Information to be considered in a weight-of-evidence-based PBT/vPvB assessment of chemicals (Annex XIII of REACH)

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EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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SUMMARY

The 2011 amendment of Annex XIII of the EU REACh Regulation introduced new information and a 'weight-of-evidence' approach to assess whether a chemical meets previously existing criteria for persistence, bioaccumulation and toxicity (PBT), or is regarded as very persistent and very bioaccumulative (vPvB). Starting from existing REACh guidance on PBT assessment, an ECETOC task force has reviewed the recent literature in this area and developed an integrated evaluation strategy in accordance with the amended Annex XIII. While some recent findings can contribute to the identification of PBT or vPvB properties, the task force has recommended further research on other topics where the science is not sufficiently developed to allow regulatory conclusions to be drawn.

This report focuses on certain aspects of persistence and bioaccumulation assessment, as sufficient guidance on the toxicity endpoints is already available. Several endpoints have been addressed in other ECETOC reports. The evaluation strategy starts at the screening level. Higher-tier assessment and/or further testing are only necessary in cases where screening does not indicate that the substance is unlikely to have PBT or vPvB properties. Particular attention is paid to the weight-of-evidence analysis that will strongly depend on the available information. Several hypotheses may be formulated for each of the criteria and several lines of evidence evaluated. Care should be taken that all relevant data and information are evaluated in a consistent manner.

Specific aspects were reviewed as follows. With regard to the Annex XIII requirement to assess relevant degradation products and impurities down to a level constituting 0.1% of the parent compound, this may be unrealistic. It is noted that the currently available methods often already pose challenges to determine amounts of 10% or higher.

In general, it is recommended to clarify upfront which is (are) the 'compartment(s) of concern' to which most of the substance would be initially partitioning, in order to confine the PBT assessment primarily to this (these) compartment(s).

On persistence, the tiered assessment starts with biodegradation models and ready biodegradation testing, advancing – in the case no ready biodegradation was observed – via enhanced ready tests to inherent biodegradability testing at the screening level. If there is insufficient degradability observed upon screening, higher-tier simulation testing is recommended, preferably in the compartment(s) of concern. It is noted that research on the improvement and interpretation of simulation studies is ongoing. A refinement of the persistence assessment may need to be considered once the results of these research projects are available.

The possible role of non-extractable residues in a PBT assessment was addressed following a number of recent publications that clarified the definition and characterisation of non-extractable residues. Non-extractable residues are strongly bound to sediment or soil and while absorbed they are protected from degradation and are not bioavailable. Therefore, in the context of the PBT/vPvB assessment, non-extractable residues NERs should be considered as neither persistent, nor bioaccumulative, nor toxic.

On bioaccumulation, the information relevant for assessment may vary widely between substances, depending on the volume and use of a particular chemical. The task force has reviewed different elements

that can be taken into consideration in a weight-of-evidence analysis. However, many of those data may not be suitable to come to a definitive decision on the bioaccumulation potential.

The screening level assessment of the bioaccumulation potential typically uses physico-chemical properties, such as solubility in water and octanol, octanol-water and octanol-air partition coefficients, results of in vitro studies and model calculations. Although it is also useful to consider the compartment of concern for the bioaccumulation assessment, the usual starting point is the assessment of bioaccumulation in the aquatic compartment. The B assessment in the terrestrial compartment is at present still complicated by a lack of scientifically based threshold levels for terrestrial bioaccumulation and the scarcity of experimental methods and their interpretation with regard to the terrestrial bioaccumulation assessment.

Higher-tier assessment information for B can comprise a number of different data, starting from classical bioconcentration studies in fish, fish dietary biomagnification studies, determination of laboratory and field bioaccumulation factors, field studies on biomagnification and trophic magnification in food chains. The report discusses the different parameters and current approaches to come to a comparable evaluation of the different study results. The use of fugacity-normalised data seems to be a promising approach for non-ionic chemicals. This report discusses the methodology in terms of advantages and limitations.

Several aspects are important when evaluating study results on bioaccumulation. These are discussed in detail in this report. All data need to be considered in the context of the respective study design that needs to be evaluated. For example, in field studies on trophic magnification, consideration should be given to the proper balancing of samples across different trophic levels, the correct allocation of species to trophic levels and the respective food chain. Biomagnification studies should include the determination of the concentration in the relevant environmental compartments constituting all, or at least the most prominent, contributions to exposure.

Organ-specific bioconcentration factors must used with caution and take into consideration the contribution of the respective organs to the total body burden, the function of the organ and existing data on extrapolation factors to whole-body concentrations. The latter depend on the class of substances as well as species and organ-specific characteristics.

The task force has reviewed the state of the science in terrestrial bioaccumulation assessment following the results of an ILSI/HESI workshop on this subject. It is noted that the data available to date do not allow the setting of definitive criteria for terrestrial bioaccumulation. Additional research in this area is needed before any specific guidance can be developed.

The use of biomonitoring and environmental monitoring data for the bioacccumulation assessment is usually limited in the view of the task force. The detection of a substance in an organism is not *per se* an indication of a bioaccumulation potential. It is of paramount importance to correlate the levels of a substance in an organism with those in the surrounding environment and all potential sources of exposure before drawing any conclusions on a possible bioaccumulation. Results of biomonitoring and environmental monitoring studies could be used to consider the feasibility of a well-designed field study in the respective compartments of concern to determine a trophic magnification factor.

Results of mammalian repeated dose and toxicokinetic studies can contribute on a case-by-case basis to the assessment of the bioaccumulation potential. The task force has discussed some of the factors that

can be considered for the assessment. Classical mammalian toxicokinetic studies give valuable information on the absorption, distribution, metabolism and elimination of a substance in particular for the assessment of bioaccumulation in terrestrial food chains. Some models have been described in the literature that associate toxicokinetic parameters, like elimination half-life, with the bioaccumulation behaviour of a substance. All models are dependent on relevant input parameters, that could be obtained from toxicokinetic studies, and have certain limitations and applicability domains. However, there remains a need for further research in this area.

Finally, a short list of research recommendations is given at the end of the report.

1. INTRODUCTION AND PURPOSE

1.1 Background - REACh Annex XIII

The original REACh regulation from 2006 (EU, 2007) was amended in 2011 to introduce additional information that must be considered when assessing the persistent, bioaccumulative and toxic (PBT) nature of (organic) chemical substances. The particulars are described in the amended Annex XIII, reproduced here from the official publication (EC, 2011) and appended to the present report (Appendix A). Prior to the amendment, the bioaccumulation endpoints were largely reflective of the freshwater aquatic compartment. The new information is clearly intended to extend the scope of the bioaccumulation assessment to terrestrial species, including humans.

It should be noted that the actual criteria for the identification of PBT substances, and very persistent and very bioaccumulative (vPvB) substances, have not changed (apart from refined wording) and still mostly refer to the aquatic environment. In addition, the numerical 'cut-off' (minimum limit, threshold or trigger) values have remained the same. The criteria are detailed in Section 1 of Appendix A.

The amended Annex XIII makes a distinction between information that is relevant for (simple) 'screening' and for (higher-tier) 'assessment' (Appendix A3.1, A3.2, respectively). The two steps are in line with scientific practice (ECETOC, 2003a). For example if at the screening level, it appears that biodegradation takes a long time (as calculated by using a mathematical model or measured in a standard test), the substance in question may be persistent (P) (A3.1.1). The positive P indication ('screened in') still requires confirmation by more detailed and elaborate investigations such as simulation tests, field or monitoring studies, in a subsequent assessment (Section A3.2.1). Similarly, when screening for bioaccumulation (B), an octanol-water partition coefficient between 4.5 and 10 (log value) may give a hint that a substance has the potential to bioaccumulate (A3.1.2) pending proper assessment of the results from studies on bioaccumulation and biomagnification along the food chain, data on human and environmental biomonitoring, or findings of chronic toxicity in (laboratory) animals (A3.2.2). Briefly, screening for toxicity (T) is usually based on short-term toxicity in algae, daphnia or fish (A3.1.3). This is followed by assessment of long-term toxicity tests conducted in environmental organisms, supplemented by (existing) classification of certain human health hazards (A3.2.3). Lastly, substances that are 'screened out' for P, B or T do not require further testing or assessment. If no definitive conclusion can be drawn from screening, additional information needs to be generated for more in-depth assessment (A2.1).

Furthermore, the amended Annex XIII calls for the application of expert judgement while weighing the evidence from 'all relevant and available information listed', not only for assessment (A3.2), but also for screening (A3.1) as explained in the guidance (ECHA, 2014a,b). Weight of evidence (WoE) is the only way to assess information when the criteria (A1) are "not directly applicable", viz. descriptive and lacking cut-off values. The WoE approach is set out briefly in the introduction of Annex XIII (Appendix A).

Finally, the amendment of REACh (EC, 2011) prompted a formal update of the original REACh guidance on PBT (ECHA, 2008a,b,c). No adaptation to technical (and scientific) progress was attempted and the update will be published shortly (EC, 2012; 2014a,b,c,d). Meanwhile, a group of PBT experts from EU member states and ECHA identified a number of science topics for further work to be addressed in the guidance. Topics of

mutual interest included 'screening assessment', 'dietary bioaccumulation' and 'compartment of concern'. A special ECETOC contribution was invited on 'WoE', 'screening for B', 'use of TMFs + field BMFs' and 'whole-body vs organ-specific values'. All of these topics are covered in this report, together with other items such as 'relevant conditions (temperature correction)' and 'degradation products, impurities'. The remit is stated in Section 1.2, and the scientific topics are reviewed in the remainder of the report. The report is provided as a contribution to ongoing discussions between PBT experts, especially to the PBT working group of ECHA with a view to updating the REACh guidance on PBT assessment. A representative of the task force has participated in the group since its inception (Section 1.2, term 9).

1.2 Terms of reference and formation of task force

The amended Annex XIII provides largely descriptive language, while the recommendations in the latest Technical Guidance Document (ECHA, 2008a,b,c) are considered insufficient to accurately assess the PBT status of chemicals.

These considerations led the Scientific Committee to agree terms of reference and establish a task force of experts nominated by ECETOC member companies. The composition of the task force and the Scientific Committee are specified at the end of the report. At its first meeting in April 2013, the task force adopted a slightly revised, final version of its remit as follows.

Terms of reference

1. Review and analyse the scientific literature to determine the environmental and human health relevance of the new Annex XIII assessment information.
2. Review the availability of reliable and relevant test methods and/or models for providing the data required for application of the new assessment information. Identify the advantages, disadvantages and difficulties associated with the application of the new assessment information.
3. Develop guidance as to what screening information is sufficient for a decision to conclude that a substance does not fulfil the criteria.
4. Develop intelligent evaluation strategies for the most common outcomes of the screening assessments where a PBT/vPvB conclusion cannot be reached, based on the available data.
5. Address factors influencing the results of field studies on biomagnification and trophic magnification factors (BMFs, TMFs), as well as laboratory studies on BMFs.
6. Advise on how to interpret biomonitoring (BM) information with regard to the criteria.
7. Review and analyse the scientific literature related to P, B and T descriptors (other than those listed in the amended Annex XIII) that might warrant consideration for regulatory purposes.
8. Identify the need for further research and potentially draft RfPs for projects (e.g. within the CEFIC LRI programme) on the development of alternative information to assess P, B, or T properties.
9. Serve as a channel for dialogue with ECHA and its PBT expert group, in order to:
    - contribute to the development of guidance as to how the new Annex XIII criteria should be interpreted and implemented;
    - comment on, and provide input to, the drafts of future revisions of those sections of the REACh guidance pertaining to PBT criteria;

- propose the adoption, for regulatory purposes, of novel approaches and assessment information that might usefully complement those in the current Annex XIII.

10. Write a 'Special Report' for publication by ECETOC.

2. GENERAL CONSIDERATIONS FOR THE WEIGHT-OF-EVIDENCE ASSESSMENT

This chapter refers to the introduction of Annex XIII (Appendix A).

2.0 General principles on the use of weight-of-evidence approaches

ECHA guidance R.4 (ECHA, 2011a) makes a general reference to the WoE approach in the evaluation and integration of all available information, but gives little guidance on how to practically perform this assessment in a consistent and transparent manner. ECHA's Practical Guide 2 (ECHA, 2010) gives some guidance on how to document WoE in a registration dossier, but also gives very little indications of the scientific process behind these considerations. Similarly a Consensus Panel organised by the School of Public and Environmental Affairs, Indiana University (Abelkop et al, 2013) states that in the case of PBT assessment WoE does not have a precise definition. It continues that in this area WoE is commonly understood as a more flexible approach compared to strict numerical cut-off values ('bright-line approach') by using all available data including evaluation of data reliability and relevance. The report quotes a number of examples where WoE approaches have been used in PBT assessment. This includes a case where two expert judgements on the same substance and dataset came to diverging conclusions using WoE. Consequently the report promotes more guidance on the use of WoE in PBT assessment to limit subjectivity in expert judgement and increases transparency.

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) issued a memorandum on how to perform a WoE assessment in 2012 (SCENIHR, 2012). This document, although primarily referring to human health risk assessment, presents a general approach that is considered relevant by the task force for the use of WoE considerations in the assessment of possible PBT or vPvB properties. The most important considerations in this paper are summarised here.

The application of WoE considerations typically starts with the identification of different sources of data and data gaps in relation to the aim of the assessment.

The second and third steps consist of the collection and screening followed by the evaluation of all relevant publications and data for the respective purpose. SCENIHR proposes in this step the following criteria for a publication to be considered relevant in this context: the scientific quality using essentially the Klimisch et al (1997) reliability criteria and the appropriateness of the scientific methodology and conduct of the respective study as well as repeatability considerations and the relevance of the study for the assessment of the property under consideration. Only papers that are considered of sufficient quality and relevance should be used in the further WoE approach. Kase et al (2012, 2013) have proposed a checklist-approach to judge the reliability, relevance and plausibility of a given study or publication to come to a more transparent and uniform evaluation of the underlying information. Ågerstrand et al (2011) have done this specifically for ecotoxicity data of pharmaceuticals.

The fourth step is to establish the lines of evidence that need to be considered.

SCENIHR (2012) suggests classifying the respective references into those that indicate the presence of the respective property, those that indicate an absence of the respective property and those consistent with either presence or absence of the respective property. In this step, the proposed WoE approach looks at the consistence of the references considered for the different lines of evidence. A high consistence means that most studies point in the same direction, a medium consistency would indicate that the majority of the references report a mixture of findings that could be related to either outcome (the presence or absence of an effect or property) or a low consistence if there is little agreement between the studies. In this weighing of the individual lines of evidence, the reasons for consistent and different results need to be analysed on a case-by-case basis. Some more detailed general rules are outlined in the SCENIHR document. Again, the relevance of the conditions under which certain findings were obtained for the purposes of the evaluation also needs to be assessed.

The fifth step consists of the weighing of the totality of the evidence in combining the assessments of the different lines of evidence into an overall assessment. This involves verbal justification for each step and the final conclusion. Uncertainties and knowledge gaps should be addressed here as well. This can be done either qualitatively or quantitatively and the committee suggested some detailed guidance for comparable and consistent wording.

SCENIHR proposes to characterise the overall WoE with a scoring system:

- Strong overall WoE: coherent evidence from different data sources and lines of evidence leading to the same conclusion.
- Moderate overall WoE: good evidence from a primary line of evidence, but evidence from several other lines is missing (important data gaps).
- Weak overall WoE: weak evidence from the primary lines of evidence (severe data gaps).
- Uncertain overall WoE: conflicting information from different lines of evidence that cannot be explained in scientific terms.
- Weighing of evidence not possible: no suitable evidence available.

Overall, the approach proposed by SCENIHR in 2012 is considered by the task force as a suitable and consistent approach for the process and documentation of WoE considerations in the PBT assessment. The following report sections will indicate how the different elements of information can be used in a tiered WoE driven assessment.

Borgert et al (2011) propose a quantitative WoE approach for the evaluation of data on endocrine disruption, but also lays out general principles that could be adapted for a quantitative weighing approach. The first precondition is the definition of a clearly defined hypothesis that can be tested. Each endpoint considered may need a series of hypotheses to be defined. Studies and publications to be evaluated can then be selected due to the given hypothesis. The single references are then, similar to the SCENIHR approach first evaluated for their scientific validity, fulfilling three basic principles: “first the identity and authenticity of scientific measurements must be verifiable within a defined range of precision. Second, measurements and observations must not be confounded by extraneous factors and influences known to corrupt their accuracy and precision. Third, the measurements and observations must be replicable in independent hands.” Borgert et al (2011) refer to secondary validity when evaluating the detail of reporting comparable with the Klimisch (1997) reliability criteria. They define as tertiary validity criterion the relevance and probative power of the study as well as causality to address the endpoint investigated. For all the above

criteria, the authors propose to develop quantitative or ranked weighting factors, combine these using a predefined algorithm to an overall weighting factor and finally produce an overall weighting factor for all studies considered to test the initial hypothesis to document the strength of the evidence supporting or rejecting the hypothesis.

Hope and Clarkson (2014) described a strategy for using weight-of-evidence methods in ecological risk assessments. The authors review existing WoE approaches and propose a strategy similar to those above, starting with a clear scoping and problem formulation phase defining the goals and assessment endpoints of the analysis. A conceptual model is then formulated including a series of hypotheses to address each assessment endpoint. Depending on the available information those hypotheses may be tested via one or several lines of evidence. The studies supporting one line of evidence are evaluated with regard to their quality, methodology, specificity for the problem addressed and representativeness for the endpoint. The authors also include an assessment of the exposure-response relationship with five attributes. One is assessing how closely the biological effect observed is linked to the exposure, how responsive the effect endpoint is to the exposure and how closely can the effect endpoint be related to the exposure to the stressor, how specific is the effect related to the stressor in comparison to other stressors also present, to what extent can the response be quantified. Such considerations may also be important for elements of the assessment of PBT properties, in particular when evaluating field studies. Each line of evidence is then weighed by the sum of supporting studies times their respective weighting factors. Finally, the weighed lines of evidence supporting or rejecting the respective hypothesis are evaluated. The weights of different evidence groups are then integrated in a matrix to evaluate the hypotheses with which they were associated to help to interpret the outcome of the WoE analysis. This strategy may in particular be helpful for the PBT assessment of data rich substances for which a lot of often contradictory information for a number of elements contributing to the assessment of P, B or T properties is available.

With regard to the use of WoE in the identification of PBT properties in the context of REACh two cases can be differentiated. Firstly WoE considerations are used in the assessment of screening information using the available data that can inform the decision if higher-tier assessment is needed to derive a conclusion as to whether a substance is likely to fulfil all three criteria for P, B and T or the criteria for P and B. Secondly, according to REACh Annex XIII a WoE approach needs to be applied when exploiting any available ‘assessment information’, in addition to comparison to the Annex XIII criteria, in order to reach a conclusion regarding the P/vP, B/vB or T characteristics of the substance to be assessed. This will in particular be the case where the available information is contradictory or where several different metrics are used in parallel for a given property, e.g. for bioaccumulation.

Solomon et al (2013) have for example outlined which lines of evidence could be considered in the assessment of persistence of plant protection products. The endpoints of consideration and lines of evidence per endpoint, include the identification of the compartment(s) of concern (see below) and relevant data on abiotic degradation (hydrolysis, photodegradation) and biodegradation in water, water/sediment and soil, if applicable. Available data can comprise laboratory studies, semi-field and field data as well as model predictions. The authors also suggest that in a data-rich situation WoE assessment could result in the combination of multiple acceptable studies for the same endpoint by deriving an arithmetic mean for normally distributed values and a geometric mean for log normally distributed values.

Nichols et al (2009) proposed a WoE approach for bioaccumulation assessment using predictive approaches. This would start with collecting information on a chemical that is used as input information for bioaccumulation models, i.e. physical chemical properties, chemical structure information, fate information (e.g. rate of hydrolysis), biodegradation rates, metabolism rates if available. The authors then propose to run several bioaccumulation models and then verify their output for reliability including the domain of applicability and uncertainty bounds. As an example the authors recommend to examine the results against the physico-chemical structure and fate data to determine if the compound may be difficult to model. As an example they mention a solid compound with a high melting point, a low solubility in octanol and/or water and a high cross sectional diameter that is not expected to follow the equilibrium-based passive diffusion principles on which the models are based. If the applicability of the models is established, a line of evidence argument could be made based on the degree of consensus between the different models. Care has to be taken not to use model approaches that are sensitive to the same error. The authors further explain how to approach divergent model outputs by semi-quantitative or quantitative (statistical) weighing methods on a case-by-case basis.

The following report will indicate which different elements of information can be used in a tiered WoE driven assessment in particular in the assessment of persistence and bioaccumulation.

The following subchapters under section 2 elucidate some specific information that may be relevant to consider in a WoE assessment of PBT or vPvB properties as outlined in REACh Annex XIII.

2.1 Relevant conditions (temperature, moisture)

When testing chemical substances regarding their fate in the environment (e.g. biodegradation) temperature may play a major role in the interpretation of the results. The correlation between temperature and degradation rates is commonly described by the Arrhenius equation. Based on this equation, the European Food and Safety Authority (EFSA, 2007) has – for the purpose of environmental risk assessment – recommended a factor of 2.58 to increase the soil degradation rate constant when the temperature decreases from 20 to 10°C. The correction does not apply for comparison with the P criterion. Under environmental conditions, a microbial community will be naturally adapted to the ambient temperature, for example by a higher abundance of thermophilic or psychrophilic taxa. The EFSA view is supported by ECHA in its REACh Guidance Document R.7b chapter 7.9.4.1 'Temperature correction' (ECHA, 2008b). The systematic correction via the Arrhenius equation might therefore underestimate biodegradation rates as it does not take into account the differences in the abundance of psychrophilic, mesophilic and thermophilic taxa under different temperate climate conditions and, subsequently, the differences in biodegradation behaviour. Boethling et al (2009) concluded that it is not sound scientific practice to correct degradation rates to other temperatures than those used in the test. The Scientific Committee on Health and Environmental Risks (SCHER) in their opinion for the herbicide aclonifen disapproved any deviation from the test temperature for two reasons: the PBT criteria do not include temperature correction to 12°C (Appendix A.1) and the test guidelines generally prescribe a room temperature at around 20°C (SCHER, 2011). The FOCUS document on estimating persistence and degradation kinetics from environmental fate studies on pesticides postulated 20°C as the reference temperature (FOCUS, 2006). Solomon et al (2013) also recommended using a temperature of 20°C.

For testing on biodegradation in soil, normalisation of soil moisture can also be recommended as it significantly influences bacterial activity. The FOCUS document gives a soil moisture of 100% of the field capacity of the respective soil which corresponds to a pF of 2 (pF = decadic logarithm of the soil moisture tension or soil water column, respectively). The OECD (2002a) guideline 307 for testing on biodegradation in soil also recommends maintaining soil moisture between pF 2 and 2.5 in the test.

In conclusion, there is no need for temperature and/or soil moisture normalisation of degradation testing results for the identification of PBT properties of a chemical in accordance with Annex XIII criteria (Appendix A1). Correction for relevant temperature and/or moisture conditions may be appropriate in a risk-based approach, when evaluating special applications under significantly different environmental conditions. ECETOC (2003a) reviewed the paucity of available literature and pointed to the natural operating temperature of micro-organisms, e.g. sewage treatment inocula. An ECETOC (2013c) workshop recommended investigating the representativeness of laboratory conditions for the field situation, e.g. a literature review on the topic.

2.2 Degradation products and impurities

The current guidance suggests that a ≥ 0.1% (w/w) threshold applies for a constituent or a degradation product of a substance to be assessed for PBT/vPvB properties. In practice, this is impossible to achieve using current test guidelines recommended for environmental degradation. The most relevant guidelines for performing transformation studies are the current OECD 307, 308, 309 and 314 guidelines (OECD, 2002a,b; 2004a; 2008). These guidelines state that the analytical method applied in the test should have a limit of detection (LOD) for the parent compound and possible degradation products of 0.01 mg/kg of soil or sediment (as test substance) or 1% of the applied dose. They also require that attempts should be made to identify major transformation products occurring at an amount of > 10% of the initial test substance concentration at any time during the test, or transformation products which accumulate over the test period and might themselves be persistent.

Identifying minor transformation products present at < 10% is technically challenging. These studies require a ^{14}C radiolabel and significant analytical resources. The initial test substance concentration is usually low in order to perform the test with an environmentally relevant test concentration and to avoid toxic effects on the microbial community. A lower limit of 10% of the initial parent compound concentration for the characterisation of transformation products is considered technically feasible in most cases as well as remaining environmentally relevant. Lower levels of detection might be required on a case-by-case basis if the transformation product is relevant for the further assessment of the substance, e.g. because it increases in concentration over the test period in the test system (Boethling et al, 2009). Technical limitations of the analytical methods applied might limit the possible detection of transformation products in such tests particularly where a substance has low water solubility or is toxic at low concentrations.

As technical limitations vary from study to study, depending on various parameters like substance properties and initial parent compound concentration, it is suggested that transformation products in degradation studies should be characterised on the basis of being present at > 10% of the initial dose. Justification for not characterising a transformation product for persistence assessment might include its ‘short term presence in the test system’. For example, if a transformation product is only present on one measurement occasion

(even if it is > 10%) during a study it is likely to be an intermediary transformation product and its concentration declines suggesting 'not P'.

The testing laboratory should attempt to identify transformation products unless it is technically not feasible. Software exists to predict likely transformation products for example, the 'University of Minnesota biocatalysts / biodegradation pathway prediction software' (Ellis and Wackett, 2012) or the commercially available CATALOGIC software (Dimitrov et al, 2011a,b). However, these approaches should be used with caution as their predictions are not always reliable and they are susceptible to combinatorial explosion, i.e. when a structurally simple compound (e.g. benzamide) follows several (5) transformation pathways and breaks down into many (44) products (Fenner et al, 2008). The use of a radiolabel followed by mass spectroscopy analysis to identify particular molecules is the current recommended approach. Further research could focus on developing methods to improve identification of transformation products (ECETOC, 2010).

In summary, when using the simulation studies currently available there are considerable difficulties associated with identifying transformation products present at < 10% of the original parent compound. It is understood that attempts should be made to identify the transformation products where the parent compound is rapidly transformed and to assess such products for their persistence in the environment (Boethling et al, 2009). However, the identification of degradation products at 0.1% of the parent compound is an unrealistic expectation given the available tools.

2.3 Compartment of concern

PBT assessment is currently not performed in an environmental compartment specific manner, and data used in the assessments can be relevant for water, sediment and/or soil. As bioavailability may vary significantly between the environmental compartments it is recommended to clarify upfront which is (are) the compartment(s) of concern to which most of the substance would initially partition. If a substance partitions almost exclusively (> 95% following ECETOC, 2003a) to one compartment, the assessment can be confined to that compartment.

The compartment of concern is directly linked to the physico-chemical properties of a substance such as the log K_{OW}, log K_{OC}, Henry's Law Constant, water solubility, and pKa. Depending on these properties, the substance will partition in various amounts to the different major environmental compartments (water, soil, sediment, air). Although the values of these properties can give an indication of which compartment(s) might be of concern, the use of partitioning modelling is preferred.

The environmental partitioning of a substance is commonly evaluated by means of the fugacity-based equilibrium criteria (EQC) Level I and III models (Mackay et al, 1996; Mackay, 2001). In the Level I model, a fixed quantity of a supposedly non-degradable chemical is introduced into a closed evaluative environment and equilibrium achieved between the various environmental compartments (air, water, soil, sediment). The Level III model simulates a situation in which a chemical is emitted at a constant rate into one or more of the compartments, in each of which it may degrade; the steady-state distribution between compartments is then calculated. Due to the resistance to mass transfer between compartments, the various phases are not in equilibrium and the steady-state partitioning depends on its 'mode of entry', i.e. the compartment(s) into which the chemical is injected.

The EQC models differ in the amount of input data needed and the complexity and environmental relevance of the results. The most basic model is the Level I model which requires molar mass, temperature, water solubility, vapour pressure, melting point and log K_{OW} as input data, all of which can significantly influence the results. Partitioning to different environmental compartments is then generated by the model and should be used as a decision basis for the choice of the appropriate test. Due to the limited input data, the Level I model can only give a rough estimation on partitioning, as it does not take into account the route of environmental exposure. To get a more detailed estimation of environmental distribution the Level III model should be applied as one could also consider the route of exposure in this model if such data are available.

Special attention is required for substances that exist as charged or dissociated molecules under environmental conditions. Due to the electrostatic forces, the environmental partitioning behaviour of such substances is different compared to the uncharged species. For example, the water solubility normally increases for such a substance; however, adsorption on soil particles like clay and organic molecules such as humic acids might also increase due to cation exchange capacity in soil. In such cases models like the above mentioned Level I could lead to false results if the input data are not adequately chosen for the environmentally relevant pH value. Despite this limitation the Level I model is considered a relevant tool to identify the compartment of concern if the user is actively verifying the input parameters as well as the results. The level III model incorporates complex environmental processes such as transfer between compartments and biodegradation. Therefore the results of this model are more difficult to link to the substance's physico-chemical properties.

For certain chemicals that have multiple environmental discharges and undergo multi-media transport, single media persistence assessments may not reflect the overall fate and behaviour of that chemical in the environment. For these types of chemicals concepts of overall persistence (Pov) have been developed. These concepts have been reviewed by ECETOC (2007; 2011) and are not discussed further in this report.

In summary, the compartment(s) of concern can be identified by means of the above-mentioned EQC partitioning models. If a substance partitions almost exclusively (> 95%) to one compartment, PBT assessment can be confined to that compartment. For the assessment of persistence, one can compare half-life (= DT_{50}) in each relevant compartment with the criteria from Annex XIII (Appendix A1.1.1). Only in some cases where multimedia distribution is expected, concepts of overall persistence may be of use.

2.4 Complex substances and substances with surface active properties

The WoE assessment under the REACh regulation is also deemed to support the PBT assessment for complex substances known as 'Unknown or Variable composition, Complex reaction products or Biological materials' (UVCBs). This concept has been developed to cover substances that cannot be qualitatively or quantitatively defined. For all those substances, as well as for the 'multi-constituents' (substances consisting of several main constituents present at concentrations generally ≥ 10% and < 80% w/w), the PBT status has to be defined for each constituent. However, in the end the PBT/vPvB properties are actually addressed for all registered substances, whatever their nature: mono-constituent, multi-constituent or UVCBs. The strategies to detect PBT/vPvB properties have been developed mainly for mono-constituent substances. For the other complex substances, the test methods are not suitable, in particular for the following properties: solubility,

octanol-water partition coefficient (log K_{OW}), and adsorption potential. A preliminary solution consists in deriving weighted average values for those properties where individual components can be assessed. However, this solution is sometimes not feasible. Therefore, for those complex innovative substances and WoE approaches have to be developed on a case-by-case basis. The same applies to 'surface active substances'.

3. SCREENING INFORMATION

This chapter comments on the information to be considered when screening for P, vP, B and vB properties, following Section 3.1 of the amended Annex XIII of REACh (EC, 2011) (Appendix A).

The amended REACh Annex XIII introduced screening as the first step of assessment to categorise chemicals as PBT or vP/vB. Some physico-chemical properties and (Q)SAR predictions can be used for screening.

Based on a survey of the latest scientific literature, this chapter tries to identify possible indicators deduced from some physico-chemical properties and relevant models, including newly established ones especially for terrestrial B assessment. Some indicators have no numerical threshold value at this time because of limited experimental data, so they are recommended to be used in combination with read-across of experimental data on structurally or mechanistically similar chemicals.

3.1 Persistence (P and vP properties)

It should be borne in mind that P in the environment can only be inferred from the continued presence (in the absence of continued input) of a substance in the environment. For (organic) chemicals, degradation is usually the main removal process, but this cannot be measured in practice (under all environmental conditions and compartments). Many regulators have therefore opted for extrapolation from laboratory tests for which they developed criteria, and P is regarded as a substance property (ECETOC, 2003a).

A first indication of P and vP properties of a substance can be gleaned from results of biodegradation (Q)SAR models, ready biodegradation tests, or other screening tests. Other information may also be suitable, following Annex XIII (Appendix A3.1.1).

3.1.1 Biodegradation (Q)SAR models

There are several QSAR models that can be used when screening a substance for possible P/vP properties (Nendza et al, 2013).

Especially useful for prediction of ready biodegradability are the combinations of BIOWIN2 (non-linear model) and BIOWIN3 (ultimate biodegradation timeframe), or BIOWIN6 (MITI non-linear model) and BIOWIN3. P/vP is indicated by the following joint outputs from BIOWIN2: “does not biodegrade fast (probability < 0.5)” and BIOWIN3: “ultimate biodegradation timeframe prediction ≥ months (Value < 2.2)” or the same results jointly for BIOWIN6 and BIOWIN3. Conversely, a substance may be regarded to be non-P/vP when BIOWIN3 states “ultimate biodegradation timeframe prediction ≤ months (Value ≥ 2.2)” and either BIOWIN2 or BIOWIN6 states “biodegrade fast (probability ≥ 0.5)”.

Other models reviewed by Nendza et al (2013) could also be employed for screening purposes, but are generally regarded less suitable due to the restricted relevance and/or reliability of their (individual) predictions. For example, PBT Profiler predicts biodegradability less reliably when used alone. These models may be considered for generating WoE (Table 3.1).

Table 3.1: Other models for P prediction

Prediction	Model	Reference
Direct, definite persistence	PBT Profiler [a], BIOHCWIN, START, CORAL	Howard et al, 2005; ECETOC, 2011; 2012; Toropov et al, 2012
	Pov (e.g.OECD Pov and LRTP Screening Tool)	Hollander et al, 2008
Ready biodegradability	BIOWIN [a], OASIS CATALOGIC (formally, CATABOL), VEGA, Danish QSAR database (containing MCASE), START	ECETOC, 2012; Pizzo et al, 2013
Abiotic degradation	HYDROWIN for hydrolysis, AOPWIN for atmospheric oxidation	ECETOC, 2012

[a] PBT Profiler (version 2.000 released in 2012) uses the calculation module from BIOWIN (EPIsuite version 4.1).

Some characteristics of the above models are as follows: BIOWIN has been used extensively for the prediction of ready biodegradability, for which it has six subprograms. The combination of BIOWIN3 and BIOWIN5 with applied Bayesian theorem should give enhanced predictive power in comparison to individual subprograms (Boethling et al, 2004).

The commercial OASIS CATALOGIC model predicts not only ready biodegradability, but also degradation pathways. P/vP properties are evaluated by taking into account the profile of the degradation products. Furthermore, CATALOGIC is used for read-across analysis, comparing the degradation pathways of target and source analogues (Patlewicz et al, 2013).

Overall persistence (Pov) can be estimated by multimedia box or mass balance models, e.g. the OECD Pov and LRTP Screening Tool. The two models are among the most advanced and permit direct prediction of definite persistence (Boethling, 2009; Scheringer et al, 2009; ECETOC, 2011). No definite numerical threshold for screening purposes has been proposed to date, although Scheringer et al (2009) tried to do so. Further studies are needed to clarify (i) under which environmental conditions such a value should be calculated, and (ii) the relevance and adequacy of the model calculations compared to actual observations on existing P/vP chemicals.

AOPWIN is the most commonly used model to estimate the atmospheric half-life of an organic chemical during photodegradation by OH radicals and ozone. If a chemical were mainly distributed into air, an atmospheric half-life of 2 days would indicate the chemical's potential for P and long-range transport according to the Stockholm Convention (UNEP, 2001).

In summary, the combined use of BIOWIN3 with either BIOWIN2 or BIOWIN6 is preferred when screening for P/vP. The other models predict definite persistency, ready biodegradability or abiotic degradation, which adds to a WoE evaluation.

3.1.2 Ready biodegradation tests

The OECD (1992a) 301 series and the OECD (2006b) 310 CO2-Headspace Test guideline are the principal initial tests for biodegradation. Setup of these tests is relatively simple and they are easy to perform as normally no radioactive material has to be applied and no direct analysis of the test substance is required. Indirect analysis of dissolved organic carbon (DOC), oxygen consumption or CO2 formation is used to demonstrate degradation. If the trigger for ready biodegradation of 70% DOC elimination or 60% oxygen consumption/CO2 formation after 28 days is not achieved, this indicates P/vP potential and the substance is

screened in for further investigation and testing. Assuming the inoculum is known and there has been no pre-exposure to the test chemical a positive result in a ready biodegradation test should be sufficient to assign a chemical as 'not P' even if there have been negative effects in previous studies. This is sufficient to demonstrate that the chemical will biodegrade.

If a chemical does fail the ready biodegradation test, results from other screening tests (modified or enhanced ready tests, tests on inherent biodegradation) may be useful additional testing to show a substance is not P. In some cases it may be justifiable to go directly to an enhanced test design, e.g. if QSAR modelling, the substance's molecular structure or physical properties indicate that the substance will not degrade to a significant extent in a standard biodegradation test in accordance with OECD 301 guidelines. Different methods for the enhancement or modification of a standard biodegradation test are available to demonstrate that a substance is not persistent.

3.1.3 Enhanced ready biodegradation tests

Enhanced biodegradation screens are designed to improve the environmental relevance of the OECD 301 tests (OECD, 1992a). For example, it is argued that the ability of a test system to biodegrade a specific substance may depend on the specific inocula used in the system (Thousand et al, 2011). The use of these 'enhancements' is not to demonstrate ready biodegradation but to show that the substance can be degraded within a specified timeframe and therefore should not be classified as persistent.

There are a number of 'enhancements' that can be done to prove a substance can be degraded. For example, extending the test duration, i.e. prolonging the duration of the OECD 301 test to up to 60 days in order to show that degradation does take place over this time period. This would be justifiable where a substance is poorly water-soluble and the lag phase is too long to show a sufficient degree of degradation under a standard 28-day test. Such a substance should not be considered 'P' as long as it achieves the relevant criteria within 60 days. Another justification for extending the study duration would be for marine studies where it is widely accepted that the lower bacterial levels in this test system delay the biodegradation process (Section 3.1.5).

Other enhancements include dispersing the substance or adding a surfactant so as to improve the bioavailability (OECD 301 guidance), increasing the vessel size or increasing the microbial biomass concentration. It is also possible to adapt the inocula (either low level pre-adaptation or semi-continuous adaptation) to the test substance to help facilitate biodegradation. Further research to understand the ecological significance of pre-exposure of inocula to a test substance to support guidance for enhanced biodegradation studies has been suggested following the ECETOC persistence workshop (ECETOC, 2013c). This workshop also suggested that a task force be set up to evaluate the predictive value of enhanced or modified screens. This task force would also consider whether the current biodegradation screens are complex enough to include the major processes.

Some enhancements (or modifications) of the ready biodegradation test may result in lower concentrations of chemical being tested. In such a scenario, the standard indirect measure of degradation may need to be replaced by a radiolabel or specific analysis of the test substance. Such approaches have been used for example when conducting biodegradation tests with ionic liquids (Neumann et al, 2013). If these endpoints are used there is currently no guidance on 'how much' loss of parent compound constitutes 'readily

biodegradable' or how much degradation is required to constitute 'not P'. If the parent compound is analysed there may also be a requirement, on a case-by-case basis, to consider transformation products, which adds to the analytical complexity and difficulty in interpreting these studies.

In conclusion, if enhanced studies are conducted they should fulfil the relevant criteria for the respective guideline (where available) within the 60-day time period in order to be considered acceptable to demonstrate 'not P'. For the assessment it does not matter which enhancement method was applied. According to REACh Guidance Document R.11 a positive result should be considered conclusive to assess a substance as 'not P'. However, if the substance is potentially P then further studies should be considered, e.g. inherent biodegradation tests (Section 3.1.4) or simulation studies (Section 4.1).

3.1.4 Inherent biodegradability tests

Inherent biodegradation tests are usually only conducted when the ready biodegradation study has failed. The theory is that inherent biodegradation tests generally allow more favourable conditions for biodegradation than the ready biodegradation tests by allowing higher concentrations of inocula and, for example in the OECD 302A test (OECD, 1981a), continued input of inocula throughout the test. The criteria for assessing persistence in these studies are therefore more stringent. For example, if less than 20% degradation occurs in one of the OECD 302 series tests then this is enough to confirm 'P' without further simulation testing. However, if a simulation test shows the substance to be 'not P' then the simulation result should out-weigh the inherent test result.

Confirmation of 'not P' in an inherent degradation study is less clear. Biodegradation > 70% after 28 days (measured as BOD, DOC removal or COD) or > 60% (ThOD) indicates 'not P'. With appropriate justification, biodegradation levels slightly below these thresholds may be considered 'not P'. The shape of the degradation curve should be considered to ensure that test compound is not being lost due to adsorption or volatilisation. As discussed for the enhanced ready biodegradation tests (Section 3.1.3), if disappearance of parent compound is measured directly rather than via DOC removal (for example in the OECD 302A test [OECD, 1981a]) then there are currently no criteria for what is considered 'not P'.

While the higher inocula levels in inherent studies may facilitate degradation, the higher test substance concentrations often used in these studies result in a similar food: micro-organism ratio to a ready test and may not favour biodegradation of the test substance. This would be especially relevant for substances with very low water solubility. Besides, the higher test substance concentration may cause toxicity to the microbial community, which may adversely affect the observed degradation rates. For many test substances the use of enhanced or modified ready biodegradation screens may give a better indication of degradation and should be considered if the standard ready biodegradation study fails.

Other studies for inherent biodegradation include the OECD 304A study, which might be used where the terrestrial compartment is of concern (OECD, 1981b). There is no guidance on interpreting this study for 'P/vP'. For example, there are no pass or fail criteria for 'P' over a defined time period (test durations of 32 and 64 days are allowed in the guideline). There is also the option within this study for determining the extractable residue of the test compound. Test compound that is not extracted could be considered as an NER and therefore not bioavailable as discussed in Section 4.3.

3.1.5 Marine biodegradation tests

Similar issues to those described for the ready and inherent biodegradation studies exist for the OECD 306 test on 'biodegradability in seawater' (OECD, 1992b). This is not classified as a ready biodegradation study because no inoculum is added to the test system but neither is it a simulation study as nutrients are added to the seawater. The test substance concentrations are usually high for this study to allow measurement of DOC. There are arguments that marine tests should be extended beyond 60 days because of the long lag phases before the onset of biodegradation in seawater systems (ECETOC, 2013a). It has been suggested that for marine studies the test duration should be as long as 90-120 days or at least long enough to get a meaningful half-life without extrapolation (ECETOC, 2003a). The criteria for 'not P' for this test could however be the same as for an inherent test from the OECD 302 series (OECD, 1981a), but after 60 days (or longer if it can be justified). For marine testing if a chemical fails the OECD 306 test then further testing might include simulation studies or specific testing for anaerobic biodegradation.

3.2 Bioaccumulation (B and vB properties)

Studies in aquatic species, especially fish, were previously required for assessment of B potential (B/vB properties) under REACh. The amended Annex XIII requires information on terrestrial species to be considered additionally, in particular data on B in organisms and biomagnification in food webs or human beings. It is expected that various routes of exposure of environmental organisms exist for chemicals emitted to the environment. Thus, as discussed in Chapter 2 of this report, it may be important to first identify the relevant 'Compartment of Concern' for the B assessment by taking the distribution behaviours of chemicals in the environment into account. However, in many cases, it can be very difficult to specify such a compartment definitely. Hence, possible indicators are proposed for B in aquatic and terrestrial species as far as possible.

As shown in the next Section 3.2.1, log $K_{OW} < 4.5$ is a definitive indicator of non-B in aquatic organisms forhydrophobic (lipophilic) chemicals, but other parameters could be considered as well. When several of the other parameters suggest a low B potential, it could be argued as expert judgement that the chemical is not B. The following sections explain the different screening indicators or properties.

3.2.1 Physico-chemical properties

The most frequently utilised physico-chemical property for B screening is the octanol-water partition coefficient (log K_{OW}). This is also stated in Annex XIII (Appendix A3.1.2). Other parameters include molecular weight, molecular diameter, molecular volume, octanol solubility and the octanol-air partition coefficient in particular for terrestrial organisms (Table 3.2).

Table 3.2 Physico-chemical properties and cut-off values for B screening in aquatic and terrestrial organisms

Parameter	Aquatic	Terrestrial	Reference	
			Aquatic	Terrestrial
log K_{OW}	< 4.5	< 2	ECHA, 2008	Gobas et al, 2003; Conder et al, 2012
	-	> 6 dermal	-	ECB, 2003
	> 10	-	ECHA, 2008a	Kelly et al, 2004; O'Connor et al, 2013
Solubility in octanol	0.002 mmol/l	NA	de Wolf, 2007	-
log K_{OA}	NA	< 5	-	Kelly et al, 2007; Conder et al, 2012
Molecular size				
Maximum diameter	> 1.7 nm	-	ECHA, 2008a	Arnot et al, 2009
Maximum length	> 4.3 nm	-	ECHA, 2008a	-
Molecular weight	> 800	-	Japan METI, 2011	
	> 1,100	> 1,000 GI tract	ECHA, 2008a	ECB, 2003
	-	> 500 dermal	-	ECB, 2003

-, not stated; NA, not applicable.

Octanol-water partition coefficient (log K_{OW})

From a thermodynamic viewpoint, bioaccumulation in aquatic organisms, especially bioconcentration, can be considered as a partitioning process of a chemical between fish lipid and water. Thus, bioaccumulation potential generally shows a tendency to decrease with decreasing log K_{OW} value. The chemical elimination rate via gill ventilation is on the other hand inversely related to log K_{OW} (Ehrlich et al, 2011). These tendencies should also be expected for biomagnification processes in aquatic systems. Several specific cut-off values in terms of log K_{OW} are proposed (van Wijk et al, 2009; Gobas et al, 2009) and the current REACh guidance applies the cut-off criterion of log K_{OW} of less than 4.5 because of lack of affinity for lipids of aquatic organisms (ECHA, 2008a). The lower limit of log K_{OW} is a very important and reliable indicator because it is highly related to B/vB potential, so this lower limit of log K_{OW} is recommended to be used not as one of WoE but as a definitive indicator for lipophilic chemicals in aquatic organisms, which can screen out not-B/vB chemicals on its own.

The descriptor log K_{OW} should be preferably measured, but estimation with validated models could also be applied for screening purposes. For ionisable chemicals, one of the issues regarding the estimation of bioaccumulation is the state of ionisation (i.e. the ionised form being much less accumulated than the non-ionised form). The fraction of the chemical in the ionised state is dependent on the pH of surrounding media such as water and the specific ionisation constant (pKa) for that chemical. Although there are formulas for calculating these fractions, it is recommended for screening purposes that an estimation of log K_{OW} should be made with the assumption of entirely non-ionised state of such a chemical to give a conservative prediction.

In considering the lower limit of log K_{OW} for terrestrial bioaccumulation, the above criteria for aquatic systems could not be applied directly due to the difference in exposure routes. However, chemicals with log K_{OW} of less than 2 should not normally biomagnify in terrestrial food webs because such chemicals are expected to be eliminated rapidly by urinary excretion (Gobas et al, 2003, 2009; Czub and McLachlan, 2004a; Kelly et al, 2007;

Conder et al, 2012; Arnot et al, 2014). Thus, the value (log K_{OW} < 2) could be an indicator for WoE in screening of not-B/vB for terrestrial systems. However, chemicals with log K_{OW} of more than 2 cannot be concluded to be potentially bioaccumulative because this threshold value should be very conservative and other parameters such as uptake efficiency and biotransformation rate could considerably affect their bioaccumulation behaviour.

For highly hydrophobic chemicals, the bioaccumulation potential in aquatic organisms tends to decrease with increasing log K_{OW}.

Kelly et al (2004) reported the relationship between dietary absorption efficiencies and log K_{OW} for fish and mammals including humans (Kelly et al, 2004; O'Connor et al, 2013). All the dietary absorption efficiencies of highly hydrophobic chemicals showed tendencies to decrease with increasing log K_{OW} values; chemicals with log K_{OW} > 6 to 7 showed decrease of uptake efficiency to organisms. The authors considered this to be due to the restricted permeability of the highly hydrophobic chemicals into the unstirred water layer of the gastrointestinal tract. This phenomenon could be also attributable to the increased sorption of the chemicals to the indigestible fraction in the gastrointestinal tract. For fish, a log K_{OW} of > 10 is applied as one of the indicators for not being B/vB in REACh Guidance R.11. While this upper limit for bioconcentration is empirically proposed, there is a need for further studies on dietary bioaccumulation with aquatic organisms (ECHA, 2008a). For terrestrial systems no numerical upper limit of K_{OW} could be defined at present, although the dietary absorption efficiency in terrestrial systems decreases with increasing log K_{OW} because of the limited bioavailability of the highly hydrophobic chemicals (Kelly et al, 2004; O'Connor et al, 2013). According to ECB (2003) a log K_{OW} > 6 can be considered a threshold for dermal uptake indicating limited absorption via the dermal route. This value is also a reasonable starting point for a limited oral absorption. However, oral absorption of more hydrophobic chemicals can be increased by micelle formation with bile acids. Further research is needed to better define cut-off values and criteria for screening of all substances with respect to the efficiency of dietary absorption in terrestrial systems.

Limitations of using log K_{OW} as a screening criterion

When using log K_{OW} as an indicator for non-B the following limitations need to be taken into account. Octanol may not be a satisfactory surrogate for lipid membranes in all kinds of organisms. It only mimics passive diffusion across lipid membranes, but does not predict other uptake mechanisms, such as permeation across protein channels for some water soluble or ionisable compounds or active transportation across membranes. It cannot be applied to metal ions.

Surface-active substances are capable of reducing the surface tension of a liquid in which they are dissolved. Surfactants generally contain two distinct moieties, a hydrophilic part which usually acts through hydrogen bonding or ionic interactions and a hydrophobic part which is often a straight or branched chain hydrocarbon. Surfactants adsorb at the interfaces of the aqueous and lipid phases. Commercial surfactants are not chemically pure substances, for example due to a distribution of C-chain lengths. For these reasons, surfactants are a chemical group for which it is difficult to obtain reliable partitioning values. This is not only important for the persistence assessment, but also critical for log K_{OW} determination. There are concerns that some of the methods used for log K_{OW} determination have not been fully validated and might not be applicable to 'surface-active substances'. Some regulations like the OSPAR Convention (legal instrument guiding international cooperation on the protection of the marine environment of the North-East Atlantic)

have decided that log K_{OW} could not be used for surfactants for the determination of the bioaccumulation potential (OSPAR, 2010).

At present some research is conducted to refine the log K_{OW} determination methods for surfactants. A task force on 'Hydrophobicity of Surfactants' was set up in 2011 within CESIO (Council of European Surfactants Producers), under the ERASM (Environment and health Risk Assessment and Management) programme, i.e. a research partnership of the Detergents and Surfactants Industries in Europe.

The aims of this task force are:

- To address the suitability of existing methods to determine the log K_{OW} of surfactants. The traditional 'shake flask' method according to OECD test guideline 107 (OECD, 1995) is considered wholly inappropriate for the determination of log K_{OW} values of surfactants due to emulsion formation and uncertainties with regard to phase behaviour of the surfactant in the two solvents. Consequently, during phase 1 and 2 of the REACh registration, different methods were used to determine log K_{OW} for surfactants: the 'slow stir' following OECD guideline 123 (OECD, 2006a), HPLC detailed in OECD guideline 117 (OECD, 2004a) and computational procedures referred to by OECD guideline 107 (OECD, 1995), the latter using the n-octanol / water solubility ratio and QSAR considerations. Some of these methods have not been fully validated for surfactants and might not be applicable to surface-active substances. The task force has therefore initiated a comprehensive review of all these methods as applied to a selection of non-ionic, anionic, cationic and amphoteric surfactants.
- To assess and recommend an alternative approach or methodology for the determination of hydrophobicity of surfactants. Among those alternative approaches, both modelling and new measurements of the hydrophobicity are addressed. In particular, the task force has considered the following approaches:
  - Liposome-water partitioning to determine $K_{lip\text{-}water}$ for soluble fractions.
  - Determination of phospholipophilicity using Immobilised Artificial Membranes (IAM).
  - Use of Solid Phase Microextraction (SPME).
  - Use of various Centrifugal Partition Chromatographic (CPC) techniques.
  - The pH-metric method for ionisable substances following OECD guideline 122 (OECD, 2000b).

It is nevertheless not sure if log K_{OW} is still the right parameter to be used to assess the bioaccumulation potential for surfactants. And even if log K_{OW} is a suitable parameter, the usual threshold values may need to be updated for these particular compounds. The lack of an integrated methodology for this group of substances suggests considering the different steps in the bioaccumulation process: sorption processes, transportation processes in the organisms (i.e. distribution and elimination) and finally metabolisation.

Similarily, for very hydrophobic substances and chemicals that are of very low solubility in both water and octanol and other non-polar solvents, the log K_{OW} is difficult to determine with the current test methods. For such substances estimations of log K_{OW} may vary over several orders of magnitude. All estimates and model calculations based on log K_{OW} as one of the determining parameters need therefore to be treated with caution for these chemicals.

Solubility in octanol for aquatic systems

Octanol is a reasonable surrogate for fish lipids and chemicals with low octanol solubility tend to have low bioaccumulation potential in aquatic systems. Very low solubility in octanol could be used as an indicator for low bioaccumulation. Taking into account 5% lipid normalised critical body burden for fish acute toxicity and a safety factor of 10, the chemicals with measured octanol solubility of less than 0.002 mmol/l are considered to have limited bioaccumulation potential for aquatic systems, without any chronic toxicity or other indicators of bioaccumulation, because of lack of affinity for lipids of aquatic organisms (de Wolf et al, 2007). This indicator can be used for WoE of not-B/vB under the REACh regulation (ECHA, 2008a). For terrestrial systems, the same logic may be applied but since the distribution mechanism is expected to be more complex than that in aquatic systems, no specific threshold value could be defined up to the present. Further studies are needed.

Octanol-air partition coefficient (log K_{OA}) for terrestrial species

Other than log K_{OW} mentioned above, the octanol-air partition coefficient (log K_{OA}) might be taken into account when screening for B potential in terrestrial systems. The log K_{OA} describes the analogous distribution behaviour of a chemical between air and lipid of organisms, and it is expected that a chemical with high log K_{OA} should be eliminated slowly by exhalation in terrestrial organisms (Gobas et al, 2003; Czub and McLachlan, 2004a). Some scientists proposed a screening threshold for potentially B/vB in terms of log K_{OA} to be more than 5 to 6 (Gobas et al, 2009; Arnot et al, 2014) and others proposed that chemicals with log $K_{OA} < 5$ have low biomagnification potential in food webs because of rapid elimination from terrestrial organisms through respiration (Kelly et al, 2007; Conder et al, 2012). At present, log $K_{OA} < 5$ could be tentatively proposed as a conservative indicator of not-B/vB for terrestrial systems. But this tentative and conservative indicator should be verified with actual field studies. Octanol may not be a satisfactory surrogate for lipid membranes in all kinds of organisms. Therefore the limitations stated above for log K_{OW} equally apply to logK_{OA}, for example with regard to ionisable chemicals.

As K_{OA} values determined experimentally are only available for several hundred compounds, K_{OA} can be usually estimated from the octanol-water partition coefficient (K_{OW}) and Henry's law constant (H) by the following equation.

$$K_{OA} = K_{OW} (RT)/H \quad \text{..... (Eq. 3.1)}$$

where R = Universal gas constant; T = Temperature; and H = Henry's law constant.

There is a good correlation between calculated and experimental log K_{OA} in spite of some variable factors (Meylan and Howard, 2005). Other estimation methods or models could be used if they are reliable (Xiao and Wania, 2003; Li et al, 2006).

Molecular size

A chemical with an extremely large molecular size will be restricted from permeation into biological membranes because of its low rate of chemical permeation across membrane systems (ECHA, 2008a). This phenomenon can be well understood according to the Stokes-Einstein or Saffman-Delbrück relationships and

several threshold values in terms of molecular size have been proposed (Dimitrov et al, 2002; Nakai et al, 2006; Sakuratani et al, 2008). A review on various molecular-size thresholds for B assessment warned about uncertainty in these cut-off values because of limited reliability of bioconcentration measurements for larger and very hydrophobic chemicals (Arnot et al, 2009; Nendza and Müller, 2010). Therefore, these criteria are applied conservatively for regulatory purposes. For example, ECHA applied the cut-off values of an average maximum diameter ($D_{max\ aver}$) of greater than 1.7 nm and a molecular weight of greater than 1100 g/mol, or a maximum molecular length (MML) of greater than 4.3 nm as an indicator of not-B/vB (ECHA, 2008a). Under the Chemical Substance Control Law in Japan, molecular weight of greater than 800 g/mol can be applied as the criterion for not-B/vB. This criterion cannot be applied to a chemical with more than two halogen substituents as the high density of the halogens would overestimate the molecular size (Japan METI, 2011).

Although these threshold values were derived principally based on the analyses of fish bioconcentration data, they could be also applied to WoE on biomagnification or bioaccumulation as well. In calculating the 3-dimensional molecular structure and descriptors such as $D_{max\ aver}$ or MML, OASIS is considered as the most suitable tool with high reliability (Nikolov et al, 2006; ECHA, 2008a; Brooke and Cronin, 2009). Other quantum chemical or molecular mechanics calculation tools could be also used if they are fully validated and their domain properly defined.

For bioaccumulation in terrestrial systems, no threshold in terms of molecular size could be defined at present although some studies suggest the importance of molecular size. Some studies reported the relationship between molecular size and bioaccumulation potential in terrestrial systems (Yu et al, 2012). Arnot et al (2009) reviewed various studies on pharmaceuticals and nanoparticles, and suggested that molecular size should affect the uptake efficiency to mammals (Arnot et al, 2009). Lipinski's 'rule of 5' should give qualitative information on uptake limitation useful for WoE analyses. Molecular weight of greater than 1,000, as employed for polymer risk assessment by US EPA and Japan METI, might be also a possible indicator of uptake limitation in terrestrial organisms for substances without any biological reactivity or specific transport mechanisms.

According to TGD (ECB, 2003) a molecular weight of 1,000 or above is an indication of limited absorption in the gastro-intestinal tract. This cut-off can be used as a practical approach for terrestrial bioaccumulation as well. For dermal uptake the threshold for molecular weight is 500. Some higher molecular weight molecules could be taken up by specific transport mechanisms, e.g. pinocytosis or active receptor-mediated transport. If this is a known mode of absorption, it needs to be considered in the assessment.

3.2.2 Uptake, biotransformation and elimination studies

In addition to these simple physico-chemical properties, various in vitro studies in relation to absorption, distribution, metabolism / biotransformation, and excretion (ADME) processes were developed to refine the estimation of bioaccumulation potential. In vitro metabolism studies can be a useful indicator for a low B potential for easily metabolised chemicals with high log K_{OW} values. For example, if a chemical with a log K_{OW} > 4.5 is shown to be rapidly metabolised to hydrophilic metabolites.

Biomimetic extraction methods such as SPME and SPMD are useful for mimicking uptake of a chemical by biota: Low extraction would suggest limited uptake. The solid phase micro-extraction (SPME) with a thin polymer coated fibre and the semi-permeable membrane device (SPMD) with a dialysis bag are typical

methods used for this purpose. Both methods mimic uptake (absorption and distribution) behaviour of chemicals from water to aquatic organisms and the experimental results from these methods could be used as indicators of B/vB potential in aquatic systems (de Wolf et al, 2007; Nichols et al, 2009).

Rapid elimination by metabolism or biotransformation is an indication of low B potential (Mackay et al, 2013). Modelling studies suggested that chemicals with rapid biotransformation (i.e. the order of 10% per day) should not biomagnify in food webs (SETAC, 2008). Several models calculating such biotransformation rate are available, but still need further improvement (Gobas et al, 2009; Papa et al, 2014). It is possible that an in vitro assay using fish liver S9 or isolated fish hepatocytes systems could improve the estimation of BCF for chemicals with rapid biotransformation as well as an in vivo determination of the biotransformation / elimination rate of a chemical for fish: Rapid biotransformation and/or elimination would suggest low bioaccumulation (Cowan-Ellsberry, 2008; Nichols et al, 2013), but further studies for standardisation of assay conditions are needed.

In vitro or in vivo biotransformation or elimination rate for mammals, birds, or plants can be an indication of low B potential when rapid biotransformation and/or elimination can be demonstrated (ECHA, 2008a; Goss et al, 2013). Such information can also be used in the B assessment for terrestrial systems. Goss et al suggested that a low elimination half-life could be linked directly to not-B/vB potential, regardless of uptake route (Goss et al, 2013). Thus, at present, experimental results on metabolism and excretion from toxicokinetic studies in mammals could serve as indicators of B/not-B in a WoE approach.

3.2.3 Read across (category approach)

Low bioaccumulation can be estimated by the read across and/or category approach with structurally similar chemicals having reliable BCF, BAF, BMF, TMF etc, suggesting low bioaccumulation potentials (ECHA, 2008c; ECETOC, 2012; Patlewicz et al, 2013).

The read across or category approach could be applied to the B/vB assessment for both aquatic and terrestrial systems in REACh Annex XI or ECHA Guidance R.6 and R.7c (ECHA, 2008c,d). In cases where reliable bioconcentration, bioaccumulation and biomagnification data are available for reference chemicals with similar structures to the target chemical, such estimations could be applied for screening purposes.

In categorising chemicals, uptake mechanisms (e.g. hydrophobicity and molecular size) and metabolic or biotransformation routes in organisms should be considered (ECHA, 2008c). For some chemicals the specific reactivity (e.g. protein binding) could also be considered as a factor for grouping.

For example, the National Institute of Technology and Evaluation in Japan has put forward five theoretical categories for predicting the bioconcentration potential of various organic chemicals in fish (Japan NITE, 2013) for: (i) neutral organic chemicals (with simple passive diffusion), (ii) hydrogen bond acceptors, (iii) hydrogen bond donors, (iv) ionisable chemicals and (v) rapidly metabolising chemicals. Other general methods have been reviewed by ECETOC (2012) and Patlewicz et al (2013). However, grouping and read-across need to be performed carefully and assessed on a case-by-case basis.

3.2.4 Prediction by models

Models for aquatic organisms

Low bioaccumulation potential is indicated if the BCF and/or BAF estimated by well-validated and reliable QSAR models such as EPISuite, CAESAR and OASIS (Nendza, 2013) are less than 2,000 and/or 5,000, respectively. Various QSAR models for predicting bioaccumulation potential, especially BCF for aquatic organisms were proposed (Pavan et al, 2008; Nichols et al, 2009; ECETOC, 2012; Nendza et al, 2013). For these QSAR models, octanol-water partition coefficient, log K_{OW}, is one of the most frequently-used descriptors because uptake is considered a result of passive diffusion through gill membranes. In some models, the metabolic rate constant is also taken into account (Arnot et al, 2008; Dimitrov et al, 2012). When using these models for screening purposes, reliability of the model calculations should be previously checked according to the OECD validation principles (OECD, 2007) or REACh Guidance R.6 (ECHA, 2008a). The BCFBAF (formerly BCFWIN) program in the Estimation Programs Interface Suite (EPISuite) (US EPA, 2009a; Costanza et al, 2012), CAESAR (Zhao et al, 2008; Lombardo et al, 2010), and OASIS Catalogic BCF baseline model (Dimitrov et al, 2012) are some of the recommended models satisfying OECD validation principles. The BCFBAF program in EPISuite is the most widely used one for estimating a chemical's BCF and BAF. This program provides two different QSAR models based on log K_{OW}, a regression model (Meylan et al, 1999) and a mass balance model (Arnot and Gobas, 2003; Arnot et al, 2006). The commercial OASIS Catalogic BCF baseline model offers BCF estimation methodology and takes into account such mitigating factors as possible metabolism and molecular size (Dimitrov et al, 2012). Outlines of these models are given in Appendix B and more detailed explanations can be found in the literature or manual of each program.

As most of the models are very sensitive to the log K_{OW} used as an input parameter, the estimations for highly hydrophobic compounds tend to be related with a high degree of uncertainty (Section 3.2.1). To reduce the uncertainty, for example, the OASIS Catalogic BCF baseline model utilises molecular weight as a correlation factor. Garg and Smith (2014) have proposed an additional model for the prediction of bioconcentration factors that takes into account the parabolic relationship between log K_{OW} and log BCF as well as the molecular volume as an additional parameter. They derived the following equation:

$$\log BCF = 3.036 \text{ calc } \log K_{OW} - 1.97 (\text{calc } \log K_{OW})^2 - 0.808 \text{ molecular volume} \quad \text{..... (Eq.3.2)}$$

The model was derived from a relatively low number of chemicals and may need further validation. However, this may be a first step to better address the bioaccumulation behaviour of highly hydrophobic compounds at the screening level.

The predictions with these validated QSAR models are mainly relevant for non-polar organic chemicals and are not applicable to the other specific chemical groups such as metals and surface active substances. Indeed, the mechanisms of accumulation for these chemicals should be more complex (e.g. active transportation) than those for non-polar chemicals. Specific models for other groups of chemicals are under development.

A more robust estimation of B could be obtained by a combination of well-validated models in a consensus or battery approach (Gissi et al, 2013; Fernandez et al, 2012).

Reliable BCF values predicted by validated QSAR models with consideration of the applicability domain and model uncertainty can be used as good indicators for B/vB assessment in aquatic systems and will continue in the WoE analysis when screening for B properties.

Models for terrestrial organisms

BAF, BMF and TMF can be estimated by multimedia/multipathway QSAR models. Low values would suggest a low bioaccumulation potential through the food web.

Many models are available to estimate the bioaccumulation potential of chemicals through both aquatic and terrestrial food webs. These 'holistic' models simulate chemical partitioning, degradation, environmental fate and transport, and bioaccumulation through the food web. In Appendix C, several representative models are introduced. However, these models were developed mainly to prioritise chemicals for further investigation of their B potential (Arnot et al, 2006b; Mitchell et al, 2013). Uncertainty and limitation caused by lack of measured bioaccumulation data under such complicated environmental conditions and complexity in modelling the food webs make it difficult to calculate reliable and definite values of BAF, BMF and TMF (Arnot et al, 2010b; 2012). Therefore, it is recommended that these predicted results should be used only for comparative and/or qualitative assessments as exemplified below:

- To make a rough prediction of bioaccumulation behaviour, in terms of an increase or a decrease in the concentration of a target substance in the food web.
- To estimate the highest bioaccumulation potential and in which particular organism..
- To make a comparative assessment between the calculated values for the target substance and those of other chemicals whose bioaccumulation potential is well-known.

There are several models such as ACC-HUMAN (Czub and McLachlan, 2004a,b), European Union System for the Evaluation of Substances model (EUSES) (Vermeire et al, 1997; 2005) and Arctic Terrestrial Food-Chain Bioaccumulation model (Gobas et al, 2003) as reviewed by Brooke and Crookes (2007). Some other useful general models are the Risk Assessment IDentification And Ranking (RAIDAR) model (Arnot and Mackay, 2008; Arnot et al, 2006b; 2010a), Farfield Human Exposure (FHX) model (Arnot et al, 2010b), California Toxicity (CalTOX) model (Maddalena, 1995; Hertwich et al, 2001; Huijbregts et al, 2005) and UNEP-SETAC Toxicity Model (UseTox) (Rosenbaum, 2008).

These models mainly consist of two algorithms for simulating environmental distribution and bioaccumulation behaviour. For simulating distribution, almost all models employ the fugacity-based environmental fate model originally developed by Mackay by applying different environmental default conditions (Mackay, 2001). For simulating bioaccumulation, fugacity based mass balance models or simple regression-based models applying estimated environmental concentrations of the corresponding compartment are principally employed.

The difference in estimated BAF/BMF/TMF values among these models should be attributable to: (1) how they parameterise the environmental conditions (e.g. geographic and meteorological conditions such as temperature or sunlight); (2) how they construct and simulate hypothetical bioaccumulation food webs; and (3) how they parameterise the specific organisms in the food webs. Examples of the hypothetical food webs are shown in Figures 3.1 and 3.2.

Figure 3.1: Representative ecological and human food webs - Hypothetical food web in RAIDAR (from Arnot et al, 2006b)

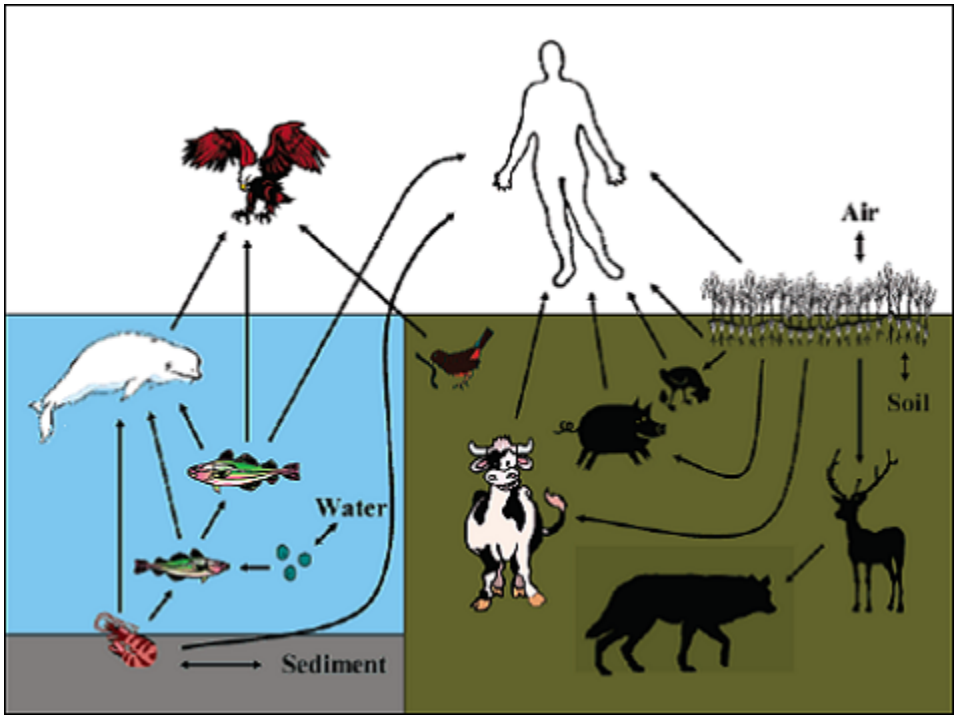
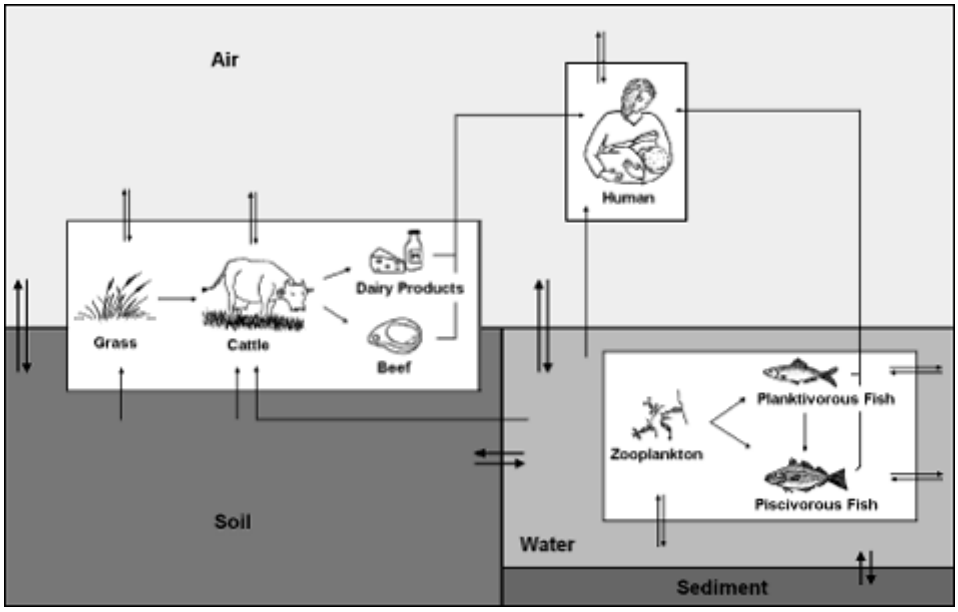


Figure 3.2: Representative ecological and human food webs - Hypothetical food web in ACC-HUMAN (from Czub and McLachlan, 2004a)



Appendix C provides short overviews of some useful terrestrial food web models. RAIDAR/FHX, ACC-HUMAN, Arctic Terrestrial Food-Chain Bioaccumulation model and EUSES can simulate bioaccumulation or biomagnification processes in several environmental organisms including humans. On the other hand, two human exposure models CalTOX and UseTOX mainly calculate chemical concentrations in human. These

models require many input parameters, such as physico-chemical properties (e.g. molecular weight, distribution parameters between different media), half-life in each medium or biota, and emission rate. A sensitivity and uncertainty analysis for validating very important input parameters and reducing uncertainty in model parameters is also recommended (Ciavatta et al, 2009; MacLeod et al, 2002). For example, the emission rate and primary biotransformation half-life in mammals are reported to be the first and second most important parameters that contribute to variance in predictions (Arnot et al, 2012).

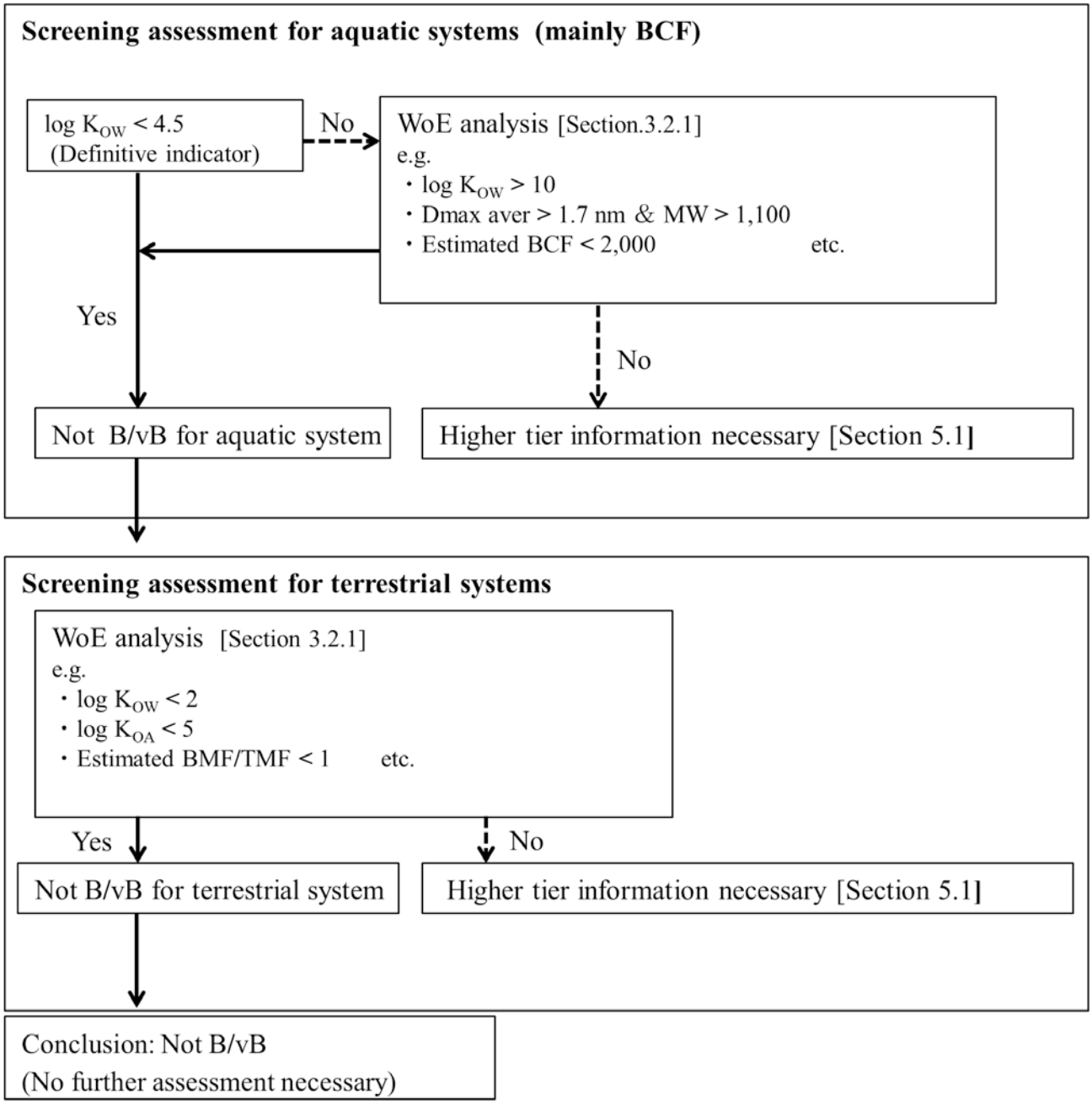
Some other models for a specific route of exposure or particular type of organism could also be used to refine these 'holistic' estimations. Examples are the models developed for foliar vegetation or crops (Trapp and Matthies, 1995; Fujisawa et al, 2002) or earthworms (Connell and Markwell, 1990). In general, many models have been developed to cover different situations. Therefore, for proper estimations, not only the hypothetical mechanisms on bioaccumulation but also default conditions such as food webs should be checked before use.

3.2.5 Summary and integrated strategy of screening B/vB assessment

Screening to confirm whether or not there is B/vB potential can be done using physico-chemical indicators/properties, results of in vitro studies, and estimations by models or read-across. It has been suggested to assess the B potential of a chemical for the (main) compartment(s) of concern as explained in Section 2.3. However, in general, it is prudent to assess the B/vB potential in aquatic organisms first, because of the specific cut-off values for B/vB that have been established so far. In general, the aquatic environment will be the main compartment of concern for many chemicals when considering their exposure route or mobility. If the target chemical can be considered 'not B/vB' in the screening assessment for the aquatic environment, then the screening for bioaccumulation potential should be investigated in terrestrial organisms. When 'not B/vB' is concluded in both systems, no further studies are required.

In summary, an integrated strategy for the B/vBscreening assessment is shown in Figure 3.3. Some indicators with numerical thresholds are recommended to be used with the combination of structurally or mechanistically similar chemicals with experimental data by read-across.

Figure 3.3: Decision tree for screening B/vB assessment



3.3 Toxicity (T properties)

3.3.1 Aquatic toxicity

There are several kinds of validated QSAR models for estimating chronic toxicity for various aquatic organisms such as fish, invertebrates and algae (ECETOC, 2012; Nendza et al, 2013). ECOSAR developed by US EPA is one of the representative models, which consists of a number of regression equations with log K_{OW} for individual chemical classes. These models should always be employed within their applicability domain and the model limitations should also be taken into account when considering expected mode-of-toxic

action of the chemicals (Robinson et al, 2014). Read across or category approach with considering structural alerts is another useful method (Kühne et al, 2013; Schüürmann et al, 2011). Several structural alerts for aquatic toxicity such as Verhaar classifications, ECOSAR classifications, and MOA by OASIS are proposed. The OECD Application toolbox with these structural alerts helps us to predict aquatic toxicity more efficiently. Combination of the results by QSAR models and the read across or category approach should improve the prediction accuracy.

3.3.2 Mammalian toxicity

Several QSAR models or qualitative estimation tools based on structural alerts have been developed for estimating mutagenicity and carcinogenicity, such as CAESAR and ChemProp (ECETOC, 2012; Nendza et al, 2013). Reproductive toxicity is currently not possible to predict by models alone. Reviews of the use of read across and categorisation for human toxicological endpoints and their limitations are given in ECETOC (2010; 2012) and Patlewicz et al (2013).

Read across or category approaches can be useful for informing intelligent testing strategies for carcinogenicity, mutagenicity, and reproductive (CMR) toxicity. The OECD Application toolbox is one of the tools that are commonly used. In the toolbox, one of the useful tools is the Hazard Evaluation Support System Integrated Platform (HESS). With this tool, the experts can screen for possible effects in rat repeated dose toxicity studies by considering the plausible mode of toxic action (MOA) or expected adverse outcome pathway (AOP) (Sakuratani et al, 2013; Yamada et al, 2013).

However, reliable predictive tools for complex mammalian toxicity endpoints, such as mutagenicity, carcinogenicity, organ toxicity after repeated exposure and toxicity to reproduction and development are not available to date. SAR or read across considerations can be developed on a case-by-case basis taking into consideration all available information on mammalian toxicity of the respective chemicals. Models revealing structural alerts can give an indication as to where toxicological testing could be targeted after expert evaluation. The T assessment for mammalian toxicity in the PBT assessment under REACh is based on the classification according to the CLP regulation. The respective guidance for toxicological hazard assessment and classification needs to be considered.

4. ASSESSMENT INFORMATION ON PERSISTENCE

4.1 Simulation testing in water, sediment and soil

Simulation studies may be required to refine the persistence assessment of a substance. These studies are considered to be more environmentally realistic than the screening studies and provide a half-life of the test substance under the given conditions that can be used for comparison with the 'P' or 'vP' criteria under REACh. In order to choose the relevant simulation study it is important to clarify the compartment(s) of concern (Section 2.3). Once the compartment(s) of concern has (have) been identified the study type can be chosen. Simulation studies would usually include any of the OECD tests following guideline 307 (soil), 308 (water/sediment) or 309 (water) (OECD, 2002a,b; 2004a) or one of the OECD 314 series of studies (OECD, 2008). It is recommended that these studies should be conducted at environmentally realistic temperatures (Section 2.1).

Although the simulation studies are supposed to reflect 'environmental relevance' this may not be considered to be true for all scenarios. The OECD 308 test was developed as a tool for assessing spray drift for pesticide applications and is not a river simulation study and therefore does not accurately represent substances released into rivers, as e.g. the water to sediment ratio is very low in the OECD 308 test design. Ericson et al (2014) have reviewed OECD 308 data from over 30 pharmaceuticals and conclude that there is a need for a more appropriate test reflecting the environmental exposure scenarios for these types of substances. Many of the pharmaceuticals reviewed dissipate from the water to the sediment over time and form non-extractable residues, making it difficult to differentiate between the parent and transformation products and making sediment half-life data difficult to calculate. It was recommended that a 'total system' half-life be used. Since the use pattern of pharmaceuticals is similar to the use pattern for other classes of chemicals (e.g. household products) the use of OECD 308 data in PBT assessments is questionable. Alternative tests such as OECD 309 or OECD 314 are suggested as more appropriate tests for these types of substance as they better simulate the environmental scenario to which these substances are released. The ECETOC workshop on Environmental Persistence (2013c) recommended that further work be done to compare the OECD 308 study with OECD 309 and 314. Alternatively, improved understanding of how changes in the water: sediment ratio and in the surface area of the sediment affect partitioning kinetics in the OECD 308 study may aid interpretation of these studies. An ongoing CEFIC LRI project on 'Improved strategy to assess chemical persistence at the water-sediment interface' may help to understand some of the variability within the OECD 308 study in the near future (Fenner, 2012).

There are also issues with the OECD 314 A-C studies (as well as the OECD 303 study) for assessing persistence as they are not currently considered acceptable by regulators to demonstrate 'not P' because they are not considered to be representative of the natural environment (they represent sewage treatment works). If, however, data are available from such studies then they should be usable in a 'WoE' approach to assess the 'P' or 'vP' potential in the environment as sewage treatment bacteria do enter surface waters and data from these studies will indicate a potential for degradation to occur. An outcome from the ECETOC workshop on Environmental Persistence (ECETOC, 2013c) was that further research should be conducted to assess whether a sewage treatment plant model can be considered to be representative of the natural environment.

In summary, under Annex XIII of REACh the simulation studies are considered the definitive tests for assessing persistence with the following criteria for 'P' or 'vP':

- While relevant tests exist there still remains considerable uncertainty in the design of these studies for the purposes they are required.
- Recent CEFIC LRI projects have been funded to try and address this uncertainty to improve the environmental realism of these simulation studies.

4.2 Anaerobic degradation

For the registration of a chemical substance under the current EU REACh legislation (EC, 2006) the environmental fate of a substance is an important part of the hazard section of the dossier. Degradation and especially biodegradation plays a major role in the assessment of a substance as it contributes to the derivation of a sound classification and labelling as well as to a proper PBT assessment. Most of the tests performed refer to biodegradability under aerobic conditions, e.g. to assess whether a substance is readily biodegradable. Nevertheless, under environmental conditions anaerobic conditions can occur in sewage treatment plants, sediments, soils and some water bodies. It is likely that due to the reductive conditions of anaerobic compartments, degradation and especially biodegradation will proceed differently compared to aerobic conditions.

If a proper assessment of the biodegradation under anaerobic conditions is needed, testing under comparable conditions is recommended, e.g. for biocides with an explicit use. SCHER (2008) investigated the relevance of anaerobic degradation for detergents, which are likely to sorb to the biosolids during wastewater treatment. These biosolids may then undergo anaerobic treatment prior to disposal, which may include use as a fertiliser in soil (where it will be exposed to anaerobic regions of the soil compartment). In the SCHER report, the authors came to the conclusion that adequate testing methodologies are available, e.g. according to OECD guidelines 307, 308 and 311 (OECD, 2002a,b, 2006c). Furthermore they assumed that poor biodegradability resulting from anaerobic studies does not substantially modify the risk for freshwater ecosystems as the surfactant may not reach anaerobic compartments due to biodegradation which occurs in aerobic compartments beforehand (e.g. sewage treatment plants, aerobic surface water bodies and sediment stratums, aerobic soil horizons). This should also hold true for most of other substances which have been proven to degrade under aerobic conditions. SCHER is also of the opinion that a general requirement for anaerobic degradation testing is not by itself an effective measure for environmental protection (SCHER, 2008). Under some circumstances modification of standard tests to include some fluctuation in redox conditions (as might occur in natural sediments) might be justifiable.

Some chemicals are more amenable to anaerobic degradation than others, for example those with halogen substituants. If anaerobic degradation is expected to be a major removal mechanism and the sorption properties indicate that it is likely to be associated with sludges or sediments then it would be justifiable to choose an anaerobic degradation test to try and show that the chemical will degrade. For most other chemicals anaerobic degradation testing would only be performed after thoroughly examining the aerobic biodegradation potential of a substance. Anaerobic data if available should be assessed in order to account for the trigger values for the different environmental compartments as given in Annex XIII of REACh (Appendix A).

Since standardised tests such as the OECD 311 guideline are not equivalent to OECD screening tests and do not have trigger values assigned to them for P or vP these tests fall into the category 'other evidence of non-persistence'.

Based on the criteria for anaerobic biodegradability contained within BioWin (US EPA, 2012), a 'pass level' of 60% mineralisation within the 56 days of the tests should be considered as a trigger for non-persistence. In terms of P and vP criteria, this test does not address the rate of primary anaerobic biodegradation but it may be feasible to modify the test design to measure primary dissipation rates and use these as triggers for P and vP (based on the sediment half-life criteria within Annex XIII of REACh (Appendix A). In the absence of such criteria, the results from anaerobic degradation studies could also be compared with existing soil and sediment half-life data.

4.3 Non-extractable residues

The extent to which chemical residues are bioavailable or bioaccessible (may become bioavailable over the long-term) continues to generate much debate. For the registration of a chemical substance under the current EU REACh legislation (EC 1907/2006) higher-tier testing on biotic and abiotic degradation is foreseen. Especially for substances with higher tonnages produced per year (> 100 t/a) these tests are foreseen, e.g. in REACh Annex IX, unless screening tests or other evidence indicate the possibility for waiving. Depending on the substance properties, the formation of so-called Non-Extractable Residues (NERs) and Bound Residues (BRs) might constitute a significant fraction of the initial amount of the parent substance in these higher-tier tests. The definition of an NER and BR is complex. The following definition was slightly adapted from IUPAC for use by the Agrochemical Industries (ECPA, 2000).

"Non-extractable residues present in the soil (sometimes referred to as bound residues) are chemical species (parent compound and metabolites, or fragments) originating from pesticides used according to good agricultural practice that cannot be extracted by methods which do not significantly change the chemical nature of these residues."

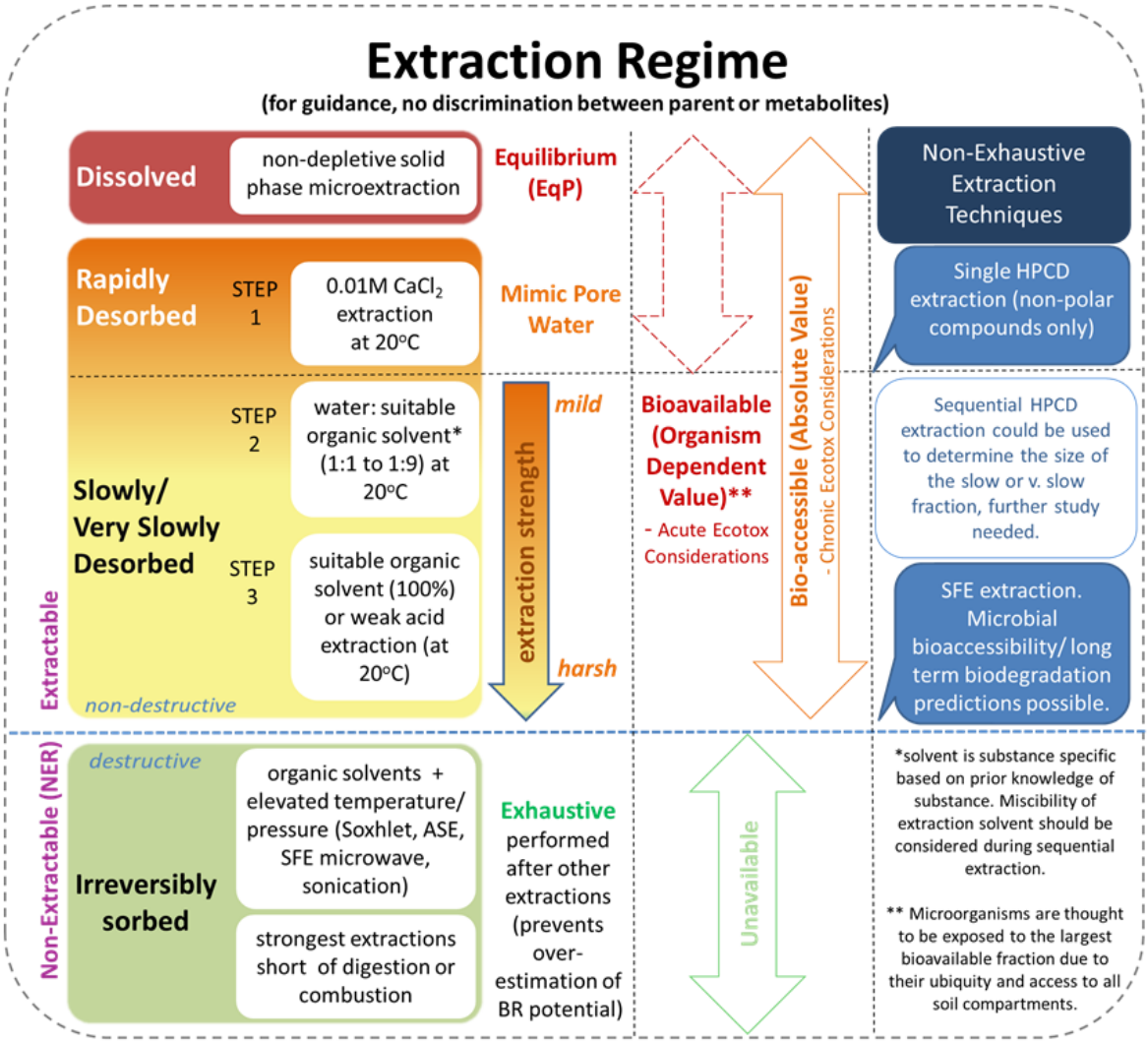
Two ECETOC task forces have considered how NERs should be treated in ERA. One task force looked at the relationship between extraction technique and bioavailability and the second one at the risk assessment of NERs. They have reviewed the literature and reported proposals on how to assess NERs (ECETOC, 2013a,b). These reports propose to use the following definitions of NER and BR based on those taken from a 2009 ECETOC workshop on Bound Residues (ECETOC, 2010):

- NER: A residue that is not extractable using 'mild' extraction methods, but extractable under harsher conditions. These conditions may include solvent extraction using methods such as refluxing, microwaves or accelerated solvent extraction (ASE). While these residues are strongly associated with the matrix, the binding may be potentially reversible; but the partitioning is very much in favour of 'binding' to components of the matrix. Therefore, for risk assessment purposes, the matrix-associated fraction is unlikely to be available to indigenous organisms.
- BR: A residue that is tightly associated with the solid matrix, often forming covalent (or similar) bonds. These residues usually cannot be released from the matrix or can only be released under extreme conditions where the integrity of the substance and/or matrix is likely to be affected. Such residues are often indistinguishable from natural organic matter e.g. humus in soil. These residues are not available

for either degradation or available for indigenous organisms and should not be considered in any impact/risk assessment.

More recently additional definitions of a Type 1, 2 and 3 NER have been proposed by UBA (Kästner et al, 2013). At the workshop on environmental persistence (ECETOC, 2013c) it was suggested that further research in this area may be required to give consensus on these definitions. The ECETOC task force which looked at the relationship between extraction technique and bioavailability has developed a framework model and extraction regime that can be used to identify the bioavailable and bioaccessible residues (ECETOC, 2013a,b).

Figure 4.1: Extraction methodology framework



According to Solomon et al (2013) NERs may be considered as if they were degraded for the purpose of PBT assessment because the risk of the NERs is low due to their reduced bioavailability. Boethling et al (2009) also move for only taking into account extractable residues in the persistence assessment. This is supported by the findings of the ECETOC workshop on "Significance of Bound Residues in Environmental Risk Assessment" as published in its report (ECETOC, 2010). Hence, bound residues that cannot be remobilised by mild extraction techniques, e.g. cold solvent-extraction, may be excluded from the mass balance and may

therefore not be considered for assessment of persistence. This is due to their limited bioavailability that makes them much less susceptible to biodegradation but also for showing harmful effects to organisms. The Scientific Committee on Health and Environmental Risks (SCHER) approved this approach in their scientific opinion for the herbicide aclonifen (SCHER, 2011).

A suggested framework for conducting risk assessments of NERs is outlined in a second ECETOC report (ECETOC, 2013b). This report builds upon a framework suggested at the ECETOC Bound Residues workshop in 2009 by introducing a tiered approach to the risk assessment of NERs (ECETOC, 2010). The framework also includes the concept of a 'soup test' to assess the sediment (or soil) ecotoxicity where real sediments or soils are used containing the NER (and/or transformation products) to understand any potential biological effects. This 'soup test' approach will be validated as part of a new CEFIC LRI project.

In conclusion, NERs are strongly bound to sediments and while adsorbed they are protected from degradation. Although these 'NERs' remain in the environment they are not bioavailable and therefore in the context of PBT assessment they should be considered equivalent to not being 'P' or 'vP'. As NERs are not bioavailable they will also not be bioaccumulative or toxic.

4.4 Field or monitoring studies

No new or additional information is available.

5. ASSESSMENT INFORMATION ON BIOACCUMULATION

5.1 Comparing bioaccumulation metrics

It is important to define the various terms and metrics used to describe bioaccumulation. The following brief definitions are adapted from those provided by the US EPA (2009b), Gobas et al (2009), and Burkhard et al (2012a). Additional details can be found in these publications.

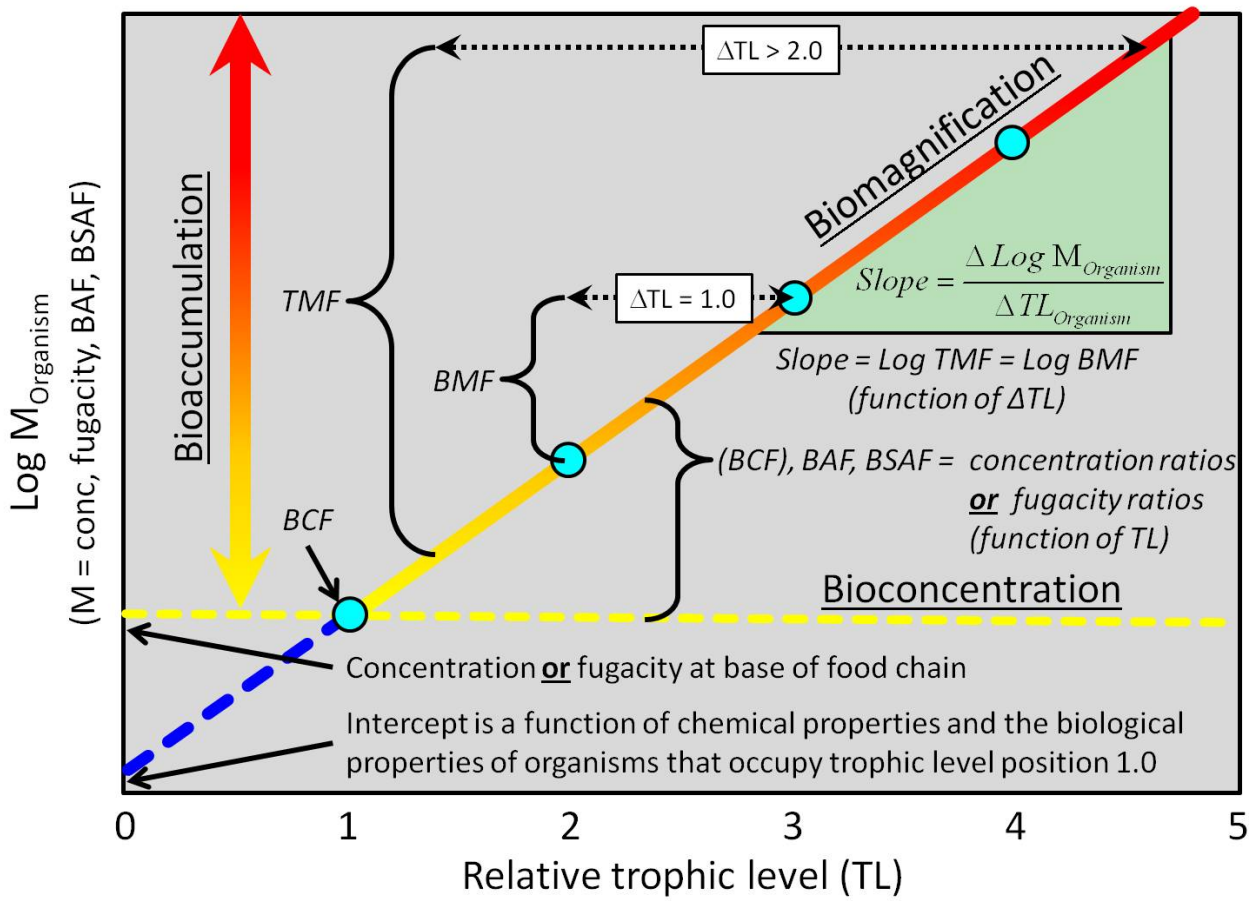
- Bioaccumulation: Net accumulation of a chemical by an organism resulting from uptake via all routes of exposure (sediment, water, and food).
- Bioconcentration: Net accumulation of a chemical by an organism resulting from uptake only through its surrounding environment via respiration and dermal routes.
- Biomagnification: Net accumulation of a chemical by an organism along a series of predator-prey associations, primarily through diet.
- Bioconcentration Factor (BCF): Ratio of the steady-state chemical concentration in an aquatic organism and the water. Exposure occurs via water.
- Bioaccumulation Factor (BAF): Ratio of the steady-state chemical concentration in an aquatic organism and the water. Exposure occurs via water and the diet.
- Biota-Sediment Accumulation Factor (BSAF): Ratio of the steady-state chemical concentration in an aquatic organism and the sediment.
- Biota-Soil Accumulation Factor (BSAF): Ratio of the steady-state chemical concentration in a terrestrial organism and the soil.
- Biomagnification Factor (BMF): Ratio of the steady-state chemical concentration in an organism and the concentration in its diet.
- Trophic Magnification Factor (TMF): The average factor by which chemical concentrations in the biota across an entire food web change per trophic level.

5.1.1 Comparing B metrics

Two recent articles highlight the use of the fugacity ratio method to collectively evaluate all bioaccumulation metrics using a WoE approach that can be graphically evaluated using a single plot (Burkhard et al, 2012a,b). This technique allows a visual interpretation of laboratory and field B measurements, if available, and provides a formal method for testing the hypothesis whether biomagnification occurs by testing whether fugacity ratios are greater than one and is described in detail below. The approach allows for an assessment of bioaccumulation potential by utilising all available data while avoiding potential errors in evaluations that rely solely upon BCF and/or K_{OW} (Arnot and Gobas, 2006; Kelly et al, 2007; Gobas et al, 2009). Burkhard et al (2013) provided a summary of the state-of-the-science on the measurement and potential applications of TMFs for the assessment of chemical bioaccumulation in food webs. The authors highlighted the relationship between the TMF, BMF, and other measures of chemical uptake and accumulation (BCF, BAF, and BSAF), as shown in Figure 5.1. The graph in Figure 5.1 illustrates how BCF/BAF and BSAF metrics describe 'points' of chemical bioaccumulation with reference to the water and sediment, respectively, whereas the BMF and TMF describe 'slopes' or changes in bioaccumulation across one or several ecological trophic levels,

respectively, which may include terrestrial food chains. A detailed description of the mathematical relationships between bioaccumulation metrics may be found in Mackay et al (2013).

Figure 5.1: Interrelationships between bioaccumulation, bioconcentration, and trophic magnification. Measures of bioaccumulation (BCF, BAF, BSAF) are individual points on the biomagnification line. Measures of trophic magnification (BMF, TMF) represent the slope of the biomagnification line, which is independent of unit terms of the y-axis if expressed on a common basis (Burkhard et al, 2013)



5.1.2 Trophic level adjustment

For field BMF values, a key issue is trophic level (TL) correction factors (Conder et al, 2012; Borgå et al, 2012), as several TLs may be involved in bioaccumulation; a well-characterised predator / prey trophic interaction is important for these metrics. The absence of such knowledge on BMF may account for the substantial variability of such measures (Gobas et al, 2009). The derivation of a field-based BMF value requires the availability of appropriate field data and may be used in situations where development of a TMF is not possible (e.g. insufficient trophic level sampling, incomplete characterisation of a food web). Field-derived biomagnification factors should be normalised for trophic level of predator and prey species (BMF_{TL}s), the same as TL- correction for biota–sediment accumulation factors ($BSAF_{TL}$s), and bioaccumulation factors (BAF_{TL}s), if data are available (Conder et al, 2012), as these measures are closely related to the biomagnification processes characterised by TMFs. Conder et al (2012) have proposed an equation for BMF that is directly comparable to TMF, as follows:

$$\mathrm{BMF_{TL}} = 10\text{^}\left[\frac{\log_{10}\left(\frac{\mathrm{C_{predator}}}{\mathrm{C_{prey}}}\right)}{\mathrm{TL_{predator}} - \mathrm{TL_{prey}}}\right] \quad \text{..... (Eq. 5.1)}$$

where $C_{predator}$ and C_{prey} are appropriately normalised chemical concentrations in the predator and prey (i.e. fugacity or lipid-normalised), respectively, while $TL_{predator}$ and TL_{prey} are the trophic levels of predator and prey, respectively.

As expressed in Equation 5.1, the BMF_{TL} represents a BMF normalised to a single trophic level increase in the food web. In food webs where the proportions of prey items in a predator's diet can be quantified (Hop et al, 2002), a weighted average C_{prey} may be used if all concentrations have been measured. An evaluation of BMF_{TL} values by Conder et al (2012) found that such values may be an appropriate surrogate when TMF data are unavailable, though BMF_{TL} values may overestimate TMFs in cases where biomagnification would not be indicated by a TMF > 1.

The biota / sediment accumulation factor (BSAF) should also be normalised by trophic level to calculate a trophic-level normalised $BSAF_{TL}$ value, as noted by Conder et al (2012) in this equation:

$$\mathrm{BSAF_{TL}} = 10\text{^}\left[\frac{\log_{10}\left(\frac{\mathrm{C_{biota}}}{\mathrm{C_{sediment}}}\right)}{\mathrm{TL_{biota}} - \mathrm{TL_{sediment}}}\right] \quad \text{..... (Eq. 5.2)}$$

where C_{biota} and $C_{sediment}$ are the appropriately lipid- and carbon-normalised biota and sediment concentrations, respectively, and TL_{biota} and $TL_{sediment}$ are the trophic levels of the sampled organism and sediment, respectively; the latter value is typically set to 1.0 (Vander Zanden and Rasmussen, 1996).

The $BSAF_{TL}$ may be more useful than the traditional BSAF values, as the former may be more readily comparable to the TMF food web. Conder et al (2012) noted a correlation coefficient (r^2) of approximately 70% ($p < 0.05$) when comparing $BSAF_{TL}$ to TMF values for phthalate esters and PCB congeners in a marine food web (data from Mackintosh et al, 2004). $BSAF_{TL}$ values were observed to be consistently smaller than TMF values, perhaps due to differences in sorptive capacity (i.e. fugacity capacity) for lipid and organic carbon (Seth et al, 1999) and/or chemical disequilibria between sediment and water (Mackintosh et al, 2004).

The bioaccumulation factor (BAF) is typically a field-measured metric and consists of the ratio of the chemical biota concentration to the water concentration or C_{biota}/C_{water}; the biota concentration can be based on either a wet-weight or lipid-weight basis. The field-derived nature of the BAF differentiates it from the laboratory-based BCF metric, as the BAF obviously involves organisms that may be exposed to all inputs of chemicals, including dietary uptake. This distinction between BAF and BCF makes it possible to infer the level of measured trophic magnification (accumulation via dietary transfer) with the calculation of a trophic level-normalised BAF_{TL} value. This method utilises a laboratory BCF value as a prey species and a field-derived BAF value for a predator species, according to this equation (Conder et al, 2012):

$$\mathrm{BAF_{TL}} = 10\text{^}\left[\frac{\log\left(\frac{\mathrm{BAF}}{\mathrm{BCF}}\right)}{\mathrm{TL_{biota}} - \mathrm{TL_{lab}}}\right] \quad \text{..... (Eq. 5.3)}$$

where BAF and BCF are the field-derived accumulation and laboratory-derived concentration factors (l/kg bw, wet, lipid or protein weight), respectively; while TL_{biota} and TL_{lab} are the trophic levels of the organism in the field or laboratory. BAF_{TL} should not be confused with the BAF.

5.1.3 Fugacity approach

Collective analysis of all bioaccumulation metrics would be useful in a WoE analysis of 'B'. One of the current difficulties in comparing BCF/BAF data to other bioaccumulation metrics such as BMF, BSAF, and TMF is the difference in numerical scale and reference media to which tissue concentrations are normalised. For example, BCFs and BAFs have values on the order of 10^0 to 10^8 while BMFs, BSAFs, and TMFs have values on the order of 10^{-4} to 10^2. BCFs and BAFs express ratios of chemical concentrations in biota to water, whereas BSAFs represent ratios of chemical concentrations in biota to sediment or suspended solids, while BMFs and TMFs reflect ratios of chemical concentrations in different predator/prey species.

It should be noted that the applicability domain of the fugacity ratio approach is limited to non-ionic organic chemicals. In addition, this methodology is not applicable to chemicals that "bind to tissues other than lipids" (e.g. proteins) or have active uptake mechanisms (Burkhard et al, 2012b).

A fugacity ratio expresses the status of chemical distribution relative to a state of chemical equilibrium, which is thermodynamically defined as a situation where the fugacity ratio is 1. A fugacity ratio greater than 1 indicates that the chemical in the organism is able to achieve a higher fugacity (or chemical activity) than that in medium to which it is exposed. In that case, the organism is able to magnify the chemical potential in its environment (Section 5.4). A fugacity ratio less than 1 implies that the chemical concentration is less than its thermodynamic equilibrium value in the medium to which it is exposed. Connolly and Pedersen (1988) were among the first ones to use fugacity ratios to determine the biomagnification of PCBs in Lake Ontario. They showed that the fugacity of high K_{OW} PCBs in organisms of the lake Ontario food-web are greater than the fugacity in the water and increase with increasing trophic level. They concluded that these high K_{OW} PCBs are mainly absorbed by organisms via their diet and biomagnify.

The application of fugacity ratios to determine the bioaccumulative nature of food-web chemicals was explored in a 2009 HESI/SETAC Workshop and the findings published by Burkhard et al (2012a,b). The authors concluded that the fugacity ratio approach is a practical framework for evidence-based decision making in chemical management. The fugacity approach has a number of advantages. First, the approach can make use of all laboratory and field data for which bioaccumulation metrics exist and it can use data from monitoring programs as well. Secondly, the fugacity ratios are able to express all bioaccumulation measures (i.e. BCF, BAF, BSAF, BMF, TMF) on a similar basis, i.e. as a dimensionless ratio. Third, the fugacity ratios can be visualised in a simple manner that allows all data to be viewed together and in relation to each other; this makes the detection of data gaps and outliers relatively easy. Most importantly, the fugacity ratio approach provides a formal method for testing the hypothesis whether biomagnification occurs, by testing whether fugacity ratios are greater than one. This approach provides a more holistic assessment of bioaccumulation potential by utilising all available data while avoiding potential errors in evaluations that rely solely upon BCF and/or K_{OW} (Arnot and Gobas, 2006; Kelly et al, 2007; Gobas et al, 2009).

The bioaccumulation endpoints of BCF and BAF can be transformed into approximate fugacity ratios by converting their standard denominators (i.e. dissolved chemical concentrations in water) to their equivalent concentrations in lipid by multiplying the denominator by the n-octanol/water partition coefficient (K_{OW}) of the chemical; in essence, dividing the lipid-normalised and freely-dissolved BCFs ($BCF_{L/fd}$) and BAFs by their substance-specific K_{OW}. With conversion of the $BCF_{L/fd}$ and other metrics to a fugacity ratio (i.e. $BCF_{L/fd}/K_{OW}$), all bioaccumulation endpoint metrics can be directly compared numerically. This is the advantage of fugacity

ratios, as they allow comparison of a chemical's bioaccumulation potential across a wide range of laboratory and field bioaccumulation metrics, thereby allowing a visual WoE evaluation of all available data.

An important caveat to note is the assumption that lipids are the only relevant phase in biota for storing hydrophobic organic chemicals. It is further assumed that lipids and octanol have equivalent fugacity capacities for an organic chemical, i.e. $Z_{lipid} = Z_{octanol}$, as Mackay (1982) proposed n-octanol to be a reasonable surrogate phase for lipids in biological organisms. However, this assumption may vary widely for different organic chemical families, for poikilothermic versus homeothermic organisms, and for different types (membrane versus storage) of lipids. The fugacity capacity issue with octanol and lipids is currently an active area of research (Seston et al, 2014).

5.2 Bioconcentration or bioaccumulation studies (aqueous and dietary)

Maintaining constant aqueous exposure concentrations in BCF tests often presents a significant obstacle. Problems are routinely encountered for chemicals that are readily biodegradable or subject to rapid abiotic loss processes. For example, the air-water partition coefficients for aliphatic hydrocarbons increase with carbon number since the water solubility decreases more rapidly than the vapour pressure. Consequently, maintaining constant aqueous concentrations for this class of chemicals under flow-through conditions is difficult (OECD, 2000b). Solvents or dispersants may be used to enhance dissolution of poorly water-soluble substances but this approach may influence test results and interpretation. Moreover, this experimental design may yield emulsions rather than true solutions, confounding the analysis of bioavailable exposure concentrations. Physical effects on the organisms may also result from this dosing procedure. Even if care is taken to prevent emulsions, the interpretation of BCF tests for poorly water-soluble substances is often complicated by bioavailability considerations of the aqueous phase measurements which typically include compound in both freely dissolved plus colloid-bound forms (Gobas and Morrison, 2000). Another challenge is extraction and analytical detection of parent compounds in fish tissue. Biotransformation processes that limit the absolute amount of analyte present in this sample matrix may further hamper analysis.

For all of the reasons mentioned above, a new dietary bioaccumulation test methodology was developed in the early 2000s, using a dosing system via the feed instead of dosing the chemicals through the dissolved phase. This methodology was validated in a ring test involving nine laboratories (OECD, 2011a) and it has been incorporated in the revision of the OECD 305 guideline (OECD, 2011b). The dietary bioaccumulation test method can be applied to highly hydrophobic substances (log K_{OW} values > 5) with very low water solubilities (< 0.01 - 0.1 mg/l), for which performing an aqueous bioaccumulation test becomes increasingly difficult.

The dietary bioaccumulation protocol allows testing the potential for bioaccumulation of substances which otherwise could not be tested, but some uncertainties remain regarding the generation and regulatory interpretation of test data:

- As for any bioaccumulation study, corrections for fish growth rate and lipid content need to be performed (Woodburn et al, 2013).

- The reliability of the generated uptake and depuration rates needs to be assessed, especially in the depuration phase if the uptake phase has not reached steady state.
- The parameter derived with a dietary bioaccumulation test is a Biomagnification Factor (BMF), which shows the potential of the substance to biomagnify in the food chain through dietary uptake. It should be realised that exposure via the water is carefully avoided in this testing method and the value obtained cannot directly be compared to BMF values from field studies, which might integrate exposure through the diet and other routes (e.g. water) (Mackay et al, 2013; Weisbrod et al, 2009).
- A kinetic BCF (BCF_k) can be estimated from the BMF obtained in the dietary study if reliable uptake and depuration rates are available. Different methods exist to estimate the BCF_k from dietary bioaccumulation data (UK Environment Agency, 2011), but it has to be kept in mind that considerable uncertainty can be associated with the resulting BCF value. Besides, the applicability of different calculation methods still needs to be thoroughly evaluated. Recent publications have proposed the use of depuration rates (k_2) obtained from bioaccumulation studies as an alternative estimate for bioaccumulation potential (Goss et al, 2013; Brooke and Crookes, 2012). This could potentially be a step forward, as it allows the use of parameters obtained directly from dietary bioaccumulation studies in substance bioaccumulation assessments.
- Regulatory discussions around the interpretation and use of dietary magnification data in the context of PBT evaluation are still being held in the EU. Currently, the only criteria for B evaluation included in Annex XIII of the REACh regulation are BCF values of 2,000 (B criterion) and 5,000 (vB criterion). A BMF of 1 has often been associated with a BCF of 5,000, indicating a high potential to bioaccumulate. Some publications are available comparing the two parameters and showing a relationship (Inoue et al, 2012, see plot); however, a systematic comparison of available data is needed in order to reach a robust conclusion that might be used in regulatory decision making. An ILSI-HESI project developing such database is already underway (Arnot, 2013).

There have been several developments that could aid the further implementation of the use of dietary bioaccumulation results in PBT assessment. An expert workshop on bioaccumulation was held in 2012 before the SETAC meeting in Berlin, in order to discuss the latest advances in the quantification of bioaccumulation potential (SETAC Globe, 2012). One of the conclusions of the workshop was that more work needs to be put into developing integrated assessment approaches to support bioaccumulation assessment. Using dietary accumulation data in conjunction with all other available (and reliable) data on a substance in a true WoE approach will help reaching scientifically sound conclusions on the bioaccumulation potential of chemicals in the environment. Besides, the OECD has organised a task force to develop guidance on the interpretation of dietary bioaccumulation results, which will possibly be available in the near future.

5.3 Use of field biomagnification factors (BMFs) and trophic magnification factors (TMFs)

An important property to evaluate for international management of possible PBT chemicals, including persistent organic pollutants (POPs), is the potential for a substance to biomagnify in food chains. Such biomagnification can result in increased exposure to wildlife and higher predators in the environment and is a concern because of the potential for increased concentrations that could result in significant adverse effects. In the initial screening of substances, the potential for biomagnification is addressed by evaluating a substance's bioaccumulation potential, using measured or estimated bioconcentration and bioaccumulation

factors, in aquatic organisms – especially fish. Research has established that several factors influence the relative importance of bioconcentration and thus the potential for biomagnification of substances, particularly in aquatic food webs.

The key processes controlling accumulation of chemicals in aquatic organisms involve respiratory and dietary uptake, gill and gastrointestinal elimination kinetics, and metabolic transformation capacity. These processes are also influenced by a variety of physical-chemical properties and various biological factors related to organism physiology (Thomann, 1989). Research has revealed that at naturally-occurring food/water concentration ratios, uptake of highly hydrophobic chemicals (i.e. $\log K_{OW} > 6$) from water into biota is generally low compared to uptake via consumption of contaminated foodstuffs, with the importance of dietary uptake increasing with increasing K_{OW} values (Thomann, 1989; Qiao et al, 2000).

Current global regulatory B criteria are based essentially on BCFs, and to a lesser extent on log K_{OW}s, with dietary BMFs (for fish) gaining recent acceptance through the addition of the dietary test guideline in the recent OECD 305 revision (OECD, 2012). Prior to the 2011 REACh Annex XIII revision, assessments were focussed solely on the aquatic food chain. The amended REACh Annex XIII introduces as 'assessment information' the item "information on the ability of the substance to biomagnify in the food chain, where possible expressed by biomagnification factors or trophic magnification factors". While Annex XIII itself does not define any numerical threshold value for BMF or TMF metrics, the relevant PBT Guidance document indicates that a substance should be considered to be bioaccumulative if its BMF or TMF values exceed 1.0 (ECHA, 2012) which is in line with a previous recommendation (Gobas et al, 2009).

Traditionally, bioaccumulation assessment of chemicals has been based on results from controlled laboratory tests, such as the BCF and BAF tests, almost exclusively in aquatic systems. It has been proposed that bioaccumulative substances should be defined as substances which biomagnify in the food web – i.e. lipid-normalised concentrations increase with higher trophic position. A 2008 SETAC POPs/PBT workshop proposed a flowchart for assessment of bioaccumulation criteria that incorporates these food web metrics. The flowchart indicates that a field TMF value is the most conclusive criterion from which to determine chemical bioaccumulation, followed by a dietary or field biomagnification factor (BMF) value, then a water BCF or BAF value, with an estimate based on physico-chemical parameters or food-web modelling generating the lowest degree of confidence (Gobas et al, 2009).

It is the intent of this document to provide constructive input to any future revision of the REACh PBT Guidance. This chapter will provide an overview of the state of the science related to TMF and BMF metrics and specifically discuss the potential relevance and usefulness of these measures as B metrics in a WoE assessment, as a complement to the well-established, often used lower tier metrics such as BCF and BAF. In addition, this chapter will identify and discuss the difficulties and pitfalls associated with designing, performing, and interpreting field BMF and TMF studies.

5.3.1 Potential advantages of field BMFs and TMFs compared to lower-tier metrics

Comparison to other B metrics

As summarised above, the tiered approach proposed by Gobas et al (2009) prioritises the various metrics according to their 'conclusiveness' as indicators of bioaccumulation (field TMF > BMF > BCF/BAF > modelling/non-testing methods). This evaluation was based on the following conclusions that (i) BCF/BAF values were no longer recognised to be good descriptors of bioaccumulation of chemical substances, particularly for highly hydrophobic substances (log K_{OW} > 6) and for higher organisms, as these metrics do not quantify biomagnification via the food web; (ii) B evaluation based on K_{OW} does not recognise biological or environmental factors and may produce numerous false positives; (iii) information from field studies (i.e. TMF and field BMF) provides the most conclusive evidence of the ability of chemicals to biomagnify in food webs, i.e. when TMF or BMF values are routinely > 1; and (iv) BCFs are viewed as less accurate than TMFs and BMFs in quantifying biomagnification (Weisbrod et al, 2009; Gobas et al, 2009; Borgå et al, 2012).

BCFs and BAFs remain useful for characterising bioaccumulation as a result of the transfer of chemicals from abiotic environmental compartments to lower trophic levels. However, laboratory BCFs are generally less accurate in predicting biomagnification, as noted previously, due to the fact that such metrics do not quantify chemical bioaccumulation via the diet, only from water; dietary bioaccumulation is responsible for biomagnification in food webs (Gobas et al, 2009). As a result, BCFs are problematic at predicting bioaccumulation behaviour of highly hydrophobic chemicals (i.e. log K_{OW} > 6), where food digestion and absorption concentrates the chemical in the gastro-intestinal tract (GIT), producing biomagnification via the diet but not necessarily via the water column (Gobas et al, 2009). The rate of biotransformation (i.e. first-order metabolism rate constant [k_m] values) can also be an important consideration, as high BCFs for some chemicals (BCF > 5,000 l / kgbw wet weight) may not necessarily be indicative of the occurrence of biomagnification due to biotransformation. For example, high fish BCF values have been observed for some chemicals (e.g. aromatic esters), which are quickly degraded in the intestinal tract after ingestion but which may be more slowly degraded by organisms after respiratory exposure (i.e. gill uptake). Such chemicals are subject to chemical transformation in the intestines, which lowers overall and hence lipid-normalised concentrations and therefore causes a reduction or absence of biomagnification. However, this is not revealed in aqueous exposure bioconcentration tests because bioconcentration tests exclude dietary exposure. In addition, BCFs with aquatic organisms have been shown to be inadequate for assessing bioaccumulation potential in food webs that include air-respiring organisms (Kelly et al, 2007; Kitano, 2007).

BMFs and TMFs allow consideration of non-aquatic systems

The early biomagnification research primarily focused on chemical distribution in aquatic food webs. In recent years, research has expanded into chemical behaviour in avian and terrestrial organisms as well. One of the key observations has been that some less hydrophobic chemicals (i.e. log K_{OW} < 5) such as chlorobenzenes and lindane were found to exhibit high levels of biomagnification in terrestrial or marine mammal food webs (Kelly and Gobas, 2003; Kelly et al, 2007). Another useful example is provided by

perfluorooctane sulphonic acid (PFOS), a substance showing a low level of dietary biomagnification in laboratory studies with fish (Martin et al, 2003), but for which field BMFs greatly exceeding 1.0 have been reported for predators that are terrestrial mammals or avian species (e.g. Tomy et al, 2004; Haukås et al, 2007; Müller et al, 2011) and for which high TMFs have also been published for food chains in which such species are the top predators (Tomy et al, 2004; Müller et al, 2011). Nevertheless, high field BMFs and/or TMFs were also reported for PFOS in many studies on the purely aquatic segments of food chains (Tomy et al, 2004; 2009; Houde et al, 2006; Powley et al, 2008; Kelly et al, 2009).

TMF values represent an average over a range of trophic levels

TMF values can be considered as an 'average' indication of a chemical's biomagnification within a food web, as the values are integrated over a wide range of tropic levels. The TMF approach assumes that diet is the major route of exposure to contaminants, and that trophic level status is the main driver for relative accumulation of contaminants in organisms within a food web. The principal advantage of the TMF is that it quantifies the chemical biomagnification behaviour as it occurs in the field. Field-derived TMFs are considered to represent a more conclusive, decisive, and holistic measure of biomagnification than laboratory metrics or BMF values from single predator-prey relationships (Gobas et al, 2009; Borgå et al, 2012; Weisbrod et al, 2009). However, regulators or interested stakeholders often must evaluate biomagnification data of several TMF studies. The results of numerous studies should be weighted according to the statistical power of the results, and the data should be considered in the context of the practicalities of the study design. Considerations include a proper balancing of samples among multiple trophic levels in a food web (e.g. benthic versus pelagic), and other considerations such as age, feeding strategy, life history, etc). Borgå et al (2012) have provided examples and recommendations regarding study design, data treatment, and statistical analysis.

Summary

In brief, there are numerous valid scientific reasons why a proposed hierarchy generally exists in the realm of 'B' metrics, with TMF > BMF > BCF/BAF > modelling/non-testing methods. This perspective should be considered when evaluating data on 'B' with regard to a chemical under review.

5.3.2 Difficulties and pitfalls associated with designing, performing, and interpreting field BMF and TMF studies

Recent research has produced an assortment of issues concerning evaluation of field BMF/TMF studies to aid in properly identifying bioaccumulative substances (Gobas et al, 2009; Conder et al, 2012; Borgå et al, 2012). Initially, food web data concerning air- and water-respiring biota should be separated due to the fundamental differences in the bioaccumulation behaviour of these organisms. Once completed, the general scientific consensus is that chemicals are considered bioaccumulative if they exhibit a TMF > 1, in either air- or water-respiring biota in the relevant food chain. However, comparison of study-derived TMF estimates to this threshold value should be based on statistical analyses such that variability is quantified and errors in bioaccumulation classification are minimised.

Non-applicability

Field BMFs and TMFs clearly cannot be determined prospectively, for new substances that have not yet been emitted to the environment, or have not been present long enough for the analytical limit of quantification to have been reached.

Non-achievement of steady-state concentrations

When a BMF is derived from the ratio of the concentration in the predator to that in its prey, it is tacitly assumed that a steady-state concentration is attained in each organism. If this is not the case, the calculated BMF will become an increasingly less accurate (i.e. more meaningless) predictor of B, the greater the departure from steady state in the predator and/or prey (which is hardly even knowable).

A steady state may not be achieved in practice, in the case of field studies, on account of varying emissions and hence environmental concentrations of a chemical. In addition, the steady state is driven by physiological and behavioural changes in the predators and prey, including growth, reproduction (transfer of contaminants to offspring through placenta or milk), and seasonal variations. For instance, the feeding rate (and hence contaminant intake) varies considerably throughout the year for hibernating animals, while the nature of the diet varies with season for many species.

Poorly understood or misinterpreted feeding ecology

Studies often report BMFs calculated by uncritically dividing the concentration of a substance in a predator or consumer organism (C) by that in a prey or diet organism (D) at a lower trophic level, assumed to be the (sole) source of food for C. For a given C, the study may report BMFs calculated for a number of distinct Ds, leading to an array of erroneous BMF values. In such a multiple-prey situation, the diet concentration to be applied should be an average for all the Ds, weighted according to their respective contributions to the diet. Some authors correctly take this into account, but many do not.

Scavenging of a predator on contaminated anthropogenic waste materials, rather than on its natural preys, may bias the reported BMF.

Predators and preys may not be co-located in time and space. For instance, some studies report levels of a chemical in samples of C and D collected in different years. Migration (e.g. of birds) can also bias the calculated BMFs, since, although the predators may be temporarily co-located with their prey at the time the study samples are taken, their body burdens of a chemical may have built up (or down) during their seasonal absence from the habitat studied, with a resulting change in diet.

Metabolism of precursor compounds

The presence of a given compound in predator and prey organisms may not result solely from the uptake of this compound from environmental media and with diet, up a trophic chain. It may also be due to metabolism of structurally related precursor compounds in the predator and/or prey. Since this confounding

phenomenon (like several others mentioned here) is almost impossible to take into account, it is generally ignored.

Variation of BMFs with trophic level and ability to predict TMFs

BMF values are not expected to be for every predator/prey pair in a food web, even if the different pairs considered are all separated by a unit TL difference. Indeed, it can be demonstrated mathematically (Goss et al, 2013) that the BMF will depend on (i) the elimination half-life in the various predators, which depends in turn on their particular physiology; (ii) the predators' average specific feeding rates, which may be much greater for mammals and birds than for fish or, more generally, for homeotherms compared to poikilotherms; and (iii) the uptake efficiencies of the chemical by the various predators.

A consequence of the postulated variation of BMF with TL is that the plots used to derive TMFs (logarithms of concentrations versus TL) will not be linear.

Conder et al (2012) examined the predictive ability of trophic level-adjusted BMF values (Eq. 5.1) to predict TMF values greater than or less than one; the analysis included data for phthalate esters, PFOS, polycyclic aromatic hydrocarbons (PAHs), bromodiphenyl ether (BDE) congeners, polychlorinated biphenyl (PCB) congeners, and methylmercury from studies conducted in a variety of aquatic ecosystems. The calculated BMF_{TL} values were generated from predator-prey relationships for fish and invertebrate species, which differed in trophic level position by 0.5 or more. The BMF_{TL} values showed a large variability for any particular chemical but in the majority of cases the range in BMF_{TL} values included the measured TMF. This is not unexpected, as the TMF may be viewed as the average BMF for the food web under examination. For datasets that exhibit a TMF that is significantly greater than 1.0 (biomagnification), a majority of calculated BMF_{TL} values were also greater than 1.0. For datasets that exhibit a TMF that is not significantly greater than 1.0 or may even be lower than 1.0 (biodilution), there was less agreement, with 15 of 18 datasets having a BMF_{TL} value greater than 1.0, i.e. these are 'false positives' for predicting an accurate TMF value. Similar conclusions regarding the accuracy of BMF_{TL} values to accurately predict TMF values have been reached by other researchers. In Arctic marine food web studies, 70 to 90% of invertebrate/fish BMF_{TL} values concurred with the corresponding TMF values in terms of greater or less than 1.0 (Fisk et al, 2001; Hop et al, 2002; Hoekstra et al, 2003). The discrepancies between TMF and BMF_{TL} values likely reflect the difficulty of a single predator-prey relationship accurately representing the overall degree of biomagnification or biodilution occurring across an entire food web.

In summary, the review of the available BMF_{TL} versus TMF values indicates that BMF_{TL} values may be an acceptable estimate of chemical ecosystem behaviour when TMF data are unavailable, though BMF_{TL}s may overestimate TMFs in cases where biomagnification would not be indicated by TMF > 1. As a result, the use of BMF_{TL} to estimate bioaccumulation potential is best performed within a WoE approach.

Normalisation to lipid or protein levels

For lipophilic chemicals, it is customary to normalise the concentrations of contaminant in predator and prey to their respective lipid levels. For proteinophilic chemicals (such as the perfluoroalkyl substances), normalisation to protein levels has been advocated and sometimes applied. But there does not appear to be

consensus on whether/how to apply this practice (for instance, some chemicals may be neither unequivocally lipophilic nor proteinophilic).

Statistics

Virtually all statistical analyses needed for B assessment may be performed using the Data Analysis Tool Package, as provided with Microsoft Excel 2003 (version 11.0), and a Type I error (α) of 0.05 is commonly used as a benchmark to judge the significance of statistical tests (Fisk et al, 2001; Gobas et al, 2009). Key concentration data typically involve naturally-occurring biota and sediment samples, and a single-factor analysis of variance (ANOVA) may be used to test for differences, for example, in mean concentrations of chemical concentration within a trophic food web structure. If the ANOVA omnibus F value indicates significant differences between the mean values, a Tukey's HSD multiple comparisons test (for equal sample sizes) or a Tukey's-Kramer multiple comparisons test (for unequal sample sizes) may be used to compare individual mean values. For TMF evaluation, logarithmic transformation of concentration data is used to generate normally distributed data for regression and correlation analyses. A more detailed description of statistical issues associated with TMF and other B measures may be found in Conder et al (2012).

As discussed in detail by Borgå et al (2012) and Conder et al (2012), the total number of samples taken, unbalanced sampling of the food web, the range of TL values, and uneven replication of samples may significantly influence the statistical determination of a compound's TMF value. In brief, not all observations will have an equal contribution to the TMF regression; as one or more observations may have substantial influence on overall conclusions. This was recently demonstrated by McGoldrick et al (2014), which showed that the number and overall range of TLs included in the TMF regression significantly affected the slope of the regression line for cyclic siloxane materials in the Lake Erie food web. Exclusion of the lowest and highest members of the food web (e.g. plankton and walleye) caused the calculated TMF values to change from < 1 (biodilution) to > 1 (biomagnification).

An issue considered critical to many regulators and stakeholders is whether or not a TMF exceeds the biomagnification threshold value of 1.0. Statistical hypothesis testing can often be useful in helping to characterise and quantify the uncertainty of datasets used for evaluating biomagnification potential. Borgå et al (2012) performed an extensive evaluation of approximately 80 TMF studies on a wide range of chemicals, primarily in North American aquatic ecosystems. The authors noted that variability of the slope of the log (Lipid-adjusted concentration) versus trophic level regression was not consistently related to sample size, TMF value, or chemical class. They observed that study designs with N = 30 to 40 samples (total) would only have been able to detect regression slopes with an absolute value greater than 0.3 to 0.5 (equivalent to TMF values of less than 0.3 to 0.5 or greater than 2.0 to 3.2) as being statistically different from a slope of zero. As a result, such study designs would have been unable to detect significant (i.e. $p < 0.05$) regression slopes for chemicals with apparent TMF values in the range of 0.5 to 2.0. Such results indicate that the variability of past study designs is a key issue in TMF evaluation, and that only large sample sizes (N > 60 - 100) are anticipated to consistently detect significant regression slopes for chemicals with apparent TMFs in the range of approximately 1.5 to 2.0. Conversely, experimental designs with fewer than 30 to 40 samples and a standard level of variability are unlikely to detect statistically significant regression slopes for contaminants with TMF values near the lower limits of regulatory relevance, i.e. 0.5 to 2.0.

5.3.3 Calculation of TMF

TMF values have been traditionally determined based on equations reported in Jardine et al (2006). Trophic level assignment for each food web species is calculated relative to a baseline species, typically a freshwater zooplankton or benthic invertebrate species. Trophic level for all macroinvertebrates and fish is generally determined by analysing the stable nitrogen isotopes ^{15}N and ^{14}N and measure the ratio ^{15}N:^{14}N to characterise the composition of their diet (further explained below, under 'Use of benchmark chemicals'). The shift or deviation of the ratio ^{15}N:^{14}N ($\delta^{15}N$) is then used as a continuous variable to calculate TL as follows:

$$TL_{Consumer} = TL_{Base} + [(\delta^{15}N_{Consumer} - \delta^{15}N_{Base})/\Delta^{15}N\ EF] \quad \text{(Eq. 5.4)}$$

where TL is the trophic level of the consuming (predator) organism, $\delta^{15}N$ is the deviation of the stable isotope ^{15}N:^{14}N ratio of its diet from the baseline ratio (of the prey), and $\Delta^{15}N$ EF is the trophic enrichment factor (EF) constant for $\delta^{15}N$ per unit TL ascent in the food web.

For aquatic poikilothermic food webs, the trophic EF constant for $\delta^{15}N$ ($\Delta^{15}N$ EF) used to calculate TL ranges from 3.0 to > 5.0 ‰ per trophic level step (Jardine et al, 2006; Post, 2002; Minagawa and Wada, 1984; Deniro and Epstein, 1981). Typically, a value of 3.4‰ per trophic level step has been recommended for constructing food webs without prior knowledge of $\Delta^{15}N$ enrichment or the ecology of the system (Deniro and Epstein, 1981; Minagawa and Wada, 1984; Post, 2002). Linear regressions of log-transformed, lipid-normalised chemical concentrations versus TL are often used to determine TMF values, as shown below:

$$\log_{10}[C_{Lipid}] = \alpha + m_{TL}TL \quad \text{(Eq. 5.5)}$$

where C_{Lipid} is the chemical concentration in biota, corrected for lipid content, TL is the trophic level assigned to the species under analysis (Equation 5.4), and α and m_{TL} are the intercept and slope of the linear regression line C_{Lipid} versus TL, respectively.

The slope *m* is defined as follows:

$$m_{TL} = \log C_{Lipid(TL = N)} - \log C_{Lipid(TL = 1)} / \Delta TL \quad \text{(Eq. 5.6)}$$

The slope (m) is then used to calculate TMF as:

$$\log TMF = m = \log C_{Lipid\ (TL = N)} - \log C_{Lipid\ (TL = 1)} / \Delta TL \quad \text{(Eq. 5.7)}$$

The slope (*m*) of a linear regression model is independent of unit terms of the y-axis when expressed on a common basis. Consequently, the slope of the biomagnification regression model, i.e. log TMF (Equation 5.7) may be obtained by regressing log-transformed concentrations (C_{Lipid} or CONC) or fugacities ($f_{Organism}$ or FUG) or concentration ratios (BAF or BSAF) on trophic level (TL). TMF values are generally independent of unit basis, as depicted by the relationship:

$$TMF = TMF_{CONC} = TMF_{FUG} = TMF_{BAF} = TMF_{BSAF} \quad \text{(Eq. 5.8)}$$

where TMF_{CONC}, TMF_{FUG}, TMF_{BAF}, and TMF_{BSAF} refer to TMF values calculated via lipid-normalised concentrations, fugacities, biota/water concentration ratios, and biota/sediment concentration ratios, respectively.

However, TMF values may be skewed or biased as a result of:

- Localised hot-spots of chemical exposure (point-source emissions).
- Exposure across concentration gradients (sediment-water interface).
- Variable inputs of chemical into food web (omnivorous feeding).
- Migrating species exposed to different conditions than local food web.

It is possible to correct for such bias by calculating TMF on basis of BAF and BSAF, which correct for varying aqueous and sediment concentrations, respectively, of the solute of interest. As a result, within a homogenous system: $TMF_{BAF} = TMF_{BSAF} = TMF_{FUG}$ and within a heterogenous system: $TMF_{BAF} = TMF_{BSAF} \neq TMF_{FUG}$. In brief, the calculation of TMF in a heterogenous system can result in misleading TMF values if it is simply based on a lipid-normalised or fugacity basis without correcting for potential biasing issues. Use of water- or sediment-based biota concentration ratios (i.e. BAF or BSAF) in Eq. 5.7 will produce TMF values that correct for potential bias (Powell, 2012).

Use of benchmark chemicals

In this section, two key issues are discussed: (i) the use of a benchmark chemical, such as PCB180, to calibrate the ^{15}N enrichment factor on a food web; and (ii) the use of a positive control chemical, such as PCB153, to verify the calibration with calculation of a TMF value within the accepted range for that chemical. The former issue concerns the widespread use of a $\delta^{15}N$ enrichment factor of 3.4‰ as a default value for calculation of TL values. This enrichment factor value is influenced by food web structure, ecosystem dynamics, climate, and other factors. Use of both benchmarking and positive control chemicals aids the researcher in proper TMF evaluation and calculation.

Field bioaccumulation metrics (e.g. BMF, BSAF, and TMF) are dependent upon accurate trophic level (TL) assignment and TL values are generally determined through the use of stable isotope analysis of biota tissue (Equation 5.1). For TL analysis, a majority of carbon and nitrogen atoms exist as ^{12}C and ^{14}N, and a small percentage of these elements exist as stable isotopes, ^{13}C and ^{15}N, respectively, known as heavy isotopes due to the extra mass of their additional neutrons. In nature, all materials, including the tissues of organisms, contain some mixture of light and heavy isotopes. Stable isotope ratios can be used to solve many problems in ecology, among them the dynamics of feeding relationships in natural food webs. In general, the increase in nitrogen ratios (^{15}N:^{14}N $\delta^{15}N$) shows that “you are what you eat, +3‰ heavier”. In other words, a predator’s tissues will be enriched by approximately 3‰ in ^{15}N over the ratio in prey items. This results from the fact that the lighter isotope, ^{14}N, undergoes amino acid deamination and transamination reactions more easily to produce lighter metabolites, leaving the remaining nitrogen pool more enriched in ^{15}N (Vander Zanden and Rasmussen, 2001; Mill et al, 2007). At each successive trophic link, the consumer’s diet contains prey that are therefore more isotopically enriched in ^{15}N, so that $\delta^{15}N$ values of different organisms in a food chain reflect their relative trophic position. Typically, consumers are +2‰ to +5‰ (average 3.4‰) enriched in ^{15}N relative to their diet/prey. As noted previously, an average enrichment value of 3.4‰ per trophic level step has been recommended for food webs without prior knowledge of ^{15}N enrichment or the ecology of the system (Deniro and Epstein, 1981; Minagawa and Wada, 1984; Post, 2002). However, the absolute magnitude of this trophic step isotope ^{15}N enrichment can be influenced by many factors, including nutritional stress, diet quality, body size, excretory mechanisms and feeding rate (Hobson and Welch, 1995; Ponsard and Averbuch, 1999; Overman and Parrish, 2001; Pinnegar et al, 2001; Vanderklift and Ponsard, 2003).

Given that ^{15}N fractionation may vary significantly from ecosystem to ecosystem, a method for calibrating the ^{15}N EF value for a system involves the use of chemical benchmarking. In this application, a well-recognised bioaccumulative chemical with known biomagnifying behaviour is used to derive a site-specific $\Delta^{15}N$ EF, allowing TL values to be determined explicitly for the aquatic ecosystem in question. The quantitative assignment of TL values using Equation 5.1 is often based on the generic $\Delta^{15}N$ EF increasing by 3.4 ‰ with each trophic level; this technique has been developed from data with a small number of predator/prey relationships and food webs and the applicability of a generic $\Delta^{15}N$ EF may be inadequate for use in universal coverage for all food web organisms. For example, the $\Delta^{15}N$ EF can vary with species, physiology, and trophic ecology. Deniro and Epstein (1981) reported a range in measured $\Delta^{15}N$ EF from −0.5 ‰ to +9.2 ‰ and McCutchan et al (2003) noted values from −2.1‰ to 5.4‰. Secondly, characterisation of $\delta^{15}N$ values for primary producers is challenging, due to spatial and temporal variability. The value of the site specific $\Delta^{15}N$ EF may be determined by benchmarking the behaviour of the isotopic composition in biota against the *in situ* behaviour of a well-known legacy chemical, e.g. PCB180. The biomagnifying characteristic of this hydrophobic (log K_{OW} = 7.29) chemical has been documented in numerous aquatic food webs and an average TMF value may be chosen (e.g. 4.65 for PCB180) as a reasonable mean TMF in aquatic foodwebs (Houde et al, 2008; Mackintosh et al, 2004; Wu et al, 2009). Combining Equations 5.1, 5.4, and 5.6 produces the following relationship on $\Delta^{15}N$ EF:

$$\Delta^{15}N\ EF = \log TMF_{RM} / Slope\ RM_{\delta^{15}N} \quad \text{(Eq. 5.9)}$$

where $\Delta^{15}N$ EF is the $\delta^{15}N$ enrichment factor for biota, TMF_{RM} is the TMF for the reference material (e.g. PCB180), and Slope $RM_{\delta 15N}$ is the slope of the log reference material (lipid-corrected) concentrations in biota versus $\delta^{15}N$.

This benchmarking approach allows an investigator to calibrate the $\Delta^{15}N$ EF value for the aquatic ecosystem under review, which may or may not be similar to the generic $\Delta^{15}N$ EF value of 3.4‰ with each trophic level. Equation 5.4 is then used to establish TL values for the organisms under examination, and the TMF value for the chemical/ecosystem under review is then determined using Equations 5.4 and 5.6.

Once the aquatic food web under review has been calibrated with regard to its TL assigned values via benchmarking, the TMF calibration may be checked by use of a positive control chemical, such as PCB153 or PCB180, to verify the TMF value is within the accepted range for that chemical.

TMFs are typically calculated (Equation 5.5, 5.6 and 5.7) using univariate deterministic methods (Borgå et al, 2012) that are based on linear, least-squares regression analysis — an approach that ignores the natural, temporal, and spatial variability in aquatic environments, and is susceptible to bias resulting from experimental design, food web structure, and variable exposure. Alternate approaches for calculation of TMF, shown in Table 5.1, incorporate multivariate probabilistic methods that were developed to correct for these sources of bias and to incorporate natural, temporal, and spatial variability (Powell, 2011; 2012; Powell et al, 2011; 2012; 2013). Probabilistic approaches offer a more comprehensive overview of TMF and provide the ability to conduct probabilistic uncertainty analyses to identify the contributing factors having the most influence on the TMF value. From a probabilistic perspective, TMF may be viewed as a continuous distribution for a food web, with mean and median values that have an associated certainty of exceeding a value of 1.0 (or any other defined endpoint). Trophic magnification factors may be calculated using the standard univariate approach or probabilistic approaches developed to minimise or eliminate bias that may result from using univariate deterministic methods (Table 5.1).

Table 5.1: Parameters used to calculate TMFs using the standard equation: log TMF = Slope × $\delta^{15}N$

Method	Type	Bias Correction	Parameters [a,b]	Equation
Standard	Deterministic		Slope = log C on $\delta^{15}N$ (individual samples) $\Delta^{15}N$ EF = 3.4‰ (standard value)	Eq. 5.9
Probabilistic	Probabilistic	Experimental design	Slope = log C on $\delta^{15}N$ (PDF; mean, stdev) $\Delta^{15}N$ EF=3.4‰ (standard value)	Eq. 5.11
Benchmark	Probabilistic	Food web structure	Slope = log C on $\delta^{15}N$ (PDF; mean, stdev) $\Delta^{15}N$ EF = derived using PCB180 as a TMF benchmark (TMF = 4.0)	Eq. 5.11 Eq. 5.10
Corrected benchmark	Probabilistic	Exposure	Slope = log BSAF on $\delta^{15}N$ (PDF; mean, stdev) $\Delta^{15}N$ EF = derived using PCB180 as a TMF benchmark (TMF = 4.0)	Eq. 5.12 Eq. 5.10

[a] TMF = trophic magnification factor; Slope = regression slope; C = concentration in the organism (µg/kg lipid); $\Delta^{15}N$ EF = $\delta^{15}N$ enrichment factor per unit trophic level for the food web; PDF = probability density function (defined by the mean and standard deviation); mean = the mean value for the species; stdev = the standard deviation of the mean value; BSAF = biota-sediment accumulation factor (kg OC/kg lipid).

[b] The food web $\delta^{15}N$ EF may be derived from a benchmark material using the equation:

$$\Delta^{15}\mathrm{N}_{\mathrm{benchmark}} = \frac{\log \mathrm{TMF}_{\mathrm{benchmark}}}{\mathrm{Slope}_{\mathrm{benchmark}}}$$

BSAF (units of kg OC/kg lipid) describes the uptake and accumulation of a chemical from all sources relative to the amount of chemical stored in the sediment compartment. Calculated as:

$$\mathrm{BSAF} = \frac{\mathrm{C}_{\mathrm{organism}}\ (\mu\mathrm{g/kg\ lipid})}{\mathrm{C}_{\mathrm{water}}\ (\mu\mathrm{g/kg\ Organic\ Carbon})}$$

BSAF represents individual points of bioaccumulation. TMF represents the slope of bioaccumulation, which is independent of unit terms when expressed on a common basis.

$$\mathrm{TMF}_{\mathrm{C}} = \mathrm{TMF}_{\mathrm{BSAF}}$$

Standard approach

Typically, TMF is calculated as the antilog of the slope (*m)* of the linear model of log-transformed lipid-normalised concentrations regressed on TL across all samples (Equation 5.7). Equation 5.7 may be combined with the equation used to calculate relative trophic level position (Equation 5.4) so that TMF may be directly calculated from the slope *m* of the linear model of log-transformed concentration regressed on $\delta^{15}N$ ($m_{\delta^{15}\mathrm{N}}$) and the ^{15}N enrichment factor for the food web ($\Delta^{15}N$ EF) using the equation:

$$\log \mathrm{TMF} = m_{\delta^{15}\mathrm{N}} \times \Delta^{15}\mathrm{N\ EF} \quad \text{..... (Eq. 5.10)}$$

As shown by Equation 5.10, TMF values calculated using the standard approach (Equation 5.7) are a direct multiple of the $\Delta^{15}N$ EF value used to calculate TL (Equation 5.4). By using Equation 5.10 to calculate TMF directly, it is not necessary to identify a baseline consumer or estimate the specific TL that the baseline consumer occupied in the food web.

Probabilistic approach

This approach applies probabilistic methods to the standard approach for calculation of TMF (Equation 5.10) to correct for bias resulting from experimental design (Sheskin, 2000). This may include unbalanced designs (unequal replication), lack of statistical power (insufficient replication; pooling of samples), collection of

samples (easier to collect some species), left censored data (results less than detection limit), and heteroscedastic data (unequal variability).

The probabilistic approach incorporates probabilistic methods into the standard approach (Equation 5.10) to generate probability distributions for TMF, showing what is the likelihood for certain TMF values to occur in a given food web. This may be accomplished by probabilistic regression of species-specific probability density functions (PDFs) for lipid-normalised concentrations on species-specific PDFs for $\delta^{15}N$ (Equation 5.7). Probabilistic regression analysis by Monte Carlo sampling of each PDF may be used to generate probability distributions (PDs) for the slope of the regression line (i.e. $\mathrm{PD}_{m_{\delta^{15}\mathrm{N}}}$), which was substituted for $m_{\delta^{15}\mathrm{N}}$ in Equation 5.10 and used to calculate TMF by the equation:

$$\log \mathrm{TMF} = \mathrm{PD}_{m_{\delta^{15}\mathrm{N}}} \times \Delta^{15}\mathrm{N\ EF} \quad \text{..... (Eq. 5.11)}$$

Probability distributions assign a probability to the outcome of a statistical inference test (for example, slope of a regression line) that is based on a distribution of continuous random variables (e.g. concentration or TL) defined by a PDF. The PDFs used for the probabilistic regression analysis may be defined, by species, as log-normal distributions that are described by the mean and standard deviation for each species in the sampled food web. Probabilistic TMFs may be calculated using the standard $\Delta^{15}N$ value of 3.4‰ per TL.

Benchmark approach

This approach applies benchmarking to the probabilistic approach for calculation of TMF (Equation 5.11) to correct for bias resulting from food web dynamics and trophic level structure (Caut et al, 2009; Layman et al, 2012), which may include: the dynamic nature and variability in food web and trophic structure, seasonal variability (especially for short-lived species), confounded food webs (benthic versus pelagic), omnivorous feeding by consumers, and uncertainty associated with $\Delta^{15}N$ EF value used for the calculation.

Trophic transfer across an aquatic food web may be benchmarked by rearranging Equation 5.11 and using the probability distribution for the regression slope of log concentration of a benchmark (BM) chemical on $\delta^{15}N$ (i.e. $\mathrm{PD}_{m_{\delta^{15}\mathrm{N_{BM}}}}$) and the TMF of the benchmark chemical (TMF_{BM}) to derive a benchmarked $\Delta^{15}N$ EF value for the food web (i.e. $\Delta^{15}N_{BM}$ EF), as shown by the equation:

$$\Delta^{15}\mathrm{N_{BM}\ EF} = \frac{\log \mathrm{TMF_{BM}}}{\mathrm{PD}m_{\delta^{15}\mathrm{N_{BM}}}} \quad \text{..... (Eq. 5.12)}$$

Benchmarked probabilistic TMF values may then be calculated for the food web by substituting the calculated food web-specific $\Delta^{15}N_{BM}$ EF from Equation 5.12 for $\Delta^{15}N$ EF in Equation 5.11, as shown by the equation:

$$\log \mathrm{TMF} = \frac{\mathrm{PD}m_{\delta^{15}\mathrm{N}}}{\mathrm{PD}m_{\delta^{15}\mathrm{N_{BM}}}} \times \log \mathrm{TMF_{BM}} \quad \text{..... (Eq. 5.13)}$$

As shown in Equation 5.13, a benchmarked log TMF value is the ratio of the regression slopes (log chemical $\mathrm{concentration_{Lipid}}$ on $\delta^{15}N$) of the chemical and the benchmark (BM) multiplied by log TMF of the benchmark compound. Calculation of TMF using the benchmark approach (Equation 5.13) generates benchmarked TMF values for the calibrated food web that are independent of the $\Delta^{15}N$ value used for calculation of TMF by the

standard approach (Equation 5.10). In addition, $\Delta^{15}N_{BM}$ EF obtained from Equation 5.12 may be used to verify the internal consistency of food web structure and dynamics by comparison to the accepted range of $\Delta^{15}N$ EF values for the type food web under evaluation.

Corrected benchmark approach

This approach applies exposure correction to the benchmark approach for calculation of TMF (Equation 5.13) to correct bias resulting from variable exposure (Borgå et al, 2012; Burkhard et al, 2013), which may include: multiple sources of contaminant into the food web (water, sediment, food), omnivorous feeding by consumers, and non-uniform migration across concentration gradients. This is a concern especially for chemicals having point-source emissions (for example, pharmaceutical and personal care products disposed to wastewater) that create exposure gradients across both water and sediment.

Because log TMF is a slope (Equation 5.7), it is independent of unit terms when the dependent variable used for the regression analysis (i.e. concentration) is expressed on a common basis. Consequently, concentration ratios may be used as the dependent variable in the regression analysis to correct TMF for variable exposures across a food web (Equation 5.8). Concentration ratios are commonly used as measures of bioaccumulation because they take into consideration that pathways of exposure occur through various sources (i.e. water, sediment, diet, and air). For aquatic organisms, BAF and BSAF are concentration ratios that describe the amount of chemical in an organism relative to the amount of chemical stored in the water and sediment compartments, respectively. These measures of bioaccumulation may thus be used to correct biased or skewed TMF values that result from variable exposures across the food web.

The primary source of exposure in an aquatic environment is presumed to be from the water column for a pelagic food web and from the sediment for a benthic or demersal food web. As such, the preferred approach to correct for variable exposure across a pelagic food web would be to normalise concentrations in the pelagic organisms to concentrations in water and use BAF as the dependent variable for the regression analyses. Similarly, the preferred approach to correct for variable exposure across a demersal food web would be to normalise concentrations in the benthic and demersal organisms to concentrations in sediment and use BSAF as the dependent variable for the regression analyses. Unfortunately, concentrations in water may not be detectable or available for use as measures of exposure for calculating BAF. In such cases, concentrations in surface sediment may be applied as relative indicators of exposure for normalising concentrations in the organisms, and BSAF used as the dependent variable in the regression analyses. Concentrations in surface sediment may be used as relative indicators of exposure based on the assumption that concentrations in the water column and sediment were in equilibrium at any given location over the long term.

Benchmarked probabilistic TMF values may be corrected for variable exposure by substituting the probability distributions for the slopes of the regression lines of log concentration on $\delta^{15}N$ in Equation 5.11 (i.e. $\mathrm{PD}_{m_{\delta^{15}\mathrm{N}}}$ and $\mathrm{PD}_{m_{\delta^{15}\mathrm{N_{BM}}}}$) with the probability distributions for the slopes of the regression lines of log BSAF on $\delta^{15}N$ (i.e. $\mathrm{PD}_{m_{\delta^{15}\mathrm{N}}}[\mathrm{BSAF}]$ and $\mathrm{PD}_{m_{\delta^{15}\mathrm{N}}}[\mathrm{BSAF_{BM}}]$), as shown by the equation:

$$\log \mathrm{TMF} = \frac{\mathrm{PD}_{m_{\delta^{15}\mathrm{N}}}[\mathrm{BSAF}]}{\mathrm{PD}_{m_{\delta^{15}\mathrm{N}}}[\mathrm{BSAF_{BM}}]} \times \log \mathrm{TMF_{BM}} \quad \text{..... (Eq. 5.14)}$$

Concentrations used to estimate BSAF values required for Equation 5.14 may be calculated from concentrations measured in surface sediments in combination with the migration patterns of each species across the defined study area. Sediment residue heterogeneity and fish migration patterns may require the use of species-specific BSAF values, as per Equation 5.14, to generate 'corrected' TMF values for chemicals demonstrating variable sediment conditions, such as those materials discharged from waste water treatment plants, originating in agricultural runoff. Fish migration patterns may also be used as weighting factors that were applied to the sediment concentrations such that relative exposure concentrations used to calculate BSAF were estimated as weighted mean concentrations for each species.

Summary and discussion

Standard trophic magnification factors (TMFs) are generally calculated using univariate methods based on linear, least-squares regression analysis and assume a homogeneous aqueous and sediment environment within the food web under study. Multivariate probabilistic methods may be used to calculate TMFs that incorporate natural variability and minimise biases associated with the $\Delta^{15}N$ EF (used to estimate relative trophic level position), omnivorous feeding, migration, and variable exposure across concentration gradients. Probabilistic uncertainty analyses may be used to analyse the likelihood of TMF values > 1 occurring across the food web under study.

Lipophilic substances are transferred within food webs, generally moving with the lipid flow or energy transfer to higher trophic-level consumers. TMF studies of aquatic food webs have shown that concentrations of persistent, bioaccumulative chemicals in aquatic biota are significantly related to their trophic position and that the magnitudes of a TMF appear to be related to the octanol-water partition coefficient (K_{OW}). For example, TMF values for persistent hydrophobic chemicals in 17 lakes in Canada and the north-eastern United States were significantly correlated with log K_{OW} (Houde et al, 2008), implying that the rate of accumulation along the food web was dependent on hydrophobicity and recalcitrance to degradation. However, trophic magnification (i.e. bioaccumulation and biomagnification) is also dependent on the metabolic capacity of each organism at the different trophic levels and the physiological properties of the chemical substance. For instance, Tomy et al (2007) found that the greatest concentrations of dechlorane plus in Lake Ontario were associated with lower trophic level organisms and that concentrations progressively decreased with increasing trophic level. Similarly, trophic dilution has also been observed in an aquatic food web in Bohai Bay, China where TMF values ranged from 0.11 for fluoranthene to 0.45 for acenaphthylene (Wan et al, 2010).

This review has examined the statistical strength needed to verify TMF values > 1 or < 1 and that only large sample sizes (total N > 60 - 100) are anticipated to consistently detect significant regression slopes for chemicals with apparent TMFs slightly greater than 1.0. Experimental designs with fewer than 30 to40 samples and a standard level of variability in chemical concentration data are unlikely to detect statistically significant regression slopes for contaminants with TMF values near the lower limits of regulatory relevance, i.e. 0.5 to 2.0. In addition, this review has shown the importance of benchmarking in determining appropriate TL values for species in the ecosystem of interest and the preference for validating TMF evaluation by use of a positive control chemical. As a result, uncritical use of the bright-line 'BMF/TMF > 1' regulatory threshold for 'B' categorisation is strongly discouraged on account of the several factors (listed above) that introduce variability into field-derived BMFs and TMFs, such as non-achievement of steady-state

concentrations, poorly understood or misinterpreted feeding ecology, uncertainties introduced by metabolism of precursor compounds, and statistical design issues. Hopefully, some of the techniques outlined in this document, such as the use of benchmarking and corrected probabilistic TMF analysis, may help encourage the development of consistent and sound methodologies for future BMF/TMF studies.

5.4 Fugacity ratios and bioaccumulation

For assessing a chemical's bioaccumulation ('B') potential, the n-octanol/water partition coefficient (K_{OW}), bioconcentration factor (BCF), and bioaccumulation factor (BAF) are frequently the measurements or 'metrics' used by global regulatory agencies. Threshold values of 1,000 to 5,000 l/kg and 100,000 (10^5) are often used for the BCF-BAF and K_{OW} metrics, respectively (Gobas et al, 2009). Other bioaccumulation assessment metrics are available from field, mesocosm, and laboratory studies, including the biota/sediment accumulation factor (BSAF), biomagnification factor (BMF), and trophic magnification factor (TMF). These additional 'B' measures provide valuable insights for characterising the bioaccumulation potential of non-ionic organic chemicals in environmental organisms. Recently, Gobas et al (2009) suggested an improved bioaccumulation assessment framework that gave preference to TMF and BMF data over BCF, BAF, and K_{OW} data, as the TMF and BMF are more appropriate for assessing the potential for a substance to biomagnify up a natural food chain. One of the current difficulties in comparing BCF/BAF data to other bioaccumulation metrics is the difference in numerical scale and reference media to which tissue concentrations are normalised. For example, BCFs and BAFs have values of the order of 10^0 to 10^8 while BMFs, BSAFs, and TMFs have values of the order of 10^{-4} to 10^2. BCFs and BAFs express ratios of chemical concentrations in biota to water, whereas BSAFs represent ratios of chemical concentrations in biota to sediment or soil, and BMFs and TMFs reflect ratios of chemical concentrations in different predator/prey species.

Fugacity can be regarded as the 'escaping tendency' of a chemical substance from a phase or medium (Mackay and Paterson, 1981). The concentration of a chemical in medium 'm' (C_m, in mol/m^3) is the product of the compound's fugacity, f (Pa), times the medium's fugacity capacity (Z_m, in mol/m^3/Pa):

$$C_m = f \times Z_m, \text{ or } f = C_m / Z_m \quad \text{(Eq. 5.15)}$$

Fugacity ratios are ratios of the chemical fugacity in biota to the chemical fugacity in the medium to which the biota are exposed. For example, the biota-water fugacity ratio, $F_{biota\text{-}water}$, of a chemical in a laboratory test or in the field can be expressed as:

$$F_{biota\text{-}water} = f_{biota}/f_{water} \quad \text{(Eq. 5.16)}$$

where f_{biota} is the chemical fugacity in biota (Pa) and f_{water} is the fugacity of the chemical in water (Pa).

The advantages and limitations of this fugacity ratio approach have been discussed above (Section 5.1.3), where it is concluded that it may provide a formal method for testing the hypothesis whether biomagnification occurs by testing whether fugacity ratios are greater than unity (1.0).

A fugacity ratio expresses the status of chemical potential relative to a state of chemical equilibrium, which is thermodynamically defined as a situation where the fugacity ratio is 1, i.e. at equilibrium, fugacities are equivalent and, therefore, so are the chemical potentials in each of the phases for each component. A

fugacity ratio greater than 1 therefore indicates that the chemical in the organism is able to achieve a higher fugacity (or chemical activity) than that in the medium to which it is exposed. In that case, the organism is able to magnify the chemical potential in its environment. A fugacity ratio less than 1 implies that the chemical concentration in the organism is less than its thermodynamic equilibrium value relative to the medium to which it is exposed. A fugacity ratio less than one can occur if the chemical is quickly biotransformed or eliminated in the organism, or it lacks sufficient accumulation uptake dynamics. In such a case, the chemical in the organism cannot achieve its equilibrium value and hence will occur at a thermodynamic potential that is less than that in the medium (i.e. water, sediment) to which it is exposed.

Lipid-adjusted bioconcentration/bioaccumulation endpoints of BCF_L and BAF_L can be transformed into approximate fugacity ratios by converting their standard denominators (i.e. dissolved chemical concentrations in water) to their equivalent concentrations in lipid by multiplying the denominator by the n-octanol/water partition coefficient (K_{OW}) of the chemical; in essence, dividing the lipid-normalised and freely-dissolved (f_d) BCFs (BCF_L/f_d) and BAFs by their substance-specific K_{OW} value. With conversion of the BCF_L/f_d and other metrics to a fugacity ratio (i.e. $BCF_{L/fd}/K_{OW}$), all bioaccumulation endpoint metrics can be directly compared numerically. This is the advantage of fugacity ratios, as they allow comparison of a chemical's bioaccumulation potential across a wide range of laboratory and field bioaccumulation metrics, thereby allowing a visual WoE evaluation of all available data.

5.4.1 Calculating biota/media fugacity ratios

The calculation of biota fugacity capacity is based on the assumption that lipid is the only relevant phase in tissue or biota for storing hydrophobic organic chemicals and that octanol and lipid have equivalent storage capacities for an organic chemical, i.e. $Z_{octanol} = Z_{lipid}$; Mackay (1982) has proposed n-octanol to be a reasonable surrogate phase for lipids in biological organisms. Hence, the biota/water partition coefficient ($K_{biota/water}$, m^3-water/m^3-biota) can be calculated as % Lipid × d_{biota}/d_{lipid} × K_{OW}. The calculation of the fugacity capacity of sediment is based on the assumption that the only relevant phase for the storage of nonionic hydrophobic organic chemicals is organic carbon (OC). Hence, $K_{sediment/water}$ (m^3-water/m^3-sediment) can be calculated as % OC × $d_{sediment}$ × K_{oc}. Fugacity capacities may be calculated as

$$Z_{water} = 1/H \text{ (mol/m}^3\text{/Pa)} \quad \text{(Eq. 5.17)}$$

where H is the Henry's Law constant for a chemical.

$$Z_{biota} = K_{biota/water} \times Z_{water} = \% \text{ Lipid} \times d_{biota}/d_{lipid} \times K_{OW} \times Z_{water} \quad \text{(Eq. 5.18)}$$

where $K_{biota/water}$ is the biota/water partition coefficient (m^3-water/m^3-biota), % Lipid is the mass fraction of lipid in biota, and d_{biota} and d_{lipid} are the density measurements for biota and lipid, respectively, in kg/m^3 (Mackay and Paterson, 1981).

$$Z_{sediment} = K_{sediment/water} \times Z_{water} = \% \text{ OC} \times d_{sediment} \times K_{OC} \times Z_{water} \quad \text{(Eq. 5.19)}$$

where $K_{sediment/water}$ is the sediment/water partition coefficient, % OC is the mass fraction of organic carbon in sediment, $d_{sediment}$ is the density measurement for sediment (kg/m^3), and K_{OC} is the organic carbon/water partition coefficient, in l-water/kg-organic carbon (Mackay and Paterson, 1981).

So, using the biota/sediment fugacity ratio ($F_{biota/sediment}$) as an example,

$$\text{Fugacity(biota)} = f_{biota} = Conc_{Biota}/Z_{biota} = Conc_{Biota} \times d_{Biota}/(\%\ Lipid \times d_{Biota}/d_{Lipid} \times K_{OW} \times Z_{water}) \quad \text{(Eq. 5.20)}$$

$$\text{Fugacity(biota)} = Conc_{Biota} \times d_{Lipid}/(\%\ Lipid \times K_{OW} \times Z_{water}) \quad \text{(Eq. 5.21)}$$

$$\text{Fugacity(sediment)} = Conc_{Sediment}/Z_{Sediment} = Conc_{Sediment} \times d_{sediment}/(\%\ OC \times d_{sediment} \times K_{OC} \times Z_{water}) \quad \text{(Eq. 5.22)}$$

$$\text{Fugacity(sediment)} = Conc_{Sediment}/(\%\ OC \times K_{OC} \times Z_{water}) \quad \text{(Eq. 5.23)}$$

So, the biota/sediment fugacity ratio or Fbiota/sediment is:

$$F_{biota/sediment} = BSAF_{OC/L} \times d_{lipid} \times K_{OC}/K_{OW} \quad \text{(Eq. 5.24)}$$

where $BSAF_{OC/L}$ is the OC/Lipid normalised BSAF value for a compound.

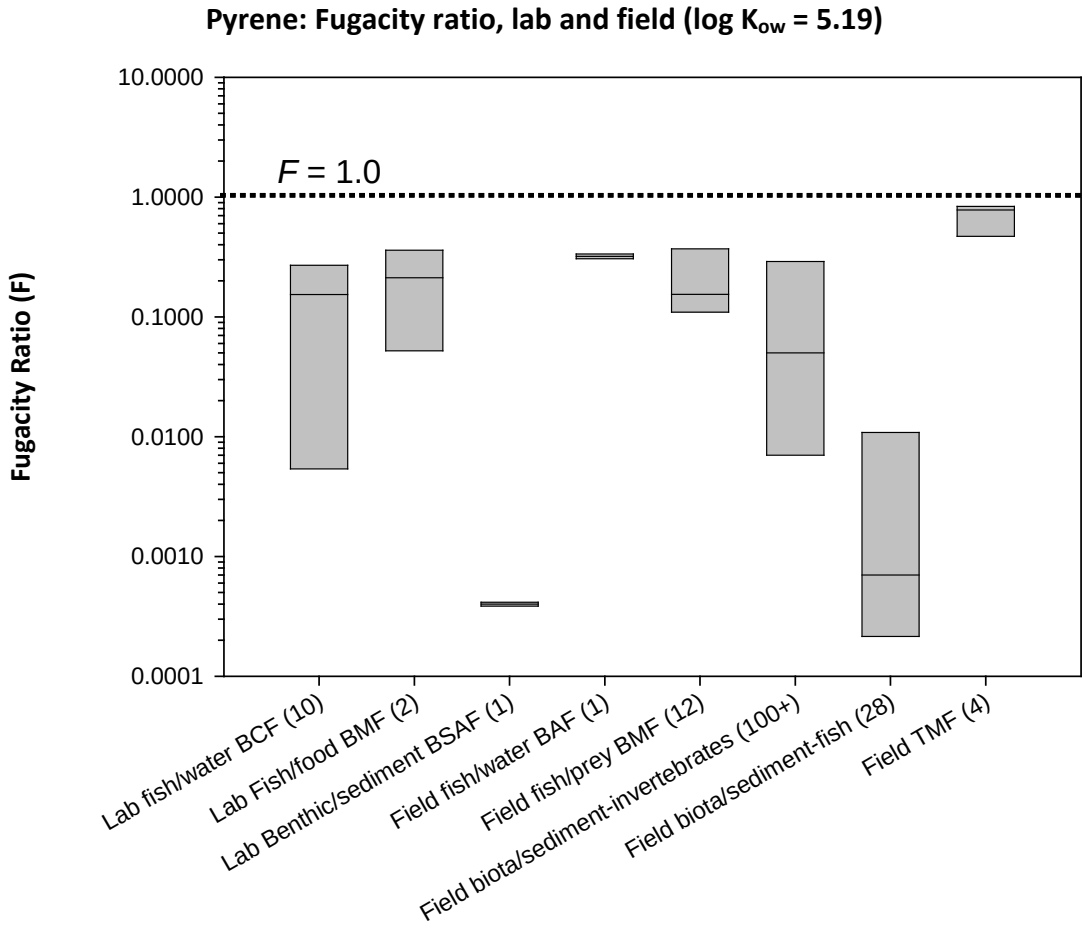
Summary and discussion

The fugacity ratio approach has the capability to identify chemical substances that biomagnify; Burkhard et al (2012a,b) outline use of the method based on a number of independent studies for a wide range of chemicals. As noted in Section 5.1.3, the applicability domain of the fugacity ratio approach is limited to non-ionic organic chemicals. In addition, this methodology is not applicable to chemicals that demonstrate high-affinity binding to proteins or that have active uptake mechanisms (Burkhard et al, 2012b).

A key advantage of the fugacity ratio approach is that it allows different bioaccumulation metrics to be expressed on a common basis and test a single hypothesis, i.e. whether the chemical is a bioaccumulative substance with fugacity ratios consistently and significantly > 1. The approach allows all available data to be used, rather than a single metric or observation (e.g. BCF). It thereby provides a WoE that increases confidence in the outcome of the assessment.

A graphical example of the use of the fugacity ratio approach with regard to bioaccumulation potential is shown in Figure 5.2 for pyrene, a metabolisable hydrocarbon (Arnot et al, 2009).

Figure 5.2: The steady-state fugacity ratio of pyrene (dimensionless) in laboratory bioconcentration/bioaccumulation tests in fish, laboratory biomagnification tests in fish, laboratory biota/sediment accumulation tests in benthic invertebrates, and in field-based studies involving benthic invertebrates, fish, and natural food webs [a]



[a] On the X-axis, the numbers following the study type descriptions are the number (N) of experimental data points per study type. The B data are taken from Burkhard et al (2012). The length of the box indicates the difference between the 10th and 90th centile distribution for that endpoint, with the line indicating the median (i.e. 50th centile) value.

As expected, the laboratory B metrics for fish are well below unity. The laboratory metrics for invertebrates are higher; this may be due to the lower metabolic capacity of these organisms, when compared to vertebrates. Field metrics, including TMFs, are below unity for pyrene, consistent with the concept of trophic dilution due to the attenuating role of biotransformation.

A more substantial and detailed explanation regarding the use of fugacity ratios in a WoE B assessment may be found in Burkhard et al (2012a,b).

As stated in Section 5.1.3, an important caveat to note is the assumption that lipids are the only relevant phase in biota for storing hydrophobic organic chemicals. It is further assumed that lipids and octanol have equivalent fugacity capacities for an organic chemical, i.e. $Z_{lipid} = Z_{octanol}$, as Mackay (1982) proposed n-octanol to be a reasonable surrogate phase for lipids in biological organisms. However, this assumption may vary widely for different organic chemical families, for poikilothermic versus homeothermic organisms, and for different types of lipids (e.g. membrane versus storage lipids). The fugacity capacity issue with octanol and lipids is currently an active area of research (Seston et al, 2014).

5.5 Whole-body versus organ-specific

For bioconcentration, bioaccumulation and/or biomagnification assessments, organ-specific concentration data are used in some cases. Indeed, it can be difficult or even impossible to obtain whole-body concentration data, either for practical or for ethical reasons. An organism may simply be too large for complete homogenisation (e.g. larger fish) making it impractical to determine a whole-body BCF, or ethical considerations can make non-lethal tissue sampling a good alternative (e.g. dolphins, polar bears). On the other hand, in some cases lipid rich organs – in most cases the liver – are harvested and analysed as hydrophobic contaminants are accumulated up to higher levels in these tissues. It is often easier to homogenise separate organs and the higher contaminant concentrations increase both the accuracy of the chemical analysis as well as the chance that a contaminant is detected. Such an approach is for example used in the routine monitoring of hydrophobic contaminants in flatfish.

While in some cases it can make sense to use organ-specific bioconcentration data – e.g. in the case of a substance known to cause organ-specific toxicity or when a substance is known to bioaccumulate in a specific organ (Franke et al, 1994) – there are also several drawbacks. Firstly, the criterion for bioconcentration used in REACh is based on whole-body concentration data. When only an organ-specific concentration is available, a direct comparison with this criterion is difficult. Secondly, when deriving biomagnification factors, sometimes the ratio of organ-specific concentrations in a predator (e.g. flatfish liver) to that in its entire diet (e.g. shrimp) is calculated. This may yield a different result compared to what would be obtained with whole-body concentrations for both organisms. The main reasons for this are that (i) the analysed organ of the predator (e.g. the liver) may not represent the same body fraction in the prey, and that (ii) other tissues in the prey may contribute more extensively to the intake of these chemicals due to their higher relative mass in the prey.

In the following subsection, it will be discussed to what extent organ-specific BCFs are representative of whole-body BCFs (for potential comparison with the REACh BCF threshold). The next subsection reviews the potential impact of the use of organ-specific predator instead of whole-body oncentration data for the derivation of BMFs and TMFs.

5.5.1 Organ-specific BCFs

Studies in which BCF data are calculated for specific tissues are rare (e.g. Taniyasu et al, 2003; Martin et al, 2003; Lindholst et al, 2000; Ankley et al, 2005; Gomez et al, 2010; Satyanarayan et al, 2005) as in most studies in which organ-specific concentrations are measured, exposure data are usually not taken into account. The most common organs in which contaminant levels are measured, are the liver (e.g. Taniyasu et al, 2003; Martin et al, 2003; Lindholst et al, 2000; Ankley et al, 2005; Gomez et al, 2010; Satyanarayan et al, 2005) and muscle tissue (e.g. Martin et al, 2003; Lindholst et al, 2000; Satyanarayan et al, 2005) and to a lesser extent gills (e.g. Gomez et al, 2010; Satyanarayan et al, 2005), intestines (e.g. Satyanarayan et al, 2005) and kidney (e.g. Satyanarayan et al, 2005). The BCF data used for direct bioaccumulation assessments under REACh, should be based on whole-body measurements and the data should ideally be normalised to lipid content (a default lipid level of 5% is most commonly used; ECHA, 2008c). Only when an organ-specific bioconcentration value can accurately represent a whole-body BCF, can it be reliably used to assess a substance's bioaccumulation potential. To this end, for substances that partition strongly to lipids

(i.e. hydrophobic contaminants), knowledge on the lipid fraction in the analysed organ is crucial. Unfortunately, in the literature cited above, information on the separate organs' lipid fractions is not available.

The available literature on organ-specific BCF data is presented in Appendix D. When comparing data between organs and tissues, it is apparent that the difference is generally within an order of magnitude. The highest accumulation generally occurs in the liver, the lowest in muscle tissue. As these organs/tissues contain respectively the highest and lowest lipid fraction within an organism, this can be expected. In the study by Lindholst et al (2000), the difference between BCF values in muscle tissue and liver was on average 9.6. While measurements of lipid content were absent in this study, differences in lipid fraction between fish muscle and liver can be an order of magnitude (unpublished data by Claessens et al, 2013).

As organ-specific BCF data are scarce, it is valuable to take a look at organ-specific concentration data. While such data – originating either from laboratory experiments or from field monitoring studies – offer no direct assessment of a substance's bioaccumulation potential due to a lack of (adequate) exposure data, they can help to elucidate the variation that exists between organs in terms of bioaccumulation patterns. This variation will inevitably increase the uncertainty of any whole-body bioaccumulation metric that is deduced from organ-specific data. In what follows, the results of a number of studies reporting organ-specific concentration data for different chemical classes and different (aquatic and terrestrial) organisms will be discussed.

5.5.2 Organ-specific concentrations

Yankovich et al (2010) performed an extensive literature review to report on whole-body to tissue concentration ratios for a large number of metals. The main aim of that study was to present guidance on how to derive whole-body activity concentrations of both radionuclides and stable elements using tissue-specific values for different organisms. To this end, Yankovich et al estimated the whole-body to tissue concentration ratios as:

$$CR_{wh:t} = SUM(C_t \times f_t)/C_t \quad \text{(Eq. 5.25)}$$

With $CR_{wh:t}$ as the whole-body to tissue-concentration ratio, C_t as the concentration of the element in the tissue (mg/kg fresh mass) and f_t as the fractional mass of a given tissue or organ relative to the fresh mass of the whole body.

Yankovich et al calculated $CR_{wh:t}$ for a wide range of elements and included organisms of different trophic levels. The authors thereby grouped organisms by type (i.e. molluscs, gastropods, marine crustaceans, fish, amphibians, turtles, other reptiles and mammals) and made no distinction at species level. In this ECETOC report, for clarity reasons the results for only a limited number of elements – a number of important metals (i.e. Cd, Cu, Fe, Ni, Pb and Zn) – will be briefly discussed.

Yankovich et al (2010) reported the mean, minimum and maximum values of $CR_{wh:t}$ as calculated from the data of a wide range of scientific research papers. As a compilation of all the underlying data was absent, a limited investigation has been made of the variability of $CR_{wh:t}$ by comparing the minimum and maximum values of this parameter and calculating the following ratio:

$$CR_{wh:t,range} = CR_{wh:t,max}/CR_{wh:t,min} \quad \text{(Eq. 5.26)}$$

Thus, $CR_{wh:t,range}$ gives an idea of the range of $CR_{wh:t}$ within a certain group of organisms (i.e. the within the groups as defined by Yankovich et al, 2010). The results for this parameter (Table 5.2) indicate that while in 59% of the cases the range stays within an order of magnitude, these cases cover 74% of the cases in which the dataset contains less than 10 data points. The groups which exhibit a small $CR_{wh:t,range}$ in general are marine molluscs, crustaceans, amphibians and reptiles (excluding turtles). The data for marine molluscs and crustaceans were all derived from a single study (Takata et al, 2010) and the number of datapoints is small (less than 10 in all cases). For amphibians the number of studies could not be readily derived from the study of Yankovich et al (2010). For the organism groups amphibians, fish and mammals reference was made to a book – containing data compilations – that was not available: Yankovich and Beaton, 2000 quoted by Yankovich et al, 2010), but given the low number of data points (Table 5.2) the number of studies was most likely small. For reptiles, the data in total are for one snake species (*Nerodia sipedon*) studied in the US (Campbell et al, 2005; Burger et al, 2005; Burger et al, 2007) and 5 crocodile species studied in – depending on the species – the US, Asia or Africa (Swanepoel et al, 2000; Xu et al, 2006; Burger et al, 2000; Markich et al, 2002; Martin et al, 1998). With the exception of Pb (for which the data of 4 crocodile species and the snake species were combined), the data for reptiles never originated from more than 3 species and as many different studies (see also Wood et al, 2010 for a compilation of the underlying data for reptiles). For turtles on the other hand, the underlying data originate from 20 different studies and cover 8 different species (freshwater, brackish water as well as sea turtles). From these results and subsequent observations it would be difficult to conclude that the variation of whole-body to tissue concentration ratios is low for molluscs, crustaceans, amphibians and reptiles given that the underlying data are rather limited. On the contrary, it can be expected that this variation would increase up to at least the levels witnessed in turtles, fish and mammals if more species from more diverse locations would be included.

What this all means in practice, is that when assessing the bioaccumulation potential of any of these metals based on its concentration in an organ or a specific tissue, a considerable amount of uncertainty can be expected when extrapolating this to a whole-body concentration. This is especially true when data on such extrapolations are not yet available for the species under investigation and one must rely on estimations of the ratio such as the ones derived by Yankovich et al (2010). Based on all the above, it does not seem unreasonable to expect uncertainties between 1 and 2 orders of magnitude in such cases.

Table 5.2: $CR_{wh:t,range}$ values for 6 metals in different organs/tissues of aquatic and terrestrial organisms (Yankovich et al, 2010)[a]

		Marine mollusc		Crustacean		Fish		Amphibians	Turtles	Reptiles	Mammals
	Organ	B[b]	G[b]	S[b]	L[b]	F[b]	M[b]				
Cd	Bone	--	--	--	--	10.5 (46)	--	--	--	--	491.2 (29)
	Kidney	--	--	--	--	35.4 (44)	--	--	108.9 (287)	1.2 (96)	176.9 (66)
	Liver	--	--	--	--	19.2 (45)	55.5 (3)	--	25.3 (339)	2.1 (96)	90.9 (66)
	Muscle	1.6 (3)	20 (8)	6 (2)	3.9 (4)	--	--	--	68.4 (322)	1.2 (96)	25.3 (22)
Cu	Bone	--	--	--	--	8.6 (48)	--	1.9 (7)	--	--	10.1 (21)
	Kidney	--	--	--	--	61.5 (46)	--	2.1 (7)	3.8 (206)	9 (14)	0.6 ()
	Liver	--	--	--	--	15.2 (47)	28.5 (2)	14.6 (7)	21.8 (251)	2.4 (14)	9.5 (47)
	Muscle	--	0.7 (8)	1.1 (4)	1.8 (4)	4.8 (49)	1.5 (2)	3.8 (7)	142.1 (236)	1.3 (14)	2.7 (18)
Fe	Bone	--	--	--	--	66.6 (43)	1.9 (6)	1.8 (7)	--	--	--
	Kidney	--	--	--	--	100 (41)	--	2.8 (7)	8.8 (193)	19.7 (14)	--
	Liver	--	--	--	--	15.2 (42)	120.4 (8)	2.3 (7)	42.3 (242)	3.1 (14)	--
	Muscle	3 (3)	1.7 (8)	4.9 (2)	1.6 (3)	13.1 (44)	2.6 (8)	1.6 (7)	6.3 (238)	2.2 (14)	--
Ni	Bone	--	--	--	--	8.2 (44)	--	3.2 (2)	--	--	--
	Kidney	--	--	--	--	39.6 (41)	--	--	97.3 (136)	1.4 (10)	--
	Liver	--	--	--	--	15.2 (39)	--	--	33.8 (156)	1.1 (10)	--
	Muscle	1.8 (3)	0.95 (8)	1.4 (2)	1.4 (4)	5.2 (43)	--	2.3 (2)	5.8 (156)	1 (10)	--
Pb	Bone	--	--	--	--	7.1 (92)	3.1 (6)	7.8 (7)	2 (80)	--	42.6 (45)
	Kidney	--	--	--	--	14.6 (43)	--	4.2 (4)	14.2 (161)	5.4 (100)	127.2 (67)
	Liver	--	--	--	--	267.7 (47)	161.9 (7)	17 (7)	13.5 (178)	9.7 (100)	116.6 (70)
	Muscle	2 (3)	2.2 (8)	1.7 (4)	1.1 (4)	6 (91)	3.3 (7)	1.9 (7)	16.5 (182)	1 (100)	33.3 (30)
Zn	Bone	--	--	--	--	3.5 (55)	2.4 (3)	2.1 (7)	1.9 (80)	--	7.5 (16)
	Kidney	--	--	--	--	23.2 (52)	--	3.1 (7)	43.6 (245)	4.4 (14)	9 (42)
	Liver	--	--	--	--	16.4 (53)	92.8 (4)	2.6 (7)	13.6 (268)	4.4 (14)	4.1 (42)
	Muscle	--	--	--	--	5.9 (55)	2.5 (6)	1.2 (7)	3.3 (251)	1.1 (14)	1.7 (7)

[a] The values in brackets indicate the number of data points on which the original $CR_{wh:t,min}$ and $CR_{wh:t,max}$ values were based.

[b] B: bivalves, G: gastropods, S: small, L: large, F: freshwater, M: marine.

As opposed to metals, no such overview exists for organic chemicals and not many studies are available in which contaminant burdens are assessed in different organs of the same specimen. Therefore, the following discussion on organic chemicals is case-by-case.

Yordy et al (2010) studied the tissue-specific distribution of different groups of persistent organic pollutants in stranded Bottlenose dolphins (*Tursiops truncatus*) found on different beaches in the USA. These authors analysed the concentrations of DDTs, PCBs and polybrominated diphenyl ethers (PBDEs) in 13 different tissues and reported lipid contents of each of these matrices, allowing lipid normalisation. Similar as in the study on metals discussed above (Yankovich et al, 2010), $CR_{wh:t,range}$ values were calculated by subsequently applying Equations 5.25 and 5.26 to the data of Yordy et al (2010). The results are presented in Table 5.3.

Table 5.3: $CR_{wh:t,range}$ values calculated for the different individuals studied in Yordy et al, 2010

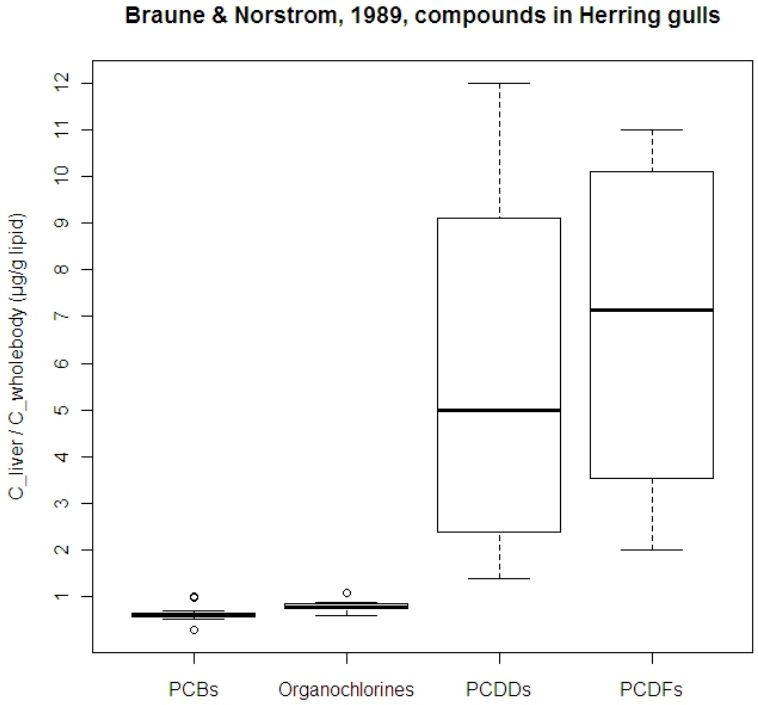
	n	Sum PBDEs	Sum PCBs	Sum DDTs
Blubber	4	2.0	2.0	2.0
Brain	4	7.4	6.9	9.1
Heart	4	4.6	2.6	3.1
Kidney	4	6.0	4.5	4.5
Liver	3	10.3	7.1	8.6
Lung	4	12.0	8.9	10.6
Mammary gland	2	3.2	3.2	4.1
Melon	4	8.4	7.9	8.0
Muscle	4	2.3	2.3	2.7
Spleen	3	2.5	8.4	11.2
Testis	2	5.0	4.5	7.6
Thymus	2	9.0	5.7	7.3
Thyroid	3	1.6	2.1	1.7
Uterus	2	23.5	17.4	13.1

While the thus calculated ranges were expected to be low, given that the data originated from four individuals of the same species sampled in the same area (see discussion above), some interesting observations can be made. Firstly, the range was most narrow for the thyroid (1.79 ± 0.24) and the blubber (1.99 ± 0.03). This suggests that – at least for Bottlenose dolphins sampled in this area – blubber or thyroid could be the best choice of organ for the purpose of extrapolating contaminant burdens to a whole-body concentration. Secondly and more importantly, further analysis indicated that the $CR_{wh:t}$ values for the three different compound groups included in Yordy et al (2010), were practically equal within the same specimen (i.e. 0.220 ± 0.004; 0.438 ± 0.003; 0.321 ± 0.001; 0.240 ± 0.002 for 4 specimens, respectively). This suggests that the same extrapolation factor can be used to derive whole-body concentrations of PCBs, DDTs and PBDEs from their concentration in Bottlenose dolphin blubber. This observation is reinforced by the data of Tanabe et al (1981), who performed a similar study on one specimen of striped dolphin (*Stenella coeruleoalba*) collected on the Pacific coast of Japan. Interestingly, $CR_{wh:t}$ was equally similar (0.220 ± 0.006) for different contaminants (PCBs, DDTs, HCHs and HCB) in blubber, the average value of 0.220 being within the range of this parameter for the data of Yordy et al. The reason for blubber seemingly being such a good surrogate for whole-body concentrations is most likely due to the fact that it has a high lipid content and contributes significantly to the total body mass of dolphins (McLellan et al, 2002), thereby contributing typically > 90% to the whole-body burden of contaminants in cetaceans (Tanabe et al, 1981). The latter explains why blubber concentrations seem to translate to whole-body burdens that well.

Braune and Norstrom (1989) determined the concentrations of different groups of organochlorine compounds in herring gulls (*Larus argentatus*) caught at Lake Ontario, USA. The data from this study are of particular interest as these authors determined the whole-body concentrations as well as the concentrations in liver. The ratio of the lipid normalised contaminant concentrations in liver to the whole- body concentrations (see Figure 5.3) illustrate that not all groups of contaminants exhibit the same bioaccumulation behaviour within the same species. For instance, the accumulation of PCBs is low in the liver as compared to the whole-body concentrations, while for example for PCDFs the opposite is true.

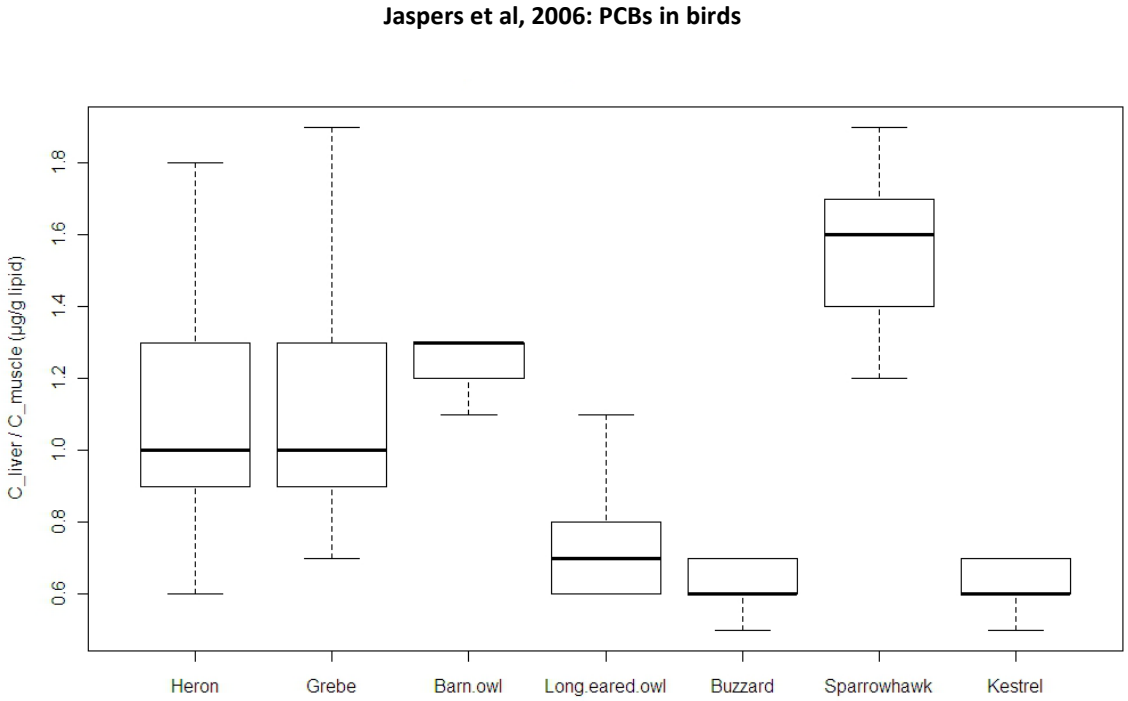
Moreover, the variation between PCDD as well as PCDF congeners is markedly larger than for PCB congeners. Whatever the underlying reason may be (e.g. differences in metabolisation rate or selective deposition), these observations warrant caution when extrapolating organ-specific (in this case liver) concentrations to whole-body concentrations using extrapolation factors derived for a specific group of chemicals. Given that in this specific example the average liver to whole-body concentration ratio of PCDFs is a factor of 10.8 higher than this ratio for PCBs, doing so may lead to substantial errors. As for the 5 different PCDD congeners included in Braune and Norstrom (1989) this ratio spans almost an order of magnitude, similar caution is needed within a group of chemicals.

Figure 5.3 The ratio of lipid-normalised concentrations of different contaminants in livers of Herring gulls to whole-body concentrations (Braune and Norstrom, 1989).



Jaspers et al (2006) determined the concentration of organochlorines in different tissues of seven bird species (Figure 5.4). The results illustrated differences in bioaccumulation patterns between the different species. Some species exhibited similar lipid normalised concentrations of PCBs in liver and muscle tissue (e.g. heron and grebe) while others showed a higher accumulation in either the liver (e.g. sparrowhawk) or muscle tissue (e.g. kestrel). In general, the differences are rather small (up to a factor of 2), but significant. Using a similar extrapolation factor across species can lead to errors as well.

Figure 5.4: The ratio of lipid-normalised concentrations of different contaminants in livers to the concentrations in muscle tissue of different bird species collected in Belgium (Jaspers et al, 2006)



In summary, the cases discussed in this section imply that when using organ-specific concentration data to assess the bioaccumulation potential of a (group of) substance(s), it is important to possess the following information when attempting to extrapolate to a whole-body value:

- Contribution of the organ/tissue to the total body burden, whereby a large contribution is better (e.g. blubber in cetaceans).
- An extrapolation factor that is specific for the (group of) substance(s), the species (group) under investigation as well as the specific organ.

Of course, in many if not most of the cases such information will be incomplete or maybe totally missing. This does not mean that the available data are useless. Much will depend for instance on the severity of the observed accumulation. If accumulation of a certain substance in an organ is observed up to levels that would lead to conclude a high B potential (regardless of the variation of the relevant extrapolation factor), such data can be used in a WoE assessment (given adequate quality of the analytical methods and other procedures used).

5.5.3 Organ-specific concentration data used in BMF/TMF calculations

Organ-specific concentration data are also used in the derivation of BMF and TMF values for the same reasons mentioned above (e.g. ethical considerations and/or impracticalities related to large organisms). However, when organ-specific data are used for the top-level consumers (e.g. marine mammals and birds) but the concentration in the (smaller) prey are measured in whole-body homogenates, this can lead to errors in the calculation of BMF and TMF values (Borgå et al, 2012). Since the organs of choice are almost

always the organs to which the contaminants under investigation preferentially partition to (thus leading to higher concentrations in this organ as compared to the rest of the body), this error typically leads to an overestimation of the bioaccumulation (Houde et al, 2006). In their study of the biomagnification of perfluoroalkyl compounds in Bottlenose dolphin food webs, Houde et al (2006) compared TMF values based on perfluoroalkyl concentrations in plasma to TMF values based on whole-body burden estimations. At a sampling location in Charleston – where they analysed nine different perfluoroalkyl compounds – they found that TMFs based on dolphin plasma were on average a factor of 1.7 ± 0.5 higher than TMFs based on estimated whole-body burdens. Based on data from Tomy et al (2004) – who studied biomagnification of fluorinated compounds in an Eastern Arctic marine food web – Houde et al (2006) made similar calculations of TMFs based on concentrations of PFOA and PFOS measured in the liver and estimated in the whole body of beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*). The TMF based on liver concentrations was on average a factor of 5.3 and 3.8 higher than the whole-body TMF for PFOA and PFOS, respectively. Care must be taken when the datasets used to derive BMFs and/or TMFs contain only organ-specific data for one or more of the included species. If this is the case, it will be important to assess the extent to which the organ in question can be representative of a whole-body burden, i.e. whether it contributes to a high extent to the total body burden such as blubber in cetaceans (see also the previous section) and/or accumulates the contaminant(s) in question up to a higher (or lower) extent compared to other organs/tissues. The knowledge or lack thereof should be taken into account when using TMF and/or BMF values in a WoE assessment of bioaccumulation. This is especially important when the TMF or BMF value is close to 1.

5.6 Bioaccumulation in terrestrial species

Bioaccumulation assessment has traditionally focused on aquatic systems, with fish being the primary receptors of interest. However, several publications have highlighted the importance of assessing bioaccumulation in terrestrial systems, particularly in sensitive Arctic systems (Kelly and Gobas, 2001, 2003). Furthermore it has been suggested that the current aquatic bioaccumulation assessment methods may not be entirely protective of terrestrial systems due to various factors in the taxa of interest, such as differences in elimination mechanisms, body temperature, and digestive tract physiology (Kelly et al, 2007). However, to-date this remains an active area of debate.

There are existing approaches that could be used to assess terrestrial bioaccumulation, several of which are described below. However, it is important to note that currently there are limited data to support setting definitive criteria for terrestrial bioaccumulation. Additional research in this area is needed before any specific guidance can be developed, and further analysis of the applicability of aquatic data, such as that generated from OECD TGs 305 and 315, to terrestrial bioaccumulation needs to be performed.

Various screening criteria for terrestrial bioaccumulation based on physico-chemical properties (e.g. K_{OW}, K_{OA}) have been suggested and are described in Section 3.2. Most of the existing modelling, laboratory, and field terrestrial bioaccumulation approaches and methodologies that could be used for terrestrial bioaccumulation assessment are limited with regard to species coverage, route(s) of exposure, and general understanding of their domain of applicability. Although models exist for terrestrial plants, invertebrates, mammals, birds, and entire terrestrial food webs (see Section 3.2.2 for a summary), these are extremely

limited in their species coverage and often require site-specific input. In addition, there is a lack of high-quality field data to properly validate these models (Gobas et al, 2013).

As is already noted in the Annex XIII guidance, the existing OECD TG 317 (Bioaccumulation in Terrestrial Oligochaetes) is currently the only existing test guideline specifically designed to assess terrestrial bioaccumulation (OECD, 2010). Although this test is more relevant for a terrestrial 'compartment of concern', definitive criteria for this test do not exist. In addition, bioaccumulation effects may be influenced by the specific properties of the test matrix (Vermeulen et al, 2009). There is potential to utilise information from other regularly-conducted laboratory tests to inform terrestrial bioaccumulation, which may include (but are not limited to) mammalian toxicokinetics data, plant uptake, translocation, and accumulation tests, and the aforementioned aquatic bioaccumulation studies. In addition, other existing chronic and reproduction tests such as those conducted on birds, algae, earthworms and fish in accordance withguideline 206, 201; 222; 210; 206 of the OECD (1984, 2001b, 2004c, 2013) could be useful on a case-by-case basis to inform terrestrial bioaccumulation with the addition of residue and depuration measurements (Hoke et al, 2014). It should be emphasised that, with the exception of the OECD (2010) test 317 on oligochaetes, this information can only be used as a screening approach at this stage.

Field data for terrestrial bioaccumulation are currently very limited, although the application of BMF and TMF approaches has been demonstrated with a small number of chemicals (Section 5.3). There are currently no standardised guidelines for the design and interpretation of field studies, and many of the same issues and difficulties associated with use of BMFs and TMFs in aquatic systems (highlighted in Section 5.3) apply to terrestrial systems as well.

A 2013 ILSI-HESI sponsored workshop on Terrestrial Bioaccumulation aimed to discuss the current state-of-the-science for terrestrial bioaccumulation, with a focus on modelling, laboratory, and field approaches. A forthcoming series of manuscripts will describe the major findings from the workshop and propose potential next steps. (Burkhard et al, 2014; Gobas et al, 2014; Hoke et al, 2014; Van den Brink et al, 2014).

5.7 Human and environmental biomonitoring

Biomonitoring can be defined as a scientific technique used to assess the exposure of humans and the environment to natural and synthetic chemicals by sampling and subsequent analysis of tissues and/or fluids of humans or organisms (Zhou et al, 2008). While the term 'biomonitoring' is not explicitly stated, the amended Annex XIII of REACh specifies two types of data that can be considered to result from biomonitoring studies, as follows (Appendix A3.2.2). Interpretation of such biomonitoring data may yield additional information for the purpose of assessment of B or vB properties, using a WoE approach.

1. Data from scientific analysis of human body fluids or tissues, such as blood, milk or fat;
2. Detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment.

The data of the former are collected through 'human biomonitoring' (HBM), the data of latter through 'environmental biomonitoring' (EBM). A notable difference between these two Annex XIII entries is that EBM data are to be compared with contaminant levels in the surrounding environment while no such comparison is explicitly mentioned for HBM data. This is remarkable as the bioaccumulation potential of a substance is

expressed as the ratio, in a steady-state situation, of its concentration in the organism to the concentration in the medium to which the organism is exposed (ECETOC, 2005a). In order to quantify the bioaccumulation potential of a substance, information must be available on both the body burden and the external exposure concentration. Logically, this also applies for HBM and any data on human body burdens should thus be accompanied by knowledge on external exposure concentrations to be useful for bioaccumulation assessments. Thus, the mere presence of a substance in human tissue or fluids does not give an indication of its bioaccumulation potential.

The appropriate weight to be given to any type of biomonitoring data (i.e. in a WoE approach) will depend on the quality and reliability of the concentration data. Guidance for the interpretation and quality assessment of human biomonitoring data is given in ECETOC (2005b; 2011). ECETOC (2005a) provides guidance on the quality, availability, interpretation and adequate design of environmental monitoring studies. In the following sections, the different types of HBM and EBM data will be discussed as well as the ways in which their quality and reliability can be assessed.

5.7.1 Human biomonitoring

In HBM, biomarkers are measured in human tissues and/or fluids to evaluate exposure of a human population to a certain chemical. Such tissues and fluids can be for example blood, fat, urine, hair, nails, sputum or milk. The analytes measured are either native chemicals, metabolites or the products of molecular interactions (e.g. with blood proteins) (Tan et al, 2012). HBM data as such are only a measure of the internal exposure to a chemical and do not provide a direct estimate of environmental exposure. This is because internal concentrations of a chemical are the result of integrated exposures from multiple sources and pathways as well as of the body's various clearance mechanisms (e.g. metabolism and excretion) (Clewell et al, 2008). Moreover, external contaminant concentrations fluctuate in time and the kinetics of the measured chemical determine the response of biomonitoring data on these variations. For example, HBM data of substances with a very short half-life (e.g. a few hours) will reflect daily variations while for substances with a long half-life (e.g. several months) such data will rather reflect concentrations averaged over time (Clewell et al, 2008).

No peer-reviewed literature is available in which HBM data are directly used to evaluate the bioaccumulation potential of substances. However, many studies exist in which attempts have been made to reconstruct human exposures based on HBM data for supporting risk assessment and risk management (Tan et al, 2012). Such studies may help to illustrate the complex relation between external and internal exposure of humans. This is necessary as the WoE approach as proposed in Annex XIII is to be used when insufficient data are available for comparison with the direct criteria (in this case of the bioaccumulation assessment). Any bioaccumulation assessment based on HBM data will depend for the most part on a comparison of these data with external exposure concentrations and on the quality of these data.

Typically, there are two types of HBM studies: population-based biomonitoring and occupational biomonitoring. In the former, samples can be obtained from the same individual at different points in time or from a group of many individuals at one time. In the latter, the biomonitoring data are usually systematically accompanied by other exposure-related data such as contaminant concentrations in air, the duration of exposure, work practices and/or use of protective gear.

Interpretation of age trends in blood levels

When the levels of a given chemical in human blood are observed to increase as a function of age, this phenomenon is sometimes referred to as evidence for bioaccumulation (see, for example: Jaga and Dharmani, 2003; Genuis et al, 2011). The use of this terminology is understandable if the increase in level occurs in a given individual as he or she grows older, since it does indeed correspond to accumulation in biota, hence referring to the phenomenon as 'bioaccumulation' may appear quite acceptable.

Nevertheless, in the context of the assessment of chemicals as '(very) bioaccumulative' under REACh or other legislations, mere increasing trends of blood levels with age are insufficient to conclude that a 'B' status should be assigned to the chemical. This section explores why this is the case and points out some of the pitfalls involved in uncritically interpreting time-trends seen in human blood (whole, plasma, serum) or in other body fluids like milk and urine, or in tissues.

First, it is obvious that when a human or other organism is first exposed to a chemical that is freshly released to the environment, and a constant exposure is subsequently maintained, the blood level of the chemical in the organism will initially rise until a steady state is eventually achieved. Clearly, the mere qualitative observation of a rise in concentration, during the approach to steady state, cannot be taken as a firm indication of 'B' status, since this phenomenon will occur with any chemical, irrespective of its intrinsic propensity to bioaccumulate.

Second, most studies on the age-dependence of the levels of chemicals in blood are so-called 'cross-sectional' ones. This means that the blood of cohorts of persons within a series of narrow age bands is sampled more or less at the same time and the average levels of a given chemical in the blood of each cohort are plotted versus age. So the levels of the chemical are generally not monitored in a given individual (or cohort of individuals) over the course of many years. The latter approach would correspond to a 'longitudinal' study, but such studies are conducted only infrequently for chemical pollutants (Tee et al, 2003). Since the different age cohorts examined in a cross-sectional study will have different time-histories of exposure to a given pollutant, the results of such studies will not be representative of how the levels of the chemical vary with aging of a given cohort, or of an individual belonging to the cohort.

Third, it should be pointed out that all commercial chemicals undergo a life cycle of technological development, market ascent, maturity and possibly decline. Release of a chemical to the environment, and hence the potential for human exposure, is likely to follow a similar, but not necessarily identical, pattern. In any case, the constantly changing exposure complicates the analysis of blood-level time-trends of a chemical in terms of its 'B' status.

This complex issue has been thoroughly investigated by Quinn and Wania (2012) in the case of PCB153, using CoZMoMAN, an environmental fate and food-chain bioaccumulation model that estimates how human body burdens of contaminants vary with age, in response to time-varying environmental emissions. In addition to simulating constantly changing environmental concentrations, this model also takes into account such factors as variations of food consumption and body weight with the age of individuals, and loss of contaminant through childbirth and breast feeding.

The modelling results confirm that the relationships between body burdens and age are not equivalent for population cross-sections and for individuals over time. Furthermore, for contaminants (such as PCB153)

that have human metabolic half-lives longer than 1 year, the factor that has the greatest influence on the shape of the concentration versus age trends is the time elapsed between the peak in environmental emissions (estimated to be 1974 for PCB153) and the moment of sample collection. Thus, the cross-sectional body burden versus age plot for PCB153 in 1968 shows a decline from 20 to 80 years of age, while the corresponding prediction for 2030 is for a steady increase in body burden over the same range of ages.

The main inference that can be drawn from the study by Quinn and Wania (2012) is that it is erroneous to uncritically assume, in a WoE 'B' assessment, that increasing human blood levels of a contaminant with age, in either cross-sectional or longitudinal studies, are a reliable indication of the propensity of the substance to bioaccumulate.

However, this does not mean that human biomonitoring data cannot be useful as additional information in a bioaccumulation assessment. Human biomonitoring data can be used to determine elimination half-lives of chemicals. In general, a longer elimination half-life of a substance may indicate a greater potential to bioaccumulate. Elimination half-lives can be estimated from existing cross-sectional biomonitoring studies that are applied to population pharmokinetic models (Ritter et al, 2011). More recently, Zhang et al (2013) showed that levels of perfluoroalkyl acids in human urine can be positively correlated with levels of the substance in blood. The advantage of this method is that urine is a less invasive sampling method than blood or some other tissues, and the substance levels in urine samples could be used to estimate the biological elimination half-lives of several PFAAs. The data obtained from such studies can be used as input for the calculation of the bioaccumulation metric based on clearance or elimination half-life and proposed by Goss et al (2013) (described in Section 5.9 on the use of toxicokinetic information).

Case study: Perfluoroalkyl acids

The anions of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulphonic acids (PFSAs), which both belong to the overall family of perfluoroalkyl acids (PFAAs), have been found in the blood of humans for many years all around the world (EFSA, 2008). The first publications indicating the presence of PFAAs in the blood of the general population date back to the early 2000s (EFSA, 2008), although some of the samples analysed in these studies were taken as early as 1974 (Olsen et al, 2005). Voluntary medical surveillance of workers at plants that produce or use PFAAs started already in 1993.

Thus human biomonitoring of PFAAs has been carried out in both occupational cohorts and the general population. For instance, regarding perfluorooctanoic acid (PFOA), the highest levels reported to date in the general population are similar to some of the lowest levels in workers exposed to PFOA occupationally. PFOA is one of the most highly studied PFAAs. It is known to have a long elimination half-life in humans (see below), on account of its binding to proteins and its resistance to metabolism, and has been measured in many biomonitoring studies on blood of the general population (EFSA, 2008) since 1974 (Olsen et al, 2005).

Several studies identified higher levels of PFOA in persons living in the proximity of fluorochemical production facilities and/or exposed to contaminated drinking water (Emmett et al, 2006; Wilhelm et al, 2008) in comparison to the control population. Moreover, analysis of the correlation between serum concentration of PFOA and personal use of water allowed identification of contaminated water as the main source of exposure in one of the studied populations (Emmett et al, 2006).

Based on biomonitoring data, the half-life of PFOA in humans has been estimated by several authors. Thus, monitoring of PFOA plasma levels of a population from Arnsberg (Germany) exposed to contaminated drinking water showed a significant decrease in PFOA levels after the introduction of a charcoal filtration system. Based on subsequent two-year biomonitoring data, Brede et al (2010) were able to calculate a mean plasma half-life of PFOA of 3.26 years. The elimination half-lives from human serum of PFOA and two PFSA homologues were also determined in retired US fluorochemical production workers, with no more occupational exposure (Olsen et al, 2007). In this study, blood sampling over a 5 year-period showed that the elimination of PFAAs from serum followed first-order kinetics, the half-life being 3.8 years in the case of PFOA.

A time-trend study on ski technicians' exposure through inhalation of fumes containing an unidentified blend of perfluoroalkyl substances from fluorinated ski wax application was performed in 2007/2008 (Nilsson et al, 2010). Levels of PFCAs in blood were analysed monthly before the ski season, during the ski season and during the unexposed post-season period. Ski technicians with initial 'low' levels of PFOA showed an increase in blood level of PFOA over the ski season, whereas the technicians with initial 'high' levels of PFOA did not show an increase in blood levels. The blood levels of perfluorohexanoic acid (PFHxA) were simultaneously analysed in the same group of ski technicians. Based on analysis of temporal biomonitoring data, the apparent half-life of PFHxA in blood of highly exposed individuals has been estimated to be between 14 and 49 days (Russell et al, 2013). In a separate study on ski waxers (Freberg et al, 2010) a significant correlation was established between the number of working years and levels of seven PFCAs in blood.

In the particular examples outlined above, in which periods of high exposure were followed by a distinct depuration phase after the cessation or drastic reduction of exposure, it was possible to use biomonitoring to determine the elimination half-lives of PFAAs. As demonstrated by Goss et al (2013), there is a direct correlation between increasing elimination half-lives and increasing biomagnification potential, so the former may be considered appropriate as a metric for bioaccumulation. This confirms the interest of biomonitoring in contributing to the B assessment.

In the case of biomonitoring studies on the general population of individuals exposed to more or less 'background' levels of PFAAs, or other contaminants, it is more difficult to come to a conclusion as to whether a substance is bioaccumulative or not. As shown by Quinn and Wania (2012) in the case of PCB153, the variations of human body burdens with age are largely determined by the time history of exposure of the individuals involved, which is generally poorly characterised.

Nevertheless, interpretation of human temporal biomonitoring data in the general population has been quoted in the REACh Annex XV dossier for PFOA as contributing to the WoE that this substance should be considered to be bioaccumulative (ECHA, 2013). First, modelling studies performed using the Ritter et al (2011) toxicokinetic approach were used to calculate the PFOA serum concentration as a function of age between 20 and 55 years old, when body weight was assumed to be constant. The exposure was also set to a constant value. For assumed half-lives of 2.3 or 5.0 years, this led to a moderate build-up of the modelled serum concentration after the age of 20 to reach a maximum (at most 20% greater than the initial level) at the age of early- to mid-40s, followed by a slight decline thereafter. This unpronounced temporal trend was claimed to be evidence for bioaccumulation. Second, two Norwegian studies (Haug et al, 2010; 2011) showing statistically significant increases of PFOA serum concentration with age in the general population were also taken as evidence for bioaccumulation, while three US studies that showed no such correlation

were discounted (Olsen et al, 2003; 2004; Calafat et al, 2007). Third, a study that indicated significantly increasing PFOA plasma concentrations with time in women after their most recent pregnancy (Brantsæter et al, 2013) was deemed indicative of bioaccumulation, on the grounds that PFOA was observed to re-accumulate in blood after excretion due to childbirth and breastfeeding.

5.7.2 Environmental biomonitoring

As mentioned above, Annex XIII describes "the detection of elevated levels of a chemical in biota compared to the levels in their surrounding environment" as information that can be included in a WoE assessment of bioaccumulation. In the best case such data allow the calculation of BAFs, BMFs and/or TMFs. Such field-derived data integrate multiple exposure routes and processes that potentially lead to bioaccumulation (and possibly biomagnification) and are the ultimate indicators of the natural bioaccumulation potential of a chemical (Weisbrod et al, 2009). However, in reality reliable BAFs (and/or BMFs and TMFs) are available for only a small percentage of industrial chemicals currently on the market. Arnot and Gobas (2006) report for instance that for only 0.3% of organic chemicals on Canada's Domestic Substances List that require a bioaccumulation assessment under the Canadian Environmental Protection Act of 1999 (Government of Canada, 1999; 2000), a BAF value is available. This was mainly due to a lack of concomitant measurement of chemical concentrations in the water phase. Of these BAFs, roughly 60% was deemed reliable. It can be reasonably assumed that there is only a small number of chemicals on the market for which adequate data are available to directly assess bioaccumulation potential based on EBM data. In cases where concentration data are available for both biota and the surrounding environment but a reliable calculation of a BAF, BMF and/or TMF is not possible, the usefulness of these data (or the weight that can be assigned to them) in a WoE bioaccumulation assessment will be proportionate to their quality or reliability as well as to their level of compatibility.

It is worth pointing out that the interpretation of 'the detection of elevated levels of a chemical in biota compared to the levels in their surrounding environment' as an indication of bioaccumulation should be approached with caution. While Annex XIII of REACh defines BCF criteria of 2,000 for bioaccumulation, the wording of the above statement suggests that any BCF > 1.0 could be interpreted as being indicative of bioaccumulation and that, if the level of a chemical in a fish species is greater than that in the surrounding aquatic environment, this can be used as evidence of bioaccumulation. This is also of particular concern when considering terrestrial or avian species; it is unreasonable to consider that the detection of higher levels of chemicals in terrestrial or avian species compared to levels in their surrounding environment (air) is evidence for bioaccumulation. The statement does not take dietary uptake routes into account.

In the context of EBM, the sources of data uncertainty are errors associated with chemical analysis (a function of the analytical technique and procedures) and the environmental and biological variability (including uncertainty regarding steady state as well as extrapolations from specific tissue concentrations inherent to the environmental system in question). That the uncertainty of EBM data can be substantial is illustrated by the observation that 95% confidence intervals of measured BAFs can encompass up to 4 orders of magnitude (Arnot and Gobas, 2006; based on BAFs from fish of two general trophic levels from the same ecosystem). This observation emphasises the need to have multiple observations – especially in case the data used are insufficient to calculate reliable BAF/BMF/TMFs – in order to substantiate a substance's bioaccumulation potential based on EBM data. In the context of the Water Framework Directive

implementation, efforts are being made to develop guidance to design and conduct environmental monitoring campaigns, which once published would be useful to minimise some of the data uncertainties.

Regarding chemical analysis, acceptable methods should be used to extract chemicals from biotic and abiotic samples and for their subsequent identification and quantification. Furthermore, these methods should be further verified with appropriate QA/QC protocols according to GLP (Arnot and Gobas, 2006). In the past, clear QA/QC procedures were not available and are (together with GLP compliance) rarely reported in the literature. Nowadays, with many journals offering the possibility to publish supporting information, QA/QC reporting can easily be incorporated in scientific papers.

But even when the uncertainty of the chemical analysis is kept to a minimum, considerable biological variability exists that contributes to the overall uncertainty in bioaccumulation assessments. Parameters that influence a chemical's bioaccumulation include age or life-stage, gender, reproductive status and size (e.g. Nichols et al, 1998; Russell et al, 1999). Organisms with a higher lipid content generally exhibit higher bioaccumulation due to a higher storage capacity (this does not hold for chemicals which partition primarily to other compartments, e.g. fluorochemicals), requiring lipid normalisation of any calculated bioaccumulation parameter to allow comparison between species. Moreover, a chemical may biomagnify in the food web. When assessing a limited amount of data that does not allow a profound assessment of the biomagnification potential, this may lead to wrong conclusions and inconsistencies between bioaccumulation assessments of different chemicals.

A summary of the factors contributing to the uncertainties involved in bioaccumulation assessments is presented in Table 5.4. Recently, attempts have been made to understand the uncertainty in bioaccumulation assessments derived from field studies and these have found that much of the variability in 'B' estimation relates to bioavailability for benthic species and to understanding the diet (both what was eaten and how much chemical was in the diet) for higher predators (Selck et al, 2012). These authors also question the BCF as the only 'B' metric for bioaccumulation.

In principle, data on all organisms are useful (terrestrial and aquatic plants, marine mammals, terrestrial organisms and birds) for demonstrating bioaccumulation and/or trophic magnification. In a review of data derived from the laboratory and the field it was considered that the use of both laboratory and field data could improve confidence in classifying bioaccumulation and data was presented to show that BSAFs (biota sediment accumulation factors) measured in the field or the laboratory for both oligocheates and molluscs did not vary more than a factor of two or three for the same substance (e.g. if steady-state BCFs were compared) (Burkhard et al, 2012a,b). The use of a trophic magnification factor (TMF) as a tool for understanding biomagnification across food webs has also been advocated as a holistic approach for understanding bioaccumulation from field studies (Conder et al, 2012).

Recent evidence suggests that the focus in regulations on aquatic bioaccumulation may have led to issues related to terrestrial ecosystems (such as soil bioavailability, atmospheric deposition and spatial variance in occurrence and uptake of chemicals) not being accounted for in some circumstances. Environmental biomonitoring therefore while being retrospective is one way in which 'unusual cases' can be identified.

Table 5.4: Important information and sources of uncertainty and variability which need to be assessed and taken into account when establishing the overall reliability (and weight) of EBM data to be used in a WoE bioaccumulation assessment.

Chemical analysis	Concentrations in biota	Concentration in surrounding environment (e.g. water or sediment)
Criteria for GLP	Temporal variability	Temporal variability
Use of QA/QC protocols	Spatial variability	Spatial variability
Use of blanks	(Lipid) normalisation	OC content
Use of proper reference materials	Age/life stage of the organism(s)	
Detection limit/span>	Reproductive status	
Quantification limit	Steady state assumption	
	Trophic level	
	Migratory behaviour	

5.8 Chronic toxicity in animals

The amended Annex XIII published in March 2011 reports that results from chronic toxicity studies in animals can be used as additional information in support of WoE bioaccumulation assessments. The amended draft guidance R.11 currently states that "the complete absence of effects in the long term is an indication that the compound is either chronically non-toxic and/or that it is not taken up to a significant extent" and it is specified that toxicity tests on mammals are targeted. Such findings from chronic toxicity studies on mammals may be used to exclude the bioaccumulation potential of a chemical. The aim of this chapter is therefore to explore the potential to extract information on bioaccumulation from classical toxicity laboratory studies aiming primarily to determine chronic toxicity in mammals. In what follows, some general considerations that should be made when using such data in a WoE bioaccumulation assessment will be discussed, followed by a few examples.

5.8.1 General considerations

Chronic toxicity studies that can provide useful information on the bioaccumulation potential of a substance should yield endpoints that can be indicative of bioaccumulation. For instance, endpoints related to carcinogenicity or skin irritation are not *per se* indicators of a substance's bioaccumulation potential, so the absence of these effects alone may not be used to exclude it.

For data-rich substances repeated dose studies ideally in the same species and with the same route of exposure are available. In this case indications for possible accumulation can be derived, if tissue levels of the substance or relevant metabolite in different tissues have been measured and are increasing, for example in fat tissues with the exposure duration at comparable dose levels. Another indication would be a disproportionately lower effect level in a study with longer duration, if the same target organ is affected, compared to standard duration extrapolation factors derived statistically from a wide range of compounds (ECETOC, 2003b). On the other hand, if for example the systemic effect levels for the same target organ are as expected for duration extrapolation or even higher in studies of longer compared to the shorter duration (e.g. 28-day and 90-day studies), this could be an indication of a low bioaccumulation potential. Similar

considerations could be made if two-generation studies were performed. If the effect levels for the same target organ were much lower in for example the F_1 generation compared to the F_0 generation, this could be an indication of a potential accumulation, whereas if the effect doses are comparable, bioaccumulation is less likely to occur. All these considerations need to take into consideration the dose levels and dose responses in the different studies.

Sometimes histopathological findings could indicate a deposition of a substance in certain organs. For instance, analysis of histopathological findings after a sub-acute or sub-chronic toxicity study in animals in target organs could give an indication in a WoE approach of bioaccumulation. Findings like liver hypertrophy generally indicate that the substance has been absorbed from the gastrointestinal tract and undergoes liver metabolism. Hypertrophy in the liver indicates enzymes induction and metabolic reactions. Together with information on the structure and possible metabolites of the substance, this information can be used to indicate the likelihood of formation of hydrophilic metabolites that are likely to limit bioaccumulation. If on the other hand there is evidence of increasing substance concentration in the liver without metabolism in the presence of hypertrophy, this could indicate a potential for accumulation of the substance itself or a metabolite in this organ. Such findings could trigger further studies on toxicokinetics that would serve to identify a possible mode of action with regard to the B assessment as well as human health hazard assessment.

On the contrary when histological effects are confined to irritative responses observed locally or systemically, an accumulation of the test item can be considered unlikely.

In any case analysis of the dose response and consideration of toxicokinetic data may help to determine the relevance of the data for inclusion in WoE considerations for the PBT assessment.

The task force identified some possible indicators of bioaccumulation from mammalian repeated dose studies. However, the interpretation depends strongly on the individual dataset and needs to be used on a case-by-case basis in a WoE approach after an in depth analysis of the available toxicological data. Due to the variabilities in the datasets general suggestions/schemes cannot be provided.

5.9 Toxicokinetic behaviour

The amended Annex XIII newly introduces the assessment of the toxicokinetic behaviour of the substance into the WoE approach for bioaccumulation. This 'endpoint' has already been subject to existing REACh guidance. Typically, toxicokinetic data may be available from mammalian studies in laboratory animals (mammals) or from studies in human volunteers or occupationally exposed workers. Increasingly, such data are also becoming available for fish.

Obviously the ADME (adsorption, distribution, metabolism and excretion) processes will determine the concentration of substances in organisms and tissues. Depending on the quality of the existing data the toxicokinetic behaviour can be assessed either qualitatively or quantitatively. If a substance is poorly absorbed, excreted mainly unchanged in the faeces and does not elicit systemic toxicity in repeated dose studies, this can be used in a qualitative way as supporting information for assuming a low potential for bioaccumulation. Additional information of relevance could be the tissue distribution, giving an indication of

a possible accumulation in fat tissue, or for example protein binding which may indicate that perhaps other tissues and modes of accumulation than partitioning into fat may need to be considered.

For a more quantitative assessment toxicokinetic modelling can be applied to determine the potential for biomagnification. The choice of the model may be influenced by the rate limiting step for the kinetics which may either be the rate of absorption or metabolism and excretion. This is particularly important if a steady-state situation is not reached during the duration of the study. Model calculations may need to be adjusted, if the absorption and elimination rates do not follow pseudo-first order kinetics, which is usually the initial assumption.

The mammalian toxicokinetic data can give an indication for a possible biomagnification in mammalian food chains. If toxicokinetic data in aquatic organisms become available, toxicokinetic models can also be used in the assessment of bioaccumulation or biomagnification in aquatic organisms. If more data become available they may also be used to derive information on a possible 'read across' from mammalian data to fish data, as many metabolic pathways are preserved in different species.

5.9.1 Elimination half-life as a metric for biomagnification potential (Goss et al, 2013)

The simplest toxicokinetic approach is one in which the predator/consumer organism is considered to be a one-compartment homogeneous mass, without any distinction being made between its constituent organs or tissues. While this assumption might be regarded as an unreasonable over-simplification, its use in connection with a bioaccumulation metric is in fact justified by reference to the definition of the biomagnification factor (BMF), (Section 5.3). Indeed, the BMF is also defined, in its simplest expression, as the ratio of the overall concentration of a substance in the predator/consumer to that in the prey/diet, without any regard to the detailed structure of the organisms involved (nevertheless, concentrations used to define BMF are, in some cases, normalised to overall lipid or protein content, or even to individual organs, tissues or body fluids, as discussed elsewhere in this report).

Such a one-compartment model has been applied by Goss et al (2013) who established a simple relationship between biomagnification of a substance from prey to predator (i.e. from diet to consumer) and the elimination rate of the substance in the predator/consumer. According to these authors a substance can be considered as bioaccumulative (BMF >1) when its elimination half-life exceeds a certain threshold value (to be discussed below).

This conclusion is quite general: it applies to any predator/prey (or consumer/diet) relationship at whatever trophic levels within a food chain, encompassing both water- and air-breathing organisms. It also applies to all categories of chemicals, not only to the lipophilic ones that partition preferentially to fatty tissues, but also to substances that exhibit other modes of interaction with biological systems, binding for example to proteins, provided that the steady state is achieved. The relationship between elimination half-life and BMF is derived by performing a simple mass balance on the substance in the predator (or consumer), assuming that a steady-state has been achieved, i.e. the concentration of the substance does not vary with time in either the predator (consumer) or its prey (diet). This is indeed the same assumption that is made in the definition of BMFs. Given this steady-state condition, it can easily be shown that:

$$C_C/C_D = (1/\ln 2) \times \alpha \times F \times EL_{0.5} \quad \text{..... (Eq. 5.27)}$$

where C_C and C_D are the mean overall concentrations (kg/kg) of the substance considered in the consumer and its diet, respectively; α is the uptake efficiency, or fraction of the chemical in the diet actually assimilated by the consumer; F is the feeding rate of the consumer, i.e. the overall amount of diet taken in per unit bodyweight per unit time (kg/kg/d); and $EL_{0.5}$ is the elimination half-life (d).

Since C_C/C_D is equal to the BMF, when the latter is expressed in its simplest form (i.e. without any normalisation of C_C and C_D to lipid or protein content of the consumer and its diet, or any correction being made to 'precise' trophic levels for predator and prey), then:

$$BMF = (1/\ln 2) \times \alpha \times F \times EL_{0.5} = 1.44 \times \alpha \times F \times EL_{0.5} \quad \text{..... (Eq. 5.28)}$$

Assuming a chemical to be bioaccumulative (or, more precisely, prone to biomagnification) if its BMF is greater than 1.0 (ECHA, 2011b), the condition for 'B' categorisation would then be:

$$EL_{0.5} > 0.7 / (\alpha \times F) \quad \text{..... (Eq. 5.29)}$$

To determine whether or not a substance fulfils the biomagnification criterion (BMF > 1.0), as expressed in terms of elimination half-life in Equation 5.29, therefore requires knowledge of three parameters, the measurement or estimation of which will be discussed below:

- α, the uptake efficiency of the substance concerned: this can either be assumed equal to 1.0, leading to the most stringent (i.e. lowest) value of the half-life criterion, or it can be measured or estimated.
- F, the feeding rate: default values may be adopted for the various categories of consumers considered (e.g. humans, rodents, fish). F will vary along a food chain.
- $EL_{0.5}$, the elimination (or clearance, or depuration) half-life. Elimination, as considered here, is the resultant of all processes leading to removal of the chemical from the predator/consumer organism, including biotransformation (metabolism) and physical excretion.

To derive an order-of-magnitude estimate of the values of the elimination half-lives likely to be characteristic of biomagnifying chemicals, Goss et al (2013) assumed that α = 1.0 ('worst-case scenario') and F = 0.01 kg/kg/d, the latter being taken as a typical feeding rate for humans and many animals. Substituting these values into Equation 5.29 leads to the following condition for confirmed biomagnification potential:

$$EL_{0.5} > 0.7 / 0.01, \text{ i.e. } EL_{0.5} > 70 \text{ days} \quad \text{..... (Eq. 5.30)}$$

If the uptake efficiency were lower than the maximum value of 1.0, say only 0.5, the $EL_{0.5}$ threshold required to meet or exceed the biomagnification criterion BMF > 1.0 would be as long as 140 days. On the other hand, consumer organisms with feeding rates greater than the 0.01 kg/kgbw/d value adopted here would have correspondingly lower $EL_{0.5}$ thresholds. However, the authors of the Goss et al (2013) paper stress the fact that their study is intended only to introduce the concept of using the $EL_{0.5}$ as a regulatory metric for bioaccumulation and not to propose a specific threshold value for categorising a substance as 'B', which requires further scientific debate.

The below sections discuss, in general terms, how the input parameters might be derived for the application of the proposed metric.

Uptake efficiency (α)

Clearly, knowledge of the uptake efficiency (α), also known as the dietary absorption or assimilation efficiency, is paramount in the estimation of $EL_{0.5}$, if the latter is not to be merely intended as a lower-limit, worst-case value. Furthermore, not only will sufficiently low values of α exclude biomagnification, but in the extreme case in which $\alpha = 0$ there would be no exposure of the organism to the chemical and hence toxic effects would be excluded.

Values of α may be determined experimentally (Kelly et al, 2004, and references therein) or estimated, for example using correlations with K_{OW} (Kelly et al, 2004; O'Connor et al, 2013). It appears that the uptake efficiency is high at intermediate values of log K_{OW}, but falls off gradually to zero when this latter parameter is high, e.g. above 6 to 7, depending on the animal species (Kelly et al, 2004), or low, e.g. below 2 to −2, depending on the hydrogen bond donor strength of the substance (O'Connor et al, 2013). Furthermore, the 'plateau' of α achieved at intermediate log K_{OW} values may reach the maximum theoretical value of 1.0, but may also be considerably lower, depending on the animal species and the properties of the substance, e.g. down to almost 0.4 in fish (Kelly et al, 2004).

Feeding rate (F)

Feeding rates vary over a rather broad range, depending on the animal species considered. Some typical values are listed below:

Species	F, kg/kgbw/d	Reference
Rainbow trout	0.009	Kelly et al, 2004
Dairy cow	0.016	
Caribou	0.021	
Ring dove	0.074	
Harp seal	0.032	
Stellar sea lion	0.050	
Wolf	0.028	
Human	0.011	
Rat	0.040	Goss et al, 2013

Elimination half-life ($EL_{0.5}$)

Elimination half-lives may be measured experimentally on animal species, e.g. from depuration experiments on fish in the OECD Guideline 305 test, from toxicokinetic studies on laboratory animals or from analysis of human body fluids after individuals are removed from exposure to a chemical following retirement from work or transfer to other activities (Olsen et al, 2007). In the case of human pharmaceuticals, clinical trials may also be a potential source of $EL_{0.5}$ data. Furthermore, $EL_{0.5}$ values can also be derived using physiologically based toxicokinetic (PBTK) models.

Categorisation procedure

Once α (depending on both the chemical and the animal species), F (depending on the animal species alone) and $EL_{0.5}$ (depending on both) have been measured or estimated, the right-hand side of Equation 5.29, namely 0.7 / ($\alpha \times$ F), is calculated, giving a result (D) in days. D may be considered to be the 'biomagnification limit elimination half-life'. If the value of $EL_{0.5}$ exceeds D, this is an indication of a potential for the substance to biomagnify.

5.9.2 Other metrics for biomagnification, especially in terrestrial organisms, including humans

Three further studies have addressed, via toxicokinetic modelling approaches, the need for bioaccumulation metrics that are either especially focused on humans, or apply generically to food chains terminating in terrestrial or avian species.

Human bioconcentration factor (hBCF)

Assessment of the bioaccumulation potential of chemicals in humans was addressed by Tonnelier et al (2012) using a more realistic approach than the ultra-simplified one of Goss et al (2013), namely application of a PBTK model, originally developed by Jamei et al (2009). This latter model was the basis for the software used by Tonnelier et al (2012), i.e. the Simcyp (2013) population-based ADME simulator, widely used in the pharmaceuticals industry for drug development. Tonnelier et al (2012) adopted the simplest version of the Simcyp software, comprising three compartments: the human liver, portal vein and systemic blood circulation.

The concept applied by Tonnelier et al (2012) for the development of a human bioaccumulation metric using the Simcyp® software was that small doses of chemical (D, mg) are constantly administered at regular intervals (T, h), so that the blood concentration of the chemical gradually increases until it reaches a quasi steady-state value C* (mg/l). In this chronic exposure mode, the 'human bioconcentration factor' (hBCF) derived from the model simulation is then defined as:

$$hBCF = b \times C^* / (D/T) \quad \text{..... (Eq. 5.31)}$$

where b, a normalisation factor that leads to a dimensionless hBCF value, is taken to be V_{PV}/t, with V_{PV} (L) being the volume of the portal vein and t unit time (1 hour).

One of the main objectives of the study by Tonnelier et al (2012) was to determine and compare hBCF values for 96 substances selected from a broad range of categories (industrial chemicals, plant protection products, pharmaceuticals and natural products) and to examine possible correlations between hBCF and substance properties. The results showed that log K_{OW} had only a relatively weak influence on hBCF, while the (fish) BCF of substances was also a poor predictor of human bioaccumulation.

On the other hand elimination was shown to have a predominant role in determining hBCF, in line with the basic tenet of the Goss et al (2013) paper. Indeed, iso-hBCF curves could be satisfactorily 'mapped' on a 2-dimensional log-log plot, in which the ordinate was the unbound intrinsic hepatic clearance (accounting for

metabolism) and the abscissa the free fraction in plasma (directly related to renal excretion), demonstrating that clearance is the main determinant of human bioaccumulation.

Furthermore, Tonnelier et al (2012) suggested not only that the hBCF of a chemical could be deduced *via* this mapping procedure, but also that the two basic input parameters could be reliably measured by two in vitro tests: one to calculate the chemical binding to plasma proteins using standard techniques such as equilibrium dialysis, ultrafiltration or ultracentrifugation, the other to estimate the liver clearance by measuring metabolite formation and/or substance depletion in the presence of human hepatocytes. This in vitro approach would be fully in line with current trends aimed at reducing in vivo animal studies.

The assessment of bioaccumulation potential expressed by the hBCF is based on the use of a PBTK model to determine the steady-state concentration reached when constant input of a chemical is maintained. As a potential alternative descriptor of bioaccumulation, Tonnelier et al (2012) also evaluated the half-life for increase in blood concentration of a substance, from the beginning of exposure until the steady state was reached. This quantity was found to be correlated with hBCF (r^2 = 0.59 in a log-log plot).

For 12 chemicals for which experimental toxicokinetic data were available, the 3-compartment Simcyp® model gave predicted results that were in fair agreement with the observations, with one notable exception – the pharmaceutical thioridazine – for which the observed half-life was 80 times shorter than the calculated one. This discrepancy highlighted a limitation of the Tonnelier et al (2012) procedure, in which the only clearance pathways considered in the model are liver metabolism and minimal renal excretion, while the main excretion route for thioridazine seems to be through the faeces. Furthermore, for some chemicals, metabolism in the gastrointestinal tract may play a role, but this too is not considered in the model.

Another potential limitation is that in vitro measurements may not adequately reflect in vivo activity. In particular, care must be taken to select test chemical concentrations that are low enough to avoid saturation when measuring substrate depletion.

Finally, a potential drawback to application of the model is that it requires access to commercial Simcyp software.

(Human) Multimedia Bioaccumulation Factor (mmBAF)

McLachlan et al (2011) adopted a somewhat different approach, from that of Tonnelier et al (2012), to bioaccumulation in humans. Building upon earlier work by the same group (Czub and McLachlan, 2004a, b), these authors used what they described as a 'holistic multimedia perspective' to assess human bioaccumulation. This involved performing simulations using a fugacity-based Mackay Level 1 environmental model linked to the bioaccumulation model ACC-HUMAN (Czub and McLachlan, 2004b), which describes how chemicals are transferred from the environment to humans through aquatic and agricultural food chains.

For each consumer organism in both food chains, the ACC-HUMAN model performs mass balances, quantifying the various routes for uptake and elimination of pollutants. The human organism is treated very simply in this model, as consisting of two compartments – lipid and water – assumed to be at equilibrium. Binding of contaminants to proteins is not considered.

The final output of the modelling exercise is the calculation of the human Multimedia Bioaccumulation Factor, mmBAF, expressed in m^2/person. This is the ratio of the body burden of a chemical in a human to the amount of the chemical present in 1 m^2 of the multimedia environment, so that it represents the area of the environment that contains the same amount of chemical as the person. A high mmBAF value therefore implies that the organism has concentrated chemical from a large area of the environment.

One of the primary goals of the study by McLachlan et al (2011) was to critically examine the often expressed belief that bioaccumulation is primarily determined by chemical partitioning properties, especially hydrophobicity. To this end, mmBAFs were calculated for a set of hypothetical chemicals, exhibiting ranges of octanol-water and octanol-air partition coefficients (K_{OW} and K_{OA}, respectively) ranging over 12 orders of magnitude. The results of this exercise demonstrated that, in the multimedia perspective considered, in which human exposure resulted from contributions from both aquatic and agricultural food chains, the partitioning properties had only a rather modest impact on the calculated mmBAFs. On the other hand, it was shown that the rate of biotransformation of a chemical in the human organism was the major determinant of its mmBAF. The model results were supported by observations reported in the literature on certain well characterised pollutants, showing that chemicals with similar partitioning properties can have widely differing bioaccumulation behaviour.

The conclusion of McLachlan et al (2011) that metabolism is paramount in determining human bioaccumulation is in line with that of Tonnelier et al (2012), even though the latter study did not refer to a multimedia environment.

It should be noted that while the metrics proposed by both McLachlan et al (2011) and Tonnelier et al (2012) establish scales enabling comparison of the bioaccumulation potentials of different chemicals, they do not directly address the question of whether the steady-state levels are greater in the predator/consumer than in the prey/diet, unlike the metric proposed by Goss et al (2013).

Generic food web model

In a similar approach to that of Czub and MacLachlan (2004a,b), Alonso et al (2008) developed a generic biomagnification model by combining toxicokinetic models of each species within a food chain, although humans were not considered explicitly.

The required input parameters for the Alonso et al (2008) model are the same as those discussed above for the Goss et al (2013) biomagnification metric, namely the uptake efficiency, the feeding rate and the elimination rate constant (or half-life).

As described in ECETOC (2011), the model consists of four steps. The first step represents the accumulation in primary producers and via non food exposure routes, mainly bioconcentration from water or exposure from sediment. The oral exposure route is considered for all consecutive trophic levels. Step 2 links individual models for the food chain with one species in each level. Step 3 segregates each level into several species and can cover different exposure patterns. The fourth step establishes complex relationships among species and defines the position of each species by the Trophic Index that is based on the feeding preferences of predators. The model was mathematically implemented through system dynamic models and Monte Carlo based probabilistic calculations using crystal ball.

The metric derived by Alonso et al (2008), termed BMF(3), is the product of three successive BMFs in the food chain. It is defined as BMF(3) = BMF (from primary producer to primary consumer) * BMF (from primary consumer to secondary consumer) * BMF(from secondary consumer to tertiary consumer).

The modelling results confirmed the paramount influence of the elimination rate constant (k_d) on biomagnification, as already reported for the other studies discussed in this section. The uptake efficiency (α) had a lesser impact, at least for α values ranging from 0.2 to 0.8.

The simulations presented in the paper by Alonso et al (2008) led to BMF(3) values of up to about 100,000 at the lowest k_d values adopted (corresponding to a half-life of 231 days). In principle the TMF over the segment of the food-chain considered would be equal to the cubic root of BMF(3) – although this was not stated explicitly in the paper – so the maximum TMF value at the lowest k_d value would exceed 45. However, the assumption that k_d was identical for all three producer organisms in the food web (except in some simulations for which k_d was divided by 10 for the primary consumers) may not have been realistic.

Nevertheless, validation of the model by comparison of the simulation results with experimental observations of the biomagnification of PCB153 in a Barents Sea food chain (from crustaceans to seabirds) gave reasonable agreement.

5.9.3 Conclusion

Classical mammalian toxicokinetic data can give valuable indications for the absorption, distribution and metabolism of a substance and possible target organs for accumulation. This may contribute to a WoE assessment in particular for terrestrial food chains. Furthermore some models have been described in the literature, that associate toxicokinetic parameters (like elimination half-life or blood half-life) with the bioaccumulation behaviour of a substance.

For example, Goss et al (2013) demonstrated a direct relationship between the biomagnification factor from prey to predator (or diet to consumer) and the elimination half-life in the predator (or consumer) organism. This general concept, which is applicable to both aquatic and terrestrial organisms, including humans, may provide a new metric for B categorisation. However, further development work is required before definite numerical threshold values for elimination half-life can be proposed for this purpose. Furthermore, such cut-off values may well depend on the specific predator (or consumer) organism considered, at different trophic levels. All models described are dependent on the existence of relevant input parameters and have certain limitations and applicability domains. If the respective data are available or can be generated these models can constitute an important contribution to a WoE assessment for bioaccumulation on a case-by-case basis.

The task force additionally identified some further research needs on the possible correlation of mammalian toxicokinetic data with field biomagnification or trophic magnification data. As information for both become increasingly available for a number of substances, further model developments and validation of existing models with experimental data can be envisaged.

6. INTEGRATED EVALUATION STRATEGY

6.1 Introduction and purpose

The previous chapters have reviewed the state of the science in the identification of PBT chemicals in accordance with Annex XIII of the REACh regulation (Appendix A). This chapter summarises the elements that can contribute to the assessment of PBT properties for a chemical substance and proposes to combine them in a tiered strategy. At the same time, it is noted that in some areas the scientific development does not yet allow to draw conclusions, e.g. due to a lack of appropriate methods on bioaccumulation in terrestrial organisms. While promoting new elements in addition to existing guidance, the latter remains a valid starting point.

Particular attention has been paid to the WoE approach as required by Annex XIII and the endpoints mentioned there (Appendix A). The task force has focused mainly on the P and B assessment. The T assessment was only considered in the screening phase, since for in-depth assessment there exists sufficient guidance for the toxicity endpoints with relevance to the PBT assessment. This is available in the form of ECHA endpoint guidance documents and other ECETOC reports, and does not need to be repeated here.

6.2 General considerations for the weight-of-evidence assessment

The task force has evaluated approaches to WoE assessments in Chapter 2 of this report. For each criterion P, B and T several hypotheses may be formulated and several lines of evidence evaluated based on the data available for the substance under consideration. It is important that all relevant data and information should be evaluated in a consistent manner including the evaluation of the reliability, quality, repeatability and relevance to the question under consideration. A number of scoring or ranking systems have been proposed that could be used to determine the relevant ‘weight’ of the individual references. The information is then combined on the basis of the different lines of evidence. The weighed lines of evidence supporting or rejecting the hypothesis are then analysed for the overall strength of the evidence. Such an approach is particularly helpful in cases of data-rich substances for which often contradictory information is available for a number of lines of evidence contributing in particular to the P and B assessment.

With regard to the use of WoE in the identification of PBT properties in the context of REACh two cases can be differentiated. Firstly WoE considerations are used in the assessment of screening information using the available data that can inform the decision if higher-tier assessment is needed to derive a conclusion as to whether a substance is likely to fulfil all three criteria for P, B and T or the criteria for P and B. Secondly, according to REACh Annex XIII a WoE approach needs to be applied when exploiting any available ‘assessment information’, in addition to comparison to the Annex XIII criteria, in order to reach a conclusion regarding the P/vP, B/vB or T characteristics of the substance to be assessed. This will in particular be the case where the available information is contradictory or where several different metrics are used in parallel for a given property, e.g. for bioaccumulation.

6.3 Degradation products and impurities

Annex XIII of REACh requires considering the assessment of the PBT/vPvB properties of relevant impurities and degradation products. With regard to degradation products the following limitations need to be taken into consideration in the assessment requirements.

The simulation studies (e.g. OECD 307, 308 and 309) available for high tier testing are usually designed to be environmentally relevant and therefore use low concentrations of test substance. This means there are often technical limitations associated with identifying transformation products. These guidelines state that the analytical method applied in the test should have a limit of detection (LOD) for the parent compound and possible degradation products of 0.01 mg/kg of soil or sediment (as test substance) or 1% of the applied dose. They also require that attempts should be made to identify major transformation products present at the guideline recommended 10% or greater of the original parent compound. Attempts should be made to identify such transformation products particularly where the parent compound is rapidly transformed in order to assess the transformation products for their persistence in the environment. However, the identification of transformation products at 0.1% of the parent compound in the recommended simulation studies is an unrealistic expectation given the available tools.

6.4 Compartment of concern

PBT assessment is currently not performed in an environmental compartment specific manner, and data used in the assessments can be relevant for water, sediment and/or soil. As bioavailability may vary significantly between these environmental compartments it is recommended to clarify upfront which is (are) the compartment(s) of concern to which most of the substance would initially partition.

Preferred EQC models have been discussed and they are recommended for use in identifying the compartment of concern (Section 2.3). Depending on the amount of input data the outcome of these estimations can vary. Only in some cases where multimedia distribution is expected, concepts of overall persistence may be of use.

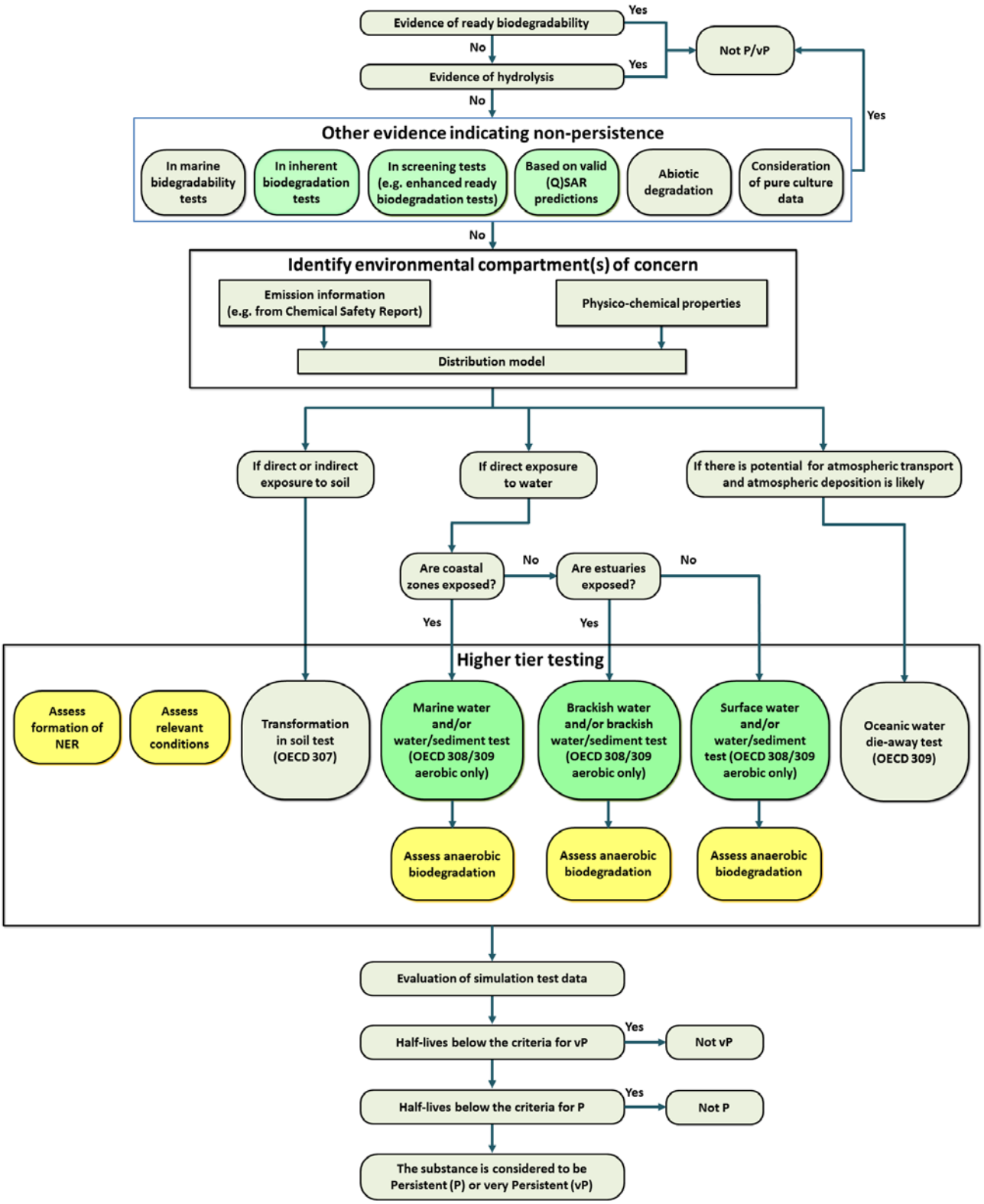
If a substance partitions almost exclusively (> 95%) to one compartment, PBT assessment can be confined to that compartment. For the assessment of persistence, one can compare the half-life (= DT_{50}) in each relevant compartment with the criteria from Annex XIII and identify the indications for bioaccumulation in the respective compartment as well. However, there may be limitations at present with regard to available methodologies and relevant criteria for bioaccumulation in particular for sediment and the terrestrial compartment.

6.5 Persistence (P and vP properties)

This report presents several general recommendations for persistence, not only related to screening methodologies but also regarding issues that could be encountered during more complex testing in a definitive persistence assessment. To aid in the practical application of the recommendations below, the

topics regarding persistence have been highlighted or added in the flowchart / decision tree, adapted from the final draft version of the PBT Assessment Guidance (ECHA, 2014) (Figure 6.1).

Figure 6.1: Adapted decision tree for P/vP, showing topics of interest [a] (after Figure R11-3 in ECHA, 2014)



[a] Green boxes are topics addressed in the existing guidance and in this report; yellow boxes are additional considerations, which have also been addressed in this report.

6.5.1 Screening information for P

The following data are typically used for the screening assessment of persistence aiming at identifying substances that are unlikely to be persistent and for which no further testing for persistence is proposed.

Biodegradation (Q)SAR models

A preliminary indication of a substance being P can be obtained by modelling based on read across and physico-chemical properties, prior to conducting a test on ready biodegradation. A combination of different models can be used as one line of evidence informing about the likelihood of a chemical to undergo rapid biodegradation. If models not equally sensitive to the same input parameters lead to the same conclusion, either the chemical is likely to not fulfil the P criterion or it may fulfil the P criterion, this can be used as one piece of evidence in a WoE assessment in the screening phase. Model predictions can also be informative for the choice of biodegradation tests to be conducted.

Ready biodegradation tests

Ready biodegradation tests are the initial screen for persistence. Where a ready biodegradation test fails a relevant modification or enhancement is recommended to investigate if the test substance will degrade under these conditions and can therefore can be identified as ‘not P’ (Section 3.1.3). If modified or enhanced studies are conducted they should fulfil the relevant criteria for the respective guideline (where available) within the 60-day time period in order to be considered acceptable to demonstrate ‘not P’. According to REACh Guidance Document R.11 a positive result should be considered conclusive to assess a substance as ‘not P’. However, if the substance is potentially P then further studies should be considered, e.g. inherent biodegradation tests or simulation studies.

Inherent biodegradation tests

The available inherent biodegradation tests have been summarised in chapter 3.1.4 of this report and where possible the criteria for assigning P or not P have been identified. The suitability of using inherent biodegradation studies for PBT assessment should be considered on a case-by-case basis bearing in mind that the criteria for these tests are less well defined than for the ready biodegradation tests.

Marine biodegradation tests

A specific test for screening for marine biodegradation exists, i.e. OECD 306. In some cases the marine biodegradation tests could be extended beyond 60 days because of the long lag phases before the onset of biodegradation in seawater systems. The criteria for ‘not P’ for this test could however be the same as for an inherent test from the OECD 302 series, but after 60 days (or longer if it can be justified).

6.5.2 Higher-tier assessment information for P

As indicated in Figure 6.1, the identification of the compartment(s) of concern can inform about the preferred higher-tier testing for substances that cannot be regarded as non-persistent following the screening assessment. If the substance is likely to distribute to several compartments, the relevant studies for every compartment of concern should be conducted. In these cases it may be advantageous to determine the overall persistence of the substance in the environment (Pov). This is further discussed by ECETOC (2003a; 2005a; 2011).

Simulation testing in water, sediment and soil

Simulation studies may be required to refine the persistence assessment of a substance. These studies are considered to be more environmentally realistic than the screening studies and provide a half-life of the test substance under the given conditions, which can be used for comparison with the 'P' or 'vP' criteria under REACh. Simulation studies would usually include any of the OECD 307 (soil), 308 (water / sediment) or 309 (water) studies or one of the OECD 314 series of studies. Some limitations of these tests have been identified and are subject to further research (See Section 4.1).

While relevant tests exist for the main compartments of concern there remains considerable uncertainty in the design and interpretation of these studies for the purposes of PBT assessment. Recent CEFIC LRI projects have been initiated to try and address some of the uncertainty and improve the environmental realism of these simulation studies.

Anaerobic degradation

The relevance of anaerobic degradation has been discussed in the scientific community. It will strongly depend on the partitioning behaviour of the respective chemical and is to be decided on a case-by-case basis (Section 4.2).

It is recommended that anaerobic degradation testing shall only be performed after thoroughly examining the aerobic biodegradation potential of a substance. Since standardised tests such as the OECD 311 guideline are not equivalent to OECD screening tests and do not have trigger values assigned to them for P or vP, these tests can currently only contribute to a WoE assessment of persistence in a qualitative manner.

Non-extractable residues

Depending on the substance properties so called Non-Extractable Residues (NERs) might constitute a significant amount of the initial parent test substance concentration within higher-tier tests. Different definitions and discussions on how to deal with NER in the assessment of persistence are discussed in Section 4.3 of this report.

Non-extractable residues (NERs) are strongly bound to sediment or soil and while adsorbed they are protected from degradation. Although these 'NERs' remain in the environment they are not bioavailable and therefore in the context of PBT assessment they should be considered equivalent to not being 'P' or 'vP'. As NERs are not bioavailable they will also not be bioaccumulative or toxic.

6.6 Bioaccumulation (B and vB properties)

The assessment of bioaccumulation of substances poses at present most challenges. Depending on the volume and use of a substance, the information and data that can inform about the potential of a substance to bioaccumulate may vary widely. A whole range of data needs to be considered for a WoE assessment of the bioaccumulation potential, but much of this information may not be suitable to come to a definite decision on B. The task force has reviewed the different elements that can be considered for the evaluation of the bioaccumulation potential and has identified some gaps in scientific knowledge with regard to this assessment. One of the major challenges is the comparison of different B-metrics. The use of metrics expressed in terms of fugacity ratios can help to obtain more comparable results. Application and limitations of this methodology are described in Section 5.1 of this report.

6.6.1 Screening information for B

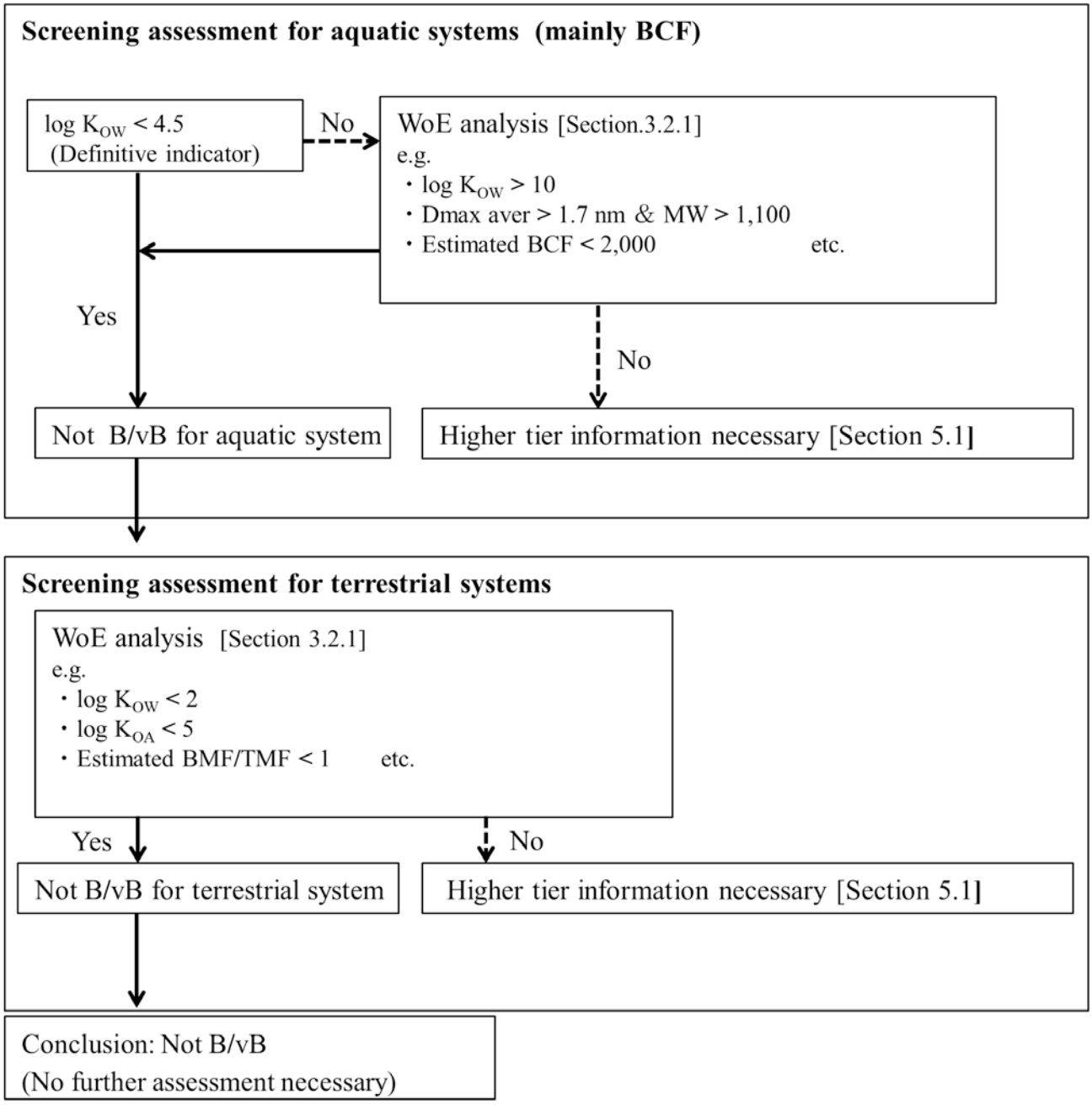
The data in Table 3.2 are typically used for the screening assessment of bioaccumulation aiming at identifying substances that are unlikely to be bioaccumulative and for which no further testing for bioaccumulation is proposed.

These data include physico-chemical indicators/properties, results of in vitro studies, and estimations by models or read-across. As in the case of persistence when assessing the bioaccumulation potential of a chemical, the relevant compartment should be considered.

However, due to the lack of scientifically based thresholds for terrestrial bioaccumulation and the scarcity of experimental methods and the interpretation of their results for the terrestrial compartment, it is recommended to assess the potential in the aquatic system first because relatively reliable thresholds already exist for the aquatic system and the aquatic system is the main compartment of concern for general chemicals based on their exposure route or mobility. If the target chemical can be considered 'not B/vB' in screening assessment for aquatic systems, then indications for a possible bioaccumulation in the terrestrial compartment should be investigated. If likely 'not B/vB' can be concluded from screening information in both systems, further studies are not required.

The following diagram (Figure 3.3 repeated here) illustrates the decision process in the screening assessment for B. Further details can be found in Section 3.2 of this report.

Figure 3.3: Decision tree for screening B/vB assessment



6.6.2 Higher-tier assessment information for B

As indicated before higher-tier information for B can comprise a number of different data, starting from the classical bioconcentration studies in fish, fish dietary biomagnification studies (OECD 305), determinations of laboratory and field bioaccumulation factors, field biomagnification studies and field studies on trophic magnification in food chains. Section 5.1 of this report discusses the different metrics and how they can be compared and assessed in a WoE approach. The different methodologies, aspects that need to be considered in the evaluation of data and design of more complex studies as well as limitations and application domains of the different methodologies are discussed in sections of Chapter 5. Further research needs in this area were also identified.

Additional information that can be considered to inform the decision on a possible bioaccumulation potential of a substance or the need for and design of further studies include data on chronic toxicity and toxicokinetics in mammalian toxicity studies, in particular when considering terrestrial bioaccumulation and information from monitoring studies. However the latter may only provide some indications for further investigations in field studies and are rarely designed in a way that allows conclusions on magnification in food chains or definitive information on bioaccumulation.

Bioconcentration or biomagnification studies (Aqueous and dietary)

Challenges related to classical bioconcentration studies in fish and principles of the dietary bioconcentration test in fish according to OECD TG 305 are discussed in Section 5.2 of this report.

There are still considerable uncertainties around the interpretation of dietary bioaccumulation results in the context of PBT assessment, mainly due to the re-calculation of BCF values from dietary BMFs. A systematic comparison of the two metrics is needed before firm conclusions can be drawn on how BMFs obtained from dietary bioconcentration studies can be evaluated against the B criterion. The TF is aware of the ongoing work of an OECD task force on guidance on the interpretation of BMF values from dietary studies and an ILSI-HESI project led by Arnot (2013) addressing these questions.

Use of TMFs and field BMFs

The key processes controlling accumulation of chemicals in aquatic organisms involve respiratory and dietary uptake, gill and gastrointestinal elimination kinetics, and metabolic transformation capacity. These processes are also influenced by a variety of physical-chemical properties and various biological factors related to organism physiology (Thomann, 1989). The authors conclude that at naturally-occurring food / water concentration ratios, uptake of highly hydrophobic chemicals (i.e. $\log K_{OW} > 6$) from water into biota is generally low compared to uptake via consumption of contaminated foodstuffs, with the importance of dietary uptake increasing with increasing K_{OW} values (Thomann, 1989; Qiao et al, 2000).

Traditionally, bioaccumulation assessment of chemicals has been based on results from controlled laboratory tests, such as the BCF and BAF tests, almost exclusively in aquatic systems. It has been proposed that bioaccumulative substances should be defined as substances which biomagnify in the food web, i.e. lipid-normalised concentrations increase with higher trophic position. A 2008 SETAC POPs/PBT workshop proposed a flowchart for assessment of bioaccumulation criteria that incorporates these food web metrics. The flowchart indicates that a field TMF value is considered to be the most conclusive metric from which to determine chemical bioaccumulation, followed by a dietary or field biomagnification factor (BMF) value (Gobas et al, 2009).

Field BMF and TMF values will only be available for chemicals that have already been in use for many years and have been released to the environment. Results of environmental and biomonitoring studies indicating the presence of a chemical in the environment and biota, could be used as a trigger to consider the feasibility of well designed field studies to determine TMF.

For data-rich substances frequently biomagnification data of several TMF studies need to be evaluated. The results of numerous studies should be weighted according to the statistical power of the results, and the data should be considered in the context of the practicalities of the study design. Considerations include a proper balancing of samples among multiple trophic levels in a food web (e.g. benthic versus pelagic), and other considerations such as age, feeding strategy, life history). Borgå et al (2012) have provided examples and recommendations regarding study design, data treatment, and statistical analysis. The most important considerations for the evaluation of TMF and field BMF studies are described in Section 5.3 of this report.

Whole-body versus organ-specific bioconcentration factors

Organ-specific (or tissue- or fluid-specific) concentration data are collected in specific cases for ethical and/or practical reasons, mostly in the larger, top-level consumers. Using organ-specific concentration data for bioaccumulation assessments can lead to erroneous results when this concentration is not a good representative of a whole-body concentration. If whole-body values are not available, organ-specific concentration data can be used as a proxy for them for bioaccumulation assessments. In such cases it is important to take into account the following points:

- Contribution of the organ/tissue to the total body burden, whereby a large contribution is better (e.g. blubber in cetaceans).
- An extrapolation factor that is specific for the (group of) substance(s), the species (group) under investigation as well as the specific organ.

The use of organ-specific concentration data for BMF/TMF calculations can also lead to erroneous results, mostly to overestimation. The above principles can be used when attempting to extrapolate organ-specific data to whole-body values.

More details regarding organ-specific concentrations can be found in Section 5.5 and Appendix D of this report.

Terrestrial bioaccumulation potential

It has been suggested that the current aquatic bioaccumulation assessment methods may not be entirely protective of terrestrial systems due to various factors in the taxa of interest, such as differences in elimination mechanisms, body temperature, and digestive tract physiology (Kelly et al, 2007). However, to date this remains largely an untested hypothesis. The state of the science in this respect is reviewed in Section 5.6 of this report. Available terrestrial food chain models are reviewed in Appendix C.

There are existing approaches that could be used to assess terrestrial bioaccumulation; however, it is important to note that currently there are limited data to support setting definitive criteria for terrestrial bioaccumulation.

Additional research in this area is needed before any specific guidance can be developed, and further analysis of the applicability of aquatic data, such as that generated from OECD TGs 305 and 315, to terrestrial bioaccumulation needs to be performed.

Human and environmental biomonitoring

The use of monitoring data in PBT assessment is reviewed in Section 5.7 of this report.

The term 'biomonitoring' can cover many different aspects, in particular with regard to environmental biomonitoring or human biomonitoring. Interpretation of such data is sometimes difficult and could result in wrong conclusions. Guidance on the performance and interpretation of human and environmental monitoring studies has been given by ECETOC (references in Section 5.7) and this is crucial to consider. This task force deems that the mere detection of a substance being present in an organism (mammalian, human, fish) does not necessarily mean that the substance is bioaccumulative. As a first step towards reaching such a conclusion, it may be useful to correlate the levels of the substance in the organism with those in its surrounding environment or ingested food, and to analyse all the possible sources of exposure with which the organism has been in contact. On the other hand, the presence of a chemical in the environment and biota at levels that may be of concern, could trigger consideration of the feasibility of well-designed field studies to determine BMFs and TMFs.

Chronic toxicity studies

The task force identified some possible indicators of bioaccumulation from mammalian repeated dose studies that are discussed in Section 5.8 of this report. However, the interpretation depends strongly on the individual dataset and needs to be used on a case-by-case basis in a WoE approach after an in depth analysis of the available toxicological data. Due to variability in the datasets, general suggestions / schemes cannot be provided.

Toxicokinetic behaviour

The use of toxicokinetic data and models as one of the elements used in a WoE assessment of the bioaccumulation potential of a substance are reviewed in Section 5.9 of this report.

Classical mammalian toxicokinetic data can give valuable indications for the absorption, distribution and metabolism of a substance and possible target organs for accumulation. This may contribute to a WoE assessment in particular for terrestrial food chains. Furthermore some models have been described in the literature, that associate toxicokinetic parameters (like elimination half-life or blood half-life) with the bioaccumulation behaviour of a substance. All models described are dependent on the existence of relevant input parameters and have certain limitations and applicability domains. If the respective data are available or can be generated these models can constitute an important contribution to a WoE assessment for bioaccumulation on a case-by-case basis.

The task force additionally identified some further research needs on the possible correlation of mammalian toxicokinetic data with field biomagnification or trophic magnification data. As information for both become increasingly available for a number of substances, further model developments and validation of existing models with experimental data can be envisaged.

6.7 Toxicity (T properties)

As indicated in the introduction, the task force did not further consider the T assessment as sufficient guidance on the assessment of the toxicological endpoints relevant for the PBT assessment is already available. For the screening assessment reference is made to QSAR models and read across approaches that have been reviewed in previous ECETOC reports. See Section 3.3 of this report.

6.8. Gaps in knowledge and research needs

The task force identified various gaps in knowledge that may need to be filled before the REACH guidance on PBT and vP/vB assessment and Annex XIII requirements can be adapted to technical and scientific progress. Proposals for such further research could be requested from the scientific community, in particular funded through the CEFIC LRI programme. At ECETOC, several task forces could be envisaged to review the latest science. This may eventually lead to the development of alternative information to assess P, B, or T properties.

The following recommendations are put forward at present, in the order of appearance in the report.

With respect to transformation products:

1. Develop science-based criteria for the identification of degradation products and impurities with PBT properties that would be of concern for human health or the environment, in regard of their constituent level and formation over time. (Section 2.2).

With respect to screening information on P:

2. Understand the ecological significance of pre-exposure of inocula to a test substance in enhanced biodegradation tests (Section 3.1.3).
3. Evaluate the predictive value of modified or enhanced ready biodegradation tests (Section 3.1.3). (Note: Reviewing this could be combined with the next topic.)
4. Consider whether the current biodegradation screens are complex enough to include the major processes of degradation under environmental conditions (Section 3.1.3).

And to screening information on B:

5. Better define cut-off values for dietary absorption efficiency in terrestrial and aquatic organisms (Section 3.2.1).
6. Refine the log K_{OW} determination methods for surfactants (Section 3.2.1 Limitations of using log K_{OW}).

As regards higher-tier assessment for P, there is a need to:

7. Investigate whether and how a sewage treatment plant model can be used to inform about the potential for significant degradation of a substance in the natural environment, making it likely that the P or vP criteria are not met (Section 4.1).
8. Conduct research on non-extractable residues to give consensus on the definitions (Section 4.3).

And to higher-tier assessment for B:

9. On B in terrestrial species, additional research for specific guidance to be developed (Section 5.6). (Note: Reviewing this could be combined with the next topic.)
10. Further analysis of the applicability of aquatic data, e.g. from OECD TGs 305 and 315, to terrestrial B assessment (Section 5.6).
11. Correlate mammalian toxicokinetic data with field biomagnification or trophic magnification data (Section 5.9).
12. Further model development and validation of existing models with experimental BMF and TMF data (Section 5.8). (Note: This review could be combined with the previous topic).

APPENDIX A: ANNEX XIII OF REACH (EC, 2011)

This report refers to Annex XIII of REACh as amended by regulation No 253/2011 from the European Commission (EC, 2011). The full text is reproduced here from the official publication, as follows:

ANNEX XIII

CRITERIA FOR THE IDENTIFICATION OF PERSISTENT, BIOACCUMULATIVE AND TOXIC SUBSTANCES, AND VERY PERSISTENT AND VERY BIOACCUMULATIVE SUBSTANCES

This Annex lays down the criteria for the identification of persistent, bioaccumulative and toxic substances (PBT substances), and very persistent and very bioaccumulative substances (vPvB substances) as well as the information that must be considered for the purpose of assessing the P, B, and T properties of a substance.

For the identification of PBT substances and vPvB substances a weight-of-evidence determination using expert judgement shall be applied, by comparing all relevant and available information listed in Section 3.2 with the criteria set out in Section 1. This shall be applied in particular where the criteria set out in Section 1 cannot be applied directly to the available information.

A weight-of-evidence determination means that all available information bearing on the identification of a PBT or a vPvB substance is considered together, such as the results of monitoring and modelling, suitable in vitro tests, relevant animal data, information from the application of the category approach (grouping, read-across), (Q)SAR results, human experience such as occupational data and data from accident databases, epidemiological and clinical studies and well documented case reports and observations. The quality and consistency of the data shall be given appropriate weight. The available results regardless of their individual conclusions shall be assembled together in a single weight-of-evidence determination.

The information used for the purposes of assessment of the PBT/vPvB properties shall be based on data obtained under relevant conditions.

The identification shall also take account of the PBT/vPvB-properties of relevant constituents of a substance and relevant transformation and/or degradation products.

This Annex shall apply to all organic substances, including organo-metals.

1. CRITERIA FOR THE IDENTIFICATION OF PBT AND vPvB SUBSTANCES

1.1. PBT Substances

A substance that fulfils the persistence, bioaccumulation and toxicity criteria of Sections 1.1.1, 1.1.2 and 1.1.3 shall be considered to be a PBT substance.

1.1.1. Persistence

A substance fulfils the persistence criterion (P) in any of the following situations:

(a) the degradation half-life in marine water is higher than 60 days;

(b) the degradation half-life in fresh or estuarine water is higher than 40 days; (c) the degradation half-life in marine sediment is higher than 180 days;

(d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days; (e) the degradation half-life in soil is higher than 120 days.

1.1.2. Bioaccumulation

A substance fulfils the bioaccumulation criterion (B) when the bioconcentration factor in aquatic species is higher than 2 000.

1.1.3. Toxicity

A substance fulfils the toxicity criterion (T) in any of the following situations:

(a) the long-term no-observed effect concentration (NOEC) or EC10 for marine or freshwater organisms is less than 0,01 mg/l;

(b) the substance meets the criteria for classification as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B, or 2) according to Regulation EC No 1272/2008;

(c) there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: specific target-organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Regulation EC No 1272/2008.

1.2. vPvB Substances

A substance that fulfils the persistence and bioacumulation criteria of Sections 1.2.1 and 1.2.2 shall be considered to be a vPvB substance.

1.2.1. Persistence

A substance fulfils the ‘very persistent’ criterion (vP) in any of the following situations:

(a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days;

(b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days;

(c) the degradation half-life in soil is higher than 180 days.

1.2.2. Bioaccumulation

A substance fulfils the ‘very bioaccumulative’ criterion (vB) when the bioconcentration factor in aquatic species is higher than 5 000.

2. SCREENING AND ASSESSMENT OF P, vP, B, vB and T PROPERTIES

2.1. Registration

For the identification of PBT and vPvB substances in the registration dossier, the registrant shall consider the information as described in Annex I and in Section 3 of this Annex.

If the technical dossier contains for one or more endpoints only information as required in Annexes VII and VIII, the registrant shall consider information relevant for screening for P, B, or T properties in accordance with Section 3.1 of this Annex. If the results from the screening tests or other information indicate that the substance may have PBT or vPvB properties, the registrant shall generate relevant additional information as set out in Section 3.2 of this Annex. In case the generation of relevant additional information would require information listed in Annexes IX or X, the registrant shall submit a testing proposal. Where the process and use conditions of the substance meet the conditions as specified in Section 3.2(b) or (c) of Annex XI the additional information may be omitted, and subsequently the substance is considered as if it is a PBT or vPvB in the registration dossier. No additional information needs to be generated for the assessment of PBT/vPvB properties if there is no indication of P or B properties following the result from the screening test or other information.

2.2. Authorisation

For dossiers for the purposes of identifying substances referred to in Article 57(d) and Article 57(e), relevant information from the registration dossiers and other available information as described in Section 3 shall be considered.

3. INFORMATION RELEVANT FOR THE SCREENING AND ASSESSMENT OF P, vP, B, vB and T PROPERTIES

3.1. Screening Information

The following information shall be considered for screening for P, vP, B, vB and T properties in the cases referred to in the second paragraph of Section 2.1 and may be considered for screening for P, vP, B, vB and T properties in the context of Section 2.2.

3.1.1. Indication of P and vP properties

(a) Results from tests on ready biodegradation in accordance with Section 9.2.1.1 of Annex VII; (b) Results from other screening tests (e.g. enhanced ready test, tests on inherent biodegradability);

(c) Results obtained from biodegradation (Q)SAR models in accordance with Section 1.3 of Annex XI; (d) Other information provided that its suitability and reliability can be reasonably demonstrated.

3.1.2. Indication of B and vB properties

(a) Octanol-water partitioning coefficient experimentally determined in accordance with Section 7.8 of Annex VII or estimated by (Q)SAR models in accordance with Section 1.3 of Annex XI;

(b) Other information provided that its suitability and reliability can be reasonably demonstrated.

3.1.3. Indication of T properties

(a) Short-term aquatic toxicity in accordance with Section 9.1 of Annex VII and Section 9.1.3 of Annex VIII; (b) Other information provided that its suitability and reliability can be reasonably demonstrated.

3.2. Assessment Information

The following information shall be considered for the assessment of P, vP, B, vB and T properties, using a weight-of-evidence approach.

3.2.1. Assessment of P or vP properties

(a) Results from simulation testing on degradation in surface water;

(b) Results from simulation testing on degradation in soil;

(c) Results from simulation testing on degradation in sediment;

(d) Other information, such as information from field studies or monitoring studies, provided that its suitability and reliability can be reasonably demonstrated.

3.2.2. Assessment of B or vB properties

(a) Results from a bioconcentration or bioaccumulation study in aquatic species;

(b) Other information on the bioaccumulation potential provided that its suitability and reliability can be reasonably demonstrated, such as:

— Results from a bioaccumulation study in terrestrial species;

— Data from scientific analysis of human body fluids or tissues, such as blood, milk, or fat;

— Detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment;

— Results from a chronic toxicity study on animals;

— Assessment of the toxicokinetic behaviour of the substance;

(c) Information on the ability of the substance to biomagnify in the food chain, where possible expressed by biomagnification factors or trophic magnification factors.

3.2.3. Assessment of T properties

(a) Results from long-term toxicity testing on invertebrates as set out in Section 9.1.5 of Annex IX;

(b) Results from long-term toxicity testing on fish as set out in Section 9.1.6 of Annex IX;

(c) Results from growth inhibition study on aquatic plants as set out in in Section 9.1.2 of Annex VII;

(d) The substance meeting the criteria for classification as carcinogenic in Category 1A or 1B (assigned hazard phrases: H350 or H350i), germ cell mutagenic in Category 1A or 1B (assigned hazard phrase: H340), toxic for reproduction in Category 1A, 1B and/or 2 (assigned hazard phrases: H360, H360F, H360D, H360FD, H360Fd,

H360fD, H361, H361f, H361d or H361fd), specific target-organ toxic after repeated dose in Category 1 or 2 (assigned hazard phrase: H372 or H373), according to Regulation EC No 1272/2008;

(e) Results from long-term or reproductive toxicity testing with birds as set out in Section 9.6.1 of Annex X;
(f) Other information provided that its suitability and reliability can be reasonably demonstrated.'

APPENDIX B: OVERVIEWS OF REPRESENTATIVE AQUATIC QSAR MODELS

The BCFBAF (formerly BCFWIN) program in EPISuite (US EPA, 2009a) provides a regression model and a mass balance model.

B.1 BCF regression model (Meylan et al, 1999)

B.1.1 Model summary

According to the following regression equations, a BCF value in fish is calculated:

For log K_{OW} 1.0 to 7.0;
log BCF = 0.6598 log K_{OW} − 0.333 + Σ correction factors ..... (Eq. B.1)

For log K_{OW} > 7.0;
log BCF = −0.49 log K_{OW} + 7.554 + Σ correction factors ..... (Eq. B.2)

The correction factors are defined for 11 kinds of structural features such as ketones, phosphate esters, multi-halogenated biphenyls and so on. These regression equations were derived with 466 non-ionic and 61 ionic compounds (e.g. carboxylic acids, sulphonic acids) [R^2 = 0.83, s = 0.71**]**. External validation with additional 158 chemicals resulted in comparable correlation [R^2 = 0.82, s = 0.77**]**.

B.1.2 Applicability domain

The range of molecular weight and log K_{OW} for the training set chemicals are as follows:

Molecular weight 60.08 to 991.80
log K_{OW} −1.37 to 11.26 (for non-ionic compound)

No definition relevant to chemical structures are made.

B.1.3 Model uncertainty

The regression-based QSAR model dose not explicitly account for chemical-specific metabolic biotransformation. So the BCF values of chemicals should be overestimated if they are rapidly biotransformed in fish. Applicability domain of this model is defined as the range of molecular weight and log K_{OW} only. Uncertainty in calculating the descriptor log K_{OW} should be also significant especially for very hydrophobic chemicals. For some kinds of chemicals with complicated bioaccumulation mechanisms such as active transportation, the prediction with this model should have somewhat large uncertainty.

B.2 BCFBAF mass balance model (Arnot and Gobas, 2003; Arnot et al, 2006a)

B.2.1Model summary

This model estimates steady-state BCF and BAF values in three different sizes of fish under representative environmental conditions such as the dissolved organic carbon content of 5×10^{-7} g/ml and the water temperature of 10°C. This model consists of two QSAR models, the mass balance bioaccumulation model and the metabolic biotransformation rate model.

The former mass balance bioaccumulation model calculates BAF as a ratio of chemical concentration in fish to that in water according to the following equation:

$$BAF = C_B/C_W = (1 - L_B) + ((k_1\phi + (k_D\ \beta\ \tau\ \phi\ L_D\ K_{OW})) / (k_2+k_E+k_G+k_M)) \quad \text{..... (Eq. B.3)}$$

where k_1 is uptake rate constant from water, k_2 elimination rate constant, k_D dietary uptake rate constant, k_E faecal egestion rate constant, and k_G growth rate constant, being mainly estimated by log K_{OW} and fish weight.

The metabolic biotransformation rate constant k_M is estimated by the latter metabolic biotransformation rate model as explained below. Other parameters listed in Table B-1 were defined based on measured data for some poorly metabolised chemicals (e.g. PCBs) (Arnot and Gobas, 2003).

Figure B-1: A conceptual diagram representing the major routes of chemical uptake and elimination in an aquatic organism (from Arnot and Gobas, 2003)

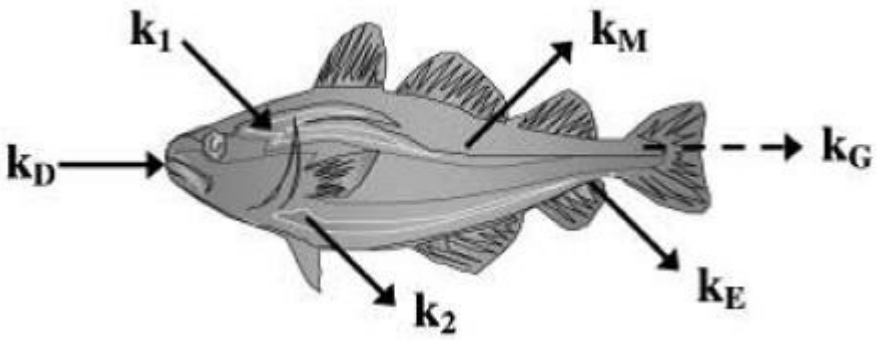


Table B-1: Parameters used to derive the Arnot & Gobas BCFBAF model (from Arnot, 2003)

Symbol	Parameter	Value, unit
T	Mean water temperature	10°C
W	Weight of organism	1 kg
L_B	Lipid content of organism	20%
L_D	Lipid content of lowest trophic level organisms	1%
χ_{POC}	Concentration of particulate organic carbon	5×10^{-7}g/ml
χ_{DOC}	Concentration of dissolved organic carbon	5×10^{-7}g/ml
ϕ	Fraction of freely dissolved chemical in water	$1/(1 + \chi_{POC} \times 0.35\ K_{OW} + \chi_{DOC} \times 0.1 \times 0.35\ K_{OW})$
β	Overall food web biomagnification factor	130
τ	Maximum trophic dilution factor	1 (default)
k_M	Metabolic transformation rate constant	0/day (default)
n	Number of trophic interactions in the food web	3 (default)
K_{OW}	Octanol-water partition coefficient	Chemical dependent
k_1	Uptake rate constant	$1/[(0.01 + 1/K_{OW}) \times W^{0.4}]$
k_D	Dietary uptake rate constant	$0.02\ W^{-0.15} e^{(0.06-T)}/(5.1 \times 10^{-8} K_{OW}+2)$
k_2	Elimination rate constant	$k_1/(L_B K_{OW})$
k_E	Faecal egestion rate constant	$0.125\ k_D$
k_G	Growth rate constant	$0.0005\ W^{-0.2}$

The k_M value in the above equation is estimated by the following metabolic biotransformation rate model (US EPA, 2009a):

$$\log k_M/\text{half-life (in days)} = 0.30734215 \log K_{OW} - 0.0025643319\ MW - 1.53706847 + \Sigma(F_i\ n_i) \quad \text{(Eq. B.4)}$$

Where $\Sigma(F_i\ n_i)$ is summation of the individual fragment coefficient values that are relevant to metabolic biotransformation structural features such as ester, amide and nitroso.

This regression equation was derived with 421 chemicals [$R^2 = 0.821$, $s = 0.494$] and an external validation with additional 211 chemicals resulted in comparable correlation [$R^2 = 0.734$, $s = 0.446$].

The overall regression correlation (93%) between the measured and calculated BAFs on the basis of bioaccumulation ratings 'not B or B/vB' of this BCFBAF model is reported to be better than that from BCFWIN model (85%) (Constanza et al, 2012).

B.2.2 Applicability domain

The ranges of molecular weight and log K_{OW} of training set chemicals are as follows:

Molecular weight 68.08 to 959.17
log K_{OW} 0.31 to 8.70

No definition relevant to chemical structures are made, but this model mainly applies to non-ionic organic chemicals. So this model should be applied with care to chemicals that appreciably ionise, such as pigments, dyes, and perfluorinated substances.

B.2.3 Model uncertainty

Constanza et al (2012) reported that this model was superior to the regression-based BCF model. In addition, Arnot and Gobas (2003) suggested that since this model gives conservative prediction compared to other log K_{OW}-based models for the whole range of log K_{OW}, it should be suitable for regulatory purposes. This model was constructed not by simple regression with log K_{OW} but by complex equation based in vivo kinetic mechanism. Thus, the uncertainty associated with model parameters should be expected if the objective conditions are markedly different from those of the model assumption.

B.3 CAESAR

B.3.1 Model summary

CAESAR, which is implemented in the VEGA platform, is a hybrid system of variety of QSAR models to predict BCF and BAF in fish (Zhao et al, 2008; Lombardo et al, 2010; CAESAR, 2011; VEGA, 2011). The descriptors for prediction are extracted by a heuristic method (HM) or genetic algorithm (GA), followed by the development of QSAR models by applying machine learning methodology such as multiple linear regression (MLR) analysis, neural network (NN) and support vector machine (SVM).

BCF and BAF are described by the following equations:

$$BCF = CB/CWD = k_1/(k_2 + k_E + k_M + k_G) \quad \text{(Eq. B.5)}$$

$$BAF = CB/CWD = [k_1 + k_D\,(CB/CWD)] / (k_2 + k_E + k_M + k_G) \quad \text{(Eq. B.6)}$$

Where CB is the chemical concentration in the organism (g/kgbw), k_1 is the chemical uptake rate constant from the water at the respiratory surface (l/kgbw/d), CWD is the freely dissolved chemical concentration in the water (g/l), k_D is the uptake rate constant for chemical in the diet (kg/kgbw/d) and k_2, kE, kM, kG are rate constants (d^{-1}) representing chemical elimination from the organism via the respiratory surface, faecal egestion, metabolic biotransformation, and growth dilution, respectively.

This system is developed on a dataset of 378 chemicals and externally validated by 95 chemicals [R^2 = 0.78, s = 0.38].

B.3.2 Applicability domain

The applicability domain is defined not only by physico-chemical properties but also by chemical structures. In this model the applicability domain is confirmed with Applicability Domain Index (ADI) of 0 (worst case) to 1 (best case). This ADI is defined based on the similarity of the test chemical with those in the training set of the model (VEGA, 2011). ADI from 0.85 to 1 is presumed to be in the applicability domain. Regardless of ADI values, 'out of domain' alert appears for chemicals with reactive functional groups.

B.3.3 Model uncertainty

This model shows good accuracy because of strictly defined applicability domain with ADI (Lombardo et al, 2010). However there is intrinsic uncertainty related to the dataset in the model. Although these data should be of high quality since they were principally derived according to official guidelines, specific variability in the experimental data should be implicitly transferred into uncertainty of the QSAR model (ECETOC, 2011). To take account of this uncertainty, the conservative criterion by an offset of 0.5 log unit is suggested for the chemicals whose predicted BCF values are near the threshold of B/vB (Lombardo et al, 2010). For example, for vB (BCF = 5,000, log BCF = 3.7), the threshold value of log (3.7 - 0.5) should be compared with the predicted value.

In addition, as this model is constructed by machine learning, mechanistic interpretation of the model should be somewhat difficult.

B.4: OASIS Catalogic BCF baseline model

B.4.1 Model summary

In contrast to the microbial metabolism, catabolic reaction for the assessment of bioconcentration shall be based on eukaryotic metabolism data. As a basis the baseline approach of the OASIS model estimates a reference curve indicating the maximum BCF possible based on the hydrophobicity of a substance. This maximum BCF implies that the substance is fully bioavailable and is not metabolised within the body. Other possible factors influencing the bioconcentration potential of a substance, e.g. ionic character and molecular size are not taken into account for the derivation of the maximum BCF. In order to deliver the user a more realistic BCF the model applies mitigating factors like molecular flexibility altering the molecular size, ionisation altering hydrophobicity or metabolism for reducing the maximum BCF derived by the base-line approach mentioned above. The computational approach for deriving metabolic pathways and their probability is the same as for the biodegradation models but rat liver data are used instead of fish data as sufficient datasets are not available yet. The mitigating factors are weighted differently. While passive diffusion contributes with 70%, metabolism is at a rate of 25% and all other mitigating factors contribute with 5%.

The current training set of the model consists of 705 chemicals derived from OECD 305 testing for which the ionisation potential was available. The mathematical formalism is based on the partition coefficient of the neutral molecule and the distribution coefficient of the ionisable substance. These two values perform better in terms of goodness of fit compared to a model which uses pKa as the key parameter to describe ionisation. This is due to the fact that the pKa assumes that only neutral species of a molecule have the ability to accumulate in organisms while the two coefficients allow a more realistic description of the partitioning of ionisable substances. These findings led to the release of the “CATALOGIC bioaccumulation BCF baseline model new ionisation term” which stands beside the normal “CATALOGIC bioaccumulation BCF baseline model” and shall be used for the BCF assessment of ionisable compounds. Further information on the accuracy of both models and the mathematical formalism are given in the publications of Dimitrov et al (2012).

B.4.2 Applicability domain

For each calculated substance, the tool itself checks if the substance lies within the applicability domain or not. First, the parametric domain is checked. The boundaries of this applicability domain are defined by the log K_{OW}, the molecular size and the water solubility. After this domain is checked, the structural domain is applied next. This domain checks whether the molecule's atom centered fragments can be correctly predicted by the tool or whether there is an increasing probability that the prediction might lead to false results.

B.4.3 Model uncertainty

As described above, the model is highly transparent and uncertainties should be well displayed for the user. For metabolic pathways some uncertainty exists because of species difference between rat liver and fish. However Dimitrov et al (2012) validated the model using measured data of 123 chemicals. As a consequence the accuracy of the tool seems to be good when the substance lies within the parametric domain.

B.5 Other models

B.5.1 Models for ionising chemicals

Some models described above also consider the correction term for ionising chemicals. Their behaviour (tissue-plasma partitioning) differs from should differ from those of neutral chemicals. So specific models for ionising chemicals have been developed (Fu, 2009; Armitage et al, 2013). The Technical Guidance Document (TGD) for chemical risk assessment in the European Union recommends to correct log K_{OW} for the degree of ionisation, or use log D instead of log K_{OW} (EC, 2003; ECHA, 2008c). Such corrections should improve the prediction accuracy based on baseline models. Meylan et al proposed a very simple estimation method by using 84 ionic compounds (Meylan et al, 1999). The dynamic cell model based on the Fick-Nernst-Plank equation, which can simulate diffusive movement and distribution of molecules in a living cell, can provide good prediction especially for moderate acids and bases with extra effects (e.g. ion trap effect or attraction by the negative potential of cytoplasm) (Fu, 2009). Recently, modified mass-balance BCF model for ionisation chemicals, which can be applied to perfluoroalkyl acids, was developed (Armitage et al, 2013).

B.5.2 Models for metals

It is very difficult to predict metal bioaccumulation potential by QSAR models because metal uptake and accumulation mechanisms in biota relate not only to passive diffusion but also to other complex processes such as sequestration, detoxification, storage and bronchial elimination (Tanaka et al, 2010). Some parameters other than octanol-water partition coefficient, for example ionic index or covalent index, should be used. There is no recommendable general model at present although some specific QSAR models for metal bioaccumulation prediction exist (Van Kolck et al, 2008; Le et al, 2011). Kinetic-based models have been recommended as one of the potential methods for predicting metal bioaccumulation (Fairbrother et al, 2007).

B.5.3 Aquatic food web models

There are some other models such as GEMCO, AQUATOX and QEADFCHN to estimate complex bioaccumulation behaviour through aquatic food web (Brooke and Crookes, 2007). Predicted results from these models cannot be compared with critical threshold values like BCF in fish for B/vB assessment, and thus, these models should be used for WoE.

APPENDIX C: OVERVIEWS OF REPRESENTATIVE TERRESTRIAL FOOD WEB MODELS

This Appendix provides short overviews of some useful terrestrial food web models. RAIDAR/FHX, ACC-HUMAN, Arctic Terrestrial Food-Chain Bioaccumulation model and EUSES can simulate bioaccumulation or biomagnification processes in several environmental organisms including human. On the other hand, two human exposure models CalTOX and UseTOX mainly calculate chemical concentrations in human. These models require many input parameters, such as physico-chemical properties (e.g. molecular weight, distribution parameters between different media), half-life in each media or biota, and emission rate. Sensitivity and uncertainty analysis for validating very important input parameters and reducing uncertainty in model parameters is also recommended (Ciavatta et al, 2009; MacLeod et al, 2002). For example, emission rate and primary biotransformation half-life in mammals are reported to be the first and second most important parameters which contribute to variance in predictions (Arnot, 2012).

Some other models specified for a specific route or an organisms, e.g. the model ‘for foliar vegetation or crops’ (Trapp, 1995; Fujisawa, 2002) or the model ‘for earthworms’ (Connell and Markwell, 1990), could also be used to refine these ‘holistic’ estimations. Many models have been developed to cover different situations. Therefore, for proper estimations, not only the hypothetical mechanisms on bioaccumulation but also default conditions such as food webs should be checked before using them.

C.1 Risk Assessment IDentification And Ranking (RAIDAR) and Farfield Human eXposure (FHX) models

Developer	Centre for Environmental Modelling and Chemistry (Trent University)
Distribution simulation algorithm	Mackay's Level II and Level III fugacity models
Bioaccumulation simulation algorithm	Fugacity-based generic bioaccumulation model (mass balance model)
Endpoint	・Concentration in each organism in hypothetical food chain ・BAF in each organism in the hypothetical food web
Hypothetical food web	Complex food web including humans: plankton, benthic invertebrate, benthic-pelagic fish, piscivorous fish, aquatic mammal, foliage vegetation, root vegetation, terrestrial invertebrate, terrestrial herbivore, terrestrial carnivore, avian small, avian scavenger, poultry, swine, cattle, bulk dairy and egg
Input parameters	Necessary to input: Chemical type, MW, temperature, half-life in air/water/soil/sediment/biota/ human, water solubility, vapour pressure, log K_{OW}, emission rate, pKa, metabolic transformation rates in organisms Calculated automatically or defined initially (allowed to input): $K_{air\text{-}water}$, $K_{soil\text{-}water}$, $K_{sed\text{-}water}$, $K_{suspended\ particlest\text{-}water}$, $K_{fish\text{-}water}$, $K_{aerosols\text{-}water}$, $K_{water\text{-}air}$, $K_{soil\text{-}air}$, $K_{sed\text{-}air}$, $K_{suspended\ particlest\text{-}air}$, $K_{fish\text{-}air}$, $K_{aerosols\text{-}air}$, concentration in air/water/soil/sediment, water concentration in biota, log_{BCF} from any system constituting hypothetical food web, respiration/feeding/drinking/growth/ urination rates of organisms, site specific parameters.
Model advantage	・Bioaccumulation behaviour is theoretically described by mass balance model. ・Each parameter in this model is defined by using empirical data.
Model limitation	・Metabolism rate constant for each biota is needed although it is difficult to be measured. ・This model is primarily designed to address non-ionizing substances. Certain chemical groups such as pigments, dyes, perfluorinated and/or ionizing substances may not be well simulated. ・This model assess the 'farfield' impact of chemical exposure (it does not consider 'nearfield' sources).
Others	・RAIDAR and FHX are very similar programs in terms of model equations and default parameters. FHX model gives human exposure estimation for each age-group, whereas RAIDAR gives that only for adult males. ・Further studies are needed in order to refine many parameters and extend the reliability of model predictions by using field monitoring data.
Literature and Website	Arnot and Gobas, 2006b; Arnot et al, 2010b http://www.arnotresearch.com/ http://www.arnotresearch.com/#!/page_RAIDAR_DL http://www.arnotresearch.com/#!/page_FHX

C-2 ACC-HUMAN model

Developer	Czub and McLachlan
Distribution simulation algorithm	Non-steady-state fugacity model
Bioaccumulation simulation algorithm	Non-steady-state fugacity-based mechanistic model
Endpoint	Concentration in each organism in hypothetical food chain especially for humans
Hypothetical food web	Complex food web including humans [males and females]: zooplankton, herring, cod, grass, cattle feed, soil in grass, milk, milk cow, beef cattle and beef
Input parameters	Necessary to input: log K_{OW}, log K_{AW} (or log K_{OA}), heat of phase transfer between octanol-water/air-water/octanol-air, concentration or fugacity in air/seawater/freshwater/soil, metabolism rate constant in humans/milk cows/beef cattle/grass/fish, emission rate. Calculated automatically or defined initially (allow to input): faeces-blood partition coefficient in humans, temperature in seawater/air/soil/freshwater, grass parameters, mass transfer coefficients for grass, organism specific parameters for milk cattle/beef cattle/fish food chain/humans
Model advantage	・Bioaccumulation behaviour is theoretically described by fugacity based mass balance model. ・Each parameter in this model is defined by using empirical data.
Model limitation	・This model is primarily designed to address non-ionising substances because the fugacity capacity of lipids in fish and mammals is assumed to be equal to that of 1-octanol and this model is evaluated with PCBs under the Swedish environment as a case study. ・Metabolism rate constant for each biota should be needed although it is difficult to measure. ・For aquatic systems, uptake from sediment into the food chain is not considered.
Others	・This model can predict variability of chemical concentration in each media or organism over time. ・Default parameters are representative of the Northern European country.
Literature and Website	Czub and McLachlan, 2004a,b http://www.itm.su.se/page.php?pid=117

C-3 Arctic Terrestrial Food-Chain Bioaccumulation model

Developer	Kelly & Gobas
Distribution simulation algorithm	Mackay's Level II and Level III fugacity models
Bioaccumulation simulation algorithm	Mechanistic mass balance equations relating to ambient concentrations in the environment such as air and snow concentrations
Endpoint	・Concentration in organisms in hypothetical food web ・Biomagnification factor
Hypothetical food web	Arctic terrestrial food chain: lichen, willows, caribou and wolf
Input parameters	Necessary to input: Molecular weight, log K_{OW}, Henry's law constant, log K_{OA}, tissue half-life in mammals, air concentration, dissolved concentration in snowpack melt water, emission rate. Calculated automatically or defined initially (allow to input): specific parameters for organisms such as weight, lipid content, respiration rate, feeding rate, urine/bile/faecal/milk excretion rate, growth rate constant, air uptake efficiency, dietary uptake efficiency and organisms-to-faeces partition coefficient, metabolic transformation rates in organisms, ambient air parameters, vegetation parameters.
Model advantage	・This model assumes the same mechanism as those of RAIDAR or ACC-HUMAN, so bioaccumulation behaviour is theoretically described. ・This model is specialised for the arctic region and terrestrial food-chain.
Model limitation	・It is difficult to apply to general locations. ・The uptake route to plant is only from air and uptake via the roots from soil is not considered.
Others	・The model is not released as a computer program but published as a series of equations in the literature.
Literature and Website	Gobas et al, 2003; Kelly, 2003.

C-4 EUSES (European Union System for the Evaluation of Substances) model

Developer	National Institute of Public Health and the Environment (RIVM) of The Netherlands, European Chemical Bureau of the European Commission
Distribution simulation algorithm	Steady-state fugacity based model
Bioaccumulation simulation algorithm	Human exposure model with regression based simple accumulation models for other organisms
Endpoint	・Concentration in organisms in hypothetical food web ・Total daily human intake
Hypothetical food web	Aquatic food chain: only transfer from water to fish, Terrestrial food chain: only transfer from soil to earthworms Human food chain: transfer from air, drinking water and food such as fish, root crops, leaf crops, meat and milk
Input parameters	Necessary to input: log K_{OW}, Henry's law constant, emission rate Calculated automatically or defined initially (allow to input): BCF/BMF by log K_{OW} based regression model, Organic-carbon water partition coefficient, half-lives in surface water/sediment/soil, metabolic half-life or rate constant in plants, result of screening test on biodegradability.
Model advantage	・Easy to use and limited input parameters. ・This model considers a generic European environment. ・Widely used for regulatory purposes.
Model limitation	・Simple food web compared with other models to simulate bioaccumulation behaviour. ・BCF and BMF are calculated by simple regression model based on log K_{OW} in a limited range.
Others	—
Literature and Website	Vermeire et al, 1997; 2005. http://ihcp.jrc.ec.europa.eu/our_activities/public-health/risk_assessment_of_Biocides/euses

C-5 UseTox (UNEP-SETAC toxicity) model

Developer	United Nations Environment Programme (UNEP) and the Society of Environmental Toxicology and Chemistry (SETAC) Life Cycle Initiative
Distribution simulation algorithm	Steady-state fugacity based model.
Bioaccumulation simulation algorithm	Human exposure model with regression based simple accumulation models for other organisms.
Endpoint	Intake fraction for humans.
Hypothetical food web	Aquatic food chain: only transfer from water to fish. Human food chain: transfer from air, drinking water and food such as fish, root crops, leaf crops, dairy products.
Input parameters	Necessary to input: Molecular weight, log K_{OW}, vapour pressure, solubility, degradation rate in air/water/sediment/soil, emission rate. Calculated automatically or defined initially (allow to input): K_{OC}, Henry's law constant, partition coefficient between dissolved organic carbon and water, bioaccumulation factor in fish/biota and root/leaf crops, biotransformation factor for meat and milk.
Model advantage	・Easy to use and limited input parameters.
Model limitation	・Calculate only concentration in humans. ・Very simple food web compared with other models. ・BCF and BMF are calculated by regression model based on log K_{OW} in a limited range.
Others	—
Literature and Website	Rosenbaum et al, 2008 http://www.usetox.org/

C-6 California Toxicity (CalTOX) model

Developer	Department of Toxic Substances Control (DTSC) of the California Environmental Protection Agency.
Distribution simulation algorithm	Eight-compartment regional and dynamic multimedia fugacity model.
Bioaccumulation simulation algorithm	Human exposure model with regression based simple accumulation models for other organisms.
Endpoint	Intake fraction for humans.
Hypothetical food web	Human exposure modelling with 23 different exposure pathways for ingestion, inhalation, or dermal intake.
Input parameters	Necessary to input: Molecular weight, log K_{OW}, melting point, vapour pressure, solubility, Henry's law constant, diffusion coefficient in air/water, Koc, emission rate. Calculated automatically or defined initially (allow to input): biotransfer factors, BCF, skin permeability coefficient, fraction dermal uptake from soil, reaction half-life in air/surface soil/root-zone soil/vadose-zone soil/ground water/surface water/sediment, partition coefficient in soil and aquatic layers, site specific parameters.
Model advantage	・Detailed human exposure model compared to other models. ・Easy to use and limited input parameters.
Model limitation	・Calculates only concentration in humans. ・Accumulation factors for fish and agricultural products are calculated by simple regression model based on log K_{OW} in a limited range. ・This model is designed for non-ionic organic chemicals and inorganic chemicals with linear and reversible distribution coefficients in soil and sediments. Thus, it is not designed for surfactants, volatile metals or ionised organic chemicals.
Others	・Human exposure model
Literature and Website	McKone, 1993 http://energy.lbl.gov/ied/era/caltox/index.html

APPENDIX D: LITERATURE DATA ON ORGAN-SPECIFIC BCF

The available organ-specific BCF data in fish are presented in Table D-1. The first study reported a field BCF value as a mean over multiple species and tissues (Taniyasu et al, 2003). At the end of the table there are also two values in mammals (Gomez et al, 2010).

Table D-1: BCF values measured[a] in freshwater fish, and two values in mammals

Species[b], tissue/ Compound	BCF	Remark	Reference
Multiple species, tissues			
PFOS	8,540 mean (range 274 - 41,600)	Field study	Taniyasu et al, 2003
Catfish, gill			
Ibuprofen	0.99 ± 0.003	From in vitro biotransformation assay	Gomez et al, 2010
Norethindrone	96.3 ± 03		
Propranolol	0.60 ± 0.002		
Catfish, liver			
Ibuprofen	0.96 ± 0.005	From in vitro biotransformation assay	Gomez et al, 2010
Norethindrone	76.8 ± 0.9		
Propranolol	0.54 ± 0.003		
Carp, gills			
Aldrin	147.2	30-day BCF	Satyanarayan et al, 2003
BHC	1.682		
DDT	24		
Dieldrin	485.5		
Carp, intestine			
Aldrin	169.2	30-day BCF	Satyanarayan et al, 2003
BHC	8.08		
DDT	61.5		
Dieldrin	478		
Carp, kidney			
Aldrin	604	30-day BCF	Satyanarayan et al, 2003
BHC	7.72		
DDT	-		
Dieldrin	459.5		
Carp, liver			
Aldrin	2,824	30-day BCF	Satyanarayan et al, 2003
BHC	31.25		
DDT	3,650		
Dieldrin	42,500		

Species [b], tissue/ Compound	BCF	Remark	Reference
Multiple species, tissues			
Carp, muscle			
Aldrin	74.2	30-day BCF	Satyanarayan et al, 2003
BHC	1.035	30-day BCF	
DDT	64	30-day BCF	
Dieldrin	64.5	30-day BCF	
Salmon, blood			
PFDA	2,700 ± 350		Martin et al, 2003
PFDoA	40,000 ± 4,500		
PFHxS	76 ± 9.7		
PFOA	27 ± 9.7		
PFOS	4,300 ± 570		
PFTA	30,000 ± 4200		
PFUnA	11,000 ± 1400		
Salmon, carcass			
PFDA	450 ± 62		Martin et al, 2003
PFDoA	18,000 ± 2,700		
PFHxS	9.6 ± 0.99		
PFOA	4.0 ± 0.6		
PFOS	1,100 ± 150		
PFTA	23,000 ± 5300		
PFUnA	2,700 ± 400		
Salmon, liver		BPA concentration (µg/l)	
BPA	22	10	Lindholst et al, 2000
BPA	25	40	
BPA	38.4	70	
BPA	10.8	100	
BPA	8.7	500	
PFDA	1,100 ± 180		Martin et al, 2003
PFDoA	18,000 ± 2,900		
PFHxS	100 ± 13		
PFOA	8 ± 0.59		
PFOS	5,400 ± 860		
PFTA	30,000 ± 6,000		
PFUnA	4,900 ± 770		
Salmon, muscle		BPA concentration (µg/l)	
BPA	3.6	70	Lindholst et al, 2000
BPA	2.2	100	
BPA	>1.7	500	

Species [b], tissue/ Compound	BCF	Remark	Reference
Multiple species, tissues			
Fathead minnow, liver			
PFOS	210	0.1 mg/l; male fish	Ankley et al, 2005
PFOS	830	0.1 mg/l; female fish	Ankley et al, 2005
Trout, gill			
Ibuprofen	0.97 ± 0.005	From in vitro biotransformation assay	Gomez et al, 2010
Norethindrone	96.1 ± 0.3		
Propranolol	0.59 ± 0.004		
Trout, liver			
Ibuprofen	0.97 ± 0.02	From in vitro biotransformation assay	Gomez et al, 2010
Norethindrone	84.9 ± 1.6		
Propranolol	0.55 ± 0.003		
Mouse, liver			
Ibuprofen	0.97 ± 0.004	From in vitro biotransformation assay	Gomez et al, 2010
Rat, liver			
Ibuprofen	0.88 ± 0.02	From in vitro biotransformation assay	Gomez et al, 2010

[a] In the laboratory.

[b] Carp, *Cyprinus carpio*; Channel catfish, *Ictalurus punctatus*; Fathead minnow, *Pimephales promelas*; Salmon, *Oncorhynchus mykiss*; Trout, *Salmo gairdneri*.

APPENDIX E: CRITERIA FOR RELIABILITY CATEGORIES (FOR ANIMAL DATA)

Adapted from Klimisch *et al* (1997).

Code of Reliability (CoR)	Category of reliability
1	**Reliable without restriction**
1a	'Good laboratory practice' guideline study (OECD, EC, EPA, FDA, etc.)
1b	Comparable to guideline study
1c	Test procedure in accordance with national standard methods (AFNOR, DIN, etc.)
1d	Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
2	**Reliable with restrictions**
2a	Guideline study without detailed documentation
2b	Guideline study with acceptable restrictions
2c	Comparable to guideline study with acceptable restrictions
2d	Test procedure in accordance with national standard methods with acceptable restrictions
2e	Study well documented, meets generally accepted scientific principles, acceptable for assessment
2f	Accepted calculation method
2g	Data from handbook or collection of data
3	**Not reliable**
3a	Documentation insufficient for assessment
3b	Significant methodological deficiencies
3c	Unsuitable test system
4	**Not assignable**
4a	Abstract
4b	Secondary literature
4c	Original reference not yet available
4d	Original reference not translated
4e	Documentation insufficient for assessment

ABBREVIATIONS

ACC-HUMAN	A non-steady-state bioaccumulation model predicting human tissue levels from concentrations in air, soil and water
ADI	Applicability domain index
ADME	Absorption, distribution, metabolism and excretion
ANOVA	Analysis of variance
AOP	Adverse outcome pathway
B	Bioaccumulation
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BDE	Bromodiphenyl ether
BM	Biomonitoring
BMF	Biomagnification factor
BOD	Biochemical oxygen demand
BR	Bound Residue
BSAF	Biota-sediment accumulation factor
BSAF	Biota-soil accumulation factor
CalTOX	California Toxicity (model)
Cefic	European Chemical Industry Council
CESIO	Council of European surfactants producers
CLP	Classification, Labelling and Packaging of substances and mixtures
CMR	Carcinogenic, mutagenic and reprotoxic
COD	Chemical oxygen demand
CPC	Centrifugal partition chromatographic (techniques)
DDT	Dichlorodiphenyltrichloroethane
DOC	Dissolved organic carbon
EBM	Environmental biomonitoring
EC	European Commission
ECB	(Former) European Chemicals Bureau
ECHA	European Chemicals Agency
ECOSAR	Ecological Structure Activity Relationships
EF	Enrichment factor
EFSA	European Food Safety Authority
EQC	Equilibrium criteria
ERASM	Environment and health Risk ASsessment and Management
EU	European Union
EUSES	European unified system for the evaluation of substances
FHX	Farfield Human Exposure (model)
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium
hBCF	Human bioconcentration factor
HBM	Human biomonitoring

HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HESS	Hazard Evaluation Support System Integrated Platform
HOCNF	Harmonised offshore chemical notification format
HPLC	High performance liquid chromatography
IAM	Immobilised artificial membrane
ILSI/HESI	International Life Sciences Institute / Health and Environmental Sciences Institute
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
LRI	Long-range Research Initiative
MML	Maximum molecular length
MOA	Mode of action
NER	Non-extractable residue
NITE	National Institute of Technology and Evaluation
OC	Organic carbon
OECD	Organisation for Economic Co-operation and Development
OSPAR	Oslo Paris Convention (legal instrument guiding international cooperation on the protection of the marine environment of the North-East Atlantic)
P	Persistence
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybromodiphenyl ether
PBT	Persistent, bioaccumulative and toxic
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo(*p*)dioxin
PCDF	Polychlorinated dibenzofuran
PD	Probability distribution
PDF	Probability density function
PFAA	Perfluoroalkyl acid
PFCA	Perfluoroalkyl carboxylic acid
PFHxA	Perfluorohexanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulphonic acid
PFSA	Perfluoroalkane sulphonic acids
Pov	Overall persistence
QA/QC	Quality assurance/Quality control
QSAR	Quantitative structure-activity relationship
RAIDAR	Risk Assessment IDentification And Ranking
REACh	Registration, evaluation, authorisation and restriction of chemicals
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SETAC	Society of Environmental Toxicology and Chemistry

SPMD	Semi-permeable membrane device
SPME	Solid phase micro extraction
T	Toxicity
TGD	Technical Guidance Document
ThOD	Theoretical oxygen demand
TL	Trophic level
TMF	Trophic magnification factor
UBA	Umweltbundesamt / German Federal Environment Agency
UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency
UseTox	UNEP-SETAC Toxicity Model
UVCB	Substance of Unknown or Variable composition, Complex reaction products or Biological materials
vB	Very bioaccumulative
vP	Very persistent
WoE	Weight of evidence

GLOSSARY

Bioaccumulation factor (BAF): Ratio of the steady-state chemical concentration in an aquatic organism and the water. Exposure occurs via water and the diet.

Bioaccumulation: Net accumulation of a chemical by an organism resulting from uptake via all routes of exposure (sediment, water, and food).

Bioconcentration factor (BCF): Ratio of the steady-state chemical concentration in an aquatic organism and the water. Exposure occurs via water.

Bioconcentration: Net accumulation of a chemical by an organism resulting from uptake only through its surrounding environment via respiration and dermal routes.

Biomagnification factor (BMF): Ratio of the steady-state chemical concentration in an organism and the concentration in its diet.

Biomagnification: Net accumulation of a chemical by an organism along a series of predator-prey associations, primarily through diet.

Biota-sediment accumulation factor (BSAF): Ratio of the steady-state chemical concentration in an aquatic organism and the sediment.

Biota-soil accumulation factor (BSAF): Ratio of the steady-state chemical concentration in a terrestrial organism and the soil.

Bound residue (BR): A residue that is tightly associated with the solid matrix, often forming covalent (or similar) bonds. These residues usually cannot be released from the matrix or can only be released under extreme conditions where the integrity of the substance and/or matrix is likely to be affected. Such residues are often indistinguishable for natural organic matter e.g. humus in soil. These residues are not available for either degradation or available for indigenous organisms and should not be considered in any impact / risk assessment.

Klimisch et al (1997) reliability criteria: Animal studies are assigned into four reliability categories: (i) Reliable without restriction, (ii) Reliable with restrictions, (iii) Not reliable, and (iv) Not assignable. Main considerations are whether a standardised test method was used and relevant details of the study design were given. The categorisation provides a practical systematic approach for quality evaluation of experimental toxicological data. The detailed criteria are shown in Appendix E.

Non-extractable residue (NER): A residue that is not extractable using ‘mild’ extraction methods, but extractable under harsher conditions. These conditions may include solvent extraction using methods such as refluxing, microwaves or accelerated solvent extraction (ASE). These residues are strongly associated with the matrix, however they may be potentially reversible; but the partitioning is very much in favour of ‘binding’ to components of the matrix. Therefore, for risk assessment purposes, the matrix associated fraction is unlikely to be available to indigenous organisms.

Overall weight-of-evidence (WoE) scores:

1. Strong, coherent evidence from different data sources and lines of evidence leading to the same conclusion.
2. Moderate, good evidence from a primary line of evidence, but evidence from several other lines is missing (important data gaps).
3. Weak, weak evidence from the primary lines of evidence (severe data gaps).
4. Uncertain, conflicting information from different lines of evidence that cannot be explained in scientific terms.
5. Not possible, no suitable evidence available.

WoE considerations or approach: Starts with the identification of different sources of data and data gaps in relation to the aim of the assessment. The second and third steps consist of the collection and screening followed by the evaluation of all relevant publications and data for the respective purpose. The fourth step is to establish the lines of evidence that need to be considered. The fifth step consists of the weighing of the totality of the evidence in combining the assessments of the different lines of evidence into an overall assessment. This involves verbal justification for each step and the final conclusion.

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