurements (i.e., values for BCF, BAF, BSAF, BMF, BSSAF, and TMF). The working group concluded that the use of both field and laboratory bioaccumulation measurements in “B” assessments will improve confidence in “B” classification decisions.

- BAF values are generally preferred as they are more ecologically relevant (field experiments, steady-state conditions, all exposure routes) than BCF values for the same species; however, BAF values are largely variable due to site-specific environmental conditions affecting their

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determination and less available than BCF values (Arnot and Gobas 2006; Weisbrod et al. 2009; Costanza et al. 2012).

- Food chain transfer and secondary poisoning are basic concerns in relation to PBT and vPvB substances, and therefore an indication of a biomagnification potential (BMF and/or TMF > 1) can on its own be considered as a basis to conclude that a substance meets the B or vB criteria. However, absence of such a detection cannot be used to conclude that these criteria are not fulfilled. This is because a field BMF only represents the degree of biomagnification in the predatory/prey relationship for which it was measured. Biomagnification will vary between predatory/prey relationships, meaning a low BMF in one predator/prey pair does not mean that it will be low in other pairs. Furthermore, the biomagnification potential also heavily depends on the exposure levels of the respective foo web. Conversely, evidence of high biomagnification in one predatory/prey relationship is cause for significant concern and it is then in accordance with a precautionary approach to assume that biomagnification may also occur in other (unmeasured) predatory/prey relationships. The same applies for bioaccumulation factors (BAF) calculated from field data (i.e. by relating concentrations in field sampled aquatic organisms to the concentration in their habitat). If such BAF values are above the criteria for B or vB it should be considered whether this information is sufficient to conclude that the substance meets the B or vB criteria.
- Monitoring and field data can provide additional information to confirm the properties of a substance already identified in frame of risk assessment. As delineated in the R.11 ECHA Guidance (ECHA, 2017a) bioaccumulation factors (BAF calculated from monitoring data, field measurements or measurements in mesocosms) or specific accumulation in food chains/webs expressed as biomagnification factors (BMFs) or trophic magnification factors (TMFs) can provide supplementary information indicating that a substance does or does not have bioaccumulation potential. Furthermore, the same information may be used to support the assessment of persistence, in particular for potential long-range transport, e.g. when considerable concentrations are found in biota in remote areas. If field data indicate that a substance is effectively transferred in the food web, this is a strong indication that it is taken up from food and that the substance is not easily eliminated (e.g. excreted and/or metabolized) by the organism (this principle is also used in the fish feeding test for bioaccumulation).

Field studies showing trophic magnification among food webs (TMF)

Though TMF assessment was not part of the Life Apex project, information and issues to be considered as addressed in the ECHA Guidance documents and literature is reviewed as follows:

- TMF is considered as the most comprehensive metric for understanding the biomagnification potential of a substance as it represents the average increase or decrease of concentration levels in a food web: a TMF higher than 1 indicates that the substance biomagnifies in the food web (i.e. concentration increases with trophic level); a TMF lower than 1 indicates that the substance undergoes trophic dilution (Weisbrod et al. 2009).
- As outlined in ECHA Guidance R.11 a relevant BMF or TMF value significantly higher than 1 (see R.11; R.7c) can also be considered as an indication of very high bioaccumulation. For aquatic organisms, this value indicates an enhanced accumulation due to additional uptake of a

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substance from food in addition to the bioconcentration from water. However, as dietary and trophic biomagnification represent different processes than bioconcentration in aquatic organisms, BMF and/or TMF values <1 cannot be directly used to disregard a valid assessment based on reliable BCF data indicating that a substance meets the numerical B/vB criteria in Annex XIII to the REACH Regulation (ECHA 2017). In these cases, all available data need to be considered together in a WoE approach.

- Difficulties in the interpretation arise especially for trophic magnification factors (TMFs), which describe the accumulation throughout the whole food web. The TMF for a food web is calculated as the exponent of the slope of the natural logarithm transformed concentrations for organisms in the food web as a function of the trophic level of these organisms. Currently, there is no standard procedure for studying TMFs. Hence, the conductance and sampling may vary considerably between different studies. As such, TMF represents the average biomagnification per trophic level within that food web. The validity of the TMF is strongly dependent on the spatial and time scales over which the samples were retrieved. The most reliable TMFs are derived from data for non-migratory species originating from a confined area and sampled in the same period, or from food webs for which low variability in time and space can be assumed (e.g. for vast remote areas). See also publications from (Borgå et al., 2012; Kidd et al., 2019)and ECETOC (2014) for discussion on uncertainties.
- The way data, on the basis of which the TMF values are calculated, are treated has a great impact on the outcome of the TMF value. Not only the magnitude of the TMF value can be impacted, but also whether biomagnification or biodilution occurs. In addition, the setup of the field study could have an influence on the resulting TMF values as well.
- These aspects cover both spatial and temporal variability in sampling, but also the selection of species belonging to the food web. Spatial variability can lead to different organisms being exposed to different environmental concentrations. Temporal differences could have a strong impact on trophic magnification as well. Such temporal variability further complicates the interpretation of the observed TMF values. Further, it appears that TMF values could be strongly dependent on the inclusion or exclusion of certain species and on which part of the food web is considered, for example pelagic species only or the benthopelagic food web. Apart from that, even from similar food web widely varying results can be obtained for the TMF (Houde et al., 2008)
- Thorough elucidation of the food-web structure (feeding ecology; determination of the trophic level). The position in the food web is quantified using relative abundances of naturally occurring stable isotopes of N (15N/14N, referred to as δ15N). However, the relative abundance of these isotopes and thus the determination of the trophic level and TMF is influenced by the physiology of the organism and its life trait history. Rapid growth with a higher protein demand for new tissue leads to lower enrichment factors than those with slower growth rates. Insufficient food supply and fasting and starvation leads to catabolism of body proteins and an increase of 15N in organisms relative to those organisms with adequate food supply. Furthermore, nitrification in agricultural areas have shown to alter the 15N/14N ratio, which may lead to a bias when assessing the trophic position of a species within a food web (Elliott et al., 2021).

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4. Quality assurance and reporting of field data in B-screening and assessment

As discussed in ECHA Guidance R.11, the uncertainties related to field data apply to all field metrics described above. If field data are available, these should be considered and reported in the assessment. In particular, if the number of field studies is low, the data should be accompanied with a comprehensive discussion on the uncertainties. Generally, bioaccumulation and biomagnification are influenced by an interplay between physicochemical properties of a chemical, source distribution, trophic interaction, species biology and many other biotic and abiotic factors which are not fully understood yet. This makes the interpretation often difficult. The precision or uncertainty of a field biomagnification factors determination is defined largely by the total number of samples collected and analysed. For practical reasons, precision of the measurements may be balanced against the costs associated with sample collection and analysis, and in many cases, pooling of samples is required to limit costs associated with the analytical analyses (ECHA, 2017b) . Gathering and reporting too little information is far worse than providing too much information. The adequacy of the data on the intended purpose depends on their quality. If there is no evidence of quality assurance or if the data are incompatible with other studies, the results should not be used in risk and hazard assessment. In addition, expert judgement will usually be required on a case-by-case basis.

Data from a field study that can be used to quantify bioaccumulation should ideally report and consider the following:

Sampling, Transport & Storage

- Sampling design (site selection, spatial resolution, frequency of determination, etc.) and details of the sampling methodology, sample handling, sample storage and delivery conditions and stability, steps taken to reduce contamination, and of all equipment being used;
- Physical details of the site, including temperature, salinity, direction and velocity of water flow, water/sediment depth and physico-chemical properties (e.g. particulate organic carbon and dissolved organic carbon levels);
- Availability and reliability of exposure relevant contextual data for each analysed sample
- Apex predators are protected species, which is why invasive monitoring campaigns are impossible. European sample collections (i.e. environmental specimen banks, research collections and natural history museums) archive many samples from dead found individuals that can be used for chemical analysis and subsequent risk assessment once appropriate quality assurance measures are established
- Alternatively, non-invasive sample matrices from apex predators such as eggs, feathers or blood (if possible) might be feasible in some for some contaminants as well.
- In general, using opportunistic samples from natural history museums, environmental specimen banks or scientific collection substantially challenges the bioaccumulation assessment, since predator-prey relationship or spatiotemporal matches between predator

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and prey species) are generally not possible for archived samples. Furthermore, the exposure levels of the sampled individuals are generally unknown.

- A more systematic sampling regime, including the analysis of water samples to screen for bioconcentration in fish e.g. within e.g. the Eater Framework Directive (WFD)is considered to increase the value of opportunistic samples for regulatory purposes. Another opportunity would be to apply non-lethal sampling methods (e.g. by establishing a systematic sampling scheme for dead found bodies). Harmonised approaches for the target species and sample matric have already been established on a pan-European scale for terrestrial predators (i.e. raptors (see Badry et al., 2020; Espín et al., 2020; Espín et al., 2016)
- Sample collection is often restricted to tissue from dead found individuals or blood from nestlings (in case of raptors) due to ethical and practical considerations.
- Further guidance on quality assurance of sampling, storage, and chemical analysis of biota samples can be found in the other Life Apex guidance documents at www.lifeapex.eu.

Species ecology

- Interspecies differences in gut physiology, diet preference, foraging strategies, environmental interactions, mobility and migration, physiological differences, and other species-specific ecological traits can have important consequences for chemical exposure, uptake and metabolism as recently reviewed e.g. for birds (Kuo et al., 2022),
- Therefore, the influence of sampling location(s) and timing(s), concentration gradients and migration behaviour need to be considered. In particular migratory behaviour might strongly impact exposure levels. In particular, care should be taken that the samples used to derive bioaccumulation factors are collected at the same time from the same location, and sufficient details provided to relocate the sampled site. Samples grabbed randomly without consideration of the organism's home range will, in high likelihood, have poor predictive ability for substance residues in the organisms because the data will not be representative of the organism's actual exposure (Burkhard, 2003);
- Details of the organisms being analysed, including species, sex, size, weight, lipid content and life history pattern (e.g. migration, diet, and food web structure which may be determined using measurements on nitrogen or carbon isotopes, and composition). For resident species, the sample collection should be fairly straightforward. Migratory species may present special challenges in determining which food, sediment, or water sample should be used to calculate the field biomagnification factor.
- Influence of species physiological characteristics (e.g. typical lipid content, whether air-inhaler or water inhaler);
    - Influence of digestion rate/diet energy content, size and growth, ability to biotransform, sex, age;
    - The influence of sampling habitat and exposure to potential point pollution
    - Influence of diet (generalist vs specialist), which might be controlled by analysing stable isotope values (d15N, d13C).

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Exposure and analytical considerations

- Description of analytical methods (including use of field blanks, procedural and instrumental blanks in analysis, laboratory pre-treatment, standard reference materials, etc.), as well as evidence of quality control procedures;
- Data on biomagnification (TMF, BMF or B-values) should be calculated based on lipid-normalised concentrations (unless lipid is not important in the partitioning process, e.g. for many inorganic compounds).
- Exposures from all relevant routes and compartments have to be considered when assessing the bioaccumulation of a chemical.
- Evidence is needed to demonstrate that the steady-state has been achieved in the considered food web, which is however challenging.
- Opportunistic feeders vary their diet and point sources may influence observed BMFs and TMFs. Additionally, apart from the diet there is always the possibility of a direct uptake of a chemical. Furthermore, the relative importance of food versus e.g. water exposure can influence the magnitude of the TMF;
- Data on the concentrations in the ambient medium and on the temporal trend of environmental inputs are often missing, which complicates the bioaccumulative assessment when only using monitoring data.
- Selck et al. (2012 showed that at lower trophic levels (mayfly and polychaete), variability in bioaccumulation is mainly driven by sediment exposure, sediment composition and chemical partitioning to sediment components, which is in turn dominated by the influence of black carbon. At higher trophic levels (yellow perch and the little owl), food web structure (i.e., diet composition and abundance) and chemical concentration in the diet became more important particularly for the most persistent compound, PCB-153. These results suggest that variation in bioaccumulation assessment can most effectively be reduced by improved identification of food sources as well as by accounting for the chemical bioavailability within the food web.
- Although tissues of top predators can be used to monitor contaminant levels in the environment, variation in diet can confound the interpretation of the results (Braune et al., 2014a; Braune et al., 2014b; Hebert et al., 2000). Therefore, an accurate B-assessment for higher trophic level predatory species such as raptors or marine mammals requires knowledge on the chemical concentration in potential prey species. If suitable models to extrapolate between tissue/organ and whole body concentrations can be established, how this would then lead to a useful value for regulatory context remains yet unaddressed. Further, when a BCF is not derived but instead e.g. a BAF, the extrapolation from these factors (e.g. BAF/BSAF/BMF) to BCF is in itself data-intensive, making the concept more impractical (Environemnet UK 2022).
- Tissue type samples is critical for interpretation of contaminant levels and an extrapolation to whole body is crucial to interpret the levels as regard to bioaccumulation. A recent report by Environment UK (2022) evaluated the

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applicability and validity of extrapolation from tissue burden to whole body concentrations. The authors suggest some promise for the use of both liver and muscle tissue concentrations for extrapolation to whole body burdens, but limitations in the dataset inhibited full interpretation, such as relying on sums of chemical classes rather than defining each individual substance. It should be stressed that, though there may be scope to extrapolate tissue to whole body concentrations, no one sampled tissue should be taken to represent whole body concentrations (i.e., it is not possible to assume muscle or liver concentrations are equal whole organism concentration).

- Many food webs are complex, and a single metric (food chain length) is unable to represent all variation in relationships (Elliott et al., 2021). Contaminant levels often vary among habitats due to processes such as long-range transport, point source pollution, microbial degradation of organic compounds, and variation in processes at the base of food webs might be as important as biomagnification for understanding contaminant levels in higher trophic level species ('habitat variation hypothesis'; (Elliott and Elliott, 2016; Lavoie et al., 2015). Additional dietary tracers associated with the spatial origin of diet ($\delta^{13}C$, $\delta^{18}O$, $\delta^{34}S$) are recommend to refine diet reconstruction (Elliott et al., 2021; Elliott and Elliott, 2016; Hobson et al., 1993). Although all three isotopes are associated with habitat, $\delta^{13}C$ also varies systematically with trophic position and $\delta^{18}O$ reflects variable contributions from diet and body water. In contrast, $\delta^{34}S$ may be a particularly useful dietary tracer of spatial origin as $\delta^{34}S$ varies little from source to consumer (Florin et al., 2011). The combined use of multiple isotopes could provide a more nuanced description of food web structure in the context of environmental pollution. In particular, the use of amino acid specific $\delta^{15}N$ and $\delta^{13}C$ values, including both those that change for predator to prey ('trophic' and 'non-essential' amino acids, respectively, for $\delta^{15}N$ and $\delta^{13}C$) and those that do not ('source' and 'essential'), may additionally refine the dietary estimate (Elliott et al., 2021).

Quality assurance considerations: Sampling and processing

When applying comprehensive when applying comprehensive analytical techniques using high-resolution mass spectrometry (HRMS) such as non-target and suspect screening, it is important to apply appropriate quality assurance measures for sampling and sample processing as HRMS can be susceptible to external trace contamination. This is particularly true for compounds that have a high risk for cross contamination such as personal care products (e.g. parabens or fragrances), veterinary antibiotics and analgesics or frequently used industrial chemicals in laboratories (e.g. plasticisers, flame retardants, etc). As a consequence, the LIFE APEX project developed protocols both for sampling and sample processing but also for extraction, instrumental analysis and digital sample freezing (Badry et al., 2022a,b). It has been argued that the application of field blanks represents the most suitable measure for detecting potential cross contamination with those chemicals. However, such measures are currently not in place in sample collection archiving predator samples such as environmental specimen banks, research collections and natural history museums. However, using harmonised sampling and processing protocols (e.g. clean air conditions and sterile equipment) can already considerably reduce the risk of cross contamination (Badry et al., 2022a; Espín et al., 2020). Lastly, archiving samples in appropriate sample containers (e.g. glass) at low temperatures (e.g. -

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80°C and lower) further increased the durability and quality of apex predator samples, especially when analysing long time series of archived samples.

Quality assurance considerations: Analytical sample preparation

This section is based on the protocols presented in Badry et al. (2022a). The described analytical methodologies such as suspect screening or wide-scope target screening are able identify and (semi) quantify thousands of chemicals with various physicochemical properties in each sample. Target analysis, suspect screening and non-target screening can all be performed in a single analytical run in case generic sample preparation protocols are applied. However, for some analytes specific extraction protocols might be necessary to reach lower detection limits and increase the method performance. For example, the full scan acquisition mode in the HRMS instrumental set-up may account for increased detection limits compared to a specifically designed method for pre-selected group of target analytes that are usually of the same chemical class (Badry et al., 2022b). In general, the sample preparation and extraction protocols play a critical role for the sensitivity and selectivity and therefore strongly affects the analytical results. There are currently no harmonised extraction protocols for environmental matrices but first efforts were made by NORMAN network and the implementation of HRMS interlaboratory studies (Badry et al., 2022a). Using internal standards further helps to identify potential losses or reproducibility issues during the sample preparation. They are furthermore recommended to control for variability among the instrumental part such as variations in injection volume or MS sensitivity. Using quality control samples (i.e. procedural blanks) is furthermore recommended to detect potential external contamination during the sample preparation steps. Performing appropriate method validation procedures represents an important step during the analytical process, which can be done by performing a smart validation for a selected number of contaminants that are included in the wide-scope target screening method. This method covers a large range of contaminants with different physicochemical properties and analytical characteristics to ensure the representativeness of the whole target list (Gago-Ferrero et al., 2020; Gil-Solsona et al., 2021). The use of an isotopically labelled standard is best practice for quantifying a contaminant. However, this is not possible for the large number of detected compounds in suspect and non-target screening due their low availability and comparable high costs. Therefore, a mix of isotopically labelled compounds covering a large range of different physicochemical properties is usually used (Badry et al., 2022a). Assessing matrix effects and determine the recovery (for wide-scope target analytes) is performed by preparing spiked samples with a mixture of known (legacy) contaminants due to low availably of certified reference material for many emerging contaminants.

Quality assurance considerations: Digital sample freezing

This section is based on the protocols presented in Badry et al. (2022a). A major advantage of using HRMS system with full scan acquisition mode is that the acquired chromatograms can be stored in online databases (e.g. digital sample freezing platform, DSFP) and can be used without additional analysis for retrospective suspect and non-target screening (Alygizakis et al., 2019). In contrast, this is not possible for target analysis using e.g. elected Reaction Monitoring (SRM) mode, which however may have the benefit of lower detection limits and %recoveries for some analytes. There is already a technical guide for digital archiving and subsequent automated suspect screening available, which was developed by the NORMAN Network (NORMAN Network, 2019). Within the DSFP, normalisation efforts such as the retention time index have been developed, which allows (1) the integration of HRMS data from different analytical set-up set-ups using a set of calibrant

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substances (Aalizadeh et al., 2021) and (2) for the semi-quantification of emerging contaminants without using reference standards (Alygizakis et al., 2022).

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