ECHA
EUROPEAN CHEMICALS AGENCY

GUIDANCE

Guidance on the Application of the CLP Criteria

Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures

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4.3. Persistent, Bioaccumulative and Toxic or Very Persistent, Very Bioaccumulative (PBT/vPvB) and Persistent, Mobile and Toxic or Very Persistent, Very Mobile (PMT/vPvM) Properties

4.3.1. Definitions and general considerations for PBT/vPvB and PMT/vPvM substances

Commission Delegated Regulation (EU) 2023/707, Annex I*: 4.3.1. and 4.4.1.* *For the purposes of Sections 4.3 and 4.4 the following definitions shall apply:*

"PBT" means a persistent, bioaccumulative and toxic substance or mixture that meets the classification criteria set out in Section 4.3.2.1.

"vPvB" means a very persistent and very bioaccumulative substance or mixture that meets the classification criteria set out in Section 4.3.2.2.

"PMT" means a persistent, mobile and toxic substance or mixture that meets the classification criteria set out in Section 4.4.2.1.

"vPvM" means a very persistent and very mobile substance or mixture that meets the classification criteria set out in Section 4.4.2.2.

"log *K*oc" means the common logarithm of the organic carbon-water partition coefficient (i.e. *K*oc).

Annex I*: 4.3.1.2.* The hazard class Persistent, Bioaccumulative and Toxic or Very Persistent, Very Bioaccumulative properties is differentiated into:

- PBT properties and,
- vPvB properties.

Annex I: 4.4.1.2. The hazard class Persistent, Mobile and Toxic or Very Persistent, Very Mobile properties is differentiated into:

- PMT properties and,
- vPvM properties.

Definitions

Persistence (P) can be defined as the resistance of chemicals to transformation by degrading processes of biological and physical origin (Mackay, 2001). Alternatively, Annex II of REACH on the requirements for the compilation of safety data sheets defines persistence *"as the lack of demonstration of degradation, as defined in Annex XIII, Sections 1.1.1 and 1.2.1."* Degradability is further defined as *"the potential for the substance or the appropriate substances in a mixture to degrade in the environment, either through biodegradation or other processes, such as oxidation or hydrolysis"*. Persistence is usually quantified by a **half-life ($t_{1/2}$)** which is used to characterise the rate of a first or pseudo-first order reaction and corresponds to a concentration decrease by a factor 2 (REACH Guidance on information requirements and chemical safety assessment, ECHA Guidance on IR&CSA, Chapter R.7b). Degradation half-life (DegT50) describes the

time for 50 % of substance to disappear from a compartment as a result of degradation processes alone.

Bioaccumulation (B) refers to the potential of the substance or certain substances in a mixture to accumulate in biota and, eventually, to pass through the food chain (REACH Annex II, 12.3) and is the net result of uptake, transformation and elimination of a substance in an organism due to all routes of exposure (i.e. air, water, sediment/soil and food) (CLP Annex I, 4.1.1.1.).

Mobility (M) refers to the affinity of a substance, once released to the environment, to spread over short or long distances and enter water bodies, including drinking water and groundwater. REACH Annex II defines mobility in soil as "*the potential of the substance or the components of a mixture, if released to the environment, to move under natural forces to the groundwater or to a distance from the site of release*". Mobile substances possess moderate to (very) low adsorption potential, as indicated by the organic carbon-water partition coefficient (i.e. *K*oc, see Section 4.3.3.3.1).

Toxicity (T) refers to the intrinsic property of a substance to cause adverse effects to humans, wildlife, plants and/or other environmental organisms as a result of the exposure to the substance itself.

CLP refers explicitly to the combination of these properties that poses concern, for example the combination of not easy to break down in the environment and tendency to accumulate in living organisms (for PBTs/vPvBs) and high persistence and high mobility (for PMTs/vPvMs). More definitions of the relevant terminology are included in the respective Sections of this Guidance.

Historical developments on PBT/vPvB and PMT/vPvM assessment

For more than 30 years, regulatory Authorities throughout the world have been assessing the hazards caused by substances that possess persistent, bioaccumulative and toxic (PBT) and very persistent, very bioaccumulative (vPvB) properties. These properties indicate that such substances break down slowly in the environment, they are toxic, they tend to accumulate in living organisms and exposure to the environment (including pristine/ remote regions and humans, amended CLP preamble 7) is difficult to reverse. Between 1994 and 2007 (the entry into force of Regulation (EC) No 1907/2006, the "REACH" Regulation), 141 risk assessments have been performed and concluded by the different Member States[1]. Since the introduction of REACH, the identification of substances with PBT and/or vPvB properties entailed the comparison with the numerical criteria stipulated in Annex XIII of REACH, where all available information is assessed in a weight of evidence determination (WoE). The same applies to the PBT/vPvB assessment under the Biocidal Products Regulation (BPR, Regulation (EU) 528/2012)).

The experience and accumulated scientific knowledge in PBT/vPvB assessment and the need of protection for the environment regarding Substances of Very High Concern (SVHCs), were the trigger for the European Commission to propose the introduction of a new hazard class (HC) in Regulation (EC) No 1272/2008 ("the CLP" Regulation) regarding substances with PBT and/or vPvB properties. Due to the similarity of their properties with

[1] https://echa.europa.eu/information-on-chemicals/information-from-existing-substances-regulation

the exception of toxicity, the Commission has proposed one single new hazard class, with differentiation, while establishing common rules for the scientific assessment of the intrinsic properties related to persistency and bioaccumulation. The overall aim of PBT/vPvB assessment undertaken either under the REACH Regulation or under CLP is to ensure a high level of protection for human health and the environment.

In recent years, substances that break down slowly in the environment and have a high environmental mobility, often reaching water resources, have received increased scientific and regulatory attention. The German authorities (UBA) first proposed to name such substances in the regulatory context of REACH as PMT/vPvMs (Neumann *et al*., 2015, Neumann and Schliebner, 2019). These substances possess persistent, mobile and toxic (PMT) and/or very persistent, very mobile (vPvM) properties, often reaching (drinking) water resources, they are only partly removed by wastewater treatment processes, they can spread over long distances and also cause difficult to reverse environmental exposures (Neumann and Schliebner, 2019, Arp and Hale, 2022). As such, the European Commission proposed a new hazard class (with differentiation) to be introduced in CLP also regarding substances with PMT and/or vPvM properties, with the overall aim being to ensure a high level of protection for human health and the environment.

The following Sections of the present Guidance document will outline the respective CLP criteria, identify the different sources of relevant information, detail the different assessment elements to be taken into account by Authorities and data holders and, importantly, compare the available information with the CLP criteria to come to a conclusion on whether classification in either of the related hazard classes apply. The following apply to single substances and their relevant constituents and/or degradation products, with further considerations on mixtures described in Section 4.3.4. As clearly indicated in CLP, the two new hazard classes (PBT/vPvB and PMT/vPvM) apply only to all organic substances, including organo-metals. The reason for that is that the PBT/vPvB assessment under REACH was defined in Annex XIII that "is generally applicable to any substance containing an organic moiety. Based on the common definition of an organic substance in chemistry, PBT and vPvB criteria are not applicable to inorganic substances" (ECHA Guidance on IR&CSA, Chapter R.11.2.1). Furthermore, inorganic substances are considered by default as Persistent.

4.3.2. Classification criteria for PBT/vPvB and PMT/vPvM substances

The following Sections (green texts) merely reproduce CLP Annex I regarding the numerical criteria for the individual properties. Further elaboration on these can be found in subsequent Sections of the Guidance (4.3.3).

4.3.2.1. Persistence criteria

Annex I: 4.3.2.1.1. and 4.4.2.1.1. A substance shall be considered to fulfil the persistence criterion (P) where any of the following conditions is met:

(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.

Annex I: 4.3.2.2.1 and 4.4.2.2.1 A substance shall be considered to fulfil the 'very persistent' criterion (vP) where any of the following situations is met:

(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.

4.3.2.2. Bioaccumulation criteria

Annex I: 4.3.2.1.2. A substance shall be considered to fulfil the bioaccumulation criterion (B) where the bioconcentration factor in aquatic species is higher than 2000.

Annex I: 4.3.2.2.2. A substance shall be considered to fulfil the "very bioaccumulative" criterion (vB) where the bioconcentration factor in aquatic species is higher than 5 000.

4.3.2.3. Mobility criteria

Annex I: 4.4.2.1.2. A substance shall be considered to fulfil the mobility criterion (M) when the log K_{OC} is less than 3. For an ionisable substance, the mobility criterion shall be considered fulfilled when the lowest log K_{OC} value for pH between 4 and 9 is less than 3.

Annex I: 4.4.2.2.2. A substance shall be considered to fulfil the 'very mobile' criterion (vM) when the log K_{OC} is less than 2. For an ionisable substance, the mobility criterion shall be considered fulfilled when the lowest log K_{OC} value for pH between 4 and 9 is less than 2.

4.3.2.4. Toxicity criteria

Annex I: 4.3.2.1.3. and 4.4.2.1.3. A substance shall be considered to fulfil the toxicity criterion (T) in any of the following situations:

(a) the long-term no-observed effect concentration (NOEC) or ECx (e.g 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 Sections 3.5, 3.6 or 3.7;

(c) there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification as specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Section 3.9;

(d) the substance meets the criteria for classification as endocrine disruptor (category 1) for human health or the environment according to Sections 3.11 or 4.2.

4.3.3. Identification and assessment of hazard information for PBT/vPvB and PMT/vPvM substances

The following Sections will present in detail the information that can be used for classification and labelling purposes on the PBT/vPvB and PMT/vPvM properties, as well as the related assessment elements (named "Interpretation of data" in the Sections of the CLP Guidance referring to aquatic hazards, Section 4.1). Before proceeding to the identification of the relevant information and its regulatory assessment, a number of general points have been assembled that are relevant for the consideration of all hazard properties discussed. These include:

(i) **data availability and quality**

CLP refers to the identification of all relevant available information for the purposes of determining whether the substance entails a physical, health or environmental hazard as set out in its Annex I. Available data should be based on methods referred to in Article 13(3) of the REACH Regulation. CLP Article 8 further expands on the scientific principles that the performance of any new tests should be followed by manufacturers, importers or downstream users before the submission of a proposal for harmonised classification and labelling for the purpose of determining whether a substance or a mixture entails a human health or environmental hazard. Furthermore, scientific information must be in accordance to standardised test methods, where available. In the presence of such information, results from reliable experimental studies conducted under Good Laboratory Practice (GLP), generally receive higher weight over estimated/predicted values for the classification and labelling of the substance.

CLP Annex I, 4.1.1.2.2 and Section 4.1.3.1.2 of this Guidance further expand on the use of other data than from standardised studies, stating that "*in practice data from other standardised test methods such as national methods shall also be used where they are considered as equivalent*". Importantly, data from non-standard studies and non-testing methods shall be considered in classification provided that they fulfil the requirements specified in Section 1 of Annex XI to Regulation (EC) No 1907/2006. Based on this firm legal provisions, RAC has previously formed opinions on the harmonsied classification and labelling of substances referring to aquatic hazards using data from non-standard test methods. In all cases, the classification should be based on the best available data (CLP Annex I, 4.1.1.2.2).

Concerning active substances in accordance with Regulation (EC) No 1107/2009 ("the PPP"

Regulation, or PPPR), Commission Communication in the framework of the implementation of Commission Regulation (EU) No 283/2013 of 1 March 2013 is setting out the data requirements and relevant test methods and guidelines for pesticides. Concerning active substances in accordance with the BPR, ECHA (2017c) further details the information requirements and relevant test methods for biocides.

CLP does not introduce any direct responsibilities to generate new information, but in case of any new testing being carried out for the purposes of the CLP Regulation, Article 7 explicitly states that any testing on animals shall only be undertaken where no other alternatives exist that would provide reliable, high quality data. In the absence of experimental information, qualitative or quantitative structure-activity relationships (QSARs), suitable *in vitro* tests, information from the application of the category approach (grouping, read-across) and other types of available information may be used in a WoE determination (see point below, but also within the Sections for the individual properties).

Furthermore, the Court of Justice of the European Court has confirmed that the application of the precautionary principle is also applicable in the context of the classification of a substance under Regulation No 1272/2008, where the assessment of the risks of that substance to the environment and to human health gives rise to uncertainty[2]. In this context, when more than one reliable experimental studies are available for the same property, in most cases the most conservative value is used in order to account for the uncertainties of the test method and differing experimental conditions. This is in line with both the long-established PBT/vPvB assessment approach used, for example, for the identification of Substances of Very High Concern (SVHC) under REACH Article 57 (d)/(e) and with the approach used for harmonised classification of substances under CLP. However, there may be exceptional specific situations where it is possible to combine study results for the same test conditions. This is discussed further below under the respective Sections 4.3.3.1, 4.3.3.2, 4.3.3.3 and 4.3.3.4. but also in other parts of this Guidance, where the conditions that need to be met for averaging results from different (but similar) reliable studies are detailed.

Sections 4.3.2.3 and 4.4.2.3 of Annex I of CLP indicate that the information used for the purposes of assessment of the PBT/vPvB and PMT/vPvM properties shall be based on data obtained under relevant conditions (see following bulletpoint).

(ii) relevant conditions

Sections 4.3.2.4 and 4.4.2.3 of Annex I of CLP state that the information used for the purposes of assessment of the PBT/vPvB properties and PMT/vPvM properties shall be based on data obtained under relevant conditions. Relevant conditions refer to those conditions that allow for an objective assessment of the PBT/vPvB/PMT/vPvM properties of a substance instead of under particular environmental or 'realistic' conditions that may vary considerably across the European Union. In other words, the purpose of the PBT/vPvB assessment has been defined by Court rulings on different Appeal cases concerning REACH substances as one that is meant to clarify the intrinsic property of the substance irrespective of the local/specific environmental conditions and taking into account the physico-chemical properties of the substance (T-176/19[3], Digest of decisions of the Bord

[2] https://curia.europa.eu/juris/document/document.jsf?text=%2522coal%2Btar%2522&docid=260991&pageIndex=0&doclang=EN&mode=lst&dir=&occ=first&part=1&cid=1798278#ctx1
[3] https://curia.europa.eu/juris/liste.jsf?language=en&td=ALL&num=T-176/19

of Appeal of the European Chemicals Agency, 2022[4]). Furthermore, a study is considered to be performed under relevant conditions if it is performed in accordance with the testing conditions provided for in the test methods Regulation, in line with Article 13(3) of the REACH Regulation and bulletpoint (i) above. These considerations also hold true for the PBT/vPvB and PMT/vPvM assessment under CLP.

Property specific considerations of relevant conditions are presented in this Guidance under each respective property, when relevant, and in ECHA Guidance on IR&CSA Chapters R.11, R.7b and R.7c.

(iii) use of QSARs and read-across/ category approaches

QSAR predictions can be used as supporting information in the WoE determination. When using QSARs to predict a substance property, an assessment of both the model and the prediction is needed. A QSAR model must be recognised as scientifically valid (using OECD principles (OECD, 2004; OECD, 2007)) and adequate and reliable documentation must be provided. A valid QSAR model does not necessarily produce a valid prediction. For a valid QSAR prediction, the input is correct, the substance falls within the applicability domain of the model, the prediction is reliable and the outcome is fit for the regulatory purpose. The validity of models and predictions can be assessed by using the OECD QSAR assessment framework (QAF). More information can be found in OECD QSAR assessment framework documents (OECD, 2023), in the Guidance on QSARs and grouping of chemicals, Chapter R.6[5] and in ECHA Practical Guide "How to use and report (Q)SARs"[6].

It has to be noted that, as reported also above, in case of available and reliable laboratory studies, these are generally preferred over predicted data.

Read-across is a technique for predicting endpoint information for one substance (target), by using data from the same endpoint from (an)other substance(s) (source). To cover the complexity of each endpoint, it needs to be clear how the read-across addresses the endpoint or property under consideration. The term "analogue approach" is used when the read-across approach is employed between a small number of structurally similar substances. As the number of substances is small, trends may not be apparent. As a result of structural similarity, a given (eco)toxicological/ environmental fate property of the source substance is used to predict the same property of the target substance. The "category approach" is used when read-across is employed between several substances that are grouped together based on defined structural similarity and allowable differences between the substances. Because of the structural similarity, the results will be either similar, or follow a regular pattern.

The basis for a prediction within the group for the target substance must be explicit (e.g. "worst case", or trend analysis). Use of the Read-Across Assessment Framework (RAAF, ECHA 2017a[7]) may help assess and, where necessary, improve the read-across. ECHA

[4] https://echa.europa.eu/documents/10162/2314761/digest_of_decisions_of_boa_en.pdf/cad5c04e-1888-9ac3-5718-eb6f17a395a8?t=1670504949902

[5] https://echa.europa.eu/documents/10162/17224/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9?t=1322594777272

[6] https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf/407dff11-aa4a-4eef-a1ce-9300f8460099

[7] https://echa.europa.eu/documents/10162/17221/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a and

developed the RAAF based on the most frequently encountered types of read-across approaches in the different ECHA-managed regulatory processes.

The documents "Practical Guide: How to use alternatives to animal testing" (ECHA 2016[8]) and "ECHA Guidance on IR&CSA, Chapter R.6: QSARs and grouping of chemicals" (ECHA 2008), developed by ECHA, give further details on how to use and report read-across.

(iv) substances with more than one constituents, additives, impurities and UVCBs

CLP Annex I, 4.3.2.3 and 4.4.2.3 refer to the identification that "*shall also take account of the PBT/vPvB and PMT/vPvM properties of relevant constituents, additives or impurities of a substance* …". PBT/vPvB and PMT/vPvM assessment are exercises usually performed on single substances with a well-defined identification. However, as discussed below, a chemical may be composed by more than one single substances in a form of its constituents. The term UVCB is defined as substances of Unknown or Variable composition, Complex reaction products or Biological materials as further detailed in Chapter 4.3 of the Guidance for identification and naming of substances under REACH and CLP (ECHA 2017b). Constituents, impurities, and additives should normally be considered relevant for the PBT/vPvB and PMT/vPvM assessment when they are present in concentration of ≥ 0.1% (w/w). This limit of 0.1% (w/w) is set based on a well-established practice recognised in European Union legislation to use this limit as a generic limit. Individual concentrations below 0.1% (w/w) normally do not need to be considered.

Importantly, a close structural similarity of individual constituents within a fraction of a UVCB substance, namely constituents with the same carbon number, chain lengths, degree and/or site of branching or stereoisomers, triggers the need to sum up the concentrations of these constituents and to compare the total concentration with the limit of 0.1% (w/w) in order to determine whether these constituents need to be covered in the PBT/vPvB assessment. This approach is also relevant for PMT/vPvM assessment, with more detailed elaboration on the criteria for grouping or read across, in other Sections of this and the REACH Guidance.

In order to comply with the CLP Annex I, 4.3.2.3 and 4.4.2.3 provisions on the PBT/vPvB and PMT/vPvM properties of the relevant constituents, a as comprehensive as possible characterisation and identification of UVCBs or fractions of impurities needs to take place. However, this may not always be possible or even necessary due to (i) the number of constituents/impurities may be relatively large and/or (ii) the composition may, to a significant part, be unknown, and/or (iii) the variability of composition may be relatively large or poorly predictable. Regardless of whether full substance identification is possible or not for the whole composition, efforts should be made for carrying out a PBT/PMT assessment for all constituents, impurities and additives present in concentrations above 0.1% (w/w). ECHA Guidance on IR&CSA, Chapter R.11: PBT/vPvB assessment includes further information on assessment of substance with complex composition.

The PBT/vPvB and PMT/vPvM assessment should be performed on each relevant constituent, impurity, and additives present in concentrations above 0.1% (w/w). It is not possible to draw an overall conclusion if, for example, the assessment of persistence has

https://echa.europa.eu/documents/10162/17228/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

[8] https://echa.europa.eu/documents/10162/17250/pg_report_qsars_en.pdf/407dff11-aa4a-4eef-a1ce-9300f8460099

been concluded for one constituent and the assessment of bioaccumulation or toxicity for another constituent.

As detailed in the ECHA Guidance on IR&CSA, Chapter R.11.4.2.2, there are three assessment approaches of substances containing multiple constituents, impurities and/or additives, namely the known constituent approach, the fraction profiling and the whole substance approach.

The **known constituent** approach can be applied when a substance is "a priori" known to contain specific constituents at relevant concentrations, these constituents are suspected based on available information to represent the worst case of these properties of all constituents of the substance, and these specific constituents can be isolated or separately manufactured. Depending on the quality and availability of information for all relevant constituents and properties, a conclusion as PBT/vPvB and/or PMT/vPvM for the whole substance may be drawn in case one or more constituent of the substance is proven to fulfil all the regulatory criteria. This approach has been applied in the SVHC identification of substances originating from coal tar distillation (e.g., coal tar pitch, high temperature; anthracene oil) and also under Substance Evaluation. Advantages and disadvantages of this and the other two approaches are reported in ECHA Guidance on IR&CSA, R.11.4.2.2.2.

The **fraction profiling** approach is applied when, due to the complexity of the substance, it is not feasible to fully identify, assess or isolate single constituents but the substance can be divided into fractions/blocks. Within these blocks, the constituents must be structurally similar and their degradation, bioaccumulation and toxicity properties can be predicted to follow a regular predictable pattern.

The **whole substance** approach considers the substance to be one, assuming that all its constituents can be justified to be very similar and, therefore, can be expected to have reasonably similar PBT/PMT properties. Same principles in establishing similarity of constituents apply for mono-constituent, multi-constituent and UVCB substances. For such similarity criteria, please refer to Chapter R.6 of the ECHA Guidance on IR&CSA, Read-Across Assessment Framework (RAAF) and advice on using read-across for UVCB substances. In a regulatory context, information from the first two approaches is preferable to the last, as these provide more certain, transparent and detailed information. Guidance R.11 further details certain circumstances that the whole substance approach can be used for certain endpoint-specific assessments.

(v) relevant transformation/degradation products

CLP Annex I, 4.3.2.3 and 4.4.2.3 refer to the identification that "*shall also take account of the PBT/vPvB and PMT/vPvM properties of …. relevant transformation or degradation products*". The PBT/PMT assessment should be performed on the substance and each of the relevant transformation/degradation products. There is currently no set w/w or molar threshold concentration for relevant transformation or degradation product in the CLP Regulation.

A transformation or degradation product can be considered relevant in the degradation tests for soil, water-sediment and water when it is detected at least ≥10% of the applied concentration of the parent substance at any sampling time (principal transformation/degradation products) or when detected ≥ 5% in at least two sequential measurements or the concentration is continuously increasing, or it seems to be stable

during a degradation study (See also Section 4.3.3.1.2.1, simulation tests in water, water-sediment and soil). Lower percentages that these may be adopted in a case-by-case basis, with the assessment accounting for the overall hazardous profile of the substance and its relevant transformation/ degradation products, including the "*the rate of generation of the more hazardous degradation product (i.e., quantity produced and time frame) should be considered*" (Section 4.1.3.3.1 of the current Guidance).

The PBT/vPvB and PMT/vPvM assessment should be carried out for each relevant transformation or degradation product. In all cases, any information that the substance may be mineralised quickly (not likely to form transformation/degradation products relevant for the assessment) or the opposite (based, for example, on results from hydrolysis studies or field data) must be carefully considered.

To provide some context of the set boundaries for the relevance of the transformation or degradation products, OECD test guideline (TG) requirements and data requirements in Regulation (EU) No. 283/2013 are shortly described below.

In simulation degradation tests, the concentration of the test substance and transformation products should be measured and reported at every sampling time. In general, transformation products detected at ≥ 10% of the applied concentration at any sampling time should be identified unless reasonably justified otherwise (OECD TGs 307, 308 and 309). OECD TGs 309 and 308 further specify that transformation products for which concentrations are continuously increasing during the study should also be considered for identification, even if their concentrations do not exceed the limit given above, as this may indicate persistence.

Regulation (EU) No. 283/2013, Section 7 specifies that data on route of degradation in soil shall be sufficient to identify:

- the individual components which in at least two sequential measurements, account for more than 5 % of the amount of active substance added;
- components present which at any time account for more than 10 % of the amount of active substance added;
- and the individual components (> 5 %) for which at the end of the study the maximum of formation is not yet reached.

Regulation (EU) No. 283/2013, Section 7 further specifies that aerobic degradation (DegT50 and 90 values) from a minimum of three different soils shall be provided for metabolites, breakdown and reaction products which occur in soil if one of the following conditions is fulfilled:

- they account for more than 10 % of the amount of active substance added at any time during the studies;
- they account for more than 5 % of the amount of active substance added in at least two sequential measurements;
- the maximum of formation is not reached at the end of the study but accounts for at least 5 % of the active substance at the final measurement;
- all metabolites found in lysimeter studies at annual average concentrations exceed 0.1 µg/L in the leachate.

(vi) Substances with nanoforms

Annex VI of REACH, on the basis of the Commission Recommendation of 18 October 2011, defines a nanform as "*a form of a natural or manufactured substance containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm, including also by derogation fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm*". When a substance fulfils the criteria of the nanoform definition, specific considerations apply, with REACH Annex I currently noting that the PBT and vPvB assessment under REACH shall address also all revant nanoforms.

ECHA Guidance on IR&CSA, Chapter R.11.4.2.1.4 reports on some key considerations regarding the PBT/vPvB assessment of substances with nanoforms. Appendices to ECHA Guidance on IR&CSA, Chapters R.7a, R.7b, and R.7c contain recommendations for assessment of nanomaterials in the context of the chemical safety assessment, under REACH. Future updates of the current CLP Guidance will include more information on the PBT/vPvB and PMT/vPvM assessment under CLP, once further experience on the regulatory handling of substances with nanoforms is gained.

(vii) assessment of "difficult" substances requiring special considerations

Some substance properties may lead to difficulties to both testing and the interpretation of study results. Thus, assessment of substances requiring special considerations refer to those that possess, for example, very high sorption potential, low solubility in octanol and water, high volatility, high instability in biotic and abiotic media, complex or multi-constituent substances including those in nanoforms, surface-active, ionisable and coloured substances. For some of these type of substances, standard test guidelines used to determine the different properties may not be directly applicable. Specific considerations for these substances are reported in ECHA Guidance on IR&CSA, Chapters R.11.4.2, but also in various Sections in R.7b and R.7c), in Section 4.1.3.2.2 of this Guidance, as well as in the "Guidance Document on the aqueous-phase aquatic toxicity testing of difficult test chemicals" (no. 23) developed by OECD.

Several considerations relating to such substances will be incorporated in subsequent Sections of this Guidance, for example, in 4.3.3.1-4.3.3.4, whilst specific considerations on ionisables are reported in detail in, among others, both the following bulletpoint and in Section 4.3.3.3.7 of this Guidance.

(viii) specific considerations for ionisable substances

Ionisable substances are molecules able to dissociate, forming ionic compounds. In general, ionised organic substances do not readily diffuse across respiratory surfaces, although other processes may play a role in uptake (e.g. complex permeation, carrier-mediated processes, ion channels, or ATPases). Dissociated and neutral chemical species can, therefore, have markedly different bioavailabilities. It is essential to know or estimate the dissociation constant pK_a to evaluate the degree of ionisation in surface waters at environmentally relevant pH (pH 4-9, ECHA Guidance on IR&CSA, Chapter R.7a) and under physiological conditions (pH 3-9) (R.7c).

The balance between dissociated and non-dissociated forms of some substances varies with the pH of the solution in which a substance is dissolved. Since dissociated and non-dissociated forms can have different solubility in water, small changes in the pH can significantly alter the bioavailability of a substance in a toxicity test. Design of toxicity tests should consider the effects on dissociation equilibrium due to changes in the pH of test solution. Information on the toxicity of the two forms of a substance from preliminary tests can help in deciding the pH of the solution in the definitive test, that should be conducted in condition where the test organisms are exposed to the most toxic form, providing that this condition allows a healthy maintenance of the test organisms. Thereby, test solutions might have to be buffered in order for the test to be "*conducted at a pH consistent with the more toxic form of the substance, whilst remaining within the range required to maintain the health of the control organisms*" (EFSA, 2013). Specific indications on how to conduct toxicity tests with ionisable substances are reported in the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (no.23).

Different Sections of this Guidance, especially the one relevant to Mobility (4.3.3.3.7), will elaborate further, more property-related considerations for ionisable substances.

4.3.3.1. Persistence assessment

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.3.2.3.1. and 4.4.2.3.1. The following information shall be considered for the 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.

Annex I: 4.3.2.4.2. and 4.4.2.4.2. In applying the WoE determination, the following information, in addition to the information referred to in Sections … 4.3.2.3.1 and 4.4.2.3.1… shall be considered as part of the scientific assessment of the information relevant for the … P, vP … properties:

(a) Indication of P or vP properties:

(i) Results from tests on ready biodegradation;
(ii) Results from other degradation screening tests (e.g. enhanced ready test, tests on inherent biodegradability);
(iii) Results obtained from well-developed and reliable biodegradation (Q)SAR models;
(iv) Other information, provided that its suitability and reliability can be reasonably demonstrated.

4.3.3.1.1. Persistence terminology

Abiotic degradation is transformation or degradation of a substance modified by non-biological mechanisms (i.e. physico-chemical processes) such as hydrolysis, oxidation and photolysis

Biodegradation is biologically mediated transformation or degradation of a substance, usually carried out by microorganisms. It can proceed in the presence of oxygen (aerobic biodegradation) or in the absence of oxygen (anaerobic biodegradation).

Degradation is a loss process by which a substance is physically transformed from one chemical species to another.

A **degradation half-life (DegT50)** is the time taken for 50% transformation of a test substance when the transformation can be described by first-order kinetics and it is independent of the concentration. The half-life and the degradation rate constant are related by the equation $t_{1/2} = \ln 2/k$ ($t_{1/2}$=half-life and k=first order or pseudo first order kinetic rate constant (d^{-1}).

Degradation products are all substances resulting from biotic and abiotic transformation

reactions of a substance.

Degradation rate constant is typically a first order or pseudo first order kinetic rate constant, k (d^{-1}), which indicates the rate of the degradation processes.

Dissipation is a result of one or more loss processes leading to the disappearance of a substance from an environmental matrix, test system or one compartment of a test system by biotic and/or abiotic processes, such as degradation processes (microbial degradation, hydrolysis and/or photolysis) and transfer processes between different compartments (such as volatilisation, and adsorption, leaching and plant/organism uptake).

DT50 is generic term to describe the time required for disappearance of 50% of the residue.

Hydrolysis is decomposition or degradation of a substance by reaction with water.

Inherently biodegradable substance is a substance that meets the agreed pass level in inherent biodegradability test (e.g. a level of 70% mineralisation (DOC removal) within 7 days, the lag phase no longer than 3 days, and the removal before degradation below 15%, no pre-adaptation in OECD TG 302B). **Inherent biodegradation** describes the potential for biodegradation under optimised aerobic conditions designed to promote biodegradation.

Mineralisation is the complete degradation of an organic compound to CO_2, H_2O under aerobic conditions, and CH_4, CO_2 and H_2O under anaerobic conditions.

Photolysis is chemical decomposition or degradation induced by light or other radiant energy.

Primary degradation is the initial structural change (transformation) of a substance resulting in the loss of the original chemical identity and property, and formation of a transformation, degradation product or metabolite.

Ultimate degradation is degradation of the substance leading to formation of inorganic end products, such as CO_2, H_2O, CH_4 or NH_3, and biomass.

Readily biodegradable substance is a substance that reaches the required pass level of 60% CO_2 evolution or O_2 demand, or 70 % dissolved organic carbon (DOC) removal within 28 days in standard ready biodegradability tests.

The 10-day window is the 10 days immediately following the attainment of 10% biodegradation in ready biodegradability tests. The 10-day window begins when the degree of biodegradation has reached 10% (DOC removal, ThOD or $ThCO_2$) and must be reached within the 28-d period of the test.

4.3.3.1.2. Data on persistence

Data on degradation of a substance may be available from standardised tests, or from other types of information, such as field and monitoring studies, screening studies or QSAR models. The interpretation of such degradation data for classification purposes often requires detailed evaluation of the (test) data.

There are three types of tests that measure biological degradation that are the most relevant for the persistence assessment:

1. Tests on simulation degradation and transformation (OECD TG 309 surface water, OECD TG 308 sediment, OECD TG 307 soil or field studies)
2. Tests on inherent biodegradation (OECD TG 302 series)
3. Tests on ready biodegradation (e.g. OECD TG 301 series, OECD TG 306, OECD TG 310 and enhanced ready test)

Simulation tests provide information on degradation kinetics, degradation half-lives, mineralisation, non-extractable residues (NERs) and transformation/degradation products. Simulation tests are the most relevant information for deriving a definitive DegT50 value, whilst tests on ready and inherent biodegradability contribute supporting information at a screening level.

Abiotic degradation tests provide also relevant information to be included in the assessment. Tests, for example, for hydrolysis and photolysis are presented in more detail in Section 4.3.3.1.2.5 of this Guidance.

The ECHA Guidance on IR&CSA, Chapters R.7b and R.11 further detail the availability, applicability, adequacy (reliability and relevance), reporting and scientific and regulatory considerations for the use of different test methods on degradation. Difficult to test substances may require additional measures in reporting and assessment of the data. For example, volatility of a substance potentially leading to dissipation of the substance plays an important role in the persistence assessment and may bring challenges in the assessment. Therefore, in interpretation of the degradability test results it is crucial to differentiate between disappearance of the substance from the test system due to degradation and other dissipation processes. It is also important to acknowledge that not all tests are applicable to volatile substaces and some modifications of the test system may be varranted. For example, OECD TG 301 describes six different methods to measure ready biodegradability but only three of the methods are applicable for volatile substances. Simulation biodegradation tests, such as OECD TGs 307, 308 and 309, have been developed for non-volatile or slightly volatile substances, but they may be adapted to volatile substances using precautions (see ECHA Guidance on IR&CSA, Chapter R.11.4.2.1.3 and ECHA (2022b) for further information).

The following Sections will also briefly summarise the key studies and considerations on their conduct and regulatory use.

The scope of P/vP assessment covers all following environmental compartments:

- fresh, estuarine and marine water
- fresh, estuarine and marine sediment and
- soil.

Once reliable and relevant information is available resulting in a half-life value in any of these environmental compartments, above the regulatory threshold(s) set for P and/or vP, the substance can be concluded as fulfilling the CLP criterion for P and/or vP, respectively. Section 4.3.3.5 of this Guidance will present the assessment of the weight or evidence determination to reach a conclusion if substance meets the CLP criteria for P/vP.

Degradation half-life (DegT50) derivation

Degradation half-life (DegT50) can be directly compared with the numerical P/vP criteria. DegT50 values are most commonly based on data derived from simulation biodegradation tests. It is important to note that a dissipation half-life (DT50) is referring to the overall process leading to the disappearance of the test substance from the test system (or one compartment of the system). If transfer processes have occurred simultaneously with degradation, the derived DT50 value is not representative of the DegT50 value.

The kinetic model that best fits and/or most appropriately describes the experimental data should be used for estimating the degradation half-life[9]. A qualitative assessment should describe whether the degradation pattern observed from the experimental data is representative of the degradation of the substance under the test conditions and not the result of experimental artefacts. The selection of a degradation kinetic model should be based on the assessment of the metrics for determining the "goodness of fit" which include visual assessment of goodness of fit, $\chi 2$ error and t-test statistical metric. Detailed description for the criteria for the acceptability of the fit is included in FOCUS guidance (2014).

When the kinetic of decline is first-order and no lag phase occurs, the degradation half-life predicted by SFO (Single First-Order Rate) kinetic model can be used for direct comparison with the P/vP criteria. When the kinetics of decline are bi-phasic, the best-fit model (e.g. DFOP, HS)[10] should be selected and used for predicting a degradation half-life $DegT_{50}$. When DFOP (Double First-Order) or the HS (Hockey-Stick) kinetic model (both models allow deriving slow phase DegT50) is selected as the best fitting model, the degradation half-life (DegT50) predicted from the slow phase should be preferred for comparison with the P/vP criteria. The First Order Multi-Compartment (FOMC) model, also mentioned in the FOCUS guidance, is a bi-phasic mechanistic model based on the soil heterogeneous nature (FOCUS, 2014). Considering the uncertainties around the DegT50 values derived using the FOMC model, this model is the less preferred one to be used for comparison to the P/vP criteria. In any case, a justification for the selection of the model should be provided with adequate and reliable documentation such as the key parameters of the kinetic analysis and assessment of the goodness of fit.

When there is no significant measurable degradation observed during the test and the kinetic model indicates that the relevant rate constant is not significantly different from zero it is still possible to reach a conclusion on persistence after carefull interpretation of the calculated degradation half-lives.

Lag phase of degradation could be occasionally observed in simulation studies. When a lag phase occurs in simulation tests the estimated length of the lag phase should be reported, together with the explanation how it is determined (e.g. based on detection limit of the method or another definition, or whether the value is derived from data analysis software). OECD TG 309 includes a lag phase definition and specific advice on the lag phase lemght estimation. In addition, efforts should be made to distinguish whether the observed lag phase can be attributed to any experimental artefacts. Justification for the treatment of

[9] In the context of the Plant Protection Products legislation (EC 1107/2009) and specifically within the FOCUS guidance (2014) a distinction is made between trigger and modelling endpoints, for the purpose of the P/vP assessment under CLP this distinction does not apply and the kinetic model that most appropriately describes the observed data should be used.

[10] The DFOP model (Double-First-Order in Parallel model, SFO in parallel- the sum of two first order equations), and HS model (Hockey-Stick model, SFO in series-two sequential first order curves).

644 the lag phase length in the DegT50 derivation should be provided. When the lag phase is
645 attributed to experimental artefact the validity of the study needs to be assessed carefully
646 as this might indicate issues related to the test design and performance.

647 Any deviations from the recommended mass balance/recovery, as they are described in
648 the corresponding testing guidelines (OECD TG 309, OECD TG 308 and OECD TG 307)
649 should be reported and justified. Further guidance on handling mass balance/recovery
650 data is provided in ECHA Guidance on IR&CSA, Chapters R.11, Section R.11.4.1.1.3 and
651 Table R.11-6.

652 A good knowledge of the degradation pathway up to the transformation/degradation
653 product is essential for deriving a reliable degradation half-life for a
654 transformation/degradation product. When a study is performed on a parent substance
655 and transformation/degradation products are formed, the pathway model approach as
656 described in the Generic Guidance Document for Estimating Persistence and Degradation
657 Kinetics from Environmental Fate Studies on Pesticides in EU Registration (FOCUS, 2014)
658 should be used. In the pathway approach, the parent and transformation/degradation data
659 is assessed together. Evaluation of the transformation/degradation products data
660 individually by using only the decline phase (Decline model) is another available option
661 and it should be used only if the pathway fit does not appropriately describe the data.

662 Further information on the degradation kinetic models, the data handling, assessment of
663 the goodness of fit and general recommendations on the kinetic analysis can be found in
664 ECHA Guidance on IR&CSA, Chapters R.11, Section R.11.4.1.1.3. and the Generic
665 Guidance Document for Estimating Persistence and Degradation Kinetics from
666 Environmental Fate Studies on Pesticides in EU Registration (FOCUS, 2014).

667
668 **4.3.3.1.2.1. Simulation tests in water, water-sediment and soil**

669 Simulation degradation tests attempt to simulate degradation in a specific environment by
670 use of indigenous biomass, media, and relevant solids (e.g. soil and sediment) in relevant
671 test conditions. As detailed in the Section 4.3.3.5 of the Guidance, degradation simulation
672 studies performed in relevant environmental media specified in Annex I (4.3.2.1.1. and
673 4.4.2.1.1.) of CLP and at relevant conditions are the tests considered as the ones with the
674 highest regulatory relevance. These tests provide a definitive degradation half-life that can
675 be compared to the numerical persistence criteria as defined in CLP. Such tests allow both
676 biotic and abiotic degradation processes to operate.

677 The following tests can be used to simulate the biodegradation of organic substances under
678 relevant conditions in soil, sediment or surface water: Aerobic and Anaerobic
679 Transformation in Soil (OECD TG 307); Aerobic and Anaerobic Transformation in Aquatic
680 Sediment Systems (OECD TG 308); and Aerobic Mineralisation in Surface Water –
681 Simulation Biodegradation Test (OECD TG 309).

682 The simulation degradation studies include two types of investigations: a) a degradation
683 pathway study where degradation products (i.e. degradation transformation/degradation
684 products) are identified and quantified, b) a kinetic study where the degradation rate
685 constants (and degradation half-lives) of the parent substance and, if applicable, of the
686 transformation/degradation products, are experimentally determined. In the simulation
687 test, the test concentration is low to anticipate that the biodegradation kinetics (first order

or pseudo-first order) obtained in the test reflect those expected in the environment. Higher concentrations of the test substance (e.g., >100 µg/L) are relevant preferably to overcome potential analytical limitations when identifying and quantifying the transformation/degradation products. The endpoints that need to be addressed and reported are primary or ultimate degradation rate and degradation half-lives (DegT50) or dissipation half-lives (DT50) for the compartments included in the test system, as well as the route of degradation, transformation/degradation products and non-extractable residues. In addition, a mass balance and quantity of possible losses from the test system during the test period need also to be reported. An incomplete mass balance will introduce severe uncertainty to the interpretation of data. This, in turn, can ultimately impede the substance assessment with sufficient certainty and to give a low weight to the test and its results in the P/vP assessment as part of a WoE approach.

The use of both radiolabelled and non-labelled test substances is accetable. For assessing total mineralisation, a ^{14}C-labelled test substance is typically used and $^{14}CO_2$ evolution is measured. If a ^{14}C-labelled substance is used, the most relevant location of the label is in the most recalcitrant part of the molecule. This must be considered in the assessment. If the used analytical method is sensitive enough to detect low concentrations applied in simulation tests, such data can be used to report on the total residual concentration of the test substance. Disappearance of the parent substance however does not necessarily imply its degradation. Other dissipation processes, for example volatilisation or adsorption, may also cause disappearance of the parent substance and they should be taken into account when assessing results on the primary degradation rate. Data on chemical analyses can be used in parallel with radiolabelling techniques. Specific chemical analyses are also needed to identify and quantify transformation/degradation products.

When a substance is not fully degraded or mineralised, the persistence of relevant transformation/degradation products must be considered in the assessment. Identity, stability, behaviour, molar quantity relative to the parent substance of the transformation/degradation products are important parameters to be included in the assessment. There is no set regulatory w/w threshold concentration for transformation/degradation products in persistence assessment under CLP. However, a transformation/degradation product has been previously considered relevant in the simulation degradation test for soil, water-sediment and surface water at least when detected at ≥10% of the applied concentration of the parent substance at any sampling time (principal transformation/degradation products) or when detected ≥ 5% in at least two sequential measurements or the concentration is continuously increasing, or it seems to be stable during a degradation study (see also section 4.3.3 (v) of this Guidance).

ECHA Guidance on IR&CSA, Chapter R.7b, Section R.7.9.4.1 "Data on degradation/biodegradation" provides guidance on the key results to be reported on each of these tests.

The radiolabelled mass balance should range from 90% to 110%, whereas the analytical accuracy should lead to an initial recovery of between 70% and 110% for non-labelled test substances. The simulation test results should be considered as not valid or at least treated with caution if the mass balance is not fulling these criteria. ECHA Guidance on IR&CSA, Chapter R.11 describes DegT50 calculation methods for studies with incomplete mass balance.

Degradation half-lives (DegT50) obtained in the simulations test conducted in the relevant conditions and in accordance with the respective test guidelines may be directly compared with the numerical P/vP criteria. In the context of simulation degradation tests, by "relevant conditions", relevant testing conditions are generally meant (see also section 4.3.3 (ii) of this Guidance. In terms of simulation test conditions among others, the following factors should be considered: temperature, test concentration, test design, physico-chemical properties of the substance etc.

The simulation test is considered relevant to derive degradation half-life when

- no pre-exposure (pre-adaptation) of the water, soil or sediment microorganism has taken place; and
- low concentration (µg/L) reflecting those expected in the environment is used: and
- study is considered to be performed under relevant conditions
- study is performed in accordance with the testing conditions provided for in the test methods Regulation, in line with Article 13(3) of the REACH Regulation.

Non-extractable residues (NERs) may be formed during the degradation simulation tests. Total NER are defined as the residues remaining in the matrix after defined exhaustive extractions. The Total NERs are considered as non-degraded parent substance in DegT50 derivation unless further characterisation of the Total NER is performed. Total NER consists of potentilly remobilisable (Type 1) and irreversibly bound (Type 2 and 3) NER. The potentially remobilisable fraction of the Total NER (NER Type 1) poses a potential risk for the environment. If the quantity of the remobilisable fraction (Type 1) is available, the total extractable fraction together with the Type I NER are considered for the DegT50 estimation. If such DegT50 is above the P/vP criterion, the half-life can be further refined by taking into account only the quantity of the parent substance concentration in the Type I NER together with extractable fraction of the parent substance. Appendix R.11-4 "Approach on non-extractable residues (NER) quantification and characterisation in persistence assessment" of ECHA Guidance on IR&CSA, Chapter R.11 provides stepwise assessment approach on how to take the different types of NERs into account.

Temperature has an influence on the degradation rate. In Europe, due to wide range of environmental temperatures this must be taken into account in the estimations of the degradation rate in different environmental compartments. According to the three OECD test guidelines (TGs 307, 308 and 309), the studies can be performed at a range of temperatures, typically between 10 and 25 °C. The average temperature in Europe is 12°C (9°C for marine environment). Degradation rates in a test conducted in the laboratory at 20-25°C are in general higher than those measured in the field in Europe.

Therefore, temperature correction to 12°C (9°C for marine environment) should be applied to the DegT50 obtained in a water, sediment or soil simulation test conducted at any other temperature (in line, for example, with the Judgement of the General Court in rulings T-177/19[11] and T-176/19[12]).

In the absence of structural substance class-specific equations/models reflecting the temperature dependence of biodegradation, the Arrhenius equation (or a similar

[11] Link to T-177/19

[12] Link to T-176/19

appropriate equation designed to normalise physico-chemical degradation rates) can be used for normalisation. This is:

$\ln k = lnA - (Ea/RT)$

Where

k = rate constant (day^{-1})
A = factor equal to the rate coefficient at infinite temperature (day^{-1})
Ea = activation energy ($kJ\ mol^{-1}$)
R = gas constant ($8.314.10^{-3}\ kJ.K^{-1}.mol^{-1}$)
T = temperature (K)

For first-order kinetics, the equation can be reformulated to:

$$DegT50env = DegT50test.e^{\left(\frac{Ea}{R}\left[\frac{1}{Tenv}-\frac{1}{Ttest}\right]\right)}$$

where

DegT50env = half-lives at environmental temperature *Tenv* (typically 285K) and

DegT50test = half-lives at test temperature *Ttest* (typically 293K).

There are potential uncertainties resulting from the use of the Arrhenius equation because:

1) It was designed for simple chemical reactions rather than biological processes

2) The specific activation energy (E_a) for a substance or a chemical group is rarely known

A generic E_a of 65.4 kJ/mol[13] has been derived by EFSA (2007). It corresponds to the median value of available pesticide E_a data. In the absence of valid substance specific data, the Arrhenius equation with the generic Ea-value should be used if temperature correction is needed.

Other relevant test conditions depend on the type of study conducted. Test dependent considerations on the relevant test conditions are further described below.

Surface water simulation test (OECD TG 309)

The purpose of the OECD TG 309 is to measure the time course of biodegradation of a test substance at low concentration in aerobic natural water and to quantify the observations in the form of kinetic rate expressions. This simulation test is a laboratory shake flask batch test to determine rates of aerobic biodegradation of organic substances in samples of natural surface water (fresh, brackish (estuarine) or marine). To ensure the presence of an active microbial population, a substance, which is normally easily degraded under aerobic conditions (e.g. aniline or sodium benzoate) should be used as reference substance.

The test is performed in batch by incubating the test substance with either surface water only ("pelagic test") or surface water amended with suspended solids/sediment of 0.01 to

[13] Fixed activation energy of 54 kJ/mol should be used for all hydrolysis reactions.

1 g/L dry weight ("suspended sediment test") to simulate a water body with suspended solids or re-suspended sediment.

Results of OECD TG 309 may be used for classification purposes, when test is

- performed at concentrations between 1 and 100 µg/L and preferably in the range of <1-10 µg/L (to ensure that biodegradation follows first order kinetics);
- inoculum is collected from natural surface water preferably containing suspended matter (SPM)/ L between 10 and 20 mg_{dw} in freshwater and c.a. 5 mg_{dw} SPM/L in marine water;
- conducted in relevant temperature in accordance with the test guideline (temperature correction applied in accordance with text above);
- determination of the degradation half-life in at least one surface water sample and at two different concentrations of the test substance.

If any other conditions are used, the relevance of the information must be justified as part of the WoE assessment.

However, for low solubility substances, even if their water solubility is within the range reported above, it is acknowledged that the feasibility of the test depends, *inter alia*, on the possibility to develop with reasonable efforts appropriate analytical methods with suitable sensitivity to detect relevant changes in concentration (including transformation/degradation products).

For the purpose of CLP, the 'suspended sediment test' is generally not preferred over pelagic test conditions as the subsequent addition of suspended matter may significantly enhance biodegradation of some substances (Ingerslev and Nyholm, 2000). This simulation test is applicable to non-volatile or slightly volatile organic substances tested at low concentrations. The relevance of the test conducted with volatile substances depends on the means taken to minimise volatilisation and maintenance of the test substance in the water phase accessible for microorganisms to the extent that a reliable degradation half-life can be determined. The volatilised fraction should be adequately trapped and quantified in order to be able to interpret the results reliably. Further information on how to address volatilisation in simulation testing and data handling can be found in ECHA Guidance on IR&CSA, Chapters R.11, Section R.11.4.2.1.3 and Appendix R.11-7,R.7, Section R.7.9.4 and ECHA (2022b).

Aerobic and Anaerobic Transformation in Aquatic Sediment Systems (OECD TG 308)

OECD TG 308 describes a laboratory test method to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems. The surface layer of aquatic sediments can be either aerobic or anaerobic, whereas the deeper sediment is usually anaerobic. These conditions in sediment may be simulated by using aerobic or anaerobic tests described in the test guidelines (OECD TG308). The aerobic test simulates an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient. The anaerobic test simulates a completely anaerobic water-sediment system.

The sediment degradation test according to OECD TG 308 includes the determination of the degradation half-lives in two different types of sediment. OECD TG 308 allows;

i. the measurement of the transformation rate of the test substance (and relevant transformation products) in a water-sediment system;

ii. the measurement of the transformation rate of the test substance (and relevant transformation products) in the water and in sediment;
iii. the measurement of the mineralisation rate of the test substance and/or its transformation products (when a ^{14}C-labelled test substance is used);
iv. the identification and quantification of transformation products in water and sediment phases including mass balance (when la abelled test substance is used); and
v. the measurement of the distribution of the test substance and its transformation products between the two phases during a period of incubation in the dark (to avoid, for example, algal blooms) at constant temperature.

The method is generally applicable to chemical substances (unlabelled or labelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is applicable to slightly volatile, non-volatile, water-soluble or poorly water-soluble compounds. The test should not be applied to chemicals which are highly volatile from water (e.g. fumigants, organic solvents) and, thus, cannot be kept in water and/or sediment under the experimental conditions of this test. Further guidance on the assessment of volatile substances is provided in ECHA Guidance on IR&CSA, Chapters R.11, Section R.11.4.2.1.3 and Appendix R.11-7, R.7, Section R.7.9.4 and ECHA (2022b).

The OECD TG 308 outcome can be affected both by test vessel and system geometry and the associated water-sediment interface size. Headspace volume and height of the water and sediment columns can influence the partitioning and consequently degradation of the test substance (Hennecke *et al*., 2014; Shrestha *et al.*, 2016), especially for volatile substances. The system geometry should be consistent with the range indicated in the OECD TG 308 (i.e. water:sediment volume ratio between 3:1 and 4:1, height of 2.5 cm (±0.5) layer and minimum weight of 50g of the sediment). Sediment spiking instead of addition of the test substance via water may, in some cases, be acceptable to ensure realistic exposure of sediment in the test. This may be the case for example for substances which would transfer significantly quicker to the atmospheric compartment via volatilisation compared to transfer to the sediment compartment.

According to the OECD TG 308, the aerobic test simulates an aerobic water column over an aerobic sediment layer with an anaerobic gradient. Aeration of the test system is needed in order to maintain aerobic conditions in the water column and surface layer of the sediment throughout the study. OECD TG 308 recommends aeration by gentle bubbling or by passing air over and gently stirring the water surface in open test vessels (for non-volatile substances), and by gentle stirring of the water surface in biometer type systems (for slightly volatile substances). When results of a closed systems test with a volatile substance is interpreted, the assessment should consider if the oxygen was distributed from the headspace to the water layer to maintain aerobic test conditions. However, any aeration method should disturb as little as possible the sediment layer and its stratification. For example, visual resuspension/cloudiness of the overlying water is one indication of disturbed sediment. Aeration methods recommended in the OECD TG 308 are acceptable. If any other method is used, its influence in stratification should be taken into account. In the OECD TG 308 shaking method is not appropriate as it may modify the stratification of the sediment, affecting the maintenance of the anaerobic layer, and therefore, may have an influence on the degradation process in the sediment simulation test.

The degradation half-lives calculated for the sediment phase and the water phase separately are less reliable than the degradation half-life calculated for the total water-sediment system. Because of the low volume and depth of water relative to the volume of sediment and the surface of the water-sediment interface used in OECD TG 308, even moderately adsorptive substances will tend to rapidly partition from the water phase to the sediment phase. Therefore, for adsorptive substances (e.g. log K_{OC}>4), the degradation half-life in the sediment can reasonably be estimated from the degradation half-life for the total water-sediment system. However, the parent substance may degrade to more soluble and less adsorptive degradation products that can be released from the sediment to the water phase. This should be taken into account in the assessment.

Generally it would be expected that an anaerobic half-life would be greater than an aerobic half-life where the main route of degradation is aerobic, i.e. if there is no oxygen, degradation will be hindered. It is not recommended to judge whether a substance has an degradation half-life exceeding the P and/or vP thresholds using only anaerobic simulation data. Nevertheless, if anaerobic water sediment data are available, they may be used as supporting information.

Aerobic and Anaerobic Transformation in Soil (OECD TG 307)

OECD TG 307 describes a method designed for evaluating aerobic and anaerobic transformation of chemicals in soil. The experiments are performed to determine (i) the rate of transformation of the test substance, and (ii) the nature and rates of formation and decline of transformation/degradation products to which plants and soil organisms may be exposed.

The soil simulation degradation test according to OECD TG 307 includes the determination of the degradation half-lives in 4 different types of soils. Aerobic and anaerobic studies with one soil type are generally sufficient for the evaluation of transformation pathways. Aerated soils are aerobic, whereas water-saturated or water-logged soils are frequently dominated by anaerobic conditions. These conditions in soil may be simulated by using aerobic or anaerobic tests described in the test guidelines (OECD TG 307). However, in the EU, solely anaerobic test conditions are not considered to be particurarily relevant scenarios for the P assessment. Nevertheless, if anaerobic soil data is available, it may be used as part of the WoE approach.

The method is applicable to all chemical substances (non-labelled or radiolabelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is applicable to slightly volatile, non-volatile, water-soluble or water-insoluble compounds. The test should not be applied to chemicals which are highly volatile from soil (e.g. fumigants, organic solvents) and thus cannot be kept in soil under the experimental conditions of this test. Further information on how to address volatilisation in simulation testing can be found in ECHA Guidance on IR&CSA, Chapters R.11, Section R.11.4.2.1.3 and R.7, Section R.7.9.4).

Degradation rate of ionisable substances can depend on the the soil pH and should thus be considered in the assessment regarding relevance of test conditions. For example, for weakly acidic substances, a faster degradation has been observed at higher pH and a slower degradation at low pH.

Other simulation tests

The data derived from simulation degradation studies other than those described above should not be used on their own to demonstrate that the substances is or is not P/vP in relevant conditions covering water, sediement and soil. These studies described below provide information on degradation during waste water treatment process and mixing zone after the release of the effluent and are more relevant for risk assessment than hazard identification, but can be considered as supporting information in the WoE.

Other simulation test standards include:

- OECD TG 303: Simulation Test - Aerobic Sewage Treatment,
  - A: Activated Sludge Unit
  - B: Biofilms

- OECD TG 314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater
  - A: Biodegradation in a Sewer System Test
  - B: Biodegradation in Activated Sludge Test
  - C: Biodegradation in Anaerobic Digester Sludge Test
  - D: Biodegradation in Treated Effluent-Surface water Mixing Zone Test
  - E: Biodegradation in Untreated Wastewater-Surface water Mixing Zone Test

The OECD TG 314 (A-E) suite aims to allow checking of the fate of a substance on its way through the sewer system and sewage treatment plant to the mixing zone in surface water. These studies are neither a screening study nor equivalent to a simulation study on degradation in the environment. They do not employ relevant environmental conditions for assessing the persistence of the substance in the compartments relevant for the PBT/vPvB or PMT/vPvM assessment, namely natural surface water, sediment or soil. Furthermore, they provide information neither on ready biodegradability nor on degradation rates in individual environmental compartments (i.e. natural surface water, sediment or soil).

4.3.3.1.2.2. Field and mesocosm studies

Field studies, mesocosm, or lysimeter experiments can provide relevant information for the persistence assessment. In contrast to laboratory studies, field studies allow degradation testing under more natural conditions and over long periods up to several years. In field studies the risk of decreasing microbiological activity is lower than in longer-lasting extended laboratory studies due to the differences in test conditions. With field studies, it is also possible to study the accumulation potential of substances over several years.

There are several Guidance documents available on how to perform and interpret terrestrial field dissipation studies. The NAFTA Guidance (Corbin *et al*., 2006) is based on the degradation behaviour of substances under realistic exposure conditions considering all possible dissipation and degradation pathways. EFSA Guidance Document (EFSA, 2014) is used for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. OECD Guidance document 232 (OECD, 2016) consideres aspects from both the NAFTA and the EFSA Guidances and guidance on how to derive DegT50 values from meso- or macrocosm studies is provided in Deneer *et al*. (2015).

Compared to laboratory studies, field studies are semi-controlled with a range of varying environmental factors and particularly dependent on local conditions including varying temperature and moisture conditions. Derivation of degradation half-lives from field dissipation studies is complicated and has uncertainties related to dissipation processes such as volatilisation, photolysis, leaching, surface run-off or uptake into plants during the test (EFSA, 2014). These uncertainties can significantly influence the disappearance of the substance from the test matrix and should be taken into account in the assessment and in considerations of the reliability of the derived DegT50 when compared to the numerical P/vP criteria under CLP. DegT50 values from field studies are generally not directly comparable with one another or laboratory tests. Information may, however, be used as part of WoE. In some cases, if dissipation e.g. due to volatilisation from soil, leaching, surface run-off or uptake into plants can be excluded, mesocosm or field studies may be used to derive reliable DegT50 (EFSA, 2014). In cases where field data clearly demonstrate that more than 50% of a compound remains in the environment for a longer period than the criteria for P/vP, even though a numerical half-life is not possible to calculate, the substance could be concluded P/vP.

Consideration should be given especially to whether temperature and moisture correction should be applied by taking into account normalisation factors to relevant conditions. Moreover, it should be considered how the formation of NER could influence the DT50 derivation.

Means to perform temperature correction are provided above in this Guidance. FOCUS Kinetics Generic Guidance (FOCUS, 2014), Chapter 9 explains the normalisation of field dissipation half-lives to the reference moisture conditions. It explains that it is useful to normalise the data not only to a reference temperature, but also at moisture conditions (i.e.: 100% FC = pF2). Normalised input parameters will allow field dissipation data collected under specific environmental conditions to be used to simulate likely behaviour under different conditions if dissipation is mainly due to degradation. The normalisation can be conducted using measured or simulated values for soil moisture content (e.g., daily experimentally measured data or calculated from standard weather data using a pesticide leaching model). These simulation models are based on Walker (1974). In order to permit the broadest possible use of field dissipation data, suitable for calculation of DegT50 by assessing the likely impact of other loss processes (volatilisation, soil surface photolysis, leaching out of the sampled soil layers and possible uptake into plants) is also described.

Lysimeter studies, which are often carried out with radiolabelled substances (OECD, 2000), can also provide useful information about the degradation behaviour of a substance to be used as supporting information. Guidance Document for the Performance of Out-door Monolith Lysimeter Studies (OECD No. 22) describes a method for obtaining information on the fate and behaviour of a chemical in an undisturbed soil under outdoor conditions. Lysimeter studies are dose-dependent, they cannot fully control the varying climatic conditions and they are not suitable to all soil types. The output of this method is a concentration, expressed as maximum of average, in µg/L. More information on lysimeter studies can be found under Section 4.3.3.3.1 Data on adsorption/desoption.

In addition to the above, see also ECHA Guidance on IR&CSA, Chapter R.7b, Section R.7.9.4.2 and Chapter R.11, Section R.11.4.1.1.4.

4.3.3.1.2.3. Monitoring studies

There are many relevant sources of monitoring data. Information may be found for example from national monitoring programmes of Member States (e.g. Swedish national monitoring data collection[14]), from European monitoring programmes (e.g. NORMAN Network[15]), Information Platform for Chemical Monitoring (IPCheM)[16] or internationally acknowledged organisations (such as OSPAR or the Danube Convention).

Findings of significant concentrations of the substance in remote and pristine environments such as the Arctic sea or Alpine lakes may be evidence of high persistence. Also, significant concentrations of the substance in higher levels of the food chain in unpolluted areas may indicate high persistence, besides the potential to bioaccumulate.

Trends of rising concentrations in environmental media or biota may be observed. The reasons for such time trends, if available, can provide relevant information when assessed against the information on the time trends of volumes, uses and releases. Archived samples from environmental specimen banks, dated sediments cores and ice cores can be used to gain understanding on temporal changes. The reliability of data from archived samples should take into account the compatibility of the methods of sample collection, processing, and storage with the known properties of the substance of interest.

Monitoring data obtained in areas closer to the sources may also be useful for P/vP assessment and can be used as one line of evidence for supporting the conclusions on persistence. Use of monitoring data in P/vP assessment encompasses several uncertainties and conclusions should be drawn on the basis of monitoring data only when there is sufficient understanding of the substance distribution and transport behaviour and under the condition that the uncertainties in the monitoring data presented are adequately addressed. The lack of detection of a substance in monitoring data should be considered carefully as it does not necessarily mean that a substance is not persistent. This is because shortcomings in analytical methods may affect monitoring of substances in the environment. Uneven distribution of the substance in the media, such as soil or sediment may also lead to lack of detection or variation in presence of the substance in the environmental samples.

Monitoring data from sewage treatment plants, a percentage of removal during the residence time in the sewage treatment plant or determination of transformation/degradation products, may provide useful information for persistence assessment. However, it cannot be considered relevant in estimating degradation rates in the environmentally relevant conditions.

Use of monitoring data in P/vP-assessment encompasses several uncertainties. All available information on distribution and transport behaviour including potential sources, trends of volume, uses and releases should be considered when evaluating the suitability of monitoring data in the P/vP assessment.

In addition to the above, see also ECHA Guidance on IR&CSA, Chapter R.11, Section R.11.4.1.1.1 and R.11.4.1.1.6.

[14] http://dvsb.ivl.se/dvss/DataSelect.aspx
[15] http://www.norman-network.net/
[16] https://ipchem.jrc.ec.europa.eu/#discovery

4.3.3.1.2.4. Screening studies

There are several standard degradation test methods that can be used in the WoE assessment in addition to the information referred to in Annex I: 4.3.2.3.1. and 4.4.2.3.1.

Short description of the available screening methods is provided below. ECHA Guidance on IR&CSA, Chapters R.7b, Section R.7.9.4.1 and Chapter R.11, Section R.11.4.1.1 provide more detailed guidance on the available screening tests and their use in persistence assessment. Sections 4.1.3.2.3.2 and II.2 of this Guidance describes the use of screening information to assess rapid degradation as part of the aquatic hazard identification.

The existing methods for testing ready biodegradability are OECD TG 301 A-F and OECD TG 310. These test guidelines are not equally applicable to all types of substances. Difficulties may especially occur during tests on substances which have low water solubility, high volatility or adsorbing properties. The applicability of the ready biodegradability tests for poorly water soluble, volatile and adsorbing substances has been summarised by the OECD (2006) and in respective TGs.

The following pass levels of biodegradation, obtained within 28 days, may be regarded as evidence of ready biodegradability: 70% DOC removal (OECD TG 301 A and TG 301 E); 60% theoretical carbon dioxide ($ThCO_2$; TG 301 B); 60% theoretical oxygen demand (ThOD; TG 301 C, TG 301 D and TG 301 F). In OECD TG 310, the CO_2 evolution resulting from the ultimate aerobic biodegradation of the test substance is determined by measuring the inorganic carbon (IC) produced in sealed test bottles, and the pass level has been defined as 60% of theoretical maximum IC production (ThIC).

If the substance is readily biodegradable, or if the criteria for ready biodegradability are fulfilled with the exception of the 10-day window, the substance may be considered as not P. However, in case of contradicting results within the WoE, screening information indicating not P and not vP may not always exclude the substance from being persistent or even very persistent. Furthermore, a negative result in a test for ready biodegradability does not necessarily mean that the substance will not be degraded under relevant environmental conditions.

Ready biodegradation studies are conducted in stringent test conditions and are known to be highly variable in measuring ready biodegradability. When faced with conflicting results on ready biodegradability, differing results always have to be assessed considering the test conditions, substance properties and reliability of the data (see also Annex II Section II.3.5 of this Guidance).

Information on enhanced ready biodegradability tests is relevant when the substance is poorly soluble and/or adsorptive and enhancement is used to compensate for poor bioavailability. The enhancements can be an extended test duration or an increased test vessel size. The test should be performed with non-pre-adapted/non pre-exposed inocula. The test duration should never be extended beyond 60 days, and the test criteria set for ready biodegradability tests should be applied, i.e. 60% or 70% degradation, depending on analyte (DOC, $ThCO_2$ or ThOD), without the 10-day window. Prolongation of the test duration up to 60 days is considered acceptable if some initial, slow but steady, biodegradation is observed not reaching a plateau by the end of the ready biodegradability test, i.e. after 28 days. Positive results from enhanced ready biodegradability tests may

be used together with other supporting information to conclude that the substance is not P/vP. If the results on enhanced test are negative, depending on the other information the substace may or may not be concluded persistent.

OECD TG 306 "Biodegradability in Seawater" includes shake flask and closed bottle tests. If the result is positive (>70% DOC removal; >60% ThOD - theoretical oxygen demand), it may be concluded that there is a potential for biodegradation in the marine environment. OECD TG 306 indicates that results are not to be taken as indications of ready biodegradability, but are to be used specifically for obtaining information about the biodegradability of chemicals in marine environments. These tests are not tests for ready biodegradability since no inoculum is added in addition to the micro-organisms already present in the seawater. Neither do the tests simulate the marine environment since nutrients are added and the concentration of test substance is very much higher than would be present in the sea. If the ratio of inoculum to substrate in the test system is enhanced by increasing the concentration of micro-organisms this also increases the degradation potential. In this case the test system does not resemble a pelagic water body anymore and is, thus, less stringent. This has consequences for interpretation of the data with respect to conclusion on ready biodegradation behaviour.

Degradation of substances in seawater has generally been found to be slower than in freshwater inoculated with activated sludge or sewage effluent due to lower amount and diversity of microorganisms. Therefore >60% ThOD or >70% DOC removal obtained in OECD TG 306 (sea water without added inoculum) after 28 day (Closed Bottle Method) or 60 day (Shake Flask Method) is indicative of potential for ultimate biodegradation in the marine environment and can also be regarded as a piece of evidence that the substance is likely to fulfil the criteria for ready biodegradability. A result of >20% ThOD or DOC removal in OECD TG 306 (sea water with no added inoculum) is indicative of a potential for primary biodegradation in the marine environment (ECHA Guidance on IR&CSA, Chapter R.7b).

Tests from the OECD TG 302 series determine the inherent biodegradability of organic substances and include three methods: the Modified SCAS Test (OECD 302 A), the Zahn-Wellens/EMPA Test (OECD 302 B) and the Modified MITI Test (II) (OECD 302 C). Inherent tests are similar to ready biodegradability tests as they usually measure the same parameters and are conducted with a high test substance concentration and an even higher microbial concentration. In general, they use more favourable, if not optimal, conditions than ready biodegradability tests (e.g. with increased biomass to test substance ratio and allowing pre-adaptation of the microbial inoculum), and are hence designed to show whether a potential for degradation exists.

Two of these methods, OECD TG 302 B or OECD TG 302 C may be used to confirm that the substance does not fulfil the criteria for P provided that the following conditions are fulfilled. In OECD TG 302B biodegradation above 70% of theoretical (measured as DOC removal or O_2 uptake) may be regarded as evidence of inherent, ultimate, biodegradability provided that ≥70 % mineralisation (DOC removal) is reached within 7 d, lag phase is no longer than 3d, removal before degradation occurs is below 15% and icoculum is not pre-adapted or ≥70 % mineralisation (O_2 uptake) is reached in OECD TG 302C within 14 d, lag phase is no longer than 3d, and icoculum is not pre-adapted. Careful interpretation of data must be performed when considering the use of DOC removal as a degradation sum parameter to ensure that elimination did not occur due to adsorption or volatilisation (both of which are physical removal processes which should not be misinterpreted as

transformation or biodegradation). If supported by other weight or evidence, lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD TG 302 series may provide sufficient information to confirm that the P-criteria are fulfilled. Additionally, in specific cases it may be possible to conclude that the vP-criteria are fulfilled with this result if there is additional specific information supporting it (e.g., specific stability of the chemical bonds). Care should be taken to the interpretation of such tests, since, for example, a very low water solubility of a test substance may reduce the availability of the substance in the test medium. These issues are discussed in more detail in ECHA Guidance on IR&CSA, Chapter R.7b, Sections R.7.9.4 and R.7.9.5.

ECHA Guidance on IR&CSA, Appendix R.7.9—1 in Chapter R.7b contains a list of the ISO and OPPTS tests that are equivalent to the OECD guidelines listed above. This Chapter also lists some of the important attributes of each test.

Results obtained from the ready biodegradability, enhanced ready biodegradability, and inherent biodegradability test can be mainly used as indication of persistence or non-persistence or as supporting information in the persistence assessment.

Interpretation of screening studies with substances containing multiple constituents, impurities and/or additives is challenging if the study is conducted with the whole substance. If the concentration of the constituents is analytically monitored during the study it may be possible to assess the degradation potential of the relevant constituents separately. If only, for example, evolved CO_2 or consumed O_2 is measured, it is not possible to demonstrate which constituens of the substance have degraded and which not.

Differences in degradation potential of constituents, impurities and additives must also be assessed as part of the biodegradation screening test results. Section 4.3.3 of this Guidance provides further insights into the assessment of substances with more than one constituents.

4.3.3.1.2.5. Abiotic degradation

Abiotic processes such as hydrolysis, oxidation and photolysis may transform substances in aquatic environments, soil and air. Abiotic transformation can be an important step in the pathway for degradation of substances in the environment (OECD, 2006b).

The following guideline exists to assess hydrolysis:

- OECD TG 111: Hydrolysis as a function of pH

In general, the hydrolysis reactions are relatively sensitive to temperature. The OECD TG 111 on hydrolysis points out that tier 2 hydrolysis tests should be carried out with a minimum of three temperatures and preferably at least one temperature below the standard reporting temperature of 25°C. For the persistence assessment purposes, the hydrolysis rate at temperature of 12°C is required. Hydrolysis temperature correction estimate may be done by using the Arrhenius equation (see Section 4.3.3.1.2.1) by applying Ea of 54 kJ/mol (Guidance BPR Vol IV B+C).

Rapid hydrolysis needs to be shown across all environmentally relevant pHs. Additional evidence is also needed to consider whether the fate properties (as adsorption) of the substance would cause attenuation of the hydrolysis rate in sediment or soil, or whether

suspended solids would similarly affect the rate in aquatic media such as river or sea water.

The degradation half-lives obtained in a hydrolysis test (OECD TG 111) can be used as supporting information in the WoE assessment. Loss of parent substance by hydrolysis alone cannot remove the concern for P/vP in relevant conditions. As abiotic degradation is primary degradation, careful consideration will need to be given to the potential formation of stable degradation products with PBT/vPvB or PMT/vPvM properties. Identified hydrolysis products should be reported in accordance with the recommendations contained in the test guidelines (e.g. OECD TG 111).

The following guidelines exists to assess phototransformation:

- OECD TG 316: Phototransformation of Chemicals in Water – Direct Photolysis;
- Draft OECD guidelines on Phototransformation of Chemicals in Water – Direct and Indirect Photolysis (draft August 2000) and on Phototransformation of Chemicals on Soil Surfaces (draft January 2002);
- US EPA 1998: Phototransformation of substances in water by indirect photolysis;
- EFSA Journal (2022): Scientific guidance on soil phototransformation products in groundwater–consideration, parameterisation and simulation in the exposure assessment of plant protection products

Data derived from abiotic studies cannot be used on their own within the persistence assessment, but may be used as part of a WoE approach. Due to the large variation in the light conditions between the different environmental compartments, the use of photolysis data is not generally recognised for the persistence assessment. This is discussed in more details in the ECHA Guidance on IR&CSA, Chapter R.7b. Nevertheless, the relevance of phototrasnformation products for the persistence assessment should be included in the assessment, if the phototransformation products are expected to be formed under relevant environmental conditions.

4.3.3.1.2.6. Non-standard biodegradation studies

In addition to the standardised data described above, there is a vast amount of non-standardised biodegradation data that has been published in the scientific literature. Many of these studies share some common principles with the standard biodegradability tests, for example the fact that the test substance is usually introduced to the microorganism or microbial community as the sole source of carbon for growth and energy. Non-standard data may be valuable, as part of a WoE assessment provided that they are relevant and reliable. Reporting and use of non-standard information, Section 4.3.3 (iv) of this Guidance provides general principles on how to use and record WoE.

The persistence assessment tool[17] (PAT) promotes standardised recording and evaluation of various lines of evidence related to non-standard information.

4.3.3.1.2.7. Databases with available data

The ECHA REACH database includes public and disseminated information on ready biodegradation and biodegradation simulation studies, from the registration dossiers, submitted by companies to ECHA in the framework of the REACH Regulation. The data is

[17] https://www.ricardo.com/en/news-and-insights/insights/persistence-assessment-tool-pat

available on ECHA's dissemination website[18] and OECD QSAR Toolbox[19]. Information on Biocidal active substances and Biocidal products is also available via the ECHA website[20]. The Japanese National Institute of Technology and Evaluation (NITE) database[21] collated experimental biodegradation, photooxidation and hydrolysis data. NITE biodegradation data is also available via the OECD QSAR Toolbox under 'Biodegradation NITE'.

The Global Portal to Information on Chemical Substances (eChemPortal)[22] provides free public access to information on properties of chemicals, and direct links to collections of information prepared for government chemical programmes at national, regional, and international levels. Access to information on existing chemicals, new industrial chemicals, pesticides and biocides is provided. eChemPortal also makes available national/regional classification results according to national/regional hazard classification schemes or according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

The information in these databases is not nessessarily curated and when used in the assessments its quality and reliability must be carefully considered.

4.3.3.1.3. Non-testing data on degradation

Quantitative Structure Activity Relationships (QSARs)

A variety of models have been developed to predict biodegradation and potential degradation products. QSAR predictions can be used as supporting information in the event that the applied model is scientifically valid, the input is correct, the substance is within the applicability domain of the model, the prediction is reliable, the outcome is fit for the regulatory purpose (see ECHA Guidance on IR&CSA, Chapter R.6, Section R.6.1, QAF), and the results are adequately reported.

Models for biodegradation estimation include:

- The EPI (Estimation Programs Interface) Suite™ is a Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by EPA's and Syracuse Research Corp. (SRC) (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface). EPI Suite™ is a screening-level tool and should not be used if acceptable measured values are available. It includes two individual models for biodegradation estimation
    - BIOWIN™: Estimates aerobic and anaerobic biodegradability of organic chemicals using 7 different models. Two of these are the original Biodegradation Probability Program (BPP™). The seventh model estimates anaerobic biodegradation potential. The MITI models BIOWIN5 and BIOWIN6 models were updated in June 2017 using a much larger dataset of experimental data. The updated model is contained in the EPI Suite update file[23].
    - BioHCwin: Estimates biodegradation half-life for compounds containing only carbon and hydrogen (i.e. hydrocarbons).

[18] https://echa.europa.eu/
[19] https://www.qsartoolbox.org/home
[20] https://echa.europa.eu/information-on-chemicals
[21] http://www.nite.go.jp/en/chem/qsar/evaluation.html
[22] https://www.echemportal.org/echemportal/
[23] https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411

    - HYDROWIN™: Estimate aqueous hydrolysis rate constant and half-life.
    - AOPWIN™: Estimates the atmospheric half-lives.
- The CATALOGIC software suite (commercial, requires licence) is a platform for models and databases related to the environmental fate of substances such as abiotic and biotic degradation, bioaccumulation and acute aquatic toxicity.
- The EAWAG Pathway Prediction System (PPS)[24] predicts degradation pathways using biotransformation rules established from the reactions compiled in the EAWAG-BBD database.
- VEGA HUB[25] is a platform offering a collection of QSAR models for (eco)toxicological and environmental fate endpoints, and an independent tool helping the user in the evaluation of the result, through the Applicability Domain Index. The QSAR prediction models derive from CAESAR, T.E.S.T., SARpy, EPISuite, Toxtree, and other tools.

The OECD QSAR Toolbox is a freely available software tool to perform transparent and reproducible hazard assessment. It includes publicly available databases for many chemical properties. Databases in the Toolbox containing experimental data relating to persistence are ECHA REACH, Biodegradation NITE, and Biodegradation in Soil Oasis. Furthermore, the QSAR Toolbox can be used to predict properties using QSAR models which have been made available via the QSAR Toolbox, or by building regression based QSAR models based on experimental information available in the QSAR Toolbox.

The above list of models is not exhaustive, and other models may also be used. With more experimental data becoming available, and a better understanding of the relationship between structure and endpoint, QSAR models are being updated or new models developed. In every case, it needs to be verified that both, the QSAR model and the prediction are valid.

QSAR estimates may be used only for a preliminary identification of substances with a potential for persistence. For this purpose, it is recommended to use combined results from three estimation models in the EPI Suite™ (US EPA, 2012; R.11).
The combined results of the three freely available estimation models BIOWIN 2, 6 and 3 in the EPI suite™ may be used as follows:

- Non-linear model prediction (BIOWIN 2): does not biodegrade fast (probability < 0.5)[26] and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value < 2.25 (to 2.75)[27]), **or**

- MITI non-linear model prediction (BIOWIN 6): does not biodegrade fast (probability < 0.5) and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value < 2.25 (to 2.75))

Borderline cases should be carefully examined, e.g. when the estimate of the ultimate degradation time predicted by BIOWIN 3 gives a result in the range of 2.25 to 2.75 (see Sections R.7.9.4 and R.7.9.5 in Chapter R.7b of the ECHA Guidance on IR&CSA). In every case, the prediction needs to be verified that both, the QSAR model and the prediction

[24] http://eawag-bbd.ethz.ch/predict/
[25] https://www.vegahub.eu/
[26] The probability is low that the substance biodegrades fast.
[27] For substances fulfilling this but BIOWIN 3 indicates a value between 2.25 and 2.75 more degradation relevant information is generally warranted.

are valid.

Transparent documentation of the validity of the models (QSAR Model Reporting Format (QMRF)) as well as for reporting information relevant for judging the reliability of predictions for individual substances (QSAR Prediction Reporting Format (QPRF)) or other comparable documentation must be provided. A QMRF displays a description of the QSAR model relative to the five OECD QSAR validation principles in a systematic and summarised way (OECD 2004, 2007). The information about the QSAR prediction is reported in the QPRF. An updated QPRF template was published in 2023 and it reflects the newly established OECD QSAR Prediction Principles (OECD, 2023). QSAR predictions can be used as part of *a WoE approach*. The use of QSAR model predictions is of particular relevance and interest when test data are lacking and when assessing multi-constituent substances for which it may often be difficult to find or even to generate test data on relevant individual constituents (including impurities) due to analytical, technical, practical and cost implications.

Further information can be found in ECHA Guidance on IR&CSA, Chapters R.6 (QSARs and grouping of chemicals), R.7b Sections R.7.9.3.1 and R.7.9.4.1, R.11 Sections R.11.4.1.1.4, and OECD (2023).

4.3.3.2. Bioaccumulation assessment

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.3.2.3.2. The following information shall be considered for the 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:

(i) results from a bioaccumulation study in terrestrial species;
(ii) data from scientific analysis of human body fluids or tissues, such as blood, milk or fat;
(iii) detection of elevated levels in biota, in particular in endangered species or in vulnerable populations or subpopulations, compared to levels in their surrounding environment;
(iv) results from a chronic toxicity study on animals;
(v) 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.

Annex I: 4.3.2.4.2. In applying the WoE determination, the following information, in addition to the information referred to in Sections … 4.3.2.3.2 … shall be considered as part of the scientific assessment of the information relevant for the … B, vB … properties:

(b) Indication of B or vB properties:

(i) Octanol-water partitioning coefficient experimentally determined or estimated by well-developed and reliable (Q)SAR models;
(ii) Other information provided that its suitability and reliability can be reasonably demonstrated.

4.3.3.2.1. Bioaccumulation introduction

Bioaccumulation is generally referred to as a process in which the chemical concentration in an organism achieves a level that exceeds that in the respiratory medium (e.g., water for a fish or air for a mammal), the diet, or both (OECD, 2012). The accumulation can be from all possible environmental sources including water, food and sediment. It is the net result of uptake versus removal processes. Bioconcentration refers to the accumulation of a substance dissolved in water by an aquatic organism.

Bioaccumulation can lead to internal concentrations of a substance in an organism that cause toxic effects over long-term exposures even when external concentrations are very low. Highly bioaccumulative substances may also transfer through the food web, which in some cases may lead to biomagnification (ECHA Guidance on IR&CSA, Chapter R.11). Biomagnification refers to accumulation of a substance via the food chain, from prey to predator. It may be defined as an increase in the '(fat-adjusted)' internal concentration of

a substance in organisms at succeeding trophic levels in a food chain (ECHA Guidance on IR&CSA, Chapter R.7c).

A range of terms are used to describe accumulation of substances in biota, as described below.

4.3.3.2.2. Bioaccumulation terminology

Annex 1 of OECD TG 305 contains the following definitions for Fish BCF (OECD, 2012):

The fish **steady-state bioconcentration factor (Fish BCF_{SS})** is the ratio of the concentration of a substance in an organism to the concentration in water once a steady state has been achieved:

$BCF_{SS} = Co/Cw$

where BCF is the bioconcentration factor (L/kg)

Co is the substance concentration in the whole organism (mg/kg, wet weight)

Cw is the substance concentration in water (mg/L)

Please note that corrections for growth and/or a standard lipid content are not accounted for in this definition of the BCF. Kinetic and steady-state BCFs should also be reported relative to a default fish lipid content of 5% (w/w), unless it can be argued that the test substance does not primarily accumulate in lipid. Fish concentration data, or the BCF, are normalised according to the ratio between 5% and the actual (individual) mean lipid content (in % wet weight). The figure of 5% lipid content has been widely used as this represents the average lipid content of fish commonly used in the OECD TG 305 (OECD, 2012).

The steady-state bioconcentration factor (BCF_{SS}) does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium being constant during this period.

The **5% lipid normalised steady-state fish bioconcentration factor (Fish BCF_{SSL})** is normalised to a fish with 5% lipid content.

The fish **kinetic bioconcentration factor (Fish BCF_K)** is the ratio of the uptake rate constant, k_1, to the depuration rate constant, k2 and can be determined under non-steady state conditions. In principle, the value should be comparable to the BCF_{SS} but deviations may occur if steady-state was uncertain or if corrections for growth have been applied to the kinetic BCF.

$$BCFk = \frac{k1}{k2}$$

The **uptake rate constant** (**k_1**) is the numerical value defining the rate of increase in the concentration of test substance in/on test fish (or specified tissues thereof) when the fish are exposed to that chemical (k_1 is expressed in L kg^{-1} day^{-1}).

The **depuration (loss) rate constant** (**k2**) is the numerical value defining the rate of reduction in the concentration of the test substance in the test fish (or specified tissues

thereof) following the transfer of the test fish from a medium containing the test substance to a medium free of that substance (k2 is expressed in day^{-1}).

The **5% lipid normalised kinetic fish bioconcentration factor (BCF_{KL})** is normalised to a fish with a 5% lipid content.

The **5% lipid normalised, growth corrected fish kinetic bioconcentration factor (Fish BCF_{KgL})** is the kinetic BCF which is corrected for fish growth observed during the study period and is subsequently normalised to a fish with a 5% lipid content. Growth correction during the study period is described in Annex 5 of the OECD TG 305 (see also ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-6).

Annexes 1 and 7 of OECD TG 305 contains the following definitions for results from a fish dietary test (OECD, 2012):

The fish **dietary biomagnification factor** (**dietary Fish BMF**) describes the result of dietary exposure test, in which exposure via the aqueous phase is carefully avoided and thus the dietary BMF from this test method cannot directly be compared to a BMF value from a field study (in which both water and dietary exposure may be combined).

$$dietary\ BMFk\ = \frac{I\ x\ \alpha}{k2}$$

$$dietary\ BMFkg\ = \frac{I\ x\ \alpha}{k2g}$$

where: α = assimilation efficiency[28] (absorption of test substance across the gut);

k2 = overall (not growth-corrected) depuration rate constant (day^{-1}), calculated according to OECD TG Annex 5

k2g = growth-corrected depuration rate constant (day^{-1});

I = food ingestion rate constant (g food g^{-1} fish day^{-1});

Dietary Fish BMFk is the kinetic dietary BMF without growth correction

Dietary Fish BMFkg is the kinetic, growth corrected dietary BMF.

The **assimilation efficiency (α)** is a measure of the relative amount of substance absorbed from the gut into the organism (α is unitless, but it is often expressed as a percentage rather than a fraction). Annex 7 of OECD TG 305 explains how to calculate it from the test results.

The **food ingestion rate** (**I**) is the average amount of food eaten by each fish each day, relative to the estimated average fish whole body weight (expressed in terms of g food/g fish/day).

The **lipid- and growth-corrected fish kinetic dietary biomagnification factor**, Fish BMFkgL, is the dietary BMF which has been growth corrected and corrected for lipid content

[28] In OECD TG305 the term "assimilation efficiency" is used. It was pointed out, however, that assimilation is not the correct term, since it refers to uptake and subsequent incorporation into tissue, i.e. it refers to uptake and transformation.

of the fish and its food. For any use of the BMFkgL, it is important that the dietary lipid content and the feeding rate are reported alongside the value.

The following definitions apply for sediment-dwelling organisms (OECD TG 315, 2008):

OECD TG 315 Bioaccumulation in Sediment-dwelling Benthic Oligochaetes indicates that the main endpoint of this test is the **sediment bioaccumulation factor, sediment BAF**.

The **steady state sediment bioaccumulation factor** (**sediment BAFss**) is the BAF at steady state and does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium (Cs as g kg^{-1} of wet or dry weight of sediment) being constant during this period of time.

$$BAFss = \frac{Ca\ at\ steady\ state\ or\ at\ day\ 28\ (mean)}{Cs\ at\ steady\ state\ or\ at\ day\ 28\ (mean)}$$

Where

Sediment BAFss = steady state sediment bioaccumulation factor [$kg_{sediment} \cdot kg^{-1}_{worm}$]

Ca = concentration in worms in g kg^{-1} wet or dry weight

Cs = concentration in sediment as g kg^{-1} of wet or dry weight of sediment

The **kinetic sediment BAF**, **sediment BAFk**, is defined as:

$$BAFk = \frac{k1}{k2}$$

where

BAFk = the kinetic bioaccumulation factor

k_1 = uptake rate constant in tissue [g sediment kg^{-1} of worm d^{-1}]

k_2 = elimination rate constant [d^{-1}]

The **biota-sediment accumulation factor** (**BSAF**) is the lipid-normalised steady state concentration of test substance in/on the test organism divided by the organic carbon-normalised concentration of the substance in the sediment at steady state.

$$BSAF = BAFk\ x\ \frac{foc}{flip}$$

where

BSAF = biota-sediment accumulation factor [kg sediment OC kg^{-1} worm lipid content]

foc = the fraction of sediment organic carbon based on dry weight, or on wet weight

flip = the fraction of worm lipid, both based either on dry weight, or on wet weight.

It should be noted that the term **biota-sediment accumulation factor** (**BSAF**) has been used in the literature to refer to bioaccumulation factors in sediment which have not been normalised to organism lipid and sediment total organic carbon content. Care should be taken to ensure it is clear what the reported value refers to.

The following definitions apply for soil-dwelling organisms (OECD TG 317, 2010):

OECD TG 317 Bioaccumulation in Terrestrial Oligochaetes indicates that the main endpoint of this test is the **soil bioaccumulation factor, BAF**.

The **steady state soil bioaccumulation factor** (**soil BAFss**) is the BAF at steady state and does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium (Cs as g kg^{-1} of wet or dry weight of soil) being constant during this period of time.

$$BAFss = \frac{Ca\ at\ steady\ state\ or\ at\ day\ 21\ (mean)}{Cs\ at\ steady\ state\ or\ at\ day\ 21\ (mean)}$$

where

Soil BAFss = steady state soil bioaccumulation factor [$kg_{soil} \cdot kg^{-1}_{worm}$]

Ca = concentration in worms in g kg^{-1} wet or dry weight

Cs = concentration in soil as g kg^{-1} of wet or dry weight of soil

The **kinetic soil BAF**, **soil BAFk**, is defined as:

$$BAFk = \frac{k1}{k2}$$

where

BAFk = the kinetic bioaccumulation factor

k_1 = uptake rate constant in tissue [g soil kg^{-1} of worm d^{-1}]

k_2 = elimination rate constant [d^{-1}]

The **biota-soil accumulation factor** (**BSAF**) is the lipid-normalised concentration of test substance in/on the test organism divided by the organic carbon-normalised concentration of the substance in the soil at steady state.

$$BSAF = BAFk\ x\ \frac{foc}{flip}$$

where

BSAF = biota-soil accumulation factor [kg soil OC kg^{-1} worm lipid content]

f_{oc} = the fraction of soil organic carbon based on dry weight, or on wet weight

f_{lip} = the fraction of worm lipid, both based either on dry weight, or on wet weight.

It should be noted that the term **biota-soil accumulation factor** (**BSAF**) has been used in the literature to refer to bioaccumulation factors in soil which have not been normalised to organism lipid and soil total organic carbon content. Care should be taken to ensure it is clear what the reported value refers to.

Field bioaccumulation metrics

The **field bioaccumulation factor** (**field BAF**) represents environmental exposure in the field to an aquatic organism from all routes and is referenced to the substance concentration in water (Arnot and Gobas, 2004; Burkhard *et al*., 2012b). The basis for the field BAF value is the ratio of the concentration in wet weight (ww) of the organism divided by the water concentration. The unit of the field BAF is $L \cdot kg_{ww}^{-1}$. It is recommended that the field BAF is reported in terms of wet weight as well as dry weight and is also normalised to lipid weight, with an explanation of how the normalisation was performed (European Commission, 2018).

Field measured biota-sediment accumulation factors (**field BSAF**) are derived by the concentration of a substance in biota divided by the concentration in the sediment (Burkhard *et al*., 2010).

The **field biomagnification factor (field BMF**) is the concentration of a substance in a predator relative to the concentration in the predator's prey (or food) originating from the same ecosystem at steady-state and in which both, water and dietary exposure may be combined the ratio of the concentration in the predator and the concentration in the prey (ECHA Guidance on IR&CSA, Chapters R.11, R.7c):

BMF = Co/Cd

where field BMF is the biomagnification factor (dimensionless)

Co is the steady-state substance concentration in the organism (mg/kg)

Cd is the steady-state substance concentration in the diet (mg/kg).

Field BMFs for substances that partition into lipids should, as far as possible, be lipid normalised to account for differences in lipid content between prey and predator. It allows for a comparison of field BMF values in a direct and objective manner.

The **trophic magnification factor** TMF describes the average increase in biota concentration per trophic level (ECHA Guidance on IR&CSA, Chapter R.7c). The TMF for a food web is calculated as the exponent of the slope of the natural logarithm transformed concentrations for organisms in the food chain as a function of the trophic level of these organisms. The TMF represents the average biomagnification per trophic level within that food web. For substances that partition into lipids the TMF should be derived from lipid-normalised biota concentrations versus trophic level.

4.3.3.2.3. Data on Bioaccumulation

4.3.3.2.3.1. Fish bioaccumulation tests - aqueous exposure

The most commonly used test guideline for fish bioaccumulation is OECD TG 305 (OECD, 2012). Detailed guidance on interpretation of OECD TG 305 fish bioaccumulation test data is provided in the related OECD Guidance document (OECD, 2017), ECHA Guidance on IR&CSA, Chapters R.11 and R.7c and current Guidance on aquatic hazards, Annex III.2. In principle, the OECD Guidance document can also apply to other aquatic bioaccumulation tests. These tests measure fish BCF. Reliable fish BCFs have been extensively used in a regulatory context to conclude that a substance meets the criteria for B or vB.

Principle of the test
To measure bioconcentration of a substance in fish, a sufficient number of fish are exposed to one or two sub-lethal concentrations of the test substance dissolved in water. Fish and water are sampled at regular time-intervals and the concentration of test substance IS measured. Tests are generally conducted using a flow-through system. After reaching an apparent steady-state tissue concentration (usually after 28 days, see paragraphs 17-18 of OECD, 2012), the remaining fish are transferred to clean water and the depuration is followed. A control group of fish is held under identical conditions except for the absence of the test substance, to relate possible adverse effects observed in the bioconcentration test to a matching control group and to obtain background concentrations of the test substance.

Where possible the bioconcentration factor is calculated both as the ratio of concentration in the fish (C_f) and in the water (C_w) at steady-state (BCF_{SS}) and as a kinetic bioconcentration factor (BCF_K), which is estimated as the ratio of the rate constants of uptake (k_1) and depuration (k_2) assuming first order kinetics. The uptake rate constant, the depuration (loss) rate constant, the bioconcentration factor (steady-state and/or kinetic), and where possible, the confidence limits of each of these parameters are calculated from the model that best describes the measured concentrations of test substance in fish and water.

Fish lipid content should be measured so that the BCF can be expressed on a 5% lipid content basis, unless the substance is not expected to accumulate primarily in lipids. The average lipid content of fish used in the OECD TG 305 is 5%.

The increase in fish mass during the test will result in a decrease of test substance concentration in growing fish (so-called growth dilution), and thus the kinetic BCF will be underestimated if not corrected for growth (see also ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-6). OECD TG 305 explains how to correct the BCF_K for growth dilution. There is currently no method to correct BCF_{SS} for growth dilution.

BCFkgL is the 5% lipid normalised, growth corrected kinetic bioconcentration factor and is the preferred result for comparison with the CLP B/vB criteria for substances accumulating mainly in lipids.

OECD TG 305 specifies the applicability of the test and the conditions which must be met for a study to be valid.

Considerations when reviewing fish BCF tests (see also current Guidance on aquatic hazards, Annex III)

Exposure concentrations should not exceed the aqueous solubility of the test substance. In cases where test exposures significantly exceed aqueous solubility (e.g. due to the use of dispersants), and the analytical method does not distinguish between dissolved and non-dissolved substance, the study data should generally be considered unreliable. An indication of the BCF might be given by assuming that the organisms were exposed at the water solubility limit. The total organic carbon and dissolved oxygen concentrations in the dilution water should be reported.

The concentration(s) of the test substance should be below its chronic effect level or 1% of its acute asymptotic LC_{50}. This is to avoid any toxic effect of the test substance during the test. The average growth in both test and control groups can be compared to check for toxic effects. Any decreased growth in the test groups would suggest toxic effects occurred. If no mortality information is provided for a study, one option is to designate the study as 'reliable with restrictions' if the exposure concentration used is at least a factor of 10 below the known or predicted fish LC_{50}.

If a radiolabelled test substance is used, total radioactivity measurements alone may overestimate the concentration of parent substance due to small amounts of radiolabelled impurities that may be present in the test substance, and/or formation of metabolites. To avoid overestimation of the BCF, it is preferable to have a substance-specific chemical analytical technique or selective clean-up procedure at the end of the exposure period. If the fish are not fed, high concentrations of (usually more polar) metabolites may build up in the gall bladder, which may lead to an overestimate of whole body levels (OECD, 2001).

The analytical method used for the quantification of the substance should be described. The recovery efficiency, precision, limits of quantification and detection and working range should be reported with an explanation of how they were determined.

The kinetic BCF should be corrected for growth dilution. Older studies may not have any information on fish growth. In this case, an assessment of the likely significance of growth on the results should be made. As noted in OECD TG 305, fish species tested during a (juvenile) life-stage with rapid growth can complicate data interpretation. For relevance and scientific justification of correction for growth dilution when deriving BCF see Appendix R.11-6 in ECHA Guidance on IR&CSA, Chapter R.11.

The whole body lipid content should ideally be reported since many organic substances partition to lipid. Where reported, the BCF should be normalised to 5% lipid to allow comparison between studies, unless it is known that the substance does not primarily partition to lipids. BCF results should specify the units and tissue type (e.g. whole body, muscle, fillet, liver, fat). Whole body wet weight measurements are preferred.

The kinetic BCF (BCF_K) is preferred for regulatory purposes since for bioaccumulative substances a real steady state is often not attained during the uptake phase. The BCF_K should be corrected for growth dilution. Where information on growth is not available, the likely significance of growth on the results should be assessed. The uncertainty in a BCF value derived from a fast-growing fish will be greater than that for a slow growing fish.

In conclusion, reliable and relevant fish BCFs can be compared directly with the numerical CLP B/vB criteria of BCF >2000 and BCF >5000, respectively.

4.3.3.2.3.2. Fish bioaccumulation tests - dietary exposure

Although they are less commonly conducted than aqueous exposure tests, dietary exposure tests may be available for some substances. The only test guideline available currently is OECD TG 305-III: Dietary Exposure Bioaccumulation Fish Test. Most studies follow the principles of this test guideline. These tests expose the fish via food only, avoiding aqueous exposure.

The primary endpoint measured in a fish dietary study is a dietary biomagnification factor (dietary BMF), which is the concentration of a substance in fish relative to the concentration in the food at steady state. Since a field BMF covers exposure from several routes (including food and water) and a dietary BMF covers exposure only via food, dietary BMFs are generally lower than field BMFs. A dietary BMF <1 does not mean that a substance is not bioaccumulative (ECHA Guidance on IR&CSA, Chapter R.11, Section R.11.4.1.2.3).

The dietary BMF cannot be directly compared with the CLP criteria which are based on BCF values but a BCF can be estimated from fish dietary studies, as explained below. Reliable fish dietary studies have been used in a regulatory context to conclude if a substance meets the criteria for B or vB in a WoE approach, using the estimated BCF from the measured depuration rate constant/half-life.

Principle of the test

In fish dietary exposure tests, a sufficient number of fish are exposed usually to one sub-lethal concentration of the test substance spiked on fish food. Both fish and experimental diet are sampled at regular time intervals and the concentration of test substance measured. An uptake phase of 7-14 days is recommended but it can be extended, if necessary. As fish may not reach steady-state during the uptake phase, the data treatment and results are usually based on a kinetic analysis of tissue residues. The depuration phase begins when the fish are fed for the first time with unspiked food and usually lasts for up to 28 days or until the test substance can no longer be quantified in whole fish, whichever is sooner. It is important to remove any uneaten food and faeces shortly after feeding to avoid the test substance partitioning to the water leading to exposure via the water.

A control group of fish is held under identical conditions and fed identically except that the commercial fish food diet is not spiked with test substance. This control group allows background levels of test substance to be quantified in unexposed fish and serves as a comparison for any treatment-related adverse effects noted in the test group (OECD, 2012).

This method allows the determination of the substance-specific half-life ($t_{1/2}$, from the depuration rate constant, k2), the assimilation efficiency (absorption across the gut; α), the kinetic dietary biomagnification factor (BMFK), the growth-corrected kinetic dietary biomagnification factor (BMFKg), and the lipid-corrected kinetic dietary biomagnification factor (BMFKL) (and/or the growth- and lipid-corrected kinetic dietary biomagnification

factor, BMFkgL) for the test substance in fish. There has been recent discussion about the appropriateness of correcting for the lipid content of fish and their food according to the method in the OECD TG 305 (Hashizume *et al*. (2018), Gobas *et al*. (2021), Environment Agency (2023)). As a result of these discussions, it is recommended to estimate the BCF based on a model predicted uptake rate constant (k_1) and the depuration rate constant (k2) determined from the dietary bioaccumulation study (uptake rate constant estimation method (Method 1) as described in Guidance document on aspects of OECD TG 305 (OECD, 2017), Chapter 4.6.3). The estimated BCF can be directly compared to the CLP criteria. In case the derivation of a BCF is not possible, the BMF5%, which is the BMFkg normalised to a fish with a 5% lipid content as recommended by Hashizume *et al*. (2018), may be useful to compare results from different studies (Environment Agency, 2023). For any use of the BMFkgL, it is important that the dietary lipid content and the feeding rate are reported alongside the value. BMF5% and BMFkgL could be used in a benchmarking exercise.

As for the aqueous exposure method, increase in fish mass during the test will result in dilution of test substance in growing fish and thus the (kinetic) BMF will be underestimated if not corrected for growth (cf. paragraphs 162 and 163). Annex 5 of OECD TG 305 explains how to perform the growth correction. OECD TG 305 specifies the applicability of the test and the conditions which must be met for a study to be valid.

Considerations when reviewing fish dietary exposure bioaccumulation tests

It is important that the spiked food is palatable to the fish. This can be checked by examining the growth of fish during the course of the study. There should be similar growth in the control and in the test groups of fish. The body burden of the test substance in the test fish should not reach a level which is sufficient to cause toxic effects.

As for the aqueous fish bioaccumulation test, if radiolabelled test substance is used, total radioactivity measurements alone may overestimate the concentration of parent substance due to small amounts of radiolabelled impurities that may be present in the test substance, and/or formation of metabolites.

The lipid content measured at least at the start and end of the uptake phase and at the end of the depuration phase should be reported, as well as the method used for its determination. The results should be expressed based on whole body, wet weight concentrations.

The fish dietary bioaccumulation test provides a BMF rather than a BCF, which is required for comparison with the CLP criteria. Whenever possible, the kinetic BCF should be estimated based on the results of fish dietary test to compare with the CLP criteria. The BCF value can be estimated from a predicted uptake rate constant and the experimentally determined depuration rate using the Dietary Exposure Test Spreadsheet of OECD TG 305[29], unless it can be demonstrated that the uptake rate constant (k_1) cannot be reliably estimated with the available methods.

A detailed description of the methods to estimate a BCF from a dietary study can be found in Annex 8 of OECD TG 305 (OECD, 2012) and the Guidance Document on Aspects of

[29] accessible at https://www.oecd.org/chemicalsafety/testing/section-3-environmental-fate-behaviour-software-tg-305.htm (last accessed: October 2022)

OECD TG 305 (OECD, 2017) in Chapter 4.6.3. The methods are 1) Uptake rate constant estimation method, 2) Relating depuration rate constant directly to BCF and 3) Correlating dietary BMF with BCF. OECD, 2017 provides further information on the applicability domain of the three estimation methods.

Besides the calculation of a BCF from the depuration phase, the dietary BMF derived from the OECD TG 305-III test can be compared with laboratory BMF values for substances with known bioaccumulation potential in a benchmarking exercise (see Correlating dietary BMF with BCF (Method 3) in OECD, 2017). For example, such an approach has been described for dietary bioaccumulation studies with carp (Inoue *et al.*, 2012). Based on a regression between BCF_L and BMF_{kgL} for nine compounds tested in this set-up, it was shown that a BCF_L value of 5000 L/kg, normalised to a lipid content of 5%, corresponds to a lipid corrected BMF_{kgL} from the dietary test of 0.31 kg food lipids/kg fish lipids, and a BCF_L of 2000 L/kg corresponds to a BMF_{kgL} of 0.10 kg food lipids/kg fish lipids.

A different benchmarking could be obtained from aqueous and dietary bioaccumulation studies for perfluorinated compounds with rainbow trout (Martin et al., 2003a, b). These studies emphasise the fact that even if a BMF from an OECD TG 305 dietary bioaccumulation study is found to be <1, it cannot be considered as a good discriminator for concluding substances not to be (very) bioaccumulative according to the BCF criteria. If benchmarking is used for comparing dietary BMF values with BMF values for substances with a known bioaccumulation potential, it must be ensured that these BMF values were obtained under similar conditions (i.e. fish species, fish weight/size, diet lipid content, feeding rate, fish lipid content and temperature).

Another endpoint from the dietary OECD 305 test is the elimination rate constant. The elimination rate constant has been proposed as an endpoint for the bioaccumulation assessment (e.g. Brooke and Crookes, 2012, Goss *et al*. 2013, Goss *et al*. 2018). For example, Brooke and Crooke (2012) presented lipid normalised depuration rate constants of 0.181 and 0.085 d^{-1} as critical values for lipid normalised BCF values of 2000 and 5000. Relating depuration rate constant directly to BCF is described as Method 2 in Guidance document on aspects of OECD TG 305 (OECD, 2017). The depuration rate constant is a useful metric for assessing bioaccumulation. However, it should be noted that the kinetics of uptake and depuration are still dependent on other factors, for example the size of the fish (e.g. Barber 2008, Brooke and Crookes, 2012). Indeed, from the analysis from Brooke and Crookes (2012) there is considerable scatter around the regression line between log BCF_L and log k_2 (lipid normalised), which may be caused by the variability in fish weight used in the underlying studies, at least partly. This implies that it is not possible to set one value for the depuration rate constant for different organisms. If aqueous bioconcentration is considered, an uptake rate constant of 520 L/kg/d could be estimated for fish with a weight of 1 g (Sijm *et al.*, 1995). The depuration rate constants that lead to bioconcentration factors of 2000 and 5000 could thus be estimated to be 0.26 d^{-1} and 0.10 d^{-1}. For fish weighing ten grams these values would be approximately half of these values (0.12 d^{-1} and 0.05 d^{-1}).

Detailed guidance on interpretation of OECD TG 305 fish bioaccumulation test data is provided in the test guideline and in the related OECD Guidance document (OECD, 2017). More information on the fish dietary bioaccumulation test and the use of the results can be found in the ECHA Guidance on IR&CSA, Chapter R.11, Section R.11.4.1.2.3.

In conclusion, reliable fish dietary tests provide useful information on bioaccumulation but the results cannot be directly compared directly with the numerical CLP B/vB criteria. The estimated BCF needs to be derived to allow a comparison with the criteria. If it is not possible to estimate the BCF, other toxicokinetic information from the study can be used in a weight-of-evidence approach to conclude on B or vB.

4.3.3.2.3.3. *Hyalella azteca* bioconcentration tests

Hyalella azteca is an epibenthic amphipod which is widespread in North and Central America and commonly used for ecotoxicity studies (Environment Canada 2013; US EPA 2000; ASTM International 2000). A draft OECD TG for the *Hyalella azteca* bioconcentration test is currently under preparation and is scheduled to be adopted in 2024[30]. This TG provides a non-vertebrate test to estimate the bioconcentration potential of substances. Since they are an aquatic species, reliable *Hyalella azteca* BCFs can be compared with the CLP criteria for B/vB.

BCF values for lipophilic chemicals determined with the benthic freshwater amphipod *Hyalella azteca* show a strong correlation with BCFs that have been determined according to the OECD TG 305 when applying a normalisation to a total lipid content of 5% (Schlechtriem *et al*. 2019). However, bioconcentration should be normalised to the species specific lipid content of 3% (based on whole body wet weight) for comparison with the criteria, where appropriate. The test is discussed further in Section Chapter R.11.4.1.2.2 of ECHA Guidance on IR&CSA.

Principle of the test

The test follows a method similar to the OECD TG 305 fish bioaccumulation test (aqueous exposure). Groups of adult male *Hyalella azteca* are exposed to one sub-lethal concentration of the test substance dissolved in water for 3-10 days until steady state is reached. Only sexually mature males (> 8 weeks old) are used to avoid reproduction during the test and due to their more uniform size and lipid content compared to female *Hyalella azteca*. Replicates of *Hyalella azteca* and water are sampled at regular time-intervals and the concentration of test substance measured. Tests may be conducted using a flow-through or semi-static system. After reaching an apparent steady-state tissue concentration, the remaining *Hyalella azteca* are transferred to clean water and the depuration is followed. The steady state BCF_{SS} and kinetic BCF_K can be derived.

A correction of the kinetic BCF for growth dilution is not necessary because adult organisms are tested and their growth will be negligible. The lipid content of the tested *Hyalella azteca* should be determined. The BCF is based on the total concentration in *Hyalella azteca* (i.e. per total wet weight of the sampled *Hyalella azteca*). Since, for many organic chemicals, there is a clear relationship between the potential for bioconcentration and hydrophobicity, there is also a corresponding relationship between the lipid content of the test *Hyalella azteca* and the observed bioconcentration of such chemicals. Thus, to reduce this source of variability in test results for those test chemicals with high lipophilicity (i.e. with log K_{ow} > 3), bioconcentration should be expressed as normalised to H. azteca with a default 3% lipid content (based on whole body wet weight). The lipid content of lab-raised *Hyalella azteca* is usually in the range of 1-3% (w/w) but may be higher in field caught *Hyalella*

[30] Once published, the Guideline will be available under: https://www.oecd.org/env/ehs/testing/test-guidelines-for-comments-section3-degradation-and-accumulation.htm

azteca (Schlechtriem *et al*. 2019, Kosfeld *et al*. 2020, Arts *et al*. 1995, Huff Hartz *et al*. 2021). Lipid measurements should be carried out for amphipods collected directly from the study. This is necessary to provide a basis from which results for different chemicals and studies can be compared against one another. The draft OECD TG specifies the applicability of the test and the conditions which must be met for a study to be valid.

Considerations when reviewing Hyalella azteca bioconcentration tests

If readily biodegradable solvents are used, they can cause problems with bacterial growth. The test substance can adsorb to the bacteria flocs which the *Hyalella* consume, leading to exposure via the dietary route.

If radiolabelled test substances are used and only total radioactive residues are measured the BCF is based on the total of the parent substance, any retained metabolites and also assimilated carbon. Separation procedures, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) or gas chromatography (GC) may be employed before analysis in radiolabelled studies in order to determine a BCF based on the parent substance. The tested concentration should be below the solubility limit of the test chemical in the test media. The selected test substance concentration for *H. azteca* should be below its chronic effect level or 1% of its acute asymptotic LC_{50} (draft OECD TG).

In conclusion, reliable *Hyalella azteca* bioconcentration tests provide a BCF which, normalised to its typical lipid content of 3%, can be directly compared with the numerical CLP B/vB criteria.

4.3.3.2.3.4. Bioconcentration tests in other aquatic invertebrates

Other standard bioconcentration tests with aquatic invertebrates are available, for example ASTM E1022-22 Standard Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks (ASTM International, 2022, previously ASTM E1022-94) and OCSPP 850.1710: Oyster Bioconcentration Factor (Crassostrea virginica) (US EPA, 2016)). These studies provide BCFs which can be compared with the CLP B/vB criteria, if they are reliable. Invertebrate species may have a lower metabolic capacity than fish species, for example as is the case for polycyclic aromatic hydrocarbons (Bleeker and Verbruggen, 2009). Bioaccumulation in invertebrates may therefore be higher than in fish under the same exposure conditions.

Principles

BCF tests with aquatic invertebrates are similar to the fish and *Hyalella azteca* bioconcentration tests where a number of organisms are exposed to sub-lethal concentrations of the test substance dissolved in water. The organisms and water are sampled at regular time-intervals and the concentration of test substance measured. After reaching an apparent steady-state tissue concentration, the organisms are transferred to clean water and the depuration is followed.

Considerations when reviewing BCF tests in aquatic invertebrates

The considerations described above relating to fish and *Hyalella az*teca tests also apply to other standard BCF tests with aquatic invertebrates, namely the test concentration should not cause significant effects, steady-state conditions should be used, the aqueous concentration in the exposure vessels should be maintained and should be below the water

solubility of the substance, if radioanalysis is used it should be supported by parent compound analysis so that the contribution of metabolites can be assessed.

Results should be reported on a whole body wet weight basis. Where measured tissue lipid concentrations are available, the measured BCF should be lipid normalised to the typical lipid content of the organism. Since bivalves such as oyster and mussel can shut and stop feeding in the presence of toxins, the study description should indicate the acute toxicity of the substance and whether closure has occurred. For test species tend which feed on particulates (including micro-organisms), the assessment of exposure concentrations may need careful consideration if the test system is not in equilibrium, especially for hydrophobic substances.

As well as BCF values for fish species or *Hyalella*, high-quality data on the BCF value for further invertebrate species may be used. For example, mussel, oyster or scallop BCF can be used as a worst case (conservative) values after careful assesssment. BCF for algae should not be used. Further information on the evaluation of these studies is available in ECHA Guidance on IR&CSA Section, R.7.10.4.1.

4.3.3.2.3.5. In vitro fish toxicokinetic tests

In vitro methods such as fish liver S9 and primary hepatocyte assays provide information on biotransformation in the organism. Because biotransformation is considered to be the dominant mechanism of elimination of hydrophobic substances, such *in vitro* clearance assays have the potential to support the assessment of bioaccumulation in a WoE approach assuming that the the substance reaches the liver (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.4). To make use of *in vitro* fish toxicokinetic data for bioaccumulation assessment, the application of *in vitro–in vivo* extrapolation (IVIVE) bioaccumulation models is needed to convert the *in vitro* biotransformation data to *in vivo* biotransformation rates and to calculate a kinetic BCF. A range of *in vitro* fish toxicokinetic tests are available in the scientific literature. Preference is given to results obtained from standard tests OECD test guidelines 319 A/B (OECD 2018b; OECD 2018c).

Principle of the test

The OECD TGs 319 A/B (OECD 2018b; OECD 2018c) describe the use of either cryopreserved rainbow trout hepatocytes or of liver S9 subcellular fractions for determining *in vitro* biotransformation kinetics in a detailed manner. In brief, the test chemical is incubated together with either hepatocytes or S9 fraction and substrate depletion is monitored over the duration of the experiment (maximum 4 hours). From the measured substrate depletion curve, the *in vitro* biotransformation kinetics can be determined. Detailed guidance on the performance of the tests is available in the test guidelines and related OECD Guidance document (OECD 2018a).

OECD TG 319 A/B specifies the applicability of the test and the conditions which must be met for a study to be valid.

Considerations when reviewing in vitro fish toxicokinetic tests

The following information should be documented and provided in an IVIVE-based bioaccumulation assessment:

- *in vitro* test conditions (measured test chemical concentration, number of time points, species from which *in vitro* material originated, S9/hepatocyte concentration, total assay volume, open or closed system, assay duration, characterisation of *in vitro* material (Ethoxyresorufin-O-deethylase (EROD), glutathione transferase (GST) activities etc.), incubation temperature);

- evidence that the depletion follows first-order kinetics or that the chemical starting concentration is below the Michaelis-Menten constant; and documentation of the behaviour of the negative control (if the negative control shows significant losses, the test should not be used);

- determined *in vitro* biotransformation kinetics (rate constants or clearances with units);

- estimated *in vivo* biotransformation kinetics (with units) and used extrapolation formalism (with reference);

- used IVIVE-bioaccumulation model (with reference).

Currently, *in vitro* tests cannot directly substitute *in vivo* data in terms of one for one replacement, for classification purposes. However, *in vitro* data can already play a role as supporting evidence in a WoE approach and there are ongoing efforts to develop and validate further in vitro methods which may add to our understanding of bioaccumulation. Although the standard guideline *in vivo* methods remain the most informative for classification and labelling purposes, all available and relevant information on bioaccumulation, including non-guideline methods, can be assessed on their own merits and carefully balanced in the overall WoE.

4.3.3.2.3.6. Bioaccumulation tests in sediment-dwelling species

Bioaccumulation studies on sediment dwelling organisms measure the accumulation in sediment organisms via several uptake routes including direct contact, ingestion of contaminated sediment particles, porewater and overlying water (OECD TG 315). The result is a bioaccumulation factor BAF which can be normalised to lipid content of organisms and organic carbon content of sediment to derive the BSAF, biota-sediment accumulation factor. These results cannot be directly compared with the CLP B/vB criteria although the BSAF in combination with K_{OW}/ K_{OC} can provide evidence of high bioaccumulation potential (ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-3). BCF values can be calculated based on measured or estimated pore water concentrations according to ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-3. If BCF values are normalised to a lipid content of 5%, they can be considered as a conservative estimate for fish, because metabolism is generally much lower in invertebrates than in fish. A case-by-case assessment based on expert judgement of the reliability and relevance of the available information is required in order to be able to give BSAF values an appropriate weight in the WoE assessment.

Other indications of a high bioaccumulation potential, such as a bioaccumulation process not reaching the steady state at the end of the exposure period of an OECD TG 315 test or a low depuration rate, both representing slow kinetics, are relevant parts of a WoE approach when considering whether the B or vB classification criteria are fulfilled. Substances with background sediment concentrations and potentially adaptable uptake mechanisms need careful consideration because sediment-dwelling organisms may have

adapted to such substances, potentially affecting the bioaccumulation process.

It should be noted that the term biota-sediment accumulation factor (BSAF) has been used in the literature to refer to bioaccumulation factors in sediment which have not been normalised to organism lipid and sediment organic carbon content. Care should be taken to ensure it is clear what the reported value refers to.

A range of sediment bioaccumulation tests may be available in the published literature. The OECD TG 315 Bioaccumulation in Sediment-dwelling Benthic Oligochaetes is the preferred test method.

Considerations when reviewing bioaccumulation tests in sediment-dwelling species
It is important that the test organisms burrow into the sediment and do not avoid the sediment since burrowing behaviour can influence the level of exposure (OECD TG 315).

OECD TG 315 recommends the use of artificial sediment. If natural sediments are used, the sediment characteristics should be specifically reported as described in the test guideline. Substances with background sediment concentrations and potentially adaptable uptake mechanisms need careful consideration because sediment-dwelling organisms may have adapted to such substances, potentially affecting the bioaccumulation process.

For lipophilic substances, BAFs often vary with the organic carbon content of the sediment. Typically a substance will have greater availability to the organism when the sediment OC is low, compared to a higher OC. It should be considered to test at least two natural sediments with different organic matter content, and the characteristics of the organic matter, in particular the content of black carbon, should be reported. To ensure comparability of results between different sediments, the normalised BSAF normalised to total organic carbon content should be derived (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.5, current Guidance on aquatic hazards, Section 4.3.3.2.2).

If a radiolabelled test substance is used, total radioactivity measurements alone may overestimate the concentration of parent substance due to small amounts of radiolabelled impurities that may be present in the test substance, and/or formation of metabolites. To avoid overestimation of the BAF, it is recommended that BAF calculations be based on the concentration of the parent compound in the organisms and not only on total radioactive residues.

It is important to consider the implications of the worm gut contents when interpreting the study results (Mount *et al*, 1999; OECD TG 315).

Many studies have shown that black carbon can substantially affect the strength of particle sorption and hence the bioavailability of a substance (Cornelissen *et al*., 2005). Observed black carbon partition coefficients exceed organic carbon partition coefficients by up to two orders of magnitude. When interpreting data where the exposure system includes natural sediments it is therefore important to account for the possible influence of black carbon partitioning to avoid underestimation of the substance's bioaccumulation potential from the freely dissolved phase (ECHA Guidance on IR&CSA, Chapter R.7.10.3.1).

In conclusion, bioaccumulation tests in sediment-dwelling organisms provide a BAF or BSAF which cannot be compared directly with the numerical CLP B/vB criteria. However,

BCF values can be estimated from the BSAF based on measured pore water concentrations or estimated pore water concentrations.

4.3.3.2.3.7. Bioaccumulation tests in terrestrial species (soil dwelling organisms)

Bioaccumulation studies on soil dwelling organisms measure the accumulation in soil organisms exposed through three phases: soil pore water, soil air and ingestion of soil. The resulting bioaccumulation factor BAF can be normalised to lipid content of organisms and organic carbon content of soil to derive the BSAF, biota-soil accumulation factor. These results cannot be directly compared with the CLP B/vB criteria. Soil dwelling species are different in their phsiology than fish and may have a lower metabolic capacity than fish species.

The soil BSAF in combination with K_{OW}/ K_{OC} can provide evidence of high bioaccumulation potential. BCF values can be calculated based on measured or estimated pore water concentrations as specified in ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-3. A case-by-case assessment based on expert judgement of the reliability and relevance of the available information is required in order to be able to give soil BSAF values an appropriate weight in the B and vB assessment.

Bioaccumulation data from terrestrial plants should not be used, because it is currently not clear how observed accumulation in plants contributes to bioaccumulation in terrestrial food webs for classification and labelling purposes.

4.3.3.2.3.8. Field data - levels in biota, biomagnification in the food chain

Field bioaccumulation factors (Field BAF calculated from monitoring data, field measurements or measurements in mesocosms) or specific accumulation in food chains/webs expressed as biomagnification factors (BMFs) or trophic magnification factors (TMFs) can provide supplementary information indicating that the substance does or does not have bioaccumulation potential.

If field data indicate that a substance is effectively transferred in the food chain, this is a strong indication that it is taken up from food in an efficient way and that the substance is not easily eliminated (e.g. excreted or metabolized) by the organism (this principle is also used in the fish feeding test for bioaccumulation), which will lead to biomagnification from prey to predator (trophic magnification). A reliable field BMF or TMF value higher than 1 can also be considered as an indication of very high bioaccumulation (ECHA Guidance on IR&CSA, Chapter R.11). For aquatic organisms, this value indicates an enhanced accumulation due to additional uptake of a substance from food along with direct accumulation from water. However, as dietary and trophic biomagnification represent different processes than bioconcentration in aquatic organisms, field BMF and/or TMF values <1 cannot be directly used to disregard a valid assessment based on reliable BCF data the numerical CLP B/vB criteria, but in this kind of case all available data need to be considered together in a WoE approach.

Monitoring data for humans and biota are available in the open literature and some data

can be accessed via the platform IPCHeM[31] or the NORMAN network[32]. It is recommended to perform a literature search and to check these databases to check for available monitoring data on a substance.

Guidance documents and recommendations for assessing the quality of biomonitoring data including interpretation of wildlife biomonitoring have been elaborated by the EU project LIFE APEX (Badry *et al*., 2022a; Badry *et al*., 2022b ; Treu *et al*., 2022a) and Guidance Document No. 32 on Biota Monitoring prepared under the Water Framework Directive 2000/60/EC (European Commission, 2014). Further guidance on the use of field data for PBT/vPvB assessment is available in ECHA Guidance on IR&CSA, Chapters R.11.4.1.2.6 and R.11.4.1.2.7.

Field bioaccumulation metrics are the field bioaccumulation factor (field BAF), field measured biota-sediment accumulation factor (field BSAF), field biomagnification factor (field BMF), trophic magnification factor (TMF). They are explained in Section 4.3.3.2.2 of this Guidance.

BCFs, BAFs express ratios of substance concentrations in biota to water, while BMFs and TMFs reflect ratios of substance concentrations in predator–prey relationships (Burkhard *et al*., 2012a). Field BAF or field BMF of a substance may be greater than what is estimated based on BCF and BMF from laboratory experiments. This is because in the laboratory tests fish are exposed either via water or via food, while under field conditions organisms are exposed to substances via all exposure routes depending on where they live (terrestrial or aquatic) and which taxa they belong to (air-breathers or water-breathers like fish).

Furthermore, apex (top) predators reflect biomagnification over the whole food chain while laboratory tests usually include only one trophic level in the biomagnification process from diet to test organism. This will ultimately lead to higher bioaccumulation in wild organisms feeding at higher trophic levels compared to the laboratory experiments for substances that are not rapidly metabolized and eliminated. The duration of exposure is expected to be substantially longer in wild animals as compared to the laboratory tests, which can play a substantial role in long-lived species such as many apex predators that accumulate hydrophobic substances over a lifetime. Bioaccumulation measurements of very hydrophobic, persistent substances that have not approached steady-state in a field study, are considered to be underestimations (Burkhard *et al*., 2012a). Despite this, wildlife monitoring data can give valuable indication of an increased bioaccumulation potential particularly for difficult to test chemicals.

Kelly *et al*. (2007) explained that apart from low rate of respiratory elimination to air, higher biomagnification of certain organic substances in air-breathing organisms is due to the greater ability to absorb and digest their diet, which is related to differences in digestive tract physiology and body temperature. In this context, field data on bioaccumulation and magnification in air-breathing biota again can provide valuable information for identifying substances that accumulate in wildlife and in human food webs (Czub and McLachlan, 2004).

Field bioaccumulation factors (BAFs/BSAFs)

If field BAF values (based on reliable information) are above the criteria for B or vB it

[31] https://ipchem.jrc.ec.europa.eu/
[32] https://www.norman-network.com/apex/

should be considered as part of the WoE approach. For comparison of a fish field BAF with the CLP criteria, BAF values should be expressed on wet weight basis for whole body with a lipid content of 5%.

Biomagnification (field BMF)

BMFs describe the increase in concentrations from prey to predator. Food chain transfer and secondary poisoning are basic concerns in relation to PBT and vPvB substances, and therefore an indication of a biomagnification potential (BMF and/or TMF > 1) can on its own be considered as a basis to conclude that a substance meets the B or vB criteria (ECHA Guidance on IR&CSA, Chapter R.11). However, absence of such a biomagnification potential cannot be used to conclude that these criteria are not fulfilled. This is because a field BMF only represents the degree of biomagnification in the specific predator/prey relationship for which it was measured. Biomagnification will vary between predator/prey relationships, so a low field BMF in one does not mean that it will be low in other predator/prey relationship. Evidence of high biomagnification in one predator/prey relationship is an indication that biomagnification may also occur in other (unmeasured) predator/prey relationships.

Substances that partition into lipids should, as far as possible, be lipid normalised to account for differences in lipid content between prey and predator. It allows for a comparison of field BMF values in a direct and objective manner. It should however be noted that non-lipophilic substances such as PFAS may bioaccumulate by other mechanisms than partitioning/binding to lipids. In such a case, another reference parameter than lipid content may be considered for normalisation, e.g. dry weight or protein content. Normalisation of measured data with respect to lipid and dry weight content is described in Guidance Document No. 32 on Biota Monitoring prepared under the Water Framework Directive 2000/60/EC (European Commission, 2014).

Trophic magnification factor (TMF)

TMF can be used to understand the biomagnification potential of a substance as it represents the average increase or decrease of concentration levels in a food web per trophic level (TL): a TMF > 1 indicates that the substance biomagnifies in the food web (i.e. concentration increases with each trophic level) and thus can on its own be considered as a basis to conclude that a substance meets the B or vB criteria; a TMF < 1 indicates that the substance undergoes trophic dilution (Weisbrod *et al*., 2009).

Currently, there is no standard procedure for studying TMFs. Hence, the conductance and sampling may vary considerably between different studies. The validity of the TMF is strongly dependent on the spatial and temporal scales over which the samples were retrieved. Assessment of TMF studies is described in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.6.

Detection of substances in wildlife

The detection of substances in wild biota (concentration or occurrence data), in particular in apex species (top predators), provides a clear indication that it has been taken up by that organism. Care should be taken if gut content and adsorption to skin contribute significantly to the measured concentration. These data could be used within a WoE approach to assess bioaccumulation of a substance case by case (depending on the

statistical power, quality and standardisation of the study). However, a detection of a substance as such does not necessarily mean that significant bioconcentration or bioaccumulation has occurred since an exposure level from the surrounding media and/or diet would be needed for such an assessment. Thus, concentrations measured in prey species or water in the surrounding media can be helpful to identify cases where bioaccumulation occurred in wild organisms. Furthermore, data from different time points as well as regions can give indications on temporal and spatial trends.

- In cases where no data is available on sources and contemporary exposure levels, a high frequency of appearance of a substance in several biota species across different compartments could indicate bioaccumulation potential. In such cases, other available evidence of the substance's bioaccumulation potential should be thoroughly examined before reaching a conclusion;
- Detection of elevated levels of a substance in biota compared to levels in their surrounding environment indicates an increased concern for bioaccumulation. Reliable monitoring data can be used as line of evidence that the substance meets the B/vB criteria.

Concentrations in biota increasing with age due to exposure and accumulation over life-time, particularly in long-lived apex species (top predators), indicate an increased concern for bioaccumulation. Finally, it is important that the quality of monitoring data (detection or quantification of a substance in biota) needs to be assessed and interpreted correctly.

4.3.3.2.3.9. Chronic toxicity tests on animals

Chronic toxicity studies with mammals (e.g. repeated dose toxicity studies, prenatal developmental toxicity studies, one/two-generation reproduction toxicity studies and carcinogenicity studies) and birds can provide information on bioaccumulation potential. The complete absence of any effects in the long-term is an indication that the substance is either non-toxic and/or that it is not bioavailable (EFSA, 2023, Section 6.5.1). Although this is only indirect information on the uptake of a substance, it may be used together with other indicators, e.g. referring to non-testing information, to conclude in a WoE approach that a substance is likely to be not B or vB (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.9).

Toxicokinetic studies in mammals can also provide useful information for assessing the bioaccumulation properties, as discussed in Section 4.3.3.2.3.10 below.

4.3.3.2.3.10. Bioaccumulation in air-breathing organisms including humans - toxicokinetics studies

Although for many substances the assessment of bioaccumulation in aquatic species is sufficient, some substances like endosulfan, beta-hexachlorocyclohexane, many perfluorinated alkyl substances or highly lipophilic substances may accumulate more than expected in air-breathing organisms and are not recognised as highly bioaccumulative if only aquatic data are used in the assessment (Kelly and Gobas, 2001, Kelly and Gobas, 2003, Czub and McLachlan, 2004). One reason may be the ability of gill-breathing organisms to eliminate substances into the water that cannot be eliminated by air-

breathing organisms by respiration as they are not volatile. For mammals and birds, bioaccumulation essentially occurs through the dietary route, associated with elimination via urination and the gastrointestinal tract, metabolism, exhalation and growth (dilution) (Kelly and Gobas, 2003, Kelly *et al.*, 2007). In this context, air-breathing organisms also include marine mammals. The main concern of bioaccumulation is that concentrations in an organism reach levels that lead to adverse effects, especially in apex predators at the top of the food chain.

Relevant assessment endpoints are the biomagnification factor (BMF), the whole-body total (or terminal) elimination rate and the biotransformation rate. Assessment of the whole-body total (or terminal) elimination rate or corresponding elimination half-life can be assessed using biomonitoring studies in humans or toxicokinetic studies with rat (e.g., OECD TG 417).

The discussion paper "Bioaccumulation assessment of air-breathing mammals" available at the ECHA website (ECHA Working group on Toxicokinetics, 2022) gives details on the scientific background.

Relevant information on toxicokinetics

OECD TG 417 'Toxicokinetics' (2010) focuses on the investigation of the biological fate of a chemical including the formation of metabolites (Phase I and II metabolites).

This complex study is commonly performed with a ^{14}C radiolabelled test substance. Single (high and low) dose with a duration of normally 7 days, repeated (low) dose studies commonly performed for at least 14 days, and so-called preconditioning repeated dose studies (14 days unlabelled test substance plus one day ^{14}C radiolabelled test substance, 14+1 day study (OECD TG 417 §57)) are possible (Hofer, 2021).

OECD TG 417 offers quite some flexibility in study design to accommodate for different regulatory needs, but it does not include guidance on how to assess accumulation. Several factors will influence the clearance rate (or the corresponding elimination half-life), thus it is not a fixed value but relates to the test conditions, rat strain, animal age (fat content), etc.

In repeated daily administration studies, clearance rates are preferably measured after steady state conditions have been reached, when the administration is stopped. The time to establish a steady state will differ depending on substance and dose. Repeated (compared to single) dosing should better ascertain a high radiolabelled substance load into peripheral organ/tissue compartments and establishment of steady state. This is because some large and/or deep organs or tissues may have slow influx rates due to little blood perfusion, unfavourable partitioning, little active or passive transport through the cell membrane or else. So-called preconditioning studies (repeated dosing with unlabelled substance followed by a single radiolabelled dose the last day (TG 417 §57) to investigate enzyme induction/inhibition, appear not appropriate for bioaccumulation assessment since the last administered radiolabelled dose (measured) will not be present at steady state conditions, and be small in comparison to repeated administration using a radiolabelled substance (Hofer *et al*., 2021).

Considerations when reviewing toxicokinetic studies

The terminal half-life is the time required for the concentration to fall by 50% during the

terminal phase studied. A field BMF of 1 can be translated into a whole-body, terminal elimination half-life of about 4 days in rat, and/or about 50 days in humans (ECHA Working group on Toxicokinetics, 2022). If the terminal elimination half-lives are assessed to be longer than these, then this is an indication that the substance has vB properties. Tissue, organ, or body fluid specific elimination half-lives may be shorter than the total (or terminal) elimination half-life and therefore should be compared to above values with care. Declining concentrations in organs/tissues is often more relevant than in blood plasma/serum, which often underrepresents elimination half-lives in organs/tissues. Elimination in blood is relevant for substances with a high blood distribution such as PFAS (Hofer *et.al*., 2021). If whole-body terminal elimination half-lives are between 2.5 and 4 days in rat, and/or 20 and 50 days in human, the assessment of the B property should be accompanied by a T assessment (PBT concern). It is noted that the derived elimination half-life thresholds for rat and human are tentative.

In conclusion, if a whole-body, terminal elimination half-life in rat is longer than 4 days in rat, and/or 50 days in humans, then this is an indication that the substance has vB properties. There may be exceptional cases where the derived elimination half-life threshold values in rats or humans cannot be used as an indicator of vB, for example where there is very low dietary absorption efficiency. Such cases require an individual assessment to determine whether the substance is vB or not.

If whole-body terminal elimination half-lives are between 2.5 and 4 days in rat, and/or 20 and 50 days in human, it is an indication that the substance has B properties for consideration in a WoE assessment.

4.3.3.2.4. Considerations for ionisable substances, surfactants, substances not partitioning to lipids

Ionisable substances

Dissociated and neutral chemical species can have markedly different bioavailability. It is therefore essential to know or estimate the pK_a to evaluate the degree of ionisation in surface waters at environmentally relevant pH (pH 4-9) and under physiological conditions (pH 3-9). When assessing an aqueous BCF test performed on an ionisable organic substance, close attention should be paid to the pH at which the study was performed and therefore which chemical species the test was performed on. BCF tests most relevant to the aquatic environment will have been performed at environmentally relevant pH (pH 4-9) at which the highest fraction of non-ionised substance was present. Further information is provided in ECHA Guidance on IR&CSA, Chapters R.7.10-3, R.7c and in OECD GD 23.

Surface active substances (surfactants)

A substance is *surface active* when it is enriched at the interface of a solution with adjacent phases (e.g. air) and when it lowers the surface tension of the medium/phase in which it is dissolved. In general, surfactants consist of an apolar and a polar moiety, which are commonly referred to as the hydrophobic tail and the hydrophilic headgroup, respectively. According to the charge of the headgroup, surfactants can be categorised as anionic, cationic, non-ionic or amphoteric (Tolls and Sijm, 2000).

It is well established that BCFs for neutral organic chemicals are positively correlated with the K_{OW}. However, K_{OW} is not a reliable parameter for predicting the BCFs of surfactants. Due to their amphiphilic properties, surfactants form aggregates in solution and have a tendency to accumulate at the interface of hydrophobic and hydrophilic phases. Surfactants can also emulsify the n-octanol/water system, making the measurement of log K_{OW} technically extremely challenging (Hodges et al., 2019).

Log K_{OW} determination is further complicated by the fact that surfactants may form micelles in water (i.e. not dissolving exclusively as single molecules), so their 'solubility' cannot be properly defined and is hard to measure. The maximum monomolecular solubility is defined as the critical micelle concentration (CMC), with formation of micelles occurring above this concentration. Although CMC is a commonly used surrogate for water solubility, CMC is not an appropriate solubility threshold, as micelles themselves are water-soluble (Hodges et al., 2019). This can cause data interpretation problems for fish BCF tests, since the actual dissolved concentration of surfactant that the fish were exposed to may be uncertain.

Measured membrane lipid-water partitioning/distribution ratios, K_{MLW}/D_{MLW} (or K_{mw}), could be suitable to predict the bioaccumulation potential of surfactants. (Droge, et al., 2021). Further information is provided in Appendix R.7.10 3 of ECHA Guidance on IR&CSA, Chapter R.7c.

Organic substances that do not partition to lipid

Bioconcentration is generally considered as a partitioning process between water and lipid, and other distribution compartments in the organism can usually be neglected (the water fraction may play a role for water-soluble substances, de Wolf *et al.*, 1994). However, proteins have been postulated as a third distribution compartment contributing to bioconcentration (SCHER, 2005), and may be important for certain types of substances (e.g. perfluorosulphonates, organometallic compounds such as alkyl- or glutathione-compounds, for instance methyl mercury, methyl arsenic, etc.). Evidence for such a role may be available from mammalian toxicokinetics studies.

Protein binding in biological systems performs a number of functions (e.g. receptor binding to activate and/or provoke an effect, binding for a catalytical reaction with enzymes, binding to carrier-proteins to make transport possible, binding to obtain/sustain high local concentrations above water solubility, such as oxygen binding to haemoglobin, etc.). In some circumstances, binding may lead to much higher local concentrations of the ligand than in the surrounding environment.

Nevertheless, the picture may be more complicated because the process is not necessarily driven purely by partitioning (binding sites may become saturated and binding could be either reversible or irreversible). Indeed, it has been postulated that measured BCFs may be concentration dependant due to protein binding. In other words, bioconcentration is limited by the number of protein binding sites rather than by lipid solubility and partitioning. Further work is needed to conceptualise how protein binding might give rise to food chain transfer across trophic levels, and assess its relative contribution compared with other (lipids and water) distribution mechanisms.

In the absence of such studies, elimination studies can be useful for comparing half-lives

of substances that may accumulate via proteins with those for other substances that are known to be bioaccumulative.

4.3.3.2.5. Databases with available bioaccumulation data

The ECHA REACH database includes public and disseminated information on bioaccumulation studies, from the registration dossiers, submitted by companies to ECHA in the framework of the REACH Regulation. Data is available on ECHA's dissemination website and the OECD QSAR Toolbox.

The Global Portal to Information on Chemical Substances (eChemPortal) provides free public access to information on properties of chemicals, and direct links to collections of information prepared for government chemical programmes at national, regional, and international levels. Access to information on existing chemicals, new industrial chemicals, pesticides and biocides is provided. eChemPortal also makes available national/regional classification results according to national / regional hazard classification schemes or according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

The Japanese National Institute of Technology and Evaluation (NITE) database collates experimental bioaccumulation data. NITE bioaccumulation data are also available via the OECD QSAR Toolbox as 'Bioconcentration and log K_{OW} NITE' database. Experimental BCF data in REACH dossiers are available in the OECD QSAR Toolbox in a normalised format as 'REACH Bioaccumulation database (normalised)'. This database is based on data up to the year 2017.

Further bioaccumulation databases available via the OECD QSAR Toolbox are:

'Bioaccumulation Canada' is an empirical database of BCF values for non-mammalian aquatic organisms (algae, invertebrates and fish) for assessing the bioaccumulation potential of organic chemicals included in the Canadian Domestic Substance List (DSL). It has been implemented in the QSAR Toolbox in 2008.

'Bioaccumulation fish CEFIC LRI' contains experimental data for fish BCF values, which has been implemented in the QSAR Toolbox in 2008. The database is also available via[33].

A further source of data is ECOTOX Knowledgebase available under ECOTOX | Home (epa.gov). ECOTOX is a comprehensive Knowledgebase providing single chemical environmental toxicity data on aquatic and terrestrial species, also including data on bioaccumulation.

The following scientific publications contain fish bioaccumulation databases including review of data:

- Jon A Arnot and Cristina L Quinn (2015) Development and Evaluation of a Database of Dietary Bioaccumulation Test Data for Organic Chemicals in Fish. *Environmental Science & Technology* **2015** *49* (8), 4783-4796. DOI: 10.1021/es506251q

[33] http://ambit.sourceforge.net/euras/

- Jon A Arnot and Frank APC Gobas (2006) A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Reviews. 257-297.

4.3.3.2.6. Indicators of B or vB properties

4.3.3.2.6.1. Octanol-water partitioning coefficient K_{OW}

In general, the potential of an organic substance to bioaccumulate is primarily related to the lipophilicity of the substance. A surrogate measure of lipophilicity is the n-octanol/water partition coefficient (K_{OW}) which, for lipophilic non-ionised and non-surface active organic substances, undergoing minimal metabolism or biotransformation within the organism, is correlated with the bioconcentration factor. Therefore, K_{OW} is often used for estimating the bioconcentration of non-ionised organic substances, based on the empirical relationship between log BCF and log K_{OW} (current Guidance on aquatic hazards, Section 4.1.3.2.3.3). Lipid normalisation of bioaccumulation metrics is often done to allow comparison of values in an objective manner.

For some groups of substances, such as organometals, ionisable substances and surface active substances, log K_{OW} is not a valid descriptor for assessing the bioaccumulation potential (Armitage et al., 2017, Hodges et al., 2019). Information on bioaccumulation of such substances should therefore take account of other descriptors or mechanisms than hydrophobicity. Guidance on consideration for bioaccumulation assessment of ionisable and surface active substances is given in ECHA Guidance on IR&CSA, Chapter R.7.10-3.

For neutral organic substances, bioaccumulation is most often driven by partitioning to storage lipid. In these cases, a log K_{OW} greater than 4.5 is used as a screening criterion for aquatic organisms, and a log K_{OW} greater than 2 together with a log K_{OA} greater than 5 as screening criteria for air-breathing organisms. If the log K_{OW} is less than 2, the substance can normally be regarded as not fulfilling the B/vB criteria. If the substance has a log K_{OW} between 2 and 4.5, but log K_{OA} is below 5, then it can be expected that the substance is neither hydrophobic enough to bioaccumulate in aquatic species, nor that it is bioaccumulating in air-breathing species, because it can be eliminated rapidly enough by exhalation. Guidance on the derivation of log K_{OW} is given in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.10 and Appendix R.11-5. For substances with very low solubility specific methods exist to derive a K_{OW}, e.g. OECD TG 123 slow stirring method. However, this method is not always applicable due to experimental constraints caused e.g. by the low solubility and the available analytical methods.

The log K_{OW} generated by the HPLC-method according to OECD TG 117 (OECD, 2022) is an estimation method that is equivalent to theoretical models using descriptive information (like chemical structure, i.e. QSARs) to estimate the log K_{OW}. These two methods are very close to each other in predictivity. QSAR gave very different results than HPLC for ionised surfactants. For sufficiently soluble non-polar substances HPLC results are generally within 1 log unit, with the applicability domain in the range of log K_{OW} 0-6. For the extremes (log K_{OW} <0 or >6) it is concluded that the molecular fragmental constants method (QSAR) is more trustworthy. The formation of intramolecular hydrogen bonds may impact the log K_{OW} by several orders of magnitude. Since EPI Suite does not consider the potential formation of intramolecular hydrogen bonds, the estimates for such substances are less

reliable (see e.g. Wang *et* al., 2011, Buser *et al.,* 2013).

When no experimental data of high quality are available, valid QSAR) results for log K_{OW} may be useful. Examples of freely available (Q)SAR software programs that include models for the prediction of log K_{OW} are EPISuite[34], OECD QSAR Toolbox and VEGA.

For some groups of substances, such as **organometals, ionisable substances and surface active substances**, log K_{OW} is not a valid descriptor for assessing the bioaccumulation potential (Armitage *et al*., 2017, Hodges *et al.*, 2019). Information on bioaccumulation of such substances should therefore take account of other descriptors or mechanisms than hydrophobicity.

Guidance on consideration for bioaccumulation assessment of ionisable and surface active substances is given in Appendix R.7.10 3 of ECHA Guidance on IR&CSA, Chapter R.7c. Furthermore, **specific binding to proteins** instead of lipids might result in an erroneously low BCF value if this value is estimated from log K_{OW}. Per- and polyfluoroalkyl substances (PFASs) are examples of such partitioning behaviour, of which perfluorooctane sulphonic acid (PFOS) is a well-known example (e.g. Kelly *et al*., 2009). Guidance on consideration for bioaccumulation assessment of organic substances that do not partition to lipid is given in Appendix R.7.10 3 of ECHA Guidance on IR&CSA, Chapter R.7c.

For organic substances, experimentally derived high-quality K_{OW} values are preferred over other determinations of K_{OW}. If multiple log K_{OW} data are available for the same substance, the reasons for any differences should be assessed before selecting a value. Generally, the most conservative valid value should take precedence.

4.3.3.2.6.2. Octanol-air partitioning coefficient K_{OA}

An indication of substances that might bioaccumulate or biomagnify in air-breathing organisms, is a combination of the octanol-water partition coefficient K_{OW} and octanol-air partition coefficient K_{OA} (Gobas *et al*., 2003). An efficiently absorbed, non-biotransformed neutral organic substance with a log $K_{OA} \geq 5$ in combination with a log $K_{OW} \geq 2$ has the potential to biomagnify in vertebrates of the terrestrial food chains and air-breathing marine wildlife as well as in humans, while the substances with log $K_{OW} < 2$ have a reduced gastrointestinal uptake or are efficiently excreted in urine, and therefore do not biomagnify even though their K_{OA} is high (Armitage and Gobas, 2007, Kelly *et al*., 2007, Gobas *et al*., 2009, McLachlan *et al*., 2011, Goss *et al.*, 2013).

Baskaran *et al*. (2021a,b) have compiled all K_{OA} values reported in the published literature. Their dataset includes more than 2500 experimentally derived values and more than 10 000 estimated values for K_{OA}, in total covering over 1500 distinct molecules. A range of techniques can be used to predict K_{OA} of organic substances and are described in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.8. K_{OA} can furthermore be calculated reliably using LFERs (Baskaran *et al.*, 2021b) and OPERA[35] (Mansouri *et al.*, 2018). Another method is based on K_{OW} and Henry's Law Constant (H) (Meylan and Howard, 2005). In case H is also unavailable, H can be estimated based on water solubility (WS), vapour pressure (VP), and molecular weight (MW) (see equation R.16-4 of ECHA, 2016b).

[34] https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
[35] https://github.com/NIEHS/OPERA

Sander (2015) published a compilation of 17350 Henry's law constants for 4632 organic and inorganic species in water, collected from 689 references, with further information made available online.

4.3.3.2.6.3. (Q)SAR models to predict BCF

BCF-QSARs and other computer models may be used to address aquatic bioconcentration, provided that the model is appropriate for the chemical class. However, assessment of B or vB properties according to CLP (4.3.2.3.2.) clearly prefers experimental BCF data where available, and QSAR BCF data can only be considered as part of a broader WoE approach. As for other endpoints derived using QSARs, careful attention should be paid to the validity of the models and predictions, which can be assessed against the newly established principles for the assessment of QSAR predictons and results presented in the OECD QSAR assessment framework documents (OECD, 2023). Further information can be found in the Guidance on QSARs and grouping of chemicals, Chapter R.6[36] and in ECHA Practical Guide "How to use and report (Q)SARs"[37].

QSAR BCFs derived using experimental input data (e.g., log K_{OW} and intrinsic clearance data from OECD TG 319A and B) should generally be given greater weight than those where the log K_{OW} and other source data is calculated. Examples of freely available QSAR software programs that include models for the prediction of log K_{OW} and BCF are EPISuite, OECD (Q)SAR Toolbox and VEGA.

A reliable BCF prediction should not be used alone to decide whether a substance meets the CLP B/vB criteria but can be considered in the WoE assessment.

4.3.3.2.6.4. Biomimetic extraction procedures

Biomimetic extraction procedures with semi-permeable membrane devices (SPMD) and solid phase micro extraction (SPME) are used to mimic the way organisms extract substances from water. These types of methods are at the moment only well described for hydrophobic substances. For more detailed information, see Section R.7.10.3.1 in ECHA Guidance on IR&CSA.

4.3.3.2.6.5. Molecular size and octanol solubility

If average molecular size, log K_{OW}, and octanol solubility are above or below certain values (as described below), they may indicate a limited bioaccumulation potential due to the lack of uptake (ECHA Guidance on IR&CSA, Chapter R.11).

However, these parameters should never be used on their own to conclude that a substance is not bioaccumulative. The information from these parameters should be accompanied by other information confirming the low uptake of the substance in living

[36] https://echa.europa.eu/documents/10162/17224/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9?t=1322594777272
[37] https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf/407dff11-aa4a-4eef-a1ce-9300f8460099

organisms, e.g. by read-across with similar substances, absence of toxicity or lack of uptake in toxicokinetic studies with mammals. Evidence of significant uptake in fish or mammals after long-term exposure implies that the indicators above will likely underestimate the real bioaccumulative potential of the substance and thus these indicator values should be considered unreliable for assessing the bioaccumulation potential.

1. an average maximum diameter (Dmax aver) of greater than 1.7 nm
2. octanol-water partition coefficient as log10 (log K_{OW}) > 10 (calculated value, preferably by several estimation programs, for substances for which log K_{OW} can be calculated and the model is reliable)
3. a measured octanol solubility (mg/L) < 0.002 mmol/L × MW (g/mol) (without observed toxicity or other indicators of bioaccumulation)

Indicator 1. recommended here as non-testing information influences uptake and distribution of substances. The log K_{OW} (2.) is a general indicator for uptake, distribution and excretion whereas the octanol solubility (3.) reflects the potential for mass storage, which might further prevent uptake in significant amounts in the organism.

It is very important to note that the calculated log K_{OW} values above 10 are used simply to indicate a degree of hydrophobicity that is extreme. Such values should not be used in a quantitative manner.

The supplementary information to confirm this limited uptake may comprise data from a chronic toxicity study with mammals (≥ 90 days, showing no toxicity), a toxicokinetic study with mammals or birds, a bioconcentration study with invertebrates, or reliable read-across from a structurally similar compound (all showing no uptake). These types of information should be examined in a WoE approach together with the non-testing information on the substance to conclude whether the B or vB criteria are met. Evidence of significant uptake of a substance in vertebrates after prolonged exposure is a contra-indication to using the above indicators.

4.3.3.3. Mobility assessment

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.4.2.3.2. The following information shall be considered for the assessment of M or vM properties:

(a) results from adsorption/desorption testing;

(b) other information, such as information from leaching, modelling or monitoring studies, provided that its suitability and reliability can be reasonably demonstrated.

Annex I: 4.4.2.4.2. In applying the WoE determination, the following information, in addition to the information referred to in Sections … 4.4.2.3.2 … shall be considered as part of the scientific assessment of the information relevant for the … M, vM … properties:

…(b) Information relevant for the M or vM properties:

(i) Organic carbon to water partition coefficient (K_{OC}) estimated by well-developed and reliable (Q)SAR models;
(ii) Other information, provided that its suitability and reliability can be reasonably demonstrated.

CLP defines the concern posed by PMT substances as a result of the combination of their persistence, mobility and toxicity, and the concern posed by vPvM substances as a result of both their high persistence and high mobility in the environment. Due to the combination of these intrinsic properties,such persistent and mobile substances may find their way into water bodies and ultimately into drinking water, as wastewater treatment processes may only partially remove them. CLP relates the criteria for M/vM to the log K_{OC} that reflects the intrinsic ability of a substance to be adsorbed on the organic fraction of environmental matrices such as soil, sludge, sediment particles and dissolved organic matter, and is therefore inversely related to the substance's potential of entering water bodies. Once reliable and relevant information is available resulting in a log *K*oc below the regulatory threshold(s) set for M and/or vM, the substance can be concluded as fulfilling the CLP criterion for M and/or vM, respectively.

Adsorption refers to the adhesion and binding capacity of a substance to a surface, while desorption refers to the release of a substance from a surface. The potential for adsorption/desorption of a chemical is an important environmental fate parameter and an indicator of partitioning of the substance in the different environmental compartments. The following Sections will only further elaborate on adsorption and the corresponding distribution coefficient and not to desorption. In general, the capacity of organic substances to adsorb to solid organic matrices can be characterised by the organic carbon-water partition coefficient (K_{OC}, cm^3/g). For inonisable substances, other matrices (e.g clay particles) may also play a role on the adsorption of a substance (4.3.3.3.7). The K_{OC} value of a substance is known to be inversely related to the mobility in the environment (Arp and Hale, 2019, Arp and Hale, 2023), it is related to the potential for sub-surface transport (e.g. in river bank filtration) and for entering ground and surface water bodies.

Different experimental and non-experimental methods are currently available for obtaining the *K*oc value of a substance from adsorption testing. The lowest available and reliable

numerical log *K*oc value within the environmentally relevant pH range 4 to 9 should directly be compared to the M/vM criteria. Other approaches include soil leaching studies, lysimeter studies, other modelling/ computational approaches, as well as analysis of monitoring data. Further, it must be noted that simulation modelling approaches (e.g. for estimating the exposure of groundwater or surface water) include use, emission and exposure elements. In these approaches *K*oc often constitutes an important input parameter for such simulation models. Therefore, the results from such approaches are not suitable on their own for hazard identification and hazard assessment and cannot be compared to the CLP criteria.

The following Sections specify the type of information that can be considered for the assessment of M/vM properties. Section 4.3.3.6 of this Guidance describes the WoE approach for concluding on these properties.

4.3.3.3.1. Experimental data on adsorption deriving a K_{OC} value

A description of the relevant studies is provided below for supporting the data holder in the collection and interpretation of such data to be used for classification purposes, with some special considerations regarding the ionisable substances presented in Section 4.3.3.3.7 of this Guidance. Some of methods in this Section include both experimental and estimation elements to a derive a K_{OC}.

OECD TG 106 (Adsorption - Desorption Using a Batch Equilibrium Method)

The OECD TG 106 is designed to evaluate the sorption of a chemical on different soil types with a varying range of organic carbon content, clay content, soil texture and pH.It is used to obtain sorption kinetics and isotherms for different soil types that are used to determine equilibrium adsorption coefficients on the selected soils as a function of different soil characteristics, such as organic carbon content, pH, clay content, soil texture, etc. As described in this document, the test comprises of three testing tiers:

Tier 1 of the test method includes a preliminary study to determine the soil/solution ratio, the equilibration time for adsorption and the amount of test substance adsorbed at equilibrium, as well as the adsorption of the test substance on the test vessels' surfaces and the test substance stability.

Tier 2 investigates the adsorption kinetics at one concentration of the test substance. The test is performed in five different soil types and the respective distribution coefficients K_d and K_{OC} are calculated. K_d is the linear adsorption coefficient which describes the distribution of a substance between a solid and aqueous matrix after equilibration and is considered to be independent of the substance concentration. After equilibrium is reached in tier 2 testing, the water (C_{Water}) and/or the soil phase (C_{Soil}) concentrations and the distribution coefficient (K_d) is calculated as the ratio of the concentration in the soil to that in water at adsorption equilibrium.

$$Kd = \frac{C_{Soil}}{C_{Water}} \ (cm^3\ g^{-1})$$

C_{Soil}: concentration of the substance adsorbed on the soil at adsorption equilibrium (µg/ g dry weight);

C_{Water}: concentration of the substance in the aqueous phase at adsorption equilibrium (µg/ cm^3).

In order to derive 'comparative' values across different soil types with varying organic carbon content, K_d can further be normalized to the fraction of organic carbon in the soil samples, by use of the following equation:

$K_{OC} = Kd \times \frac{100}{foc}$ $(cm^3\ g^{-1})$, where f_{oc} is the soil organic carbon content (%)

The K_d derived K_{OC} is appropriate for comparing with the CLP criteria.

Tier 3 investigates the adsorption isotherms and the desorption kinetics/desorption isotherms of the substance. The adsorption isotherms describe the relationship of the amount of the substance adsorbed on the soil and the concentration of substance in the solution when equilibrium has been reached at constant temperature. Tier 3 is also used to investigate desorption by means of desorption kinetics/desorption isotherms. The Freundlich adsorption isotherm equation is an empirical model that describes the asorption isotherm of a substance as:

$C_{Soil} = K_F \cdot C_{Water}^{\frac{1}{n}}$

K_F is the Freudlich adsorption coefficient, an affinity-capacity coefficient indicating the adsorption capacity of the adsorbent. Its dimension is $cm^3\ g^{-1}$ only if 1/n = 1; in all other cases, the slope 1/n is introduced in the dimension of K_F ($\mu g^{1-1/n}$ $(cm^3)^{1/n}\ g^{-1}$). The Freundlich adsorption coefficient (K_F) derived from the sorption isotherms is equal to the distribution coefficient K_d only when the Freundlich exponent 1/n is equal to 1.

n is an exponent reflecting deviation from linearity of the relationship indicating the adsorption intensity (Pignatello, 2023). The value of 1/*n* is typically below 1 (typically ranges between 0.7-1.0.) and may vary depending on the range of concentrations over which it is measured (Pignatello, 2023). Such values indicate that sorption data are slightly nonlinear and the affinity of the solute for the adsorbent surface deminishes with increasing solute concentration. In general when 1/n is below 1 the adsorption to soil is considered as favorable and can be observed for nonpolar substances with moderate hydrophobicity at low concentrations. When 1/n is above 1 the adsorption to soil is considered unfavorable due to possible competition with water for the available adsorption sites.

As the Freundlich adsorption coefficient (K_F) is dependent on the concentration of the substance, it also finds particular use as an input parameter in risk assessment modelling.

The K_F as noted above is a measure of the adsorption capacity for the solid phase and it is concentration dependent. In the same manner as the K_d the K_F can be normalised to the oganic carbon content of the soil (K_{FOC}). However, as the K_d and K_F are not equal coefficients, the calculated K_{FOC} cannot replace the K_{oc} for comparing with the CLP mobility criteria (that refer only to *K*oc) nor can it be used for deriving a K_{oc}.

The OECD TG 106 does not differentiate between physical and chemical adsorption and specific attention should be paid to poorly water soluble (water solubility below 0.1 mg/L), highly charged and volatile substances (see OECD TG 106 for more details).

Soil selection and characterisation are important steps in the adsorption testing. Specific guidance on soil selection is provided in the OECD TG 106. As specified therein, the selected soils cover soil types from temperate geographical zones, but inclusion of soils from other geographical zones is also possible. The selected soils should be characterised in terms of organic carbon content, clay content, soil texture and pH, as these parameters are considered to be largely responsible for the adsorptive capacity of non-ionisable organic substances. For ionisable substances that are present in their ionised form under environmental relevant pH (4-9), further information on the cation-exchange capacity (CEC) of the soil and the clay content and mineralogy should be provided. The specific considerations regarding the assessment of the ionisable substances are presented in the next Section of this Guidance (4.3.3.3.7).

EFSA has published the outcome of a pesticide peer review meeting on issues to be considered by evaluators during the assessment of OECD TG 106 soil batch adsorption studies[38]. The document constitutes a checklist that was developed in order to ensure consistency and increase the quality of the undertaken regulatory assessments, but also streamline guidelines for conducting the study and clarify some concepts when applying the OECD TG 106.

OECD TG 121 (Estimation of the Adsorption Coefficient ($_{KOC}$) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC))

The OECD TG 121 is an alternative approach that can derive K_{OC} values from indirect experimental measurements. It may be used when the structure of the tested chemical is similar to at least one of the standard substances with well-known K_{OC} values reported in the Appendix of the test guideline. In the absence of such data, appropriate alternative calibration substances can be selected by the data holder, if justified. OECD TG 121 is most applicable for substances that are neutral between pH 4-9, namely that are not ionisable, or have an ionic charge within this pH range.

The method derives partition coefficients from the retention times measured on a specific HPLC column. The time it takes for the target substance to travel through the HPLC column (retention time) is determined by its partitioning between the stationary phase of the column (cyanopropyl stationary phase) and the mobile phase (liquid, e.g. water and methanol). The retention time is then compared to that of reference substances with known experimentally-derived K_{OC} values and a K_{OC} value for the target substance is derived. As already reported, it is important that the reference substances used for calibration are structurally similar to the test substance and address the same mechanisms of adsorption.

This method is designed for soils and sewage sludge, it can determine log K_{OC} values between 1.5 and 5 and may also be used for UVCBs, volatile, poorly water soluble and substances with a high affinity to the surface of incubation systems (OECD TG 121). Moreover, it may prove useful for fast degrading substances (EC, 2002), even if there is no real concern for the PBT or PMT properties of such substances. However, as this is an estimation method with a limited set of reference substances, its use is not generally

[38] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2017.EN-1326

recommended (see also SCP/KOC/002 Opinion, 2002)[39]. Further, the method may not be applicable to strong acids and bases, to surface-active substances, to chemicals that react either with the mobile or the stationary phase and to those that interact in a specific way with inorganic components (for example, formation of cluster complexes with clay minerals).

OECD TG 312 (Soil leaching columns)

The OECD TG 312 is based on soil column chromatography in disturbed soil and it describes a method to determine the potential for soil leaching of both test substance and its transformation products. K_{OC} values may also be obtained by use of different estimation techniques. For example, it can be estimated by using average leaching distance or established correlations between relative mobility factors (RMF) and K_{OC} values for reference substances[40].

The test substance is introduced into soil columns of different soil properties and the leachate is collected after application of artificial rain. At the end of the leaching process, the soil is removed from the soil column for further analysis. The leaching of the substance can be evaluated in comparison with a reference substance on a relative scale using relative RMFs. The test is not applicable to volatile substances that might be lost under the experimental conditions of this test.

As with the OECD TG 106, selection of soils with varying pH, OC, soil texture, etc. must be tested in order to evaluate the soil leaching. OECD TG 312 usually derives an amount (measured as a percentage of the one initially applied) of the test substance and its transformation products as a percentage of soil depths. In other words, these types of experiments are used to determine the penetration depth, defined usually as the soil depth that half of the applied substance mass can be found. Additionally, the Mobility Classes as defined in Annex 3 of OECD TG 312 derived by the RMF are not directly comparable to the M/vM criteria under CLP and, thus, cannot be used as such. However, estimated *K*oc data based on the RMFs can be used within the WoE.

In different regulatory regimes, such studies have been used to decide whether further field testing needs to be carried out but not to predict soil leaching behaviour under field conditions. For example, under the PPPR, results from soil leaching column studies have been used in risk assessments, in a WoE approach for additional investigations of the pesticidal mobility within the overall risk assessment (Sanco, 2014). This is usually done in combination with a scenario modelling that also accounts for the use patterns and refers mainly to pesticidal-active substances with a low adsorption potential (namely K_{OC} below 25) and when no reliable K_{OC} can be obtained by OECD TG 106 (EC, 2002).

Soil thin and thick layer chromatography (TLC)

Soil thin and thick layer chromatography (TLC) studies have also been conducted in the past to observe and measure the soil leaching of labelled pesticides through different soil types (Sánchez-Camazano *et al*., 1996, Kumar *et al*. 2013). In these studies, chromatographic techniques are used to separate the substances/compounds/ constituents in the mixture and simulate the pesticide movement by the determination of

[39] https://food.ec.europa.eu/system/files/2020-12/sci-com_scp_out128_ppp_en.pdf
[40] https://www.oecd-ilibrary.org/docserver/9789264070561-en.pdf?expires=1691490605&id=id&accname=guest&checksum=F04D799468933A0FFB44B22ABD4AB1BC

a retardation/mobility factor (R_F). This factor is the ratio between the elution distance of the substance and the elution distance of the developing solvent (Mensink *et al*., 2008). A K_{OC} value can then be estimated by established correlations between retardation/mobility factors (R_F) and K_{OC} for reference substances.

Similarly to the soil column leaching studies, these studies might underestimate adsorption due to difficulties in the exact determination of the relative rates of movement, handling of the soil, possible influence of the support material, and a probable non-equilibrium situation (Mensink *et al*., 2008). Additional argumentation on the high uncertainty and potential underestimation of adsorption in soil TLC studies can be found in the EC (2002) opinion. Finally, application to volatile substances is problematic and any losses due to volatilisation need to be fully accounted for.

4.3.3.3.2. Other experimental information deriving a K_{OC} value

Field and lysimeter studies

The potential of substances for soil leaching to the groundwater may be provided by lysimeter and field studies. Verschoor *et al*. (2001) drafted some guidance on the interpretation and use of such studies for pesticidal-active substances. These studies usually resemble the environmental and field conditions better compared to lab studies. They are mostly performed under natural conditions, in a relatively large scale and over longer periods of time. Moreover, they integrate a higher number of environmental processes and interactions than laboratory soil column leaching studies. Verschoor *et al*. (2001) and references therein reported an extensive list of quality parameters that need to be reported and met in order for a lysimeter or a field study to be regarded as reliable. These include the soil type/ texture, information on the analytical method and leachate, meteorological data, mas balance and other application-specific parameters. For the purpose of classification and labelling, their suitability, reliability and relevance would need to be demonstrated.

The risk of soil leaching to the groundwater of the test substance and its metabolites is determined by the derivation of their concentrations in the groundwater and by comparing with the respective regulatory criteria of each country. Subsequently, the results from the lysimeter or field measurements are compared to those of a simulation model (for example, FOCUS PEARL, FOCUS PELMO, etc.) that allow an extrapolation to a wider range of relevant conditions and intended substance uses.

Importantly, inverse modelling techniques utilising the data from the field and leaching studies have been extensively used for pesticides to refine input parameters such as K_{OC} and degradation half-lives of exposure models like FOCUS (Mertens *et al*. 2009, Sanco, 2014). These techniques entail entering the output from soil columns, lysimeter or field studies into an exposure model, the calibration of the model output with experimental data that is then used to calculate new values for the input parameters such as K_{OC}. Sanco 2014 details the use of inverse modelling procedures for leaching assessment of pesticidal-active substances and their metabolites to groundwater in the EU. Often, non extractable residues are taken into account both in the degradation rate estimation and the sorption partition coefficient. Double-counting of the loss via the treatment of non- extractable residues data should be avoided in this type of modeling.

Both field leaching and lysimeter studies are dose-dependent and related to exposure, namely they are application scenario-specific and are introducing exposure considerations relevant to a local risk but not to an intrinsic hazard assessment. Lysimeter studies provide information on a single location and soil type which cannot cover the range of environmental conditions in the European union. They also exhibit other limitations that currently restrict their use for the purposes of hazard classification. For example, the lack of standardisation (each test needing individual set-ups), the fact that they are time consuming, affected by the local environmental conditions (Hansen *et al*. 2000) and unclarity on whether they can sufficiently represent the conditions that need to be covered, most importantly the breakthrough in river bank filtration. Thus, use of inverse modelling carries the cumulative uncertainty and assumptions of each individual model input parameter, as well as those of the associated experimental methods, resulting to their results needing to be given lower weight within the overall WoE.

However lysimeter studies may be used for regulatory purposes in order to identify additional transformation products that may have possibly not been detected in a soil simulation test according to OECD TG 307 and that may leach to the groundwater (see also Section 4.3.3.1.2.2). This is the current practice regarding pesticidal-active substance approvals according to Regulation 1107/2009, whereas all metabolites found in lysimeter studies at annual average concentrations exceeding 0.1 µg/L in the leachate need to be considered in the groundwater risk assessment.

OECD TG 22, Guidance Document for the Performance of Out-door Monolith Lysimeter Studies

Monolith lysimeters have been used in research with crop protection products for years, as one of the tools for obtaining information on the fate and behaviour of a chemical in an undisturbed soil under outdoor conditions[41]. With monolith lysimeters, mass fluxes of water and chemicals can be monitored and chemical distribution and transformation products can also be determined. The method is applicable to substances for which an analytical method with suitable accuracy and sensitivity is available and resemble field conditions closer than other laboratory studies. However, the studies are dose-dependent, they cannot fully control the varying climatic conditions and they are not suitable to all plant and soil types. OECD TG 22, finally, proposes that for a better interpretation of results from such studies, "*it would be useful to conduct studies on adsorption/desorption or soil column leaching and on aerobic transformation in the same soil as found in the top layer of the lysimeter*". Consequently, the results need to be evaluated according to the considerations regarding lysimeter and field studies.

4.3.3.3.3. Data from estimation methods (e.g QSARs) deriving a K_{OC} value

A (Q)SAR prediction for the K_{OC} value of a test substance may be used for the purpose of hazard classification. The conditions discussed earlier in the Guidance (4.3.3 (ii)) must be fulfilled, namely the ones related to the reliability and applicability domain, documentation, molecular type/ functional/ chemical groups present, etc. In every case, it needs to be verified that both, the QSAR model and the estimated *K*oc value are valid.

[41] https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282000%298&doclanguage=en

When a measured *K*oc value of a test substance based on either OECD TG 106 or other experimental methods is not available, but a measured *K*ow (octanol-water partition coefficient) value of the test substance is available, the simplest and most widely occurring approach on estimating a K_{OC} value is based on the linear relationship between the *K*oc and the *K*ow. One of the first attempts to empirically regress this relationship was from Karickhoff (1979) who, based on experiments where K_{OC} values were measured for different soil organic contents and chemicals of different octanol-water partition coefficients, proposed the following empirical equation:

$$K_{OC} = 0.41\ K_{OW}$$

This equation is applicable to neutral, non-surface active organic substances for which their environmental sorption is attributed practically entirely to organic matter, where the sorption mechanism is hydrophobic binding. For charged substances, for which there is an electrostatic component to their sorption behavior, the equation is not applicable, as the octanol molecule is uncharged in contrast to many functionalities on natural organic matter. More considerations on ionisables can be found in Section 4.3.3.3.7.

In more recent years, more sophisticated models based on the linear regression between the two partition coefficients have been developed for a variety of substances (work of Abraham and colleagues, Sabljić *et al*., 1985, references in ECETOC, 2021). Computational methods have also been developed in the absence of available physicochemical data, namely by knowledge of only molecular structure. One example is the use of molecular connectivity indices (MCI) that are associating molecular structure information (for example, molecular size, volume, branching, etc.) to K_{OC} in terms of mathematical equations. Such *in silico* approaches of estimating organic carbon – water partition coefficient (ECB, 2003) include EPISuite[42] (US EPA 2012), the OECD QSAR Toolbox, OPERA[43], QSARINS[44] and several LFER models (for example, Bronner and Goss, 2011b). Further information on the experimental derivation of the octanol-water partition coefficient can be found in several related OECD guidelines.

4.3.3.3.4. Monitoring data

The mere presence (or absence) of a substance in any given underground or surface water body cannot in itself demonstrate that a substance is mobile or not. Quantifying any substance by use of a monitoring campaign is dependent on a range of parameters, such as presence and proximity to emission sources, exposure and route of entry into the environment, local application and other conditions (for example, meteorology, geography/ topography), environmental fate, transport and inter-media distribution processes, analytical and sampling considerations/ short-comings, etc. Nevertheless, in accordance with the assessment regarding Persistence and Bioaccumulation, the presence of a substance in a remote and pristine environment may be used within the overall WoE as an additional indication for mobility. Additionally, temporal trends within the same

[42] https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
[43] https://github.com/kmansouri/OPERA
[44] https://dunant.dista.uninsubria.it/qsar/?page_id=565

monitored media may prove increasingly important. In order to consider such data, there needs to be sufficient understanding on the substance distribution and transport behaviour and the uncertainties in the monitoring data must be adequately addressed (ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.6).

4.3.3.3.5. Other estimation approaches, including modelling not deriving a K_{OC} value

Octanol-water distribution coefficient (D_{OW})

In the absence of a K_{OC} value as an assessment criterion for M/vM, the German Environment Agency (UBA, Arp and Hale, 2023) recommended screening for mobility as a means of deriving indications of a substance's M/vM properties. UBA compared their approach with the way screening information under REACH Annex XIII has been used, namely to evaluate whether a log K_{OC} value must be generated, under the appropriate regulatory contexts. The proposed screening parameter was an experimentally derived or estimated octanol-water partition coefficient (K_{OW}) for non-ionisable substances and an experimentally derived or estimated octanol-water distribution coefficient (D_{OW}) for ionisable substances.

The lowest value D_{OW} with the environmentally relevant pH range of 4-9 was proposed to be used at a screening assessment level only and can be derived by knowledge of K_{OW} and the dissociation constant (pK_a):

$D_{OW} = (1/(1+10^{(pH - pKa)}) K_{OW}$ (for monoprotic acids)

$D_{OW} = (1 - 1/(1+10^{(pH - pKa)}) K_{OW}$ (for monoprotic bases)

A log D_{OW} value below 4.5, in line with the respective screening paramet for bioaccumulation for log K_{OW} (Neumann and Schliebner, 2019), was proposed as screening information for the mobility assessment. For neutral and non-ionisable substances, the D_{OW} has the same value as the octanol-water partition coefficient (K_{OW}). For ionisable substances, this screening approach for mobility still considers pH and pK_a corrected octanol partitioning. Experimentally-derived pK_a values should normally prevail any QSAR estimated values, but, in their absence, can be estimated by use of QSARs (ChemAxon software or ACD/Labs).

It needs to be taken into consideration that D_{OW} considers the solubility of the charged and neutral species in pure water and octanol at a specific pH and octanol is used as the surrogate for the soil matrix and all molecular interactions. For ionisable substances, octanol is not a good surrogate for the soil matrix, as octanol does not contain charged groups (more discussion to follow later in the Guidance). Additionally, neither the pH dependence nor the ionic interactions between the solute and the soil matrix are accounted for in this approach (Sigmund *et al*., 2022). Consequently, this may lead to severe underestimation of *K*oc for ionisable substances. For these reasons, the D_{OW} approach may be followed in a screening level (Arp and Hale, 2023, Sigmund *et al*., 2022) and assessed

together with any other available information in the absence of a screening level assessment in CLP.

Leaching simulation modelling: Leaching Calculator model

In order to determine the leachability of chemical substances, especially pesticides, the FOCUS-PELMO model was proposed as indicator of mobility (Klein *et al*. 2023). FOCUS-PELMO 6.6.4[45] is the latest version of the FOCUS simulation models that have been used within an exposure assessment context in order to calculate the concentrations of plant protection products in groundwater and surface water in the EU review process, according to the PPPR. The model predicts leaching for nine standard scenarios across Europe, covering a wide range of soil and environmental conditions further defined in the Generic Guidance for Tier 1 FOCUS Groundwater Assessment (FOCUS, 2001). The simulation is run over 20 years and and can account for worst-case assumptions of linear adsorption (namely the Freundlich exponent is set to 1.0 and leaching is considered independent of the application rate), no volatilisation or photodegradation for a soil pore water at depth of 1m to represent groundwater (Klein *et al*., 2023). The degree of leaching is strongly dependent on K_{OC} and soil degradation half-lives that are substance-specific input parameters for the model.

The key outcome of the Leaching Calculator model is a percentage leachability, with a leachability of below 1% of the initially applied amount being proposed to consider a substance as not mobile, leachability between 1-10% as mobile and above 10% as very mobile. The 1% leachability value was proposed by the model developers as concurring with the 0.1 µg/L cut-off value in groundwater in pesticidal and biocidal risk assessment.

The regulatory applicability of this approach for the purpose of hazard classification under CLP is currently limited. Results from the Leaching Calculator cannot be directly compared to the M/vM criteria (K_{OC} is one of the model input parameters) because of the the strong influence of parametes such as degradation half-lives, vapour pressure and other exposure-related parameters, namely application rates and timing, use patterns, and crop development/crop interception to the model. Furthermore, there are currently uncertainties whether this model has updated exposure scenarios, if it can be used for substances emitted via the sewage treatment plants (STP) and if it can be applied for potential entry of chemicals to groundwater from surface water (for example, via river bank filtration), with additional calibration still needed to account for these processes.

Thus, results from such models (including FOCUS-PEARL 5.5.5[46]) should be treated with caution when used as a line of evidence under the WoE, especially in cases where there are indications of high potential for presence in groundwater, together with all other lines of evidence. The same is the case for other approaches that include both degradation and K_{OC} data in order to derive indices for pesticides leaching such as the groundwater ubiquity score (Gustafson, 1989) that are not considered relevant for Mobility under CLP and will not be further elaborated on in this Guidance.

4.3.3.3.6. Relevance of aged sorption data

The term aged sorption is used to describe the increased sorption (adsorption and absorption) of the substance to the soil over extended period of time (weeks or months),

[45] https://www.ime.fraunhofer.de/en/Research_Divisions/Division_AE/Software_E/focus-pelmo.html
[46] https://www.pesticidemodels.eu/pearl/downloads

as opposed to the much shorter time scales in a study performed according to OECD TG 106 or other. Longer time exposures allow for the slow diffusion within the pores and channels of the solid or molecular diffusion in the macromolecular organic matter (ECETOC, 2021). Such approaches are often used in conjunction with following equilibrium adsorption/desorption studies, in order to confirm the relevance of aged sorption with, for example, at least four of the aged sorption experiments showing evidence of aged sorption, according to the respective quality criteria (EFSA, 2015).

Recent regulatory and scientific progress has led to the publication of a Guidance on the conduct, impact and use of aged sorption studies in the regulatory risk assessments of pesticides (Commission Guidance Document, 2021) that includes a comprehensive list of the uncertainties associated with the use of the aged soption concept. It is clear that this approach relates to risk and not hazard assessment and incorporates a large numer of environmental transport, exposure scenario, use and modelling considerations over large time scales. Thus, K_{OC} values derived from such approaches should not be compared with the M/vM criteria, which are based on the *K*oc value derived from equilibrium adsorption/desorption studies. Moreover, any potential influence of aging is not expected to be relevant for low or non-adsorbing (mobile) substances.

4.3.3.3.7. Considerations for ionisable substances

The terms "ionisables" and "non-ionisables" will be used throughout the Guidance to indicate substances that are ionised/ionisable or not under relevant environmental conditions.The following terminology will be used in the following Sections: anionic substances for those substances that will be in the anionic form (in a percentage above 10%) and cationic substances for those substances that will be in the cationic form (in a percentage above 10%), under relevant environmental conditions (any pH from 4 to 9). Zwitterionic substances are neutral substances that contain a positive and a negative charge but will not be further expanded upon.

Ionisable substances need special scrutiny when measuring the *K*oc value in test systems due to the impact of the pH to their speciation. As defined in the M/vM criteria in CLP, it is necessary for the purpose of hazard classification to derive the lowest *K*oc value within the environmental relevant pH range of 4 to 9. Specific considerations apply when, depending on the pH, a simple test substance can either occur in a deprotonated (negatively charged due to loss of H^+), protonated (positively charged due to take up of H^+) or neutral form, under relevant environmental conditions. A key indication of the form of the substance under relevant environmental conditions is the acid dissociation constant, also known as acid dissociation constant (K_a). For consistency, dissociation of bases is expressed using the dissociation constant of the conjugate acid. Pesticides are example substances that can often occur in an ionic form, with negatively charged pesticides in a rather basic soil assumed to have a lower *K*oc value and a lower potential to adsorb than neutral or protonated pesticides (RIVM, 2008).

Schaffer and Licha (2014) provided a simplified and general guideline for the identification of ionisable functional groups for more than 30 of the most frequently encountered ionisable compound classes, including their typical pK_a values (pK_a is the negative base-10 logarithm of the acid dissociation constant). The following Figure 1 visualises the species distribution for monoprotic substances in which the acidic substances will exist in

the anionic form in a percentage above 1% for pH greater than pK_a -2 (i.e. pH 2.5) and approximately 99% or above at a pH greater than pK_a + 2. For the basic substances, the cationic form will exist in a percentage above 1% when the pH is lower than pK_a + 2 (i.e. pH 11.5) and approximately 99% or above at a pH lower than pK_a - 2. The estimation of the species distribution for compounds with more than one pK_a value is more complex and will not be further discussed in this Guidance.

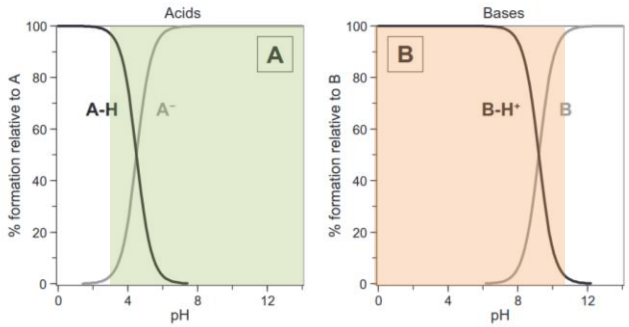


Figure 1. Visualisation of species distribution for monoprotic acidic and basic substances as adapted from Schaffer and Licha (2014). The coloured areas cover the pH range at which the substances are present in the ionic form. pKa acid = 4.5; pKa base=9.5

Relating to the mechanisms of adsorption/desorption of ionisable substances, extensive public literature exists that summarises the differences with organic non-ionisables, as well as alternative approaches to better assess their potential for adsorption (e.g. Arp and Hale, 2022; Sigmund *et al*., 2022; Henneberger and Goss, 2019; Droge and Goss, 2013; Bronner and Goss, 2011a; Mensink *et al*., 2008; Kah and Brown, 2007; Weber *et al*. 2004; Wauchope *et al.*, 2002). For neutral organic substances, soil organic matter is the key sorptive matrix (Mackay, 2001). However, the potential for adsorption for charged substances (including various pesticides, pharmaceuticals, biocides but also industrial chemicals) is usually determined by multiple adsorption/desorption mechanisms, which cannot fully be reflected by the *K*ow value (partitioning between water and the octanol phase).

The publications mentioned above, highlight the interplay of complex interactions with the soil constituents and environmental variables (e.g., pH, ionic strength, dissolved organic matter, soil texture and mineral composition), other phases present (for example, coal, black carbon), non-linear sorption mechanics and effects like aging and interface interactions that all need to be taken into account (Wauchope *et al.*, 2002, ECETOC, 2021). Depending on these processes, substance speciation as a function of the soil pH must be considered in the assessment, as well as the different interaction types included, to the degree possible. Adsorption studies on six acidic pesticides in nine soils revealed that the two strongest descriptors of the variability in adsorption were lipophilicity of the compound corrected for soil pH (Log D) and the soil organic carbon content (Kah and Brown, 2007). For cationic substances, there is evidence from the literature that the interactions underpinning their mobility may be even more complex than those for anionic substances (Kah and Brown, 2007). For example, it may, in some cases such as for soils with low organic matter content, be better characterised by adsorption to clay minerals than to soil organic matter (Sigmund *et al*., 2022, Droge and Goss, 2013, Weber *et al.*, 2004).

In general, the suitability of normalisation to soil organic carbon and, therefore, the use of octanol as a surrogate for sorption has been questioned for ionisable substances.

Instead, different approaches have been proposed including the normalisation to clay content (Hermosin *et al., 2000*) and to the estimated cation-exchange capacity (Droge and Goss, 2013), the development, validation and use of data-intensive poly parameter free energy relationships (PP-LFER) (Henneberger and Goss, 2019, Bronner and Goss, 2011b), as well as various experiments covering extended pH- and ionic strength-dependent sorption mechanisms of a wide array of soils and porewater chemistries (Sigmund *et al*., 2022, Arp and Hale, 2022). It needs to be noted that current PP-LFER approaches do not account for interactions such as electrostatic repulsion and attraction, charge-assisted H-bonding, cation bridging, etc. that may potentially be relevant for ionisable substances (Sigmund *et al*., 2022).

As can be understood from the above and is also acknowledged in the related scientific literature, none of the proposed alternatives to K_{OC}-"centric" sorption characterisation is currently available to be used for regulatory purposes to cover all types of ionic substances and interactions with soils. The currently proposed approaches usually lack harmonisation for uniform application by scientists and regulators, with no consensus having been built in agreeing on single sorption indices that can be derived under standardised experimental methods. At best, these data-intensive methods provide valuable insights into the sorption of a limited number of substances under specific soil and other environmental conditions, often containing a series of uncertainties and modelling assumptions, with limited validation datasets and with currently unaddressed complexities of extrapolating from small scales to the real hydrologic systems (Wauchope *et al*., 2002).

However, there is still an urgent need to generate and use for regulatory purposes information for ionisables that can be compared to the M/vM criteria within a hazard identification/ assessment context. Currently, recent literature still advocates the use of the organic carbon-water partition coefficient as derived from batch tests in a robust and conservative way, in order not to overestimate sorption (Arp and Hale, 2022; Sigmund *et al*. 2022). Such an approach is not context-specific as it does not take into account environmental and other exposure parameters and may be easier applied in a UN level, where some regions may have very different environmental conditions than the ones of the EU. Some supportive evidence was provided by Wauchope *et al.* (2002) who reported relatively low variance between minimum and maximum experimental K_{OC} values for a high number of most commonly used pesticides.

For **acidic substances** including, for example, carboxylic and sulfonic acids, mobility will be higher in the anionic form than in the neutral form due to their negative charge (soil is in most cases also negatively charged). In order to determine the mobility potential at all relevant conditions, testing on anionic substances needs to also include soils of high pH (when feasible, at a pH of pK_a +2) and low ionic strength (i.e. low ion concentration in solution). In such conditions, the anionic form dominates and the electrostatic repulsion with negatively charged soil moieties can increase mobility and the available cations for charge shielding and cation bridging are minimized (Sigmund *et al*. 2022). If the value of the soil pH is near the pK_a, then mobility will be sensitive to pH, as the anionic species concentration will vary as a function of the pH.

For **basic substances** including, for example, amines and amides, the adsorption behaviour could be more complex. As an example, at low pH the electrostatic repulsion increases the mobility of the cationic forms. With increasing pH, the mobility will decrease due to electrostatic attraction toward negatively charged soil moieties. At a pH > pK_a, where the neutral form dominates, the mobility can increase due to a decrease of ionic

interactions between the cationic base and the anionic surface charge of the soil (Sigmund *et al*. 2022). Thus, in order to determine the mobility potential at all relevant conditions, testing on cationic substances needs to also include soils of high pH (when feasible, at a pH of pK_a -2). If the value of the soil pH is near the pK_a then mobility will be sensitive to pH as the neutral species concentration will vary as a function of the pH. The selected soils should, thus, include soils of both low and high pH values, where both the charged and the neutral fractions can be studied.

In order to determine the mobility potential under all relevant conditions, it is recommended that testing for cationic substances should also take place in soils where sorption to clay is not dominating, namely for soils of low clay content (for example below 10%). For these soils, with the caveats discussed above, the K_{OC} value is still considered appropriate provided that the organic carbon content is within the range given in Table 1 of the OECD TG 106. In the future, the derivation of a clay- and/or CEC-normalised partition coefficient may be needed.

For performing batch equilibrium adsorption/desorption studie (OECD TG 106) with ionisable substances, soil selection and characterisation are particularly important steps, as the soil pH defines the dominant species available in the test. Depending on the nature of the ionisable substance as described above, the selected soils should also include soil(s) in which the most mobile species will be present, based on the soil pH. As recommended in the test protocol, soil pH should be measured in a 0.01 M of calcium chloride ($CaCl_2$) solution. Parameters such as the Cation Exchange Capacity, Anion Exchange Capacity as well as the clay content and mineralogy in the soil have been proposed to be reported together with organic carbon content for assessing the behaviour of such substances in the soil.

Regarding experimental results for ionisables performed according to OECD TG 121 for a compound where at least 10% of the test compound will be dissociated within pH 4-9 (note, the respective OECD guideline mentions a pH range between 5.5 and 7.5), two tests should be performed: one with the ionised form and one with the non-ionised form. The tests should be performed in appropriate buffer solutions. A suitable set of data for reference ionisable substances needs to be available for a reliable estimation of the adsorption coefficient K_{OC}.

Similarly, to the provisions above, the selected soils in a soil leaching experiment according to OECD TG 312 (Soil leaching columns) should also cover a wide range of pH, in order to evaluate the adsorption of ionisable and non-ionisable substances. The former needs to be considered only in the cases where the ionised form is present in at least 10% of the total amount of test substance within the environmentally relevant pH 4-9. In addition, as specified in the TG, at least 3 soils should have a pH at which the test substance is in its mobile form. A suitable set of data for reference ionisable substances needs to be available for a reliable estimation of the adsorption coefficient K_{OC}. Similar principles apply for testing ionisable substance using the Soil TLC (Thin Layer Chromatography). Table 1 below provides a concise overview of the impact on the mobility of the acidic and basic ionisable substances, as a function of their dissociation constant (pK_a) and the pH.

Table 1. Dominant species and Mobility of ionisable substances (adapted from Wauchope *et al*., 2002)

When test pH is:	Dominant species* and Mobility

	Acids	**Bases**
< pK_a - 2	XH (neutral) Behaves like non-ionisable substance.	$(XH)^+$ or X^+ (cation) Not mobile (clay surface and organic natter sorption)
>pK_a - 2 and < pK_a + 2	X^-/XH ratio as a function of pH If the value of soil pH is near pKa mobility will be sensitive to pH. For acids mobility increases with increasing pH.	$(XH)^+$/X or X^+/X(OH) as a function of pH If the value of soil pH is near pK_a mobility will be sensitive to pH. For bases mobility decreases with increasing pH until pKa and then increases for values above pKa
> pK_a + 2	X^- (Anion) Highly mobile in soil.	X or X(OH) (neutral) Behaves like non-ionic substance.

*X^- refers for the anionic species, XH, X, XOH refers to neutral species, $(XH)^+$, X^+ refers to cationic species of the corresponding anionic or cationic substances.

4.3.3.4. Toxicity assessment

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.3.2.3.3. and 4.4.2.3.3. The following information shall be considered for the assessment of T properties:

(a) results from long-term toxicity testing on aquatic invertebrates;

(b) results from long-term toxicity testing on fish;

(c) results from growth inhibition study on algae or aquatic plants;

(d) the substance meeting the criteria for classification as carcinogenic in Category 1A or 1B (assigned hazard statements: H350 or H350i), germ cell mutagenic in Category 1A or 1B (assigned hazard statement: H340), toxic for reproduction in Category 1A, 1B or 2 (assigned hazard statements: 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 statements: H372 or H373);

(e) the substance meeting the criteria for classification as endocrine disruptor (Category 1) for human health or the environment (assigned hazard statements: EUH380 or EUH430);

(f) results from long-term toxicity testing on terrestrial organisms; invertebrates and plants;

(g) results from long-term toxicity testing on sediment organisms;

(h) results from long-term or reproductive toxicity testing on birds;

(i) other information, provided that its suitability and reliability can be reasonably demonstrated.

Annex I: 4.3.2.4.2. and 4.4.2.4.2. In applying the WoE determination, the following information, in addition to the information referred to in Sections … 4.3.2.3.3 and 4.4.2.3.3… shall be considered as part of the scientific assessment of the information relevant for the … T … properties:

(c) Indication of T properties:

(i) Short-term aquatic toxicity (e.g. results from acute toxicity testing on invertebrates, algae or aquatic plants or fish, in vitro acute toxicity testing on fish cell line);

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

The consideration of study results from long-term toxicity testing on terrestrial organisms and sediment in the amended Annex I of CLP is a novelty related to previous Toxicity assessments, as the ones under REACH Annex XIII. The following Sections will present guidance on how information on terrestrial organisms and sediment can be assessed within the CLP context. In the absence of concrete, "real-life" examples of substances either

classified or concluded as PBT/vPvB under REACH Article 57 (SVHC identification process) solely based on such test results, the current guidance document may need to be updated in the future based on the emergence of related cases proposed for harmonised classification. Similarly, in case of a potential future introduction of new hazard classes/ criteria in CLP (or the UN GHS), a revisit of the described approach would be required.

4.3.3.4.1. Long-term aquatic toxicity

Section 4.1 and Annex I.3.2 of the current Guidance elaborate in detail on the relevant experimental and other information that can be used to conclude on long-term aquatic toxicity, in the context of the assessment of aquatic hazards under CLP. However, despite the fact that the data used in the assessment of aquatic toxicity under hazardous to the aquatic environment (CLP Annex I, 4.1) and under PBT/PMT classification are the same, the regulatory criteria are not. Keeping this in mind, the ECHA Guidance on IR&CSA, Chapters R.7b and R.11 further detail the availability, applicability, adequacy (reliability and relevance) and other scientific and regulatory considerations for the use of the different test methods on long-term aquatic toxicity for substances of varying physico-chemical properties and regulatory uses. These considerations will not be repeated in the present Guidance.

Concerning long-term toxicity data on fish, for example, these Guidance documents elaborate further on exposure during relevant life-stages to regard the tests as long-term and describe in detail relevant considerations on the conduct and regulatory use of test methods OECD TG 210, 212 and 215. Aquatic invertebrates can be tested following OECD TG 211 (*Daphnia magna* Reproduction Test), whereas long-term effects on aquatic plants and algae can be investigated by a range of tests (for example, OECD TG 201 for freshwater alga and cyanobacteria, OECD TG 221 for *Lemna sp.* and OECD TG 238 and 239 for *Myriophyllum Spicatum*).

Once reliable and relevant information is available resulting in a long-term NOEC or EC_{10} value in marine or freshwater organisms below the regulatory threshold of 0.01 mg/L, the substance can be concluded as fulfilling the CLP toxicity criterion (T). In the presence of both long-term NOEC and EC_{10} for the same experimental study, CLP gives preference to EC_{10} (OECD 2006 and current Guidance Section 4.1).

4.3.3.4.2. Carcinogenicity (Carc. 1A or 1B)

Detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for carcinogenicity can be found in Section 3.6 of this Guidance document. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in categories 1A or 1B for carcinogenicity (Carc. 1A or 1B), based on the criteria stipulated in Section 3.6.2 of CLP.

4.3.3.4.3. Germ cell mutagenicity (Muta. 1A or 1B)

Detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for germ cell mutagencitiy can be found in Section 3.5 of this Guidance document. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in categories 1A or 1B for germ cell mutagenicity (Muta. 1A or 1B), based on the criteria stipulated in Section 3.5.2 of CLP.

4.3.3.4.4. Toxic for reproduction (Repr. 1A, 1B or 2)

Detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for reproductive toxicity can be found in Section 3.7 of this Guidance document. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in categories 1A, 1B or 2 for reproductive toxicity (Repr. 1A, 1B, or 2) based on the criteria stipulated in Section 3.7.2 of CLP.

4.3.3.4.5. Specific target organ toxic after repeated dose (STOT RE 1 or 2)

Detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for specific target organ toxic after repeated exposure can be found in Section 3.9 of this Guidance document. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in categories 1 or 2 for specific target organ toxic after repeated exposure (STOT RE 1 or 2) based on the criteria stipulated in Section 3.9.2 of CLP.

4.3.3.4.6. Endocrine disruptor for Human Health (ED HH 1)

Detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for endocrine disruption for human health can be found in Section 3.11 of this Guidance document. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in category 1 for endocrine disruption for human health (ED HH 1) based on the criteria stipulated in Section 3.11.2 of CLP.

4.3.3.4.7. Endocrine disruptor for Environment (ED ENV 1)

Detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for endocrine disruption for the environment can be found in Section 4.2 of this Guidance document. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in category 1 for endocrine disruption for the environment (ED ENV 1) based on the criteria stipulated in Section 4.2.2 of CLP.

4.3.3.4.8. Long-term terrestrial toxicity

Regarding highly adsorptive substances that are likely to be present in the terrestrial environment via inter-compartmental distribution processes or direct application (e.g. via sludge), effects on terrestrial organisms provide useful insights into the toxic potential of such substances. Under REACH, terrestrial toxicity testing usually refers to testing performed on terrestrial invertebrates (usually earthworms), micro-organisms and terrestrial plants. Validated test methods are those according to OECD TG 222 (Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)), 220 (Enchytraeid Reproduction Test) and 232 (Collembolan Reproduction Test in Soil) for terrestrial invertebrates, OECD TG 216 (Soil Microorganisms: Nitrogen Transformation Test) and 217 (Soil Microorganisms: Carbon Transformation Test) for soil micro-organisms and OECD TG 208 (Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test), OECD TG 227 (Terrestrial Plant Test: Vegetative Vigour Test) and ISO 22030 (Soil Quality – Biological Methods – Chronic toxicity in higher plants) for terrestrial plants. More details can be found in the ECHA Guidance on IR&CSA, Chapter R.7.11).

Additional terrestrial tests are mentioned under the BPR, namely ISO tests 16387, 11268-1, 11267 or OECD TG 226 for terrestrial invertebrates, ISO 14238:2012, BBA guideline Part VI, 1.1 or DIN EN ISO 23753-2 for soil micro-organisms, as well as several test methods for honeybees. Regarding honeybees and other pollinators, relevant tests include, among others, ones performed according to OECD TG 245, 246 and 247. These tests are both short- and long-term and are usually referred to as "Additional Data Sets" within the BPR contex, meaning that they may be required for a certain biocidal product type, or for a certain use considering the likely exposure route, or depending on the properties of the substance. Information on non-target terrestrial arthropods is required when exposure is likely. Similar considerations and tests are also considered under the PPPR.

Considerations relative to birds are presented in Section 4.3.3.4.10, whereas no further elaboration will be provided for other toxicity study information on mammals.

As for sediment organisms (see following Section), there are currently no concrete numerical threshold criteria in CLP for the direct comparison with results from long-term terrestrial toxicity studies (expressed as mg/kg dw). Spain has previously led a UN experts sub-committee panel on the Globally Harmonized System of Classification and Labelling of Chemicals (UNSCEGHS) and developed in 2006 a proposal on 'Classification criteria for the terrestrial environment' (UN, 2006). However, the criteria proposal has not been developed any further since. Additional efforts to define approaches of dealing with terrestrial toxicity data in the framework of PBT assessment and hazard classification have been made by JRC (2014) and, more recently, by the German UBA (2022).

Until terrestrial hazard class(es) including threshold values are introduced in the regulatory framework, it is hereby proposed that a similar approach is used as for sediment organisms by use of the Equilibrium Partitioning method (EPM). As such, results from long-term terrestrial toxicity studies are used to investigate whether they lead to an aquatic toxicity that is below the regulatory classification criteria for aquatic organisms (NOEC or EC_{10} below 0.1 or 0.01 mg/L), by use of the following equation:

$$\boldsymbol{NOEC(EC10)_{porewater} = \frac{NOEC(EC10)_{soil}}{Kd}}$$

$NOEC(EC_{10})_{porewater}$ (mg/L)

K_d (L/ kg dw)

$NOEC(EC_{10})_{soil}$ (mg/kg dw)

An EFSA scientific opinion (2009) based on a literature review confirmed that for soft-bodied soil organisms (earthworms, enchytraeids, nematodes) and plants in close contact with the soil solution, porewater mediated uptake of pesticides seems mainly responsible for the effects caused, and would therefore be the relevant metric for effects assessment, and consequently also for exposure assessment.

K_d can be estimated from the K_{oc} as described in a previous pareagraph.

The method should be applied with caution where relevant and justified, exercising expert judgement depending also on the availability of other information types. This approach, when applied to sediment organisms (Section 4.3.3.4.9), has been shown to result in either an overestimation or underestimation of the toxicity to benthic organisms (Di Toro *et al*., 2005). For example, depending on the selection of soil parameters in the terrestrial toxicity test, the back calculation to aquatic organisms may not be adequate. Similarly, for pesticidal-active substances, there may be cases where the back-calculation will lead to overly conservative aquatic NOEC values. Added uncertainty comes from the limited applicability domain of the EPM, namely that it is not applicable for ionizable substances and not reliable for substances with a log *K*ow above 5. Finally, the EPM is not applicable to bees or non-target terrestrial arthropods. In all cases, this is envisaged to be the working approach until specific criteria are developed in the UN GHS level for toxicity to the terrestrial environment.

4.3.3.4.9. Long-term sediment toxicity

In cases where sediment effects assessment is necessary for substances that are known to be persistent in marine waters and may accumulate in sediments over time, tests on sediment-dwelling organisms such as *Myriphyllum Spicatum* (a submersed aquatic dicotyledon), *Chironomous sp*. (freshwater dipterans), or *Lumbriculus* (sediment-ingesting endobenthic aquatic oligochaetes) may provide useful information on the toxicity of the substance in the compartment in which it will be mainly found, namely sediment. Such validated test methods can, thus, be used for classification purposes and include OECD TG 239 (Water-Sediment *Myriophyllum Spicatum* Toxicity Test) for *Myriophyllum* species, OECD TG 218 (Sediment-Water *Chironomid* Toxicity Test Using Spiked Sediment), 219 (Sediment-Water Chironomid Toxicity Test Using Spiked Water) or 233 (Sediment-Water *Chironomid* Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment) for *Chironomids* and OECD TG 225 (Sediment-Water *Lumbriculus* Toxicity Test Using Spiked Sediment) for *Lumbriculus*. It is hereby noted that in some cases analytical verification is made in the porewater, allowing expression of results directly in mg/L porewater.

The choice of the test species depends on many factors, for example whether feeding on sediment particles takes place, for example for strongly adsorbing or binding substances with a log K_{OW} above 5 (preference on *Lumbriculus variegatus*, *Tubifex tubifex*), whether there is a specific mode of action and/or sensitivity towards a given test organism, etc. (ECHA Guidance on IR&CSA, Chapter R.7.8.10.1). More details can be found in the ECHA Guidance on IR&CSA, Chapter R.7.8.9.1, including test methods according to ASTM, US-EPA and ISO test guidelines.

Currently, neither REACH Annex XIII nor CLP include a numerical threshold value to compare to the NOEC or EC_{10} value derived from a chronic sediment toxicity, for PBT and PMT assessment purposes. As described above, a proposed approach is the use of the EPM to estimate (no-effect) concentrations expressed in mass of test substance per volume of test medium (for example, mg/L) from results of sediment toxicity test expressed in mass of test substance per mass of sediment (e.g. mg/kg of wet sediment). The estimated concentration (in mg/L) is then compared to the T criterion of 0.01 mg/L for toxicity to aquatic organisms. Further details on assumptions and considerations behind EPM are explained in ECHA Guidance on IR&CSA, Chapter R.10.5.2.1.

$$NOEC(EC10)_{porewater} = \frac{NOEC(EC10)_{sed„dw}}{Kp_{susp}}$$

$NOEC(EC_{10})_{porewater}$ (mg/L)

Kp_{susp} (L/ kg dw)

$NOEC(EC_{10})_{sed\ dw}$ (mg/kg dw)

Kp_{susp} *(L.kg^{-1} dw)* can be estimated from the K_{oc} of the substance as $Kp_{susp} = Foc_{susp} \times K_{oc}$ where Foc_{susp} is the mass fraction of organic carbon in dry suspended matter.

The same considerations for the application of this approach as for terrestrial organisms (4.3.3.4.8) are also relevant for sediments.

4.3.3.4.10. Long-term or reproductive toxicity in birds

Avian toxicity has been introduced in Annex X of the REACH Regulation to account for secondary poisoning risks to predators following chronic exposure to a substance via the fish (aquatic) and earthworm (terrestrial) food chains (R.7.10.16). The standard tests typically measure lethal effects from either short- or medium-term exposures and/or chronic lethal and reproductive effects of long-term exposures. The exposures are expressed in terms of either a concentraton or a dose. Longer-term exposure is preferred, as few (if any) scenarios are likely to lead to acute poisoning risks for birds, and evidence from pesticides (Regulation EC No 1107/2009) suggests that chronic effects cannot be reliably extrapolated or inferred from acute toxicity data (R.7.10.17).

Table R.7.10—4 from ECHA Guidance on IR&CSA provides an analytical summary of existing and proposed standardised avian toxicity tests. Additionally, *in vitro* approaches for birds are also currently under investigation, for example, Ball and Lavado (2021) who examined the use, limitations, and applications of avian cell-based models in an ecotoxicological context. Under the BPR, effects on birds based on OECD TGs 205, 206 and 223 have been required. Under the PPPR, a test for effects on reproduction in birds is currently requested in the pesticidal risk assessment, if birds are likely to be exposed during the breeding season, with two standard studies usually requested, namely based on OECD TG 206 and USEPA OCSPP 850.230027 (EFSA Journal 2023;21(2):7790).

ECHA Guidance on IR&CSA, Chapters R.7c and R.11 further clearly indicate that any results from reprotoxicity studies or other chronic data on birds (including from valid QSAR models) cannot be used on their own to directly/ numerically compare with the T criteria in REACH Annex XIII, in the absence of an agreed regulatory threshold value. This is also relevamt for the assessment under CLP. Moreover, there are uncertainties relating to lack of data in the literature, too few species tested in the laboratory, different sensitivities between industrial chemicals and pesticides, interspecies differences, uncertain extrapolation to field conditions, etc. Thus, any such data can be used within the WoE determination to conclude on the toxicity of a substance, with a NOEC value below 30 mg/kg food previously considered as a strong indicator of fulfilment of the "T" criterion (ECHA Guidance on IR&CSA, Chapter R.7.10.16.2).

4.3.3.4.11. Other suitable and reliable information

REACH Annex XIII, Section 3.1.3 considers short-term aquatic toxicity in accordance with Section 9.1 of Annex VII and Section 9.1.3 of Annex VIII as information relevant for the screening of the "T" property in PBT assessment. Section 4.1 and Annex I.3.1 of the current guidance provide details on the experimental and other information relating to acute aquatic toxicity and its use to conclude for aquatic acute classification purposes. These principles relating to the availability and assessment of such studies also apply when considering short-term aquatic toxicity as part of the different regulatory context of PBT/PMT assessment. Information from *in vitro* studies might also be considered in a WoE approach provided that they fulfil certain data quality requirements and comply with the Annex XI criteria. These quality aspects are further detailed in Guidance R.7.8.3.1 and R.7.8.4.1 (R.7b), where the availability and applicability of such *in vitro* methods is further explained.

In general, in the absence of long-term or chronic aquatic toxicity data that can be directly compared with the CLP criteria (see Section 4.3.3.4.1), acute/ short-term aquatic toxicity data may be used as an indication that the substance may fulfil the T criterion (R.11.2.2), but cannot be used for concluding definitively "not T". When acute/short-term aquatic toxicity data show that the substance is very toxic ($L(E)C_{50}$ below 0.01 mg/L), a definitive conclusion can be drawn that the substance can be classified as "T". In cases of less acute aquatic toxic substances, results from such studies may likely not provide a true measure of the intrinsic aquatic toxicity of the substance (ECHA Guidance on IR&CSA, Chapter R.7.8.2).

In addition to data from standard toxicity tests, data from reliable non-standard tests and non-testing methods may also be used if available. These data should be particularly

assessed for their reliability, adequacy, relevance and completeness (see *Chapter R.4* of the ECHA Guidance on IR&CSA). Additionally, the use of reliable QSAR predictions, as well as adequately documented and justified read-across and/or grouping approaches is allowed and assessed using expert judgement, on a case-by-case basis. The related provision in the CLP for the use of such data is "*other information, provided that its suitability and reliability can be reasonably demonstrated*". More information is included in Section 4.3.3.5.

4.3.3.5. Application of the WoE to conclude on PBT/ vPvB properties

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.3.2.3. Basis of classification

For the classification of PBT substances and vPvB substances, a WoE determination using expert judgement shall be applied, by comparing all relevant and available information listed in Section 4.3.2.3 with the criteria set out in Sections 4.3.2.1 and 4.3.2.2. That WoE shall be applied in particular where the criteria set out in Sections 4.3.2.1 and 4.3.2.2 cannot be applied directly to the available information.

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, additives or impurities of a substance and relevant transformation or degradation products.

This hazard class (Persistent, Bioaccumulative and Toxic (PBT) or Very Persistent, Very Bioaccumulative (vPvB) properties) shall apply to all organic substances, including organo-metals.

The PBT/vPvB assessment must cover a consideration of each property, namely persistence, bioaccumulation and toxicity against each respective criterion (P or vP, B or vB, and T) following the provisions and considerations that have been analytically reported in Section 4.3.3 of this Guidance. As dictated by the CLP (green text above), the decision on whether classification in the PBT/vPvB hazard class is warranted is in all cases a WoE determination using expert judgement. The following paragraphs will expand on some general principles of the WoE, with property-specific considerations further elaborated on just after the current general principles Section.

CLP refers to the comparison of all relevant and available information with the criteria, in particular in cases where these cannot be applied directly to the available information (Article 9). The current Guidance elaborates in detail on several elements to establish the **relevance** of the provided information, both in a higher level (for example as in bulletpoint (ii) in Section 4.3.3) and at an individual study level (see Sections 4.3.3.1-4.3.3.4). **Available** information refers to the one that has also been described comprehensively earlier in the Guidance and includes experimental and non-experimental information, *in vivo*, *in vitro* and *in silico* methods, monitoring and modelling data, results from studies from structurally similar substances, etc.

All available relevant information should be considered together and based on the quality of the data, the consistency of the results, the nature and severity of effects and the relevance of the information, appropriate weight should be given. In any case, expert judgement should be applied to structure the available information in such a way that integrates all relevant elements, properly weigh them and come to an overall conclusion that can be compared to the respective CLP criteria.

Separate conclusions are required for both differentiations PBT and vPvB, as well as for each of the P, B and T properties. The reason for the need for explicit separate conclusions on the individual properties is the fact that meeting the criteria for two of the criteria for being PBT leads to the substance being considered as a "Candidate for Substitution (CfS)"

under the BPR (Article 5(1)(e)) and PPPR (Annex II, 4). These Regulations also define further the regulatory implications for CfS.

In order for the PBT or vPvB criteria to be fulfilled, all respective criteria must be met for the same substance or at least one (but always the same one) individual constituent, impurity, additive or transformation/degradation product, if applicable. The criteria for (v)P, (v)B and T referred to in Annex I of CLP, 4.3 do not have to be met all in the same test compartment i.e. aquatic, soil or sediment, as the General Court of Justice has unequivocally ruled in a related Appeal case[47].

The WoE determination is not a mechanism to justify disregarding valid test data and it is not a means to average results from different sources. ECHA Guidance on IR&CSA, contains more information on specific WoE considerations including the preference on experimental results from reliable studies that can directly be compared to the criteria and their higher relevance over "screening-type" information. This does not mean that all other types of information is not taken into consideration. One example of this preference refers explicitly to the results from reliable degradation simulation studies and the fact that, in their presence, a detailed analysis of the reasons of any potential inconsistencies with the outcomes of studies with lower weight is not necessary (ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.1). The same Guidance also directs to a range of support documents that can be consulted on this topic. Additionally, ECHA has developed a template and background document intended to be used in human health and environmental hazard assessments, in order to harmonise the use of WoE and uncertainty assessment, increase transparency in regulatory decision making and facilitate the integration and use of alternative methods and all available information[48]. Similarly, EFSA (2017) has issued a Guidance on the use of the WoE approach in scientific assessments that can also be consulted[49].

Benchmarking can also be used as part of the WoE and associates the fate or behaviour of a substance to that of a similar/comparable benchmark, well-described chemical (Adolfsson-Erici *et al.*, 2012). The comparability refers to the test conditions/ set-up, test organisms of the available data, as well as the data anayliss and interpretation. More details have been included in the relevant parts of this Guidance, as well as in ECHA Guidance on IR&CSA, Chapter R.11.4.1.

Sections 4.3.3.1-4.3.3.4 have already addressed the use of non standard tests, namely that they can be considered within the WoE if deemed relevant, reliable and equivalent to other standardised methods, as well as the relevance of evidence from read-across, QSARs and monitoring data, for each individual property (P/B/T). Their use within the overall WoE per property will further be analysed in the following paragraphs, as well as how to deal with multiple studies for each property.

Finally, the conclusions of the application of the WoE to conclude on the individual PBT/vPvB properties can be one of the following:

i. Substance is P/vP/B/vB/T
ii. Substance is not P/vP/B/vB/T

It is very important that further clarifications/justifications on the reasons for a substance not meeting the P/vP/B/vB/T criteria are given, in line with the current approach of ECHA's

[47] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:62018TJ0226
[48] https://echa.europa.eu/support/guidance-on-reach-and-clp-implementation/formats
[49] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4971

Risk Assessment Committee (RAC) where the opinion documents[50] contain the exact same justifications for a substance not meeting the classification criteria. Such conclusions have in the past been based, for example, on conclusive data, on inconclusive data or on complete lack of data. Further elaborations on these are given is Section 4.3.3.7. As can easily be inferred, knowledge of the reasons for the different conclusions constitutes invaluable information for both regulators and data holders and increases the transparency of the regulatory outcome, as well as the legal robustness of the conclusion.

Persistence: The P/vP, assessment shall reach one of the conclusions described in the scheme (Figure 3). Section 4.3.3.1 of the current Guidance described the relevant experimental and computational information that may be provided as part of the WoE determination on Persistence.

The results of the degradation simulation studies are to be given more weight in the WoE assessment than the screening-level studies (Figure 2). Degradation half-life (DegT50) obtained from a simulation degradation test in water, sediment or soil, conducted in relevant conditions can be directly (numerically) compared against the respective persistence criterion of CLP to determine whether the P or vP criteria are met or not. In sediment simulation tests (OECD TG 308) where DegT50 is reported separately for water, sediment and whole system, whole system half-live obtained is preferred for comparison with the P/vP criteria. The same applies also for DegT50 values in soil, if a DegT50 in porewater has been estimated.

The reference temperature for providing DegT50 results on simulation tests or field degradation tests is 12°C for fresh or estuarine water, soil and fresh or estuarine water sediment environments and 9°C for marine water or sediment environments. Conclusion P or vP reached in one of the environmental compartments is enough to consider that the substance meets the P or vP criteria. For example the substance would be P or vP if criteria are met only for water but not for soil or sediment. In order to conclude a substance conclusively not P it must be demonstrated that the substance is not P in all of the environmental compartments listed in Annex I, Section 4.3.2.1.1, 4.4.2.1.1, 4.3.2.2.1 and 4.4.2.2.1. In general, results of a single simulation degradation study demonstrating not P in one compartment cannot be directly extrapolated to other non-tested environmental compartments. In some cases, extrapolation between compartment may be possible provided that results/bridging is backed upp by proper justification.

[50] https://echa.europa.eu/registry-of-clh-intentions-until-outcome/-/dislist/name/-/ecNumber/-/casNumber/-/dte_receiptFrom/-/dte_receiptTo/-/prc_public_status/Opinion+Adopted/dte_withdrawnFrom/-/dte_withdrawnTo/-/sbm_expected_submissionFrom/-/sbm_expected_submissionTo/-/dte_finalise_deadlineFrom/-/dte_finalise_deadlineTo/-/haz_addional_hazard/-/lec_submitter/-/dte_assessmentFrom/-/dte_assessmentTo/-/prc_regulatory_programme/-/

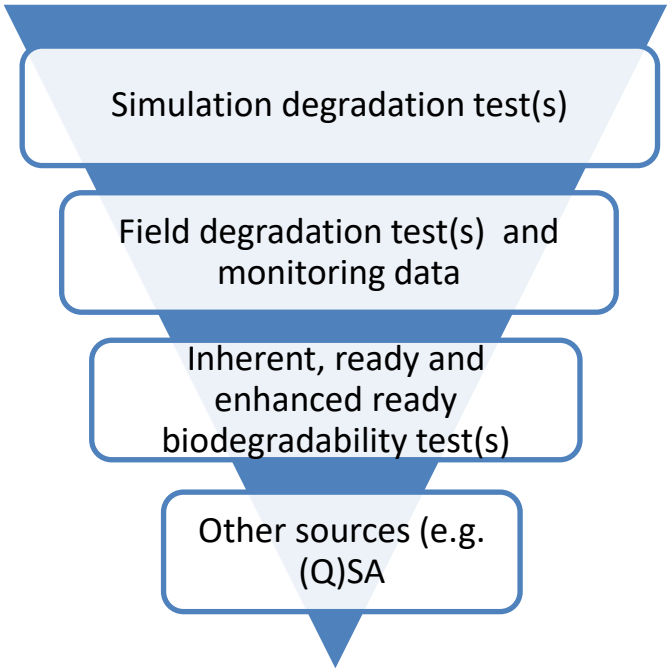


Figure 2. Simplified illustration of the relative weight of the available information (not taking into account the quality of the data) for Persistence.

If a study has not been conducted in relevant conditions, for example if much higher suspended solids concentration than allowed in the OECD TG 309 was used or sediment stratification was disturbed in an OECD TG 308 study, DegT50 values obtained in such conditions may overestimate the degradation rate. Therefore, such DegT50 values but can be used in a WoE assessment but relevance considered with care.

Tests conducted solely under fully anaerobic test conditions are considered not to be especially relevant for the P assessment as permanently anaerobic soil or sediment systems are not common in the EU. Nevertheless, if anaerobic soil data are available, they may be used as part of a WoE approach. Generally it would be expected that an anaerobic half-life would be greater than an aerobic half-life where the main route of degradation is aerobic, namely if there is no oxygen, degradation will be hindered. However, care should be taken where the anaerobic data in sediment test show rapid degradation of a substance. In such case, the OECD TG 308 may overestimate the degradation rate of some substances in the aerobic environment. This has been shown for example with nitro- containing substances, like musk xylene[51].

In the presence of a reliable degradation half-life obtained from simulation degradation test or field study, it is not necessary to analyse in detail the reasons for potentially inconsistent outcomes of the screening tests. The outcomes of a reliable and relevant simulation degradation or field study, have higher weight in the WoE than screening studies (ECHA Guidance on IR&CSA, Chapter R.11 provides further details on the WoE assessment).

With regard to persistence, it is insufficient to consider a dissipation half-life (DT50) alone, where this may simply represent removal from the test system or the transfer of a substance from one environmental compartment to another (e.g. from the water phase to

[51] SVHC SUPPORT DOCUMENT (EC 201-329-4) https://echa.europa.eu/documents/10162/909dd42e-2554-4f59-911a-729a2da1d529

the sediment). If transfer processes have occurred simultaneously with degradation, the DT50 value is not representative of the DegT50 value (CLP Annex I, 4.3 and 4.4) and thus may only serve as supporting information in the assessment. Where only primary degradation is observed, it is necessary to identify the degradation products and to assess whether they possess PBT/vPvB properties.

Where more than one acceptable degradation study (e.g. for studies on degradation kinetics in soil according to OECD TG 307 at least 4 soils should be used per study) resulting in half-life is available for the same environmental compartment, the most stringent result should be used with respect to the P/vP assessment.

When there are results from four or more simulation studies with the same environmental compartment with similar test conditions, design and degradation kinetics (e.g. SFO), aggregation (using geometric mean) of the degradation half-lives could be considered. Half-life data from different environmental compartments should not be aggregated. The type and distribution of the half-life data should be considered and any data outliers assessed and removed from the data set if appropriate. The validity of the data and comparability of the tests in terms of conditions influencing degradation potential (for example temperature, pH, organic carbon content, microbial biomass, source of the test media etc.) should be carefully considered. Only test results corresponding to similar test conditions (e.g. laboratory or field, aerobic or anaerobic, marine or fresh water) can be compared. If the data distribution does not point to use of geometric mean, use of another mean (e.g. arithmetic mean) should be considered. In all cases, the approach should be well justified and documented and should be supported by the WoE analysis. In particular, the representativeness of the test conditions should be carefully assessed for each test result. Particular scrutiny should be given if results from the tests are close to the P or vP threshold.

Field studies provided that their suitability and reliability can be reasonably demonstrated by also taking uncertainties in deriving field half-life into account may be used as assessment information (Figure 2, second entry). However, when degradation half-lives derived from field studies are compared to the P/vP criteria uncertainties related to the role of other dissipation processes such as volatilisation, leaching, etc. on the estimated half-life must be carefully considered (see also Section 4.3.3.1.2.2 of this Guidance). Influence of dissipation processes in derivation of the DegT50 is difficult to quantify and thus in many cases lowers the reliability of the estimated degradation half-live.

In addition to the simulation and field test data, existing monitoring data should be carefully examined (Figure 2, second entry). Monitoring studies provided that their suitability and reliability can be reasonably demonstrated, may be used as assessment information (see Section 4.3.3.1.2.3 of this Guidance). Howevevr, monitoring data on its own cannot demonstrate persistence because the mere presence of a substance in the environment is dependent on a range of factors other than degradation rates, namely emission and distribution rates. If monitoring data show that a substance is present in remote areas (namely, long distances from populated areas and known point sources, such as the Arctic sea or sub-Arctic/Arctic lakes in Scandinavia), it may be possible to conclude a substance as P or vP (this is especially the case for non-mobile substances) (ECHA Guidance on IR&CSA, Chapter R.11). Monitoring data obtained in areas closer to the sources may also be useful for P/vP assessment as one line of evidence for supporting the conclusions. Also, significant concentrations of the substance in higher levels of the

food chain in unpolluted areas may indicate high persistence (beside a potential to bioaccumulate).

The conclusion that a substance is not P/vP can be based on screening level information (including enhanced tests) provided that taking into account all available information in line with the Annex I of CLP, 4.3.3.2., there is no other evidence of persistence in specific compartments (Figure 2, third entry). In general, screening level information (including enhanced ready biodegradability tests) has lower weight in the WoE assessment in concluding a substance as P/vP. In some exceptional cases, if scientifically justified and supported by other available infomation, it is in principle possible to draw P/vP conclusion based on screening information. For example, if based on the structure of the substance (e.g. perfluorinated substances with covalent C-F bonds) it is known to be resistant towards degradation based on scientific evidence, screening level information would be adequate to conclude a substance as P/vP (unless other evidence indicates non-persistence).

If supported by other available evidence, lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD TG 302 series may provide sufficient information to confirm that the P-criteria are fulfilled for the purpose of persistence assessment. Additionally, in specific cases it may be possible to conclude that the vP-criteria are fulfilled with such results if there is additional specific information supporting the conclusion (e.g., specific stability of the chemical bonds). The degradation half-lives obtained in a hydrolysis test can be used only as supporting information as abiotic degradation is primary degradation, and careful consideration is needed to address the potential formation of stable degradation products with PBT/vPvB properties. Hydrolysis data always need to be considered in connection with the other properties, such as partitioning properties and the knowledge on the abiotic and biotic degradation pathways.

Similarly, data derived from other abiotic studies (e.g. photodegradation) should be considered as supporting information only in persistence assessment. Due to the large variation in the light available in different environmental compartments, the use of photolysis data is not generally recognised for persistence assessment. This is discussed in more details in the ECHA Guidance on IR&CSA Chapter R.7b.

Valid QSAR predictions can be used as supporting information in WoE determination (Figure 2, fourth entry). QSAR estimates may be used mainly for a preliminary identification of substances with a potential for persistence or non persistence for example by combining of results from three estimation models in the EPI suite (US EPA, 2012) or supporting grouping or read-across assessment (see also Section 4.3.3.1.3 of this Guidance). Degradation half-lives based on QSAR models using data from ready biodegradation tests should only be used as supporting information in the assessment as derived half-life values are only base on screening level information and not data obtained in relevant conditions.

The following decision scheme presents the decision scheme that needs to be followed on the available information, in order to come to a robust conclusion on whether the CLP criteria for Persistent and/or Very Persistent are fulfilled (Figure 3).

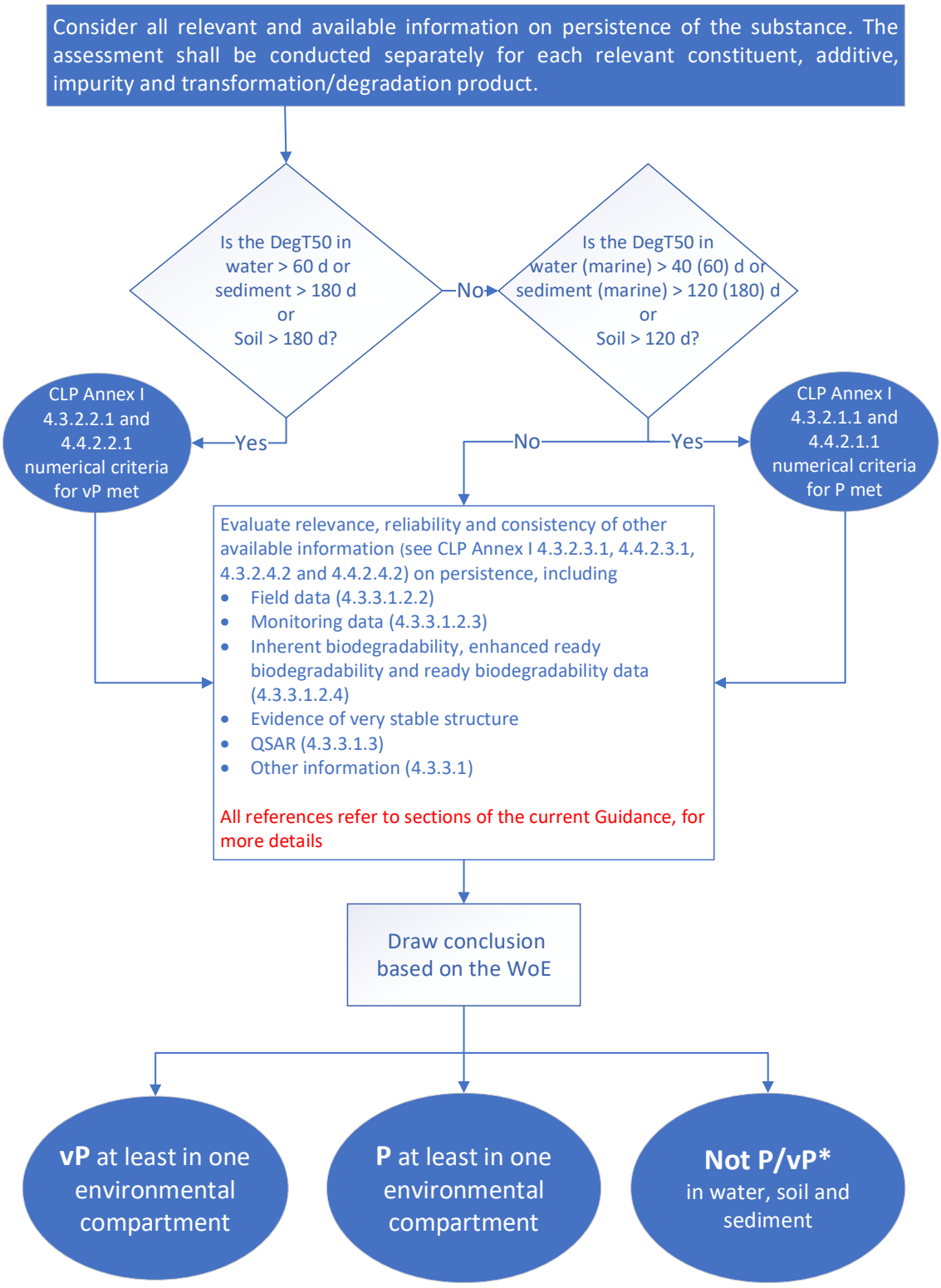


Figure 3. Decision scheme for concluding on the assessment criteria for (P/vP)

Bioaccumulation: The B/vB assessment shall reach one of the following conclusions described in the scheme (Figure 5). Section 4.3.3.2 of the current Guidance document describes the relevant experimental and computational information that may be provided as part of the WoE determination on Bioaccumulation.

The results of reliable *in vivo* bioaccumulation studies and field data are given more weight in the WoE assessment than the indicators of bioaccumulation based on physico-chemical properties and QSAR (Figure 4).

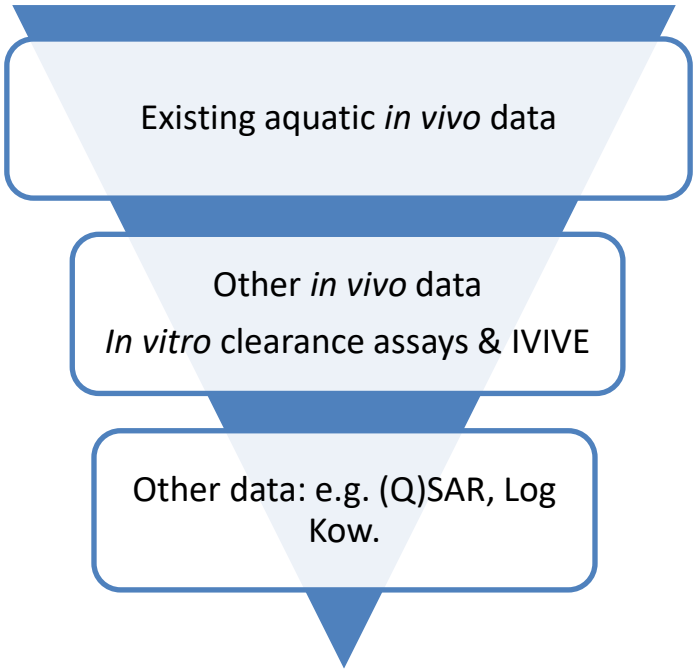


Figure 4. Simplified illustration of the relative weight of the available information (not taking into account the quality of the data) for Bioaccumulation potential

When deciding if a substance meets the B or vB classification criteria, its bioaccumulation potential in the aquatic environment, the terrestrial environment, wildlife or humans is considered.

Existing aquatic in vivo data

Each BCF study should be assessed in detail for its reliability considering the test design, exposure route, uptake and depuration periods, test species and age/life stage, test organism lipid content, test water (including pH, hardness and dissolved oxygen), test temperature, exposure concentration, analytical methods, need for growth correction and lipid normalisation and method of BCF calculation (steady-state or kinetic).

If there is a reliable aqueous bioaccumulation study available, such as an aqueous exposure fish OECD TG 305 study, or a bioaccumulation study with *Hyalella azteca* (OECD, 2023) or other aquatic invertebrate studies (e.g. mussels or oysters), the results can be directly compared to the CLP criteria for B and vB (See Figure 5). The BCF should be

growth corrected, if appropriate, then normalised to the appropriate lipid content for the organism (unless bioaccumulation is not driven by hydrophobicity).

The preferred endpoint from the OECD TG 305 dietary exposure test is the BCF value estimated from experimentally derived elimination rate constant, which can be directly compared to the numerical CLP criteria, unless it can be demonstrated that the uptake rate constant cannot be reliably estimated with the available methods. For very hydrophobic substances, k_1 estimates may become increasingly uncertain. In that case other methods (direct application of k_2, or using a correlation of dietary BMF and BCF results to interpolate other dietary BMF results) as described in OECD, 2017 should be used and the results assessed in a WoE approach.

Multiple BCF studies

Where more than one acceptable BCF study is available for the same species, the most conservative BCF value (the highest BCF value once growth corrected and lipid normalised as appropriate) may be used as the representative BCF value for that species. **BCF results for different species should not be aggregated but considered in a WoE approach.**

In the presence of **four or more** BCF studies for the **same species and life stage**, the geometric mean of the reliable BCF values may be used as the representative BCF value for that species, if the test conditions of the different studies are equivalent (for example regarding test concentration, pH, temperature, dissolved oxygen concentration, TOC, test design, etc.). The type and distribution of the BCF data should be considered and any data outliers assessed and removed from the data set if appropriate. If the data distribution does not point to use of geometric mean, use of another mean (e.g. arithmetic mean) should be considered.

There may be circumstances where a different approach is justified, for example 90^{th} percentile of BCF data on same species and lifestage where many data are available, e.g., 10 or more.

In all cases, the approach should be well justified and documented. This should include a discussion of outlying results. In particular, the representativeness of the test conditions should be carefully assessed for each test result. Particular scrutiny should be given if results from the tests are close to the B or vB threshold.

Other in vivo data

Field data

Reliable information from field studies can be used to decide if the CLP B/vB criteria are met. A reliable field field BMF >1 or field TMF >1 indicates that biomagnification of a substance occurs and can on its own be considered as a basis to conclude that a substance meets the B or vB criteria. However, absence of such a biomagnification potential cannot be used to conclude that these criteria are not fulfilled. This is because a field BMF only represents the degree of biomagnification in the specific predator/prey relationship for which it was measured. However, a field TMF represents biomagnification in the whole food web studied.

Substances that partition into lipids should, as far as possible, be lipid normalised to

account for differences in lipid content between prey and predator. It allows for a comparison of field BMF values in a direct and objective manner.

If field BAF values (based on reliable information) are above the criteria for B or vB it should be considered as part of the WoE approach. For comparison of a fish field BAF with the CLP criteria, BAF values should be expressed on wet weight basis for whole body with a lipid content of 5%.

Toxicokinetics data for mammals

If a whole-body, terminal elimination half-life is longer than 4 days in rat, and/or 50 days in humans, then this is an indication that the substance has vB properties. There may be exceptional cases where the derived elimination half-life threshold values in rats or humans cannot be used as an indicator of vB, for example where there is very low dietary absorption efficiency. Such cases require an individual assessment to determine whether the substance is vB or not.

If whole-body terminal elimination half-lives are between 2.5 and 4 days in rat, and/or 20 and 50 days in human, it is an indication that the substance has B properties.

In either case (B or vB), data indicating that the above thresholds are met should result in further consideration in a WoE assessment.

Other available data

Use of other available data is discussed in the respective sections of this guidance:

- In vitro fish toxicokinetic tests (4.3.3.2.3.5)
- Bioaccumulation tests in sediment-dwelling species (4.3.3.2.3.6)
- Bioaccumulation tests in terrestrial species (soil dwelling organisms) (4.3.3.2.3.7)
- Chronic toxicity tests on animals (4.3.3.2.3.9)
- Octanol-water partitioning coefficient K_{OW} (4.3.3.2.6.1)
- Octanol-air partitioning coefficient K_{OA} (4.3.3.2.6.2)
- (Q)SAR models to predict BCF (4.3.3.2.6.3)
- Biomimetic extraction procedures (4.3.3.2.6.4)
- Molecular size and octanol solubility (4.3.3.2.6.5)

Valid QSAR predictions for Log K_{OW} and BCF can be used as supporting information in WoE determination.

A summary of the different indicative thresholds which can be used for assessing a range of parameters for bioaccumulation is provided in Table 2 below with a link to the respective section of this guidance.

Table 2. Overview table for thresholds

Parameter	**Indicative threshold**	**Guidance Section**
Log K_{OW}	>4.5	4.3.3.2.6.1
Log K_{OA} *and*	>5 *and*	4.3.3.2.6.2

Log K_{OW}	>2	
Field TMF	>1	4.3.3.2.3.8
Field BMF	>1	4.3.3.2.3.8
Field Fish BAF	>2000/5000	4.3.3.2.3.8
Human whole body terminal elimination half-life/days	20/50 days	4.3.3.2.3.10
Rat whole body terminal elimination half-life/days	2.5 / 4 days	4.3.3.2.3.10

The Bioaccumulation Assessment Tool (BAT), accompanied by guiding principles in the BAT manual (Armitage *et al*., 2021), is a tool that promotes standardised recording and evaluation of various lines of evidence related to the endpoint bioaccumulation.

When integrating and weighing information, reliable evidence of bioaccumulation cannot be outweighed by information showing no bioaccumulation.

The following Figure presents the decision scheme that needs to be followed based on the available information, in order to come to a robust conclusion on whether the CLP criteria for Bioaccumulative and/or Very Bioaccumulative are fulfilled (Figure 5).

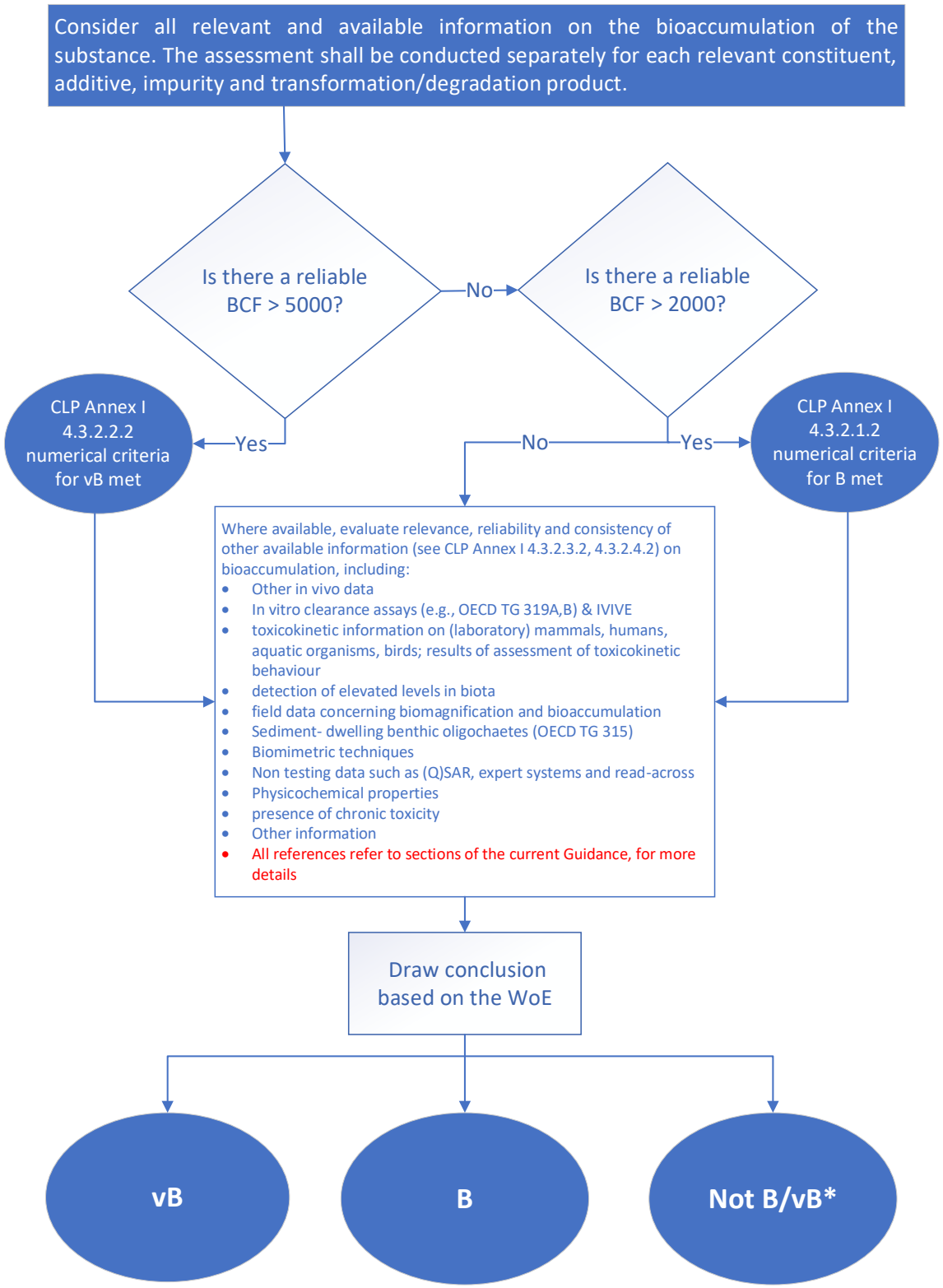


Figure 5. Decision scheme for concluding on the assessment criteria for Bioaccumulation (B/vB).

Toxicity: Section 4.3.3.4 of the current Guidance document describes the relevant experimental and computational information that may be provided as part of the WoE determination on Toxicity. Study-specific considerations on the relevance and reliability of

the individual pieces of information, as well as the conditions for meeting the criteria for classification in the different hazard classes are further analysed in the CLP Guidance. As discussed in the introduction of Section 4.3.3.5, results from studies that can directly be compared to the CLP criteria (CLP Annex I, 4.3.2.1.3 and 4.4.2.1.3 and Section 4.3.3.4 of this Guidance) are to be given higher weight in the WoE assessment (Figure 6, first entry). As always, the studies must be reliable and conducted in relevant substance and testing conditions.

Concerning results from long-term toxicity testing on terrestrial organisms and sediment, a case-by-case assessment of the study results including expert judgement should be performed (Figure 6, second entry). As discussed in a previous Section, the equilibrium partitioning method (EPM) may be used to back-calculate a NOEC or EC_{10} value of an existing sediment or terrestrial toxicity test to a corresponding aquatic NOEC or EC_{10}. This approach, as detailed in Section 4.3.3.4.8 has uncertainties and the use of any such information needs to be treated with caution, in a case-by-case basis and depending on the presence of other information types. In cases where the available environmental database is limited exclusively to studies on terrestrial organisms and/or sediment, it is highly recommended that a proposal for harmonised classification is only submitted once information generation via different REACH, PPPR, BPR or other legislative contexts has been completed and/or if other, more conclusive, information relevant for classification becomes available. This is because direct generation of information cannot be triggered under CLP. It is worth noting that in case of future scientific and regulatory agreement on the introduction of additional numerical criteria for terrestrial organisms or sediments within UN GHS, this will need to be reflected in an updated CLP and Guidance.

Concerning data for birds (Figure 6, second entry), they also cannot be directly, numerically compared with the T criteria in the absence of an agreed regulatory threshold value, but can be used in conjunction with other evidence of toxicity as part of a WoE determination. For PBT/vPvB assessment purposes under REACH, a NOEC value of below 30 mg/kg food in a long term bird study was considered as a strong indicator for a substance possessing "T" properties (ECHA Guidance on IR&CSA, Chapter R.11.4.1.3.2).

Concerning the use of short-term aquatic toxicity study results (**Error! Reference source not found.**, third entry), if such data show that the substance is very toxic ($L(E)C_{50}$ < 0.01 mg/L, ECHA Guidance on IR&CSA, Chapter R.11.2.2), a conclusion may be drawn that the substance is "T", combined with all other available information. It is hereby noted that for certain lipophilic substances, acute toxicity may not occur at the limit of the water solubility of the substance (or the highest concentration) tested, but chronic toxicity may still be exhibited.

Other available convincing information that may be used is QSARs, read-across/ grouping approaches, data from mammalian studies, monitoring data and any other data with a suitability and reliability that can reasonably be demonstrated. Only a few QSAR models predicting chronic aquatic toxicity are currently available, but further research on the QSAR prediction of chronic toxicity may increase their predictive capacities. Therefore, at the current state of the art, QSAR models generally seem not to be applicable for an unequivocal assessment of the T criterion (ECHA Guidance on IR&CSA Chapter R.11). Key considerations on important substance physical-chemical and environmental fate properties and any targeted modes of action introducing higher sensitivity to some species over others also need to be addressed.

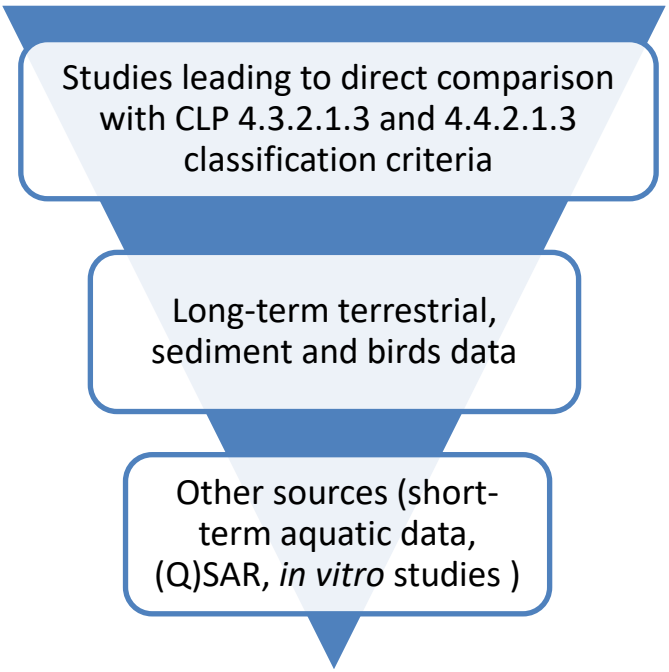
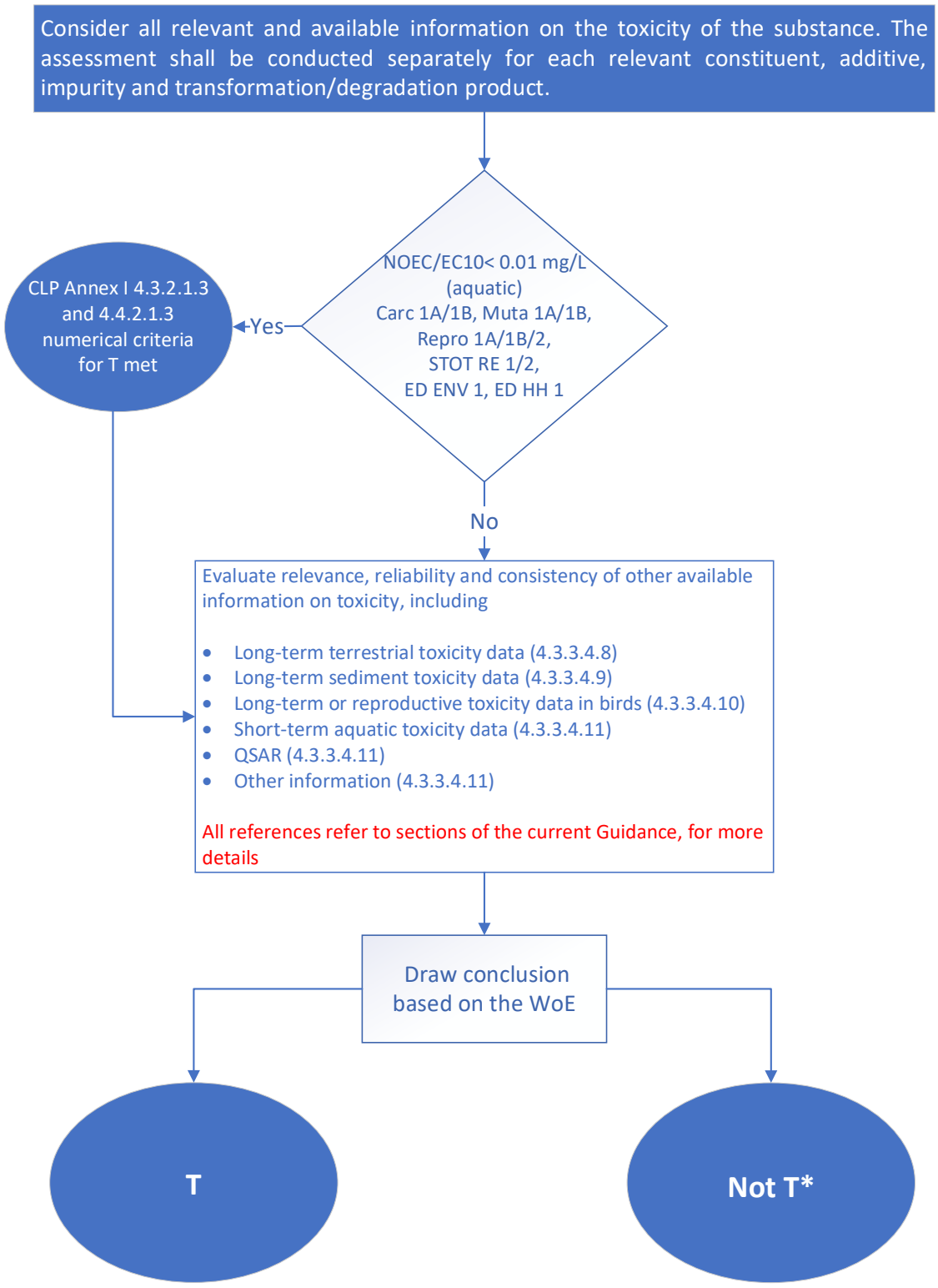


Figure 6. Simplified illustration of the relative weight of the available information (not taking into account the quality of the data) for Toxicity

In line with the CLP Guidance on aquatic hazards (Section 4.1.3.2.4.3), where more than one acceptable toxicity test results are available for the same species, the most sensitive (the one with the lowest L(E)C_{50} or NOEC/EC_{10} value) may be used as the representative toxicity value for that species. Effect concentrations for different species should not be aggregated but considered in a WoE approach. In the presence of **four or more** test results for the same species and effects endpoint, the geometric mean of the reliable toxicity values may be used as the representative toxicity value for that species, if the life stage is the same and test conditions of the different studies are equivalent (for example regarding pH, temperature, dissolved oxygen concentration, TOC, test design, duration, etc.). In case of very large data sets meeting the criteria for applying the Species Sensitivity Distribution (SSD) approach (see ECHA Guidance on IR&CSA, Chapter R.10) or other statistical techniques (e.g. HC_5 derivation, use of 10^{th} or 90^{th} percentiles, etc.) can be considered in order to estimate the aquatic toxicity reference value for classification (equivalent to using the lowest EC_{50} or NOEC), within the WoE.

In all cases, the approach should be well-justified and documented and should be supported by the WoE of evidence analysis, including a discussion of outlier results. In particular, the representativeness of the test conditions should be carefully assessed for each test result. Particular scrutiny should be given to results from tests close to the T threshold value.

The following Figure 7 presents the decision scheme for concluding on the assessment criteria for Toxicity (T).



* - Principally, due to conclusive data, inconclusive data, or a lack of data.

Figure 7. Decision scheme for concluding on the assessment criteria for Toxicity (T)

4.3.3.6. Application of the WoE to conclude on PMT/vPvM properties

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.4.2.3. Basis of classification

For the classification of PMT substances and vPvM substances, a WoE determination using expert judgment shall be applied, by comparing all relevant and available information listed in Section 4.4.2.3 with the criteria set out in Sections 4.4.2.1 and 4.4.2.2. That WoE shall be applied in particular where the criteria set out in Sections 4.4.2.1 and 4.4.2.2 cannot be applied directly to the available information.

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

The identification shall also take account of the PMT/vPvM properties of relevant constituents, additives or impurities of a substance and relevant transformation or degradation products.

This hazard class (PMT and vPvM properties) shall apply to all organic substances, including organo-metals.

The exact same considerations detailed in the introduction of Section 4.3.3.5 need also to be followed for the application of the WoE to conclude on PMT/vPvM properties. Very briefly, these refer, among others, to the need for separate conclusions for each property, the relevance and availability of the information, the fact that the criteria for P/vP, M/vM and T do not have to be met for the same environmental compartment, the higher weight placed on experimental studies that can directly be compared to the CLP criteria and the use of non standard methods. As for PBTs/vPvBs, the conclusions of the application of the WoE to conclude on the individual PMT/vPvM properties also can be one of the following:

i. Substance is P/vP/M/vM/T
ii. Substance is not P/vP/M/vM/T

, with further elaboration on the reason for the "not" conclusion needed (e.g. based on conclusive data, on inconclusive data or complete lack of data).

The general principles of identification and assessment of hazard information for PMT/vPvM have already presented in Section 4.3.3.

Persistence: See earlier Section 4.3.3.5.

Mobility: Section 4.3.3.3 of this Guidance described the experimental and non-experimental methods that are currently available for obtaining the *K*oc value of a substance from adsorption/desorption and other types of testing. Briefly, test results according to OECD TG 106, 121, 312, TLC studies and reliable QSAR methods have been described and important considerations and limitations on their use accounted for. Section

4.3.3.3.7 further presented key considerations for information provided for ionisables including recommendations on testing for K_{OC} derivation.

As for the other properties, higher weight is placed on the results from reliable experimental methods that can directly be compared to the CLP criteria. From such methods, clear preference is placed into the one conducted according to OECD TG 106 as this method derives an experimental K_{OC} value that can directly be compared to the criteria stipulated in Annex I, 4.4.2.1.2 and 4.4.2.2 (Figure 8, first entry). Furthermore, it is applicable to both non-ionisable and ionisable substances and includes testing on a range of different natural soils with varying soil types.

The second entry of Figure 8 consists of other experimental studies that, in combination with other estimation methods can derive reliable a K_{OC} values. Such test results are those performed according to OECD TG 121, information from soil leaching columns (OECD TG 312) and soil thin and thick layer chromatography (TLC) following the considerations of Section 4.3.3.3.1 of the Guidance. Regulation (EU) No 283/2013 setting out the data requirements for active substance in pesticides pointed out that, where the batch equilibrium method cannot be applied due to fast degradation, methods such as studies with short equilibration times like the HPLC method shall be considered as an alternative (see point 7.1.3.1) referring on the use of the OECD TG 121 in the related Commission Communication (2013/C 95/01). The same document (see point 7.1.4.1) refers also to the potential use of the OECD TG 312 in conditions where the batch equilibrium method cannot be applied due to weak adsorption.

Due to the fact that field and lysimeter studies incorporate a high number of uncertainties and also introduce exposure-based considerations as clearly described in Section 4.3.3.3.2, such study results have a lower weight in the WoE (Figure 8, third entry). QSARs and other estimation methods deriving a K_{OC} value follows in significance (Figure 8, fourth entry) for the reasons explained in Section 4.3.3.3.3 pursuant to the quality considerations and appropriate documentation described in Section 4.3.3 of this Guidance being fulfilled. Lastly, information from monitoring studies and other approaches not leading to a numerical K_{OC} value may also be considered, together with all other available information (Figure 8, fifth entry). Data from environmental monitoring must be treated with caution, as the absence of a chemical in a given aquatic medium may merely reflect site-specific, analytical issues, environmental fate and/or exposure considerations rather than an intrinsic tendency of the chemical not to partition to water. Also, caution should be given to monitoring data close to point sources. More details can be found in earlier Sections of the Guidance.

Finally, specific attention should be paid on outliers and/or on values that fall very close to the regulatory criteria.

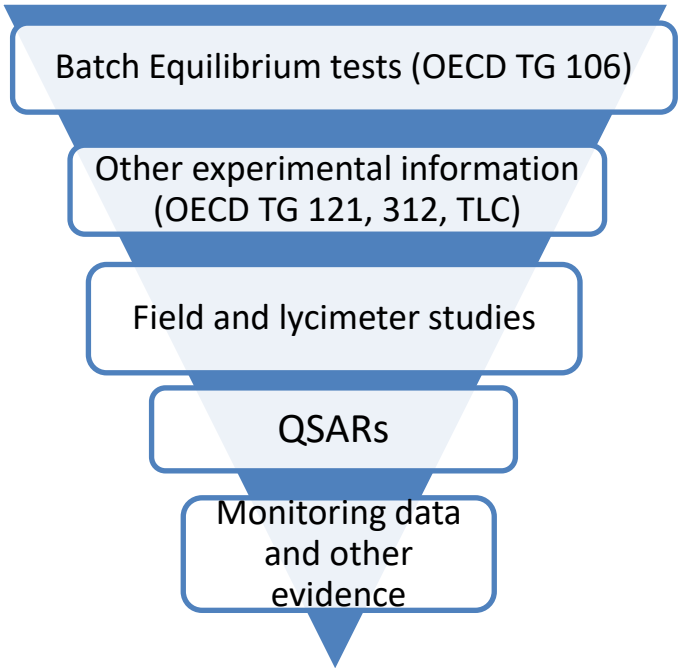


Figure 8. Simplified illustration of the relative weight of the available information (not taking into account the quality of the data) for Mobility

In the presence of several reliable studies conducted **under different** test protocols and all deriving a K_{OC} value, the overall available information needs to be weighted, with the outcome also depending on the reliability, relevance, documentation, uncertainty, any potential trends and length of the at hand dataset. As discussed above, any raised uncertainties need to be addressed by use of the precautionary principle (see bulletpoint (i) in Section 4.3.3).

In the presence of several reliable studies conducted **under the same** test protocol (for example OECD TG 106), the same principle as for persistence, bioaccumulation and toxicity is followed. Thus, normally the most conservative reliable value may be used as the representative one. This refers to the soil for which the lowest log K_{OC} value is obtained. For ionisable substances, the lowest log K_{OC} value for pH between 4 and 9 shall be considered to compare to the numerical M/vM criteria to conclude on whether a substance is mobile (M), very mobile (vM) or not mobile, for the purpose of hazard classification.

In the presence of **four or more studies** conducted according to the same test protocol, the geometric mean of the derived K_{OC} values (corresponding to an arithmetic mean for log K_{OC}) may be used. In practice, this would mean a geometric mean of 20 K_{OC} values (4 studies x 5 soils) for reliable tests performed according to OECD TG 106. Statistical computations (e.g. use of percentiles) are also possible to follow as long as there is adequate justification and documentation for their use.

The decision scheme in Figure 9 presents the proposed step-wise assessment to conclude on the assessment criteria for Mobility.

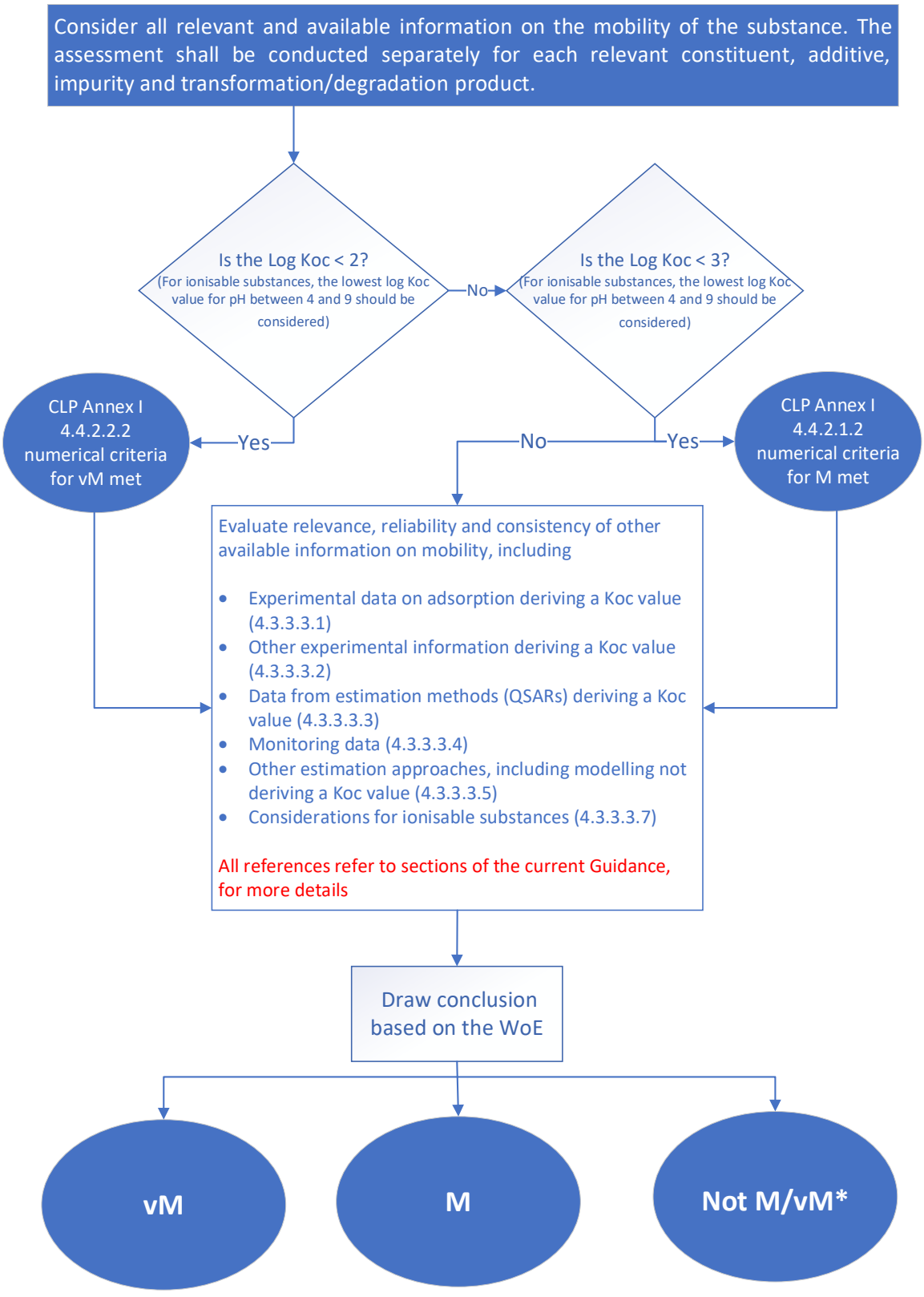


Figure 9. Decision scheme for concluding on the assessment criteria for Mobility (M/vM)

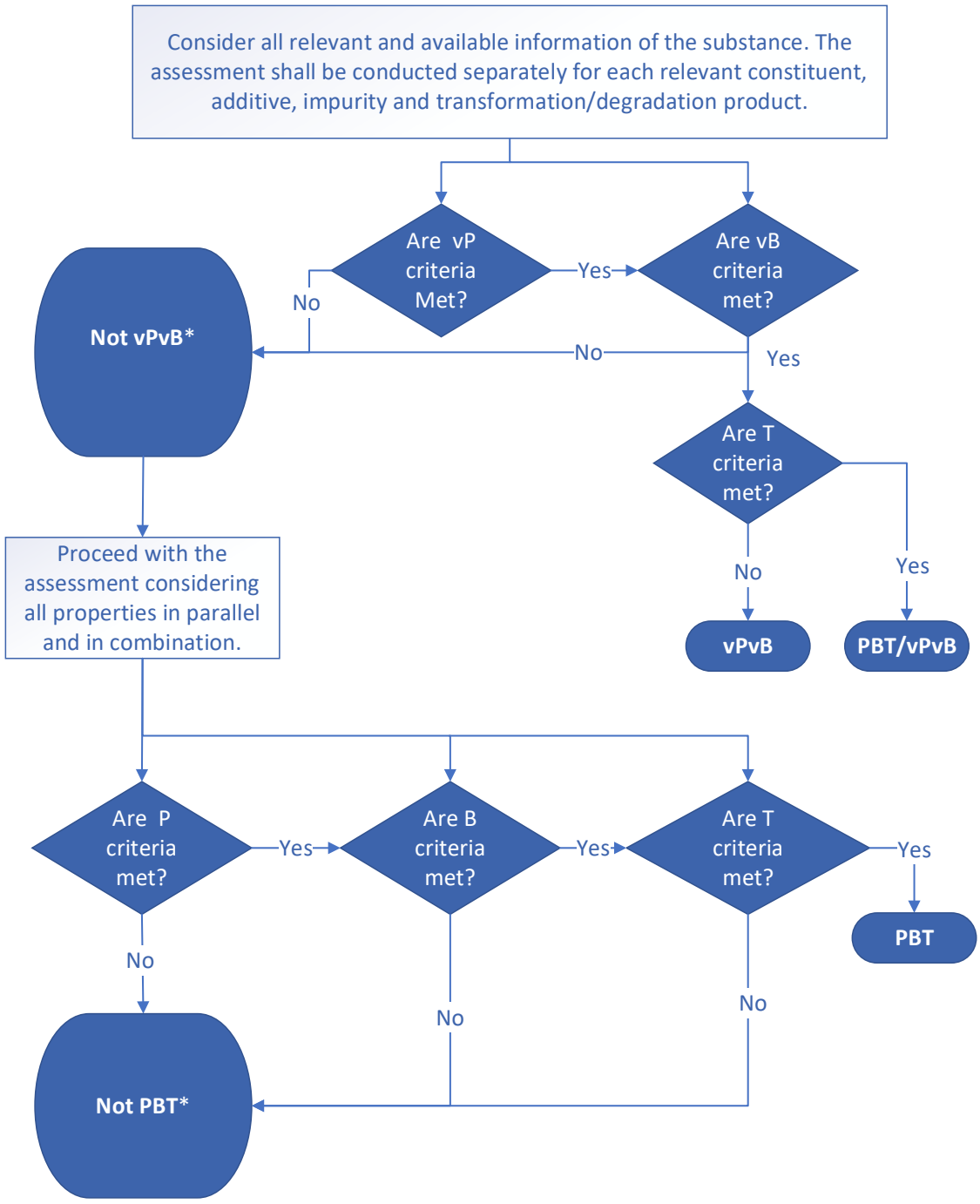
Toxicity: See earlier Section 4.3.3.5.

4.3.3.7. Overall conclusion on classification and labelling for PBT/vPvB substances

CLP Annex I, 4.3.2.4.1 states that "*the available results regardless of their individual conclusions shall be assembled together in a single WoE determination*". Therefore, on top of the conclusions drawn for the individual properties (P, B, vP, vB, T) that are also based on a WoE approach, the results must be assembled together in a single WoE determination. The assessment should also exhibit whether the relevant constituents, impurities, additives or transformation/degradation products possess PBT or vPvB properties or not (see bulletpoints (iv) and (v) in Section 4.3.3). Such a conclusion may be based on relevant data for the main constituent of a mono-constituent substance, relevant data for a constituent (or group of constituents as in 4.3.3 (iv)) and/or relevant data for one or more relevant impurity, additive or transformation or degradation product of the substance fulfilling the PBT/vPvB criteria. In all cases, the main elements that need to be included within the WoE as analysed in the previous Section 4.3.3.5, also apply for this concluding "single WoE determination".

Similarly, a conclusion that a substance and its relevant constituents, impurities, additives or transformation/degradation products does not meet all PBT/vPvB is also based on the overall WoE. If any of the criteria P, B or T are not met, the substance is not PBT. If any of the criteria vP or vB are not met, the substace is not vPvB. A conclusion that a substance does not fulfil all PBT/vPvB criteria must be followed by a statement clarifying whether this conclusion was based on conclusive, inconclusive or on lack of data. **Inconclusive** data refers to, for example, shortcomings in the provided information, uncertainties in the conduct of the study(ies) and their underlying assumptions, contradictory evidence, incomplete documentation, paucity of data, lack of statistical analysis, severe deviations from the test protocols, etc. **Lack of** data refers to a complete absence of any reliable data. As in any other case, it is at the discretion of a Dossier Submitter whether they may trigger regulatory follow-up action on such cases, depending on national priorities and other considerations.

ECHA Guidance on IR&CSA, Chapter R.11.4.1.4 presents further details on the different conclusion types for PBT/vPvB assessment and the use of constituent data. The following Figure 10 illustrates the decision scheme for concluding on the PBT/vPvB classification.



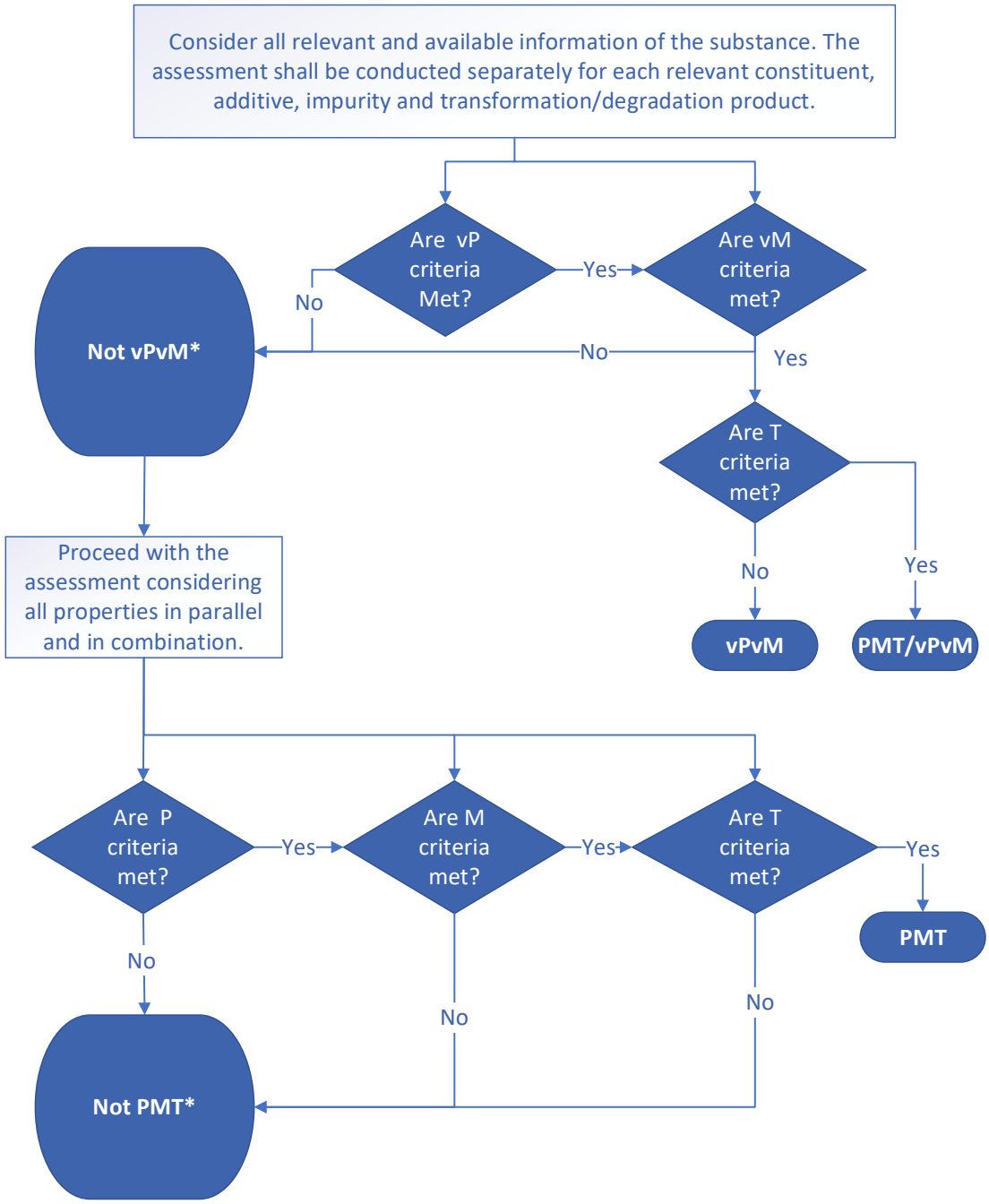
3915 * - Principally, due to conclusive data, inconclusive data, or a lack of data.

3916 **Figure 10. Decision scheme for concluding on PBT/vPvB classification.**

3917

4.3.3.8. Overall conclusion on classification and labelling for PMT/vPvM substances

Similar considerations as the ones described in Section 4.3.3.7 also apply for concluding on the PMT/vPvM hazard class,where the concept of "*the available results regardless of their individual conclusions shall be assembled together in a single WoE determination*" (CLP Annex I, 4.4.2.4.1) also applies. The following Figure 11 illustrates the decision scheme for concluding on the PMT/vPvM classification.



* - Principally, due to conclusive data, inconclusive data, or a lack of data.

Figure 11. Decision scheme for concluding on PMT/vPvM classification.

4.3.4. Classification criteria for PBT/vPvB and PMT/vPvM mixtures

Annex I: 4.3.3.1 and 4.4.3.1.

A mixture shall be classified respectively as a PBT or vPvB when at least one component contained in the mixture has been classified respectively as a PBT or vPvB and is present at or above 0,1 % (weight/weight).

A mixture shall be classified as a PMT or vPvM where at least one of its components has been classified as a PMT or vPvM and is present at or above 0,1 % (weight/weight).

The definition of "relevant components" of a mixture is similar to the one for aquatic hazards, namely those that are classified as PBT/vPvB or PMT/vPvM and are present in a concentration pf 0.1% (w/w).

Classification of mixtures shall be based on

(i) the available test data **for the individual components** of the mixture using the concentration limit of 0.1% for the components classified as PBT/vPvB or PMT/vPvM. This approach is clearly preferred in CLP and entails the application of the summation method or additivity formulas if data (either classification or toxicity) for all relevant or known components are available;

(ii) **the mixture as a whole** on a case-by-case basis, if PBT/vPvB or PMT/vPvM properties have not been established from the evaluation based on the individual components;

(iii) **bridging principles** on a case-by-case basis, in line with CLP Annex I, 1.1.3 when there are sufficient data on the individual components and similar tested mixtures. Data on similar tested mixtures shall be used only when it demonstrates classification for PBT/vPvB or PMT/vPvM, namely not to support a conclusion for no classification.

4.3.5. Hazard communication for PBT/vPvB and PMT/vPvM substances

Annex I: 4.3.4. Label elements shall be used in accordance with Table 4.3.1 for substances or mixtures meeting the criteria for classification in this hazard class (PBT and vPvB properties).

Table 4.3.1.
Label elements for PBT and vPvB properties

	PBT	vPvB
Symbol/pictogram		
Signal word	Danger	Danger
Hazard Statement	EUH440: Accumulates in the environment and living organisms including in humans	EUH441: Strongly accumulates in the environment and living organisms including in humans
Precautionary Statement Prevention	P201 P202 P273	P201 P202 P273
Precautionary Statement Response	P391	P391
Precautionary Statement Disposal	P501	P501

Annex I: 4.4.4. Label elements shall be used in accordance with Table 4.4.1 for substances or mixtures meeting the criteria for classification in this hazard class (PMT and vPvM properties)

Table 4.4.1.
Label elements for PMT and vPvM properties

	PMT	vPvM
Symbol/pictogram		
Signal word	Danger	Danger
Hazard Statement	EUH450: Can cause long-lasting and diffuse contamination of water resources	EUH451: Can cause very long-lasting and diffuse contamination of water resources
Precautionary Statement Prevention	P201 P202 P273	P201 P202 P273
Precautionary Statement Response	P391	P391
Precautionary Statement Disposal	P501	P501

A pictogram is currently unavailable for these two new hazard classes and may introduced if adopted in the context of UN GHS.

Further explanations on the precautionary statements can be found in Annex IV of CLP.

There are no additional labelling provisions for substances and mixtures classified as PBT/vPvB and PMT/vPvM.

4.3.6. Examples PBT/vPvB and PMT/vPvM substances

The following Section includes selected examples of substances that may or may not be classified as ones with PBT/vPvB and/or PMT/vPvM properties. It should be noted that the decision on classification is influenced by the strength of the overall evidence and should be decided on a case-by-case the opinion forming process of ECHA's Risk Assessment Committee (RAC) and a decision by the European Commission. As there is currently not any experience gained on dealing with such hazard classes under CLP, most of these examples broadly refer to substances that have already been concluded as SVHCs (PBT/vPvB) under REACH. The Guidance will be updated with more elaborative examples, also for PMT/vPvM substances, once more experience is gained.

In the meantime, very important reference material can be found in the following link that refers to the Candidate List of substances of very high concern for Authorisation[52], part of which comprises from substances identified as PBTs and/or vPvBs under REACH (namely, meeting the REACH Article 57(d) and (e) criteria). Finally, it is noted that one additional example substance refers to the only non approval decision taken by the European Commission for a pesticidal active substance, due to its PBT and vPvB properties. This example and the full risk assessment conducted by EFSA will not be reproduced in the current document, but the full conclusion document on the pesticide peer review is publically available[53].

List of examples included in this Section:

- 4.3.6.1. Example A: Substance meeting the REACH Article 57(d) and (e) criteria (PBT and vPvB), based on the overall WoE;
- 4.3.6.2. Example B: Substance meeting the REACH Article 57(e) criteria (vPvB), based on constituent data and on the overall WoE;
- 4.3.6.3. Example C: Substance meeting the REACH Article 57(f) criteria (ELoC), based on the overall WoE.

For each example substance, a table of all relevant data elements is presented, followed by relevant elements regarding the PBT/PMT hazard assessment, a Section showing the PBT/PMT classification, a Section with the reasoning behind the conclusions, and finally a table presenting the applicable labelling elements.

[52] https://echa.europa.eu/candidate-list-table
[53] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5085

4.3.6.1. Example A: Substance meeting the REACH Article 57(d) and (e) criteria (PBT and vPvB), based on the overall WoE

DATA ELEMENTS	Value	Test method / remarks
Physico-chemical properties and environmental fate		
Vapour pressure	2.0 10^{-5} Pa	OECD TG 104
Water solubility	0.25 mg/L	QSAR estimate
log octanol/water partition coefficient (log K_{OW})	6.3 (at 23°C)	QSAR estimate
log organic carbon/water partition coefficient (log K_{OC})	4.65	EPISuite 4.1 (K_{OW} method)
Degradation		
Ready biodegradability	2% in 28d	OECD TG 301C
Simulation studies in water-sediment	$DegT_{50,wat}$: 4-12d $DegT_{50,sed}$: 30-250d $DegT_{50,whole}$: > 180d	OECD TG 308 (for analogue susbstance in pond and river systems)
Hydrolysis	$T_{1/2}$ = 350d	OECD TG 111
Field degradation in soil	DT_{50}: 70-190d	Field study, several analogues
Monitoring studies	Presence in soils	For both substance A and analogues
QSARs	Slow degradation	BIOWIN predictions
Bioaccumulation		
Bioconcentration in fish (BCF)	6 000-12 000	OECD TG 305
Aquatic Toxicity		
Crustacea *Daphnia magna*:	3 mg/L (48h EC_{50})	OECD TG 202
Algae/aquatic plants *Lemna gibba*:	0.75 mg/L (7d E_rC_{50})	OECD TG 221
Crustacea *Daphnia magna*	0.45 mg/L (21d NOEC)	OECD TG 211
Other Toxicity		
STOT RE2 criteria met		

Hazard assessment elements:

Physico-chemical properties:

- The substance is poorly water soluble and strongly sorbing to solid matrices (log K_{OW} >6, log K_{OC} > 4.5). No information on dissociation.

Degradation:

- Hydrolysis data indicate long abiotic degradation half-lives;
- During a reliable ready biodegradation study, the substance was shown to be non-readily biodegradable (2% degradation after 28d);
- No simulation study is available for the parent substance. Water-sediment and soil field studies are available for analogue substances showing very slow degradation

in solid matrices. The whole system half-life was above 180 d. Faster degradation was exhibited for the water-phase in the water-sediment simulation test according to OECD TG 308;

- Several monitoring studies are available to indicate the presence of substance A and other structurally similar substances in sediments, many years after cessation of environmental releases.

Bioaccumulation:

- One reliable bioconcentration study on fish is available that derived high BCF values, indicating high potential for bioaccumulation. This is supported by a log K_{OW} value of 6.3.

Toxicity:

- Substance A meets the criteria for classification as STOT RE 2 as defined in the CLP Regulation. Available aquatic toxicity data indicate toxicity values below 1 mg/L for both acute and chronic toxicity.

Classification (pursuant to CLP Annex I, 4.3):

Persistent, Bioaccumulative and Toxic (PBT) properties: CLP Annex I, 4.3 **criteria met**

Very Persistent, Very Bioaccumulative (vPvB) properties: CLP Annex I, 4.3 **criteria met**

Reasoning:

- Persistence (the lines of evidence are sorted based on their respective weight from high to low weight):

(i) a water-sediment simulation study on one major metabolite (analogue substance). The read-across approach to the metabolite has been properly documented and the argumentation for its use (mainly very high structural similarity) is acceptable. The metabolite was shown to dissipate fast from the water phase to the sediment, where the degradation half-lives in both systems were above 180d, which exceeds the regulatory threshold value;
(ii) a soil field dissipation study on a very closely structurally similar substance, with dissipation half-lives as high as 190 days. Again, the read-across was comprehensively assessed and was deemed acceptable;
(iii) additional information from several monitoring studies for substance A and other structurally similar substances indicating long-term presence in sediments;
(iv) a ready biodegradation study that suggests that the substance is not subject to biodegradation (2% after 28 days);
(v) hydrolysis data indicating slow abiotic degradation rates;
(vi) validated QSAR predictions appropriate for the structure of substance A indicating slow environmental degradation.

Thus, it can be concluded that substance A fulfils the CLP Annex I, 4.3.2.1.1. (and also REACH Annex XIII 1.1.1.) **P**- and **vP**- criteria.

- Bioaccumulation:

In a BCF study on fish according to the OECD TG 305, lipid-normalized BCF values of 6 000 – 12 000 were found. As the study was protocol-compliant and was deemed scientifically reliable, it can be concluded that substance A fulfils the CLP Annex I, 4.3.2.1.2. (and also REACH Annex XIII 1.1.2.) **B**- and **vB**- criteria.

- Toxicity:

Substance A fulfils the criteria for classification as STOT RE 2 as defined in CLP Regulation Annex I, 3.9. Therefore, the substance can be concluded that substance A fulfils the CLP Annex I, 4.3.2.1.3. (c) (and also REACH Annex XIII 1.1.3.) **T** criteria.

Label elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	Danger
Hazard Statement	EUH440; EUH441
Precautionary statement(s)	P201, P202, P273

4.3.6.2. Example B: Substance meeting the REACH Article 57(e) criteria (vPvB), based on constituent data and on the overall WoE

Data for Constituent A which is present at (> 0.1 % w/w) in the UVCB substance:

DATA ELEMENTS: Constituent A	Value	Test method / remarks
Physico-chemical properties and environmental fate		
Vapour pressure	-	-
Water solubility	0.06; 0.58; 1.24 mg/L	WATERNTv1.01; WSKOW v.1.41; experimental value in Episuite
log octanol/water partition coefficient (log K_{OW})	5.52	KOWWIN v1.68
log organic carbon/water partition coefficient (log K_{OC}) pKa	5.265; 4.790 not ionisable	KOCWIN v2.00 (EPI Suite v4.11) MCI method; Kow method based on chemical structure
Degradation		
Hydrolysis	not expected	based on chemical structure
Phototransformation in air	$DegT_{50}$ 13.959 hours	AOP v1.92
Phototransformation in water	no significant decrease in concentration after 29 days	Reliability (4), Only brief study summary available
Phototransformation in soil	-	-
Ready biodegradability	-	-
Simulation studies in water; OECD TG 309 (study performed at 12°C)	$DegT_{50}$ >60 days	Reliability (2)
Simulation study in seawater	Primary $DegT_{50}$ >182 days at 20 °C	Reliability (4), raw data not available, used as supporting information
BIOWIN 2 & 3 predictions BIOWIN 3 & 6 predictions	Screens as P/vP Screens as P/vP	Reliability (2), MW of Constituent A within training set range Reliability (2), MW of Constituent A within training set range
Bioaccumulation		
Bioconcentration in fish, *O. mykiss* (BCFkgL)	12 993	Reliability 2, similar to OECD TG 305
BCF_{SSL} (5% lipid), *Cyprinus carpio*	1900 ± 300; 1100± 200	Reliability 4, No information on fish growth
BCF_K, *Lepomis macrochirus*	8148	Reliability 4, No information on lipid content or fish growth
Dietary BMFgL (5% lipid), *Oncorhynchus mykiss*	0.2	Reliability 2, depuration half life 8.1 days; estimated BCF 7241 or 8587.
BCF (QSAR estimate)	2041; 1146	Reliability 2, EPISUITE BCF BAF v 3.01 (regression; Arnot-Gobas)
Toxicity		
Crustacea *Daphnia magna*:	48h EC50 0.045 mg/L	Reliability 4
Algae	72h NOECr 1.4 mg/L	Reliability 4, OECD TG 201

Fish *Oryzias latipes* Fish *Oryzias latipes*	21-day LC50 0.025 mg/L 96-hour LC50 0.12 mg/L (95 % confidence interval: 0.053 – 0.27 mg/L).	Reliability 4, OECD TG 204 Reliability 4, OECD TG 203
Fish *Oryzias latipes*	41d NOEC: 11 µg/L	Reliability 4, OECD TG 210

DATA ELEMENTS: UVCB	Value	Test method / remarks
Physico-chemical properties and environmental fate (UVCB)		
Vapour pressure	0.002 hPa at 20 °C	calculated from experimental data at higher temperature using the Antoine equation
Water solubility	0.061 mg/L at 20 °C	
log octanol/water partition coefficient (log K_{OW})	5.3 – 6.5	OECD TG 117
log organic carbon/water partition coefficient (log K_{OC})	-	-
Degradation (UVCB)		
Hydrolysis	not expected	based on structure
Phototransformation in water	-	-
Phototransformation in soil	-	-
Ready biodegradability	14% biodegradation in 35 days (CO_2 evolution)	Reliability (2), OECD TG 301B
Simulation studies in water-sediment	-	-
Soil simulation study; similar to OECD TG 307 (temperature corrected to 12°C)	$DegT_{50}$ >218 days	
Bioaccumulation		
Bioconcentration on fish (BCF)	-	-
Crustacea *Daphnia magna*: Crustacea *Daphnia magna*	EC_{50} > 0.069 mg/L, NOEC 0.008 mg/L 21 d NOELR for reproduction < 1.0 mg/L	Reliability (2), OECD TG 202 Reliability (4), OECD TG 211

Hazard assessment elements:

Physico-chemical properties:

- Constituent A is poorly water soluble, lipophilic and it not expected to dissociate based on its chemical structure. It is present in the UVCB in the concentration range 0.2-2%.

Degradation:

- Constituent A is not expected to hydrolyse based on its chemical structure. There

is no ready biodegradability study on Constituent A but it is predicted to screen as persistent by Biowin 2, 3 and 6. A ready biodegradability study (Klimisch 2) on the UVCB reached 14% biodegradation in 35 days.

- A reliable (Klimisch 2) simulation test in river water is available for Constituent A showing that it meets the vP classification criteria, DegT50 > 60 days at temperature 12 °C. This is supported by a study in seawater performed at 20°C giving primary DegT50 >182 days. The primary DegT50 corrected to a temperature of 12°C would be even longer. The reliability of this study could not be assigned due to missing information (Klimisch 4).
- No monitoring studies are available for Constituent A or the UVCB.

Bioaccumulation:

- One reliable (Klimisch 2) fish BCF study and one reliable fish dietary study (Klimisch 2) are available for Constituent A performed on *Oncorhynchus mykiss*. Both studies point to a BCF >5000 indicating that the vB classification criterion is met. Reliable BCF QSAR predictions point to a BCF around 2000. The other BCF studies are of unassignable reliability but all point to the meeting either the B or vB criteria.

Toxicity:

- Neither the whole substance nor Constituent A meet the criteria for human health classification. The available aquatic toxicity studies for Constituent A are all of unassignable reliability (Klimisch 4) due to missing information. A reliable (Klimisch 2) long-term Daphnia study on the UVCB gives a NOEC for reproduction of 8 µg/L. However, it is not clear which constituents contributed to the toxicity so there is insufficient information to classify Constituent A as T .

Classification (pursuant to CLP Annex I, 4.3):

Persistent, Bioaccumulative and Toxic (PBT) properties: CLP Annex I, 4.3 criteria not met

Very Persistent, Very Bioaccumulative (vPvB) properties: CLP Annex I, 4.3 **criteria met**

Reasoning:

- Persistence: the WoE included results from

i. a ready biodegradation study on the whole substance that suggests that some constituents of the substance are not subject to biodegradation (14% after 35 days) (low weight as this does not bring information specifically for Constituent A);
ii. Reliable Biowin 2, 3 and 6 QSAR predictions suggest that Constituent A is not readily biodegradable and screens as P or vP. Currently there is no universally accepted definition of model domain for the Biowin models, however, the molecular weight is within the training set range for Constituent A (medium weight);
iii. A reliable simulation test in river water performed at 12°C is available for Constituent A showing that it meets the vP classification criteria in water, DegT50

> 60 days at temperature 12 °C. This values exceeds the P and vP criteria and the study is given high weight;

iv. A simulation study in seawater on Constituent A gave primary DegT50 >182 days at 12°C and 20 °C. The reliability of this study could not be assigned due to missing information but it supports the P and vP conclusion (low weight).

Thus, it can be concluded that Constituent A fulfils the CLP Annex I, 4.3.2.1.1. (and also REACH Annex XIII 1.1.1.) **P**- and **vP**- criteria. Since Constituent A is present in the UVCB Substance at >0.1%, the UVCB substance also fulfils the P and vP criteria in accordance with CLP.

- Bioaccumulation:

In a reliable fish bioaccumulation study according to OECD TG 305 a lipid-normalised, growth-corrected kinetic fish BCF of 12 993 was measured in Oncorhynchus mykiss for Constituent A (high weight). This is supported by a reliable dietary fish bioaccumulation study which gives an estimated BCF of 7241 or 8587 (medium weight). It can be concluded that Constituent A fulfils the CLP Annex I, 4.3.2.1.2. (and also REACH Annex XIII 1.1.2.) **B**- and **vB**- criteria. Since Constituent A is present in the UVCB Substance at >0.1%, the UVCB substance also fulfils the B and vB criteria in accordance with CLP.

- Toxicity:

Neither the UVCB nor its Constituent A meet the classification criteria for human health. There are insufficient reliable data on aquatic toxicity. It is not possible to conclude whether the T criteria are met.

Label elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	Danger
Hazard Statement	EUH441
Precautionary statement(s)	P201, P202, P273

4.3.6.3. Example C: Substance meeting the REACH Article 57(f) criteria (ELoC), based on the overall WoE

DATA ELEMENTS	Value	Test method / remarks
Physico-chemical properties and environmental fate		
Vapour pressure	3.5 10^{-6} Pa	
Water solubility	2.3 g/L	E Method A.6
log octanol/water partition coefficient (log K_{OW})	-1.4	ACD/ Labs
log organic carbon/water partition coefficient (log K_{OC})	1.5 1.1 0.9 1.2; 1.8 1.4 3.2	(KOCWIN v2.00) Extrapolation from log K_{OW} OECD TG 106 (pHs 4.5-7.5) FOOTPRINT Pesticides Properties Database, experimental information CompTox Chemicals Dashboard Experimental study, non-ionic species
pK_a	7.1	
Degradation		
Ready biodegradability	3% in 28 days	OECD TG 301C
Simulation studies in surface water	>80 days	OECD TG 309
Biodegradation in soil	> 3 years	ECETOC, non standard study
Abiotic degradation	Negligible degradation by hydrolysis and photodegradation	Experimental studies
Modelling studies	>40d in water, >80d in soil, >320d in sediments	Mackay Level III
Bioaccumulation		
Bioconcentration on fish (BCF)	<10, some reliability issues	OECD TG 305C
Bioconcentration on fish (BCF)	<1	Non-standard study
Bioconcentration on fish (BCF)	<0.5	Non-standard study
Log K_{OW}	-1.4	ACD/ Labs
Aquatic Toxicity		
Short and long term fish	> 10 mg/L	
Short and long term aquatic invertebrates	> 100 mg/L	
Algae and aquatic plants	> 100 mg/L	
Other Toxicity		
STOT RE 1 (H372) criteria met Carc 1B (H350) criteria met		
Other Information		
Monitoring studies	Presence in drinking and groundwater, rivers and lakes	
Modelling studies (CTD)	>2 000 km atmospheric transport potential	OECD Tool

Modelling studies (STP)	>98% in water phase for a municipal STP	SimpleTreat
Modelling studies (STP)	>90% partitioning to water	Mackay Level I; Mackay Level III

Hazard assessment elements:

Physico-chemical properties:

- The substance is very water soluble, not volatile and with very low adsorption potential. The substance can be found also at an ionised state, under relevant environmental conditions.

Degradation:

- Evidence from both abiotic degradation experimental studies (hydrolysis and photodegradation) indicates that it abiotically degrades very slowly;
- One ready biodegradability (OECD TG 301C) and one surface water simulation test (OECD TG 309) provided very low biotic degradation rates;
- The same conclusion is confirmed by both field (chemical presence in several biological wastewater treatment plants, WWTP) and modelling data (multimedia fate models deriving degradation half-lives and compartmental distribution) after cessation of environmental releases;
- Results from inherent biodegradability studies performed according to OECD TG 302B revealed <15% degradation after 28 days of incubation.

Bioaccumulation:

- One experimental study with reporting limitations (indicated that substance is not bioaccumulative to fish);
- The same conclusion also confirmed by two non-standard studies;
- No standard study on terrestrial bioaccumulation is available;
- Indication from the octanol-water partition coefficient (=-1.4) of low biomagnification potential.

Mobility:

- The substance has high water solubility;
- Experimental information (OECD TG 106) that log K_{OC} is below 1;
- Several computational studies all estimated log K_{OC} values below 2;
- Field evidence that the substance is present in several different water bodies in high concentrations;
- Modelling evidence that the substance partitions to water, does not volatilise and is slowly degraded;
- The low calculated Henry's law constant (=2 10^{-7} Pa*m^3/ mol) also provides additional evidence for low volatility from water bodies;
- Atmospheric transport over thousands of kilometres is predicted by modelling techniques.

Toxicity:

- Substance C has a harmonised classification as STOT RE 1 (H372);
- Substance C has a harmonised classification as Carc 1B (H350);
- Substance C has low aquatic toxicity.

Classification (pursuant to CLP Annex I, 4.3):

Persistent, Bioaccumulative and Toxic (PBT) properties: **No**, CLP Annex I, 4.3 criteria not met

Very Persistent, Very Bioaccumulative (vPvB) properties: **No**, CLP Annex I, 4.3 criteria not met

Persistent, Mobile and Toxic (PMT) properties: **Yes**, CLP Annex I, 4.4 criteria met

Very Persistent, Very Mobile (vPvM) properties: **Yes**, CLP Annex I, 4.4 criteria met

Reasoning:

- Persistence:

In the surface water simulation study according to OECD TG 309, the degradation half-life in surface water was higher than 60 days, therefore the substance fulfils the CLP Annex I, 4.3.2.1.1 and 4.4.2.1.1 **P** criteria, as well as the CLP Annex I, 4.3.2.2.1 and 4.4.2.2.1 **vP** criteria. Moreover, a half-life of more than 3 years was estimated for soil, supporting the conclusion for the very persistent nature of the substance. Thus, the overall WoE indicates that the substance is Persistent.

- Bioaccumulation:

The available data (BCF values below 10 , octanol-water partition coefficient -1.4) indicate that substance C does not fulfil the CLP Annex I, 4.3.2.1.2. **B** criteria nor the CLP Annex I, 4.3.2.2.2. **vB** criteria.

- Mobility

Results from several experimental and computational models have generated log K_{OC} values below 2. For the non-ionic species of the substance, a log K_{OC} of 3.2 was derived. Furthermore, the substance has high water solubilityT and low volatilisation from water potential (H= 2 10^{-7} Pa*m^3/ mol). Monitoring data reveal its wide presence in different water bodies with concentrations up to 5 µg/L in groundwater and other surface water bodies. Distribution modelling computations also confirm its affinity to water bodies and slow environmental degradation. A final statement that was considered during the SVHC process refers to the fact that the substance is not likely to be efficiently removed by adsorption to organic materials in sewage treatment plants (WWTP) or in drinking water

production. In summary, the substance can be concluded to fulfil the CLP Annex I, criteria for **M** and **vM**.

- Toxicity:

Substance C fulfils the CLP Annex I **T** criteria, as it has a harmonised classification as STOT RE 1 and Carc 1B.

Label elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	Danger
Hazard Statement	EUH450; EUH451
Precautionary statement(s)	P201, P202, P273, P391, P501

4.4. References

Adolfsson-Erici, M., Kerman, G.A., McLachlan, M.S. (2012). Measuring bioconcentration factors in fish using exposure to multiple chemicals and internal benchmarking to correct for growth dilution. Environ Toxicol Chem 31:1853–1860.

Armitage, J.M., Gobas F.A.P.C. (2007). A terrestrial food-chain bioaccumulation model for POPs. Environ Sci Technol 41:4019-25.

Armitage, J.M., Erickson, R.J., Luckenbach, T., Ng, C.A., Prosser, R.S., Arnot, J.A., Schirmer, K., Nichols, J.W. (2017). Assessing the bioaccumulation potential of ionizable organic compounds: current knowledge and research priorities. Environmental Toxicology and Chemistry 36(4) 882–897. https://doi.org/10.1002/etc.3680.

Armitage, J.M., Toose, L., Embry, M., Foster, K.L., Hughes, L., Sangion, A. Arnot, J.A. (2021). The Bioaccumulation Assessment Tool (BAT) Version 2.02. ARC Arnot Research and Consulting 6645 Inc., Toronto, ON, Canada.

Arnot J.A., Gobas, F.A.P.C. (2004). A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environmental Toxicology and Chemistry 23: 2343-2355.

Arnot J.A., Gobas, F.A.P.C. (2006). A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environmetnal Reviews 14(4): 257-297. https://doi.org/10.1139/a06-005.

Arp, H.P.H., Breedveld, G.D., Cornelissen, G. (2009). Estimating the in situ sediment-porewater distribution of PAHs and chlorinated aromatic hydrocarbons in anthropogenic impacted sediments. Environ. Sci. Technol. 43, 5576–5585.

Arp, H.P.H, Hale, S.E. (2019). REACH: Improvement of guidance and methods for the identification and assessment of PMT/vPvM substances. UBA report, texte 126/2019.

Arp, H.P.H., Hale, S.E. (2023). REACH: Guidance and Methods for the Identification and Assessment of PMT/vPvM SubstancesUBA TEXTE 19/2023. Neumann, M., Schliebner, I. [ed.], ISSN 1862-4804. German Environment Agency (UBA), Dessau-Roßlau, Germany

Arts MT, Ferguson ME, Glozier NE, Robarts RD, Donald DB (1995) Spatial and temporal 6658 variability in lipid dynamics of common amphipods: assessing the potential for uptake of 6659 lipophilic contaminants. Ecotoxicology 4(2):91-113.

ASTM International (2020) Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates E1706-20.

ASTM International (2022) Standard Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks E1022-22.

Badry, A, Rüdel, H, Göckener, B, Nika, MC, Alygizakis, N, Gkotsis G,Thomaidis, N.S., Treu, G., Dekker, R.W.R.J., Movalli, P., Walker, L.A., Potter, E.D., Cincinelli, A.,Martellini, T., Duke, G., Slobodnik, J., Koschorreck, J. (2022a). Making use of apex predator sample collections: an integrated workflow for quality assured sample processing, analysis and digital sample freezing of archived samples. Chemosphere 309, 136603. https://doi.org/10.1016/j.chemosphere.2022.136603.

Badry, A., Slobodnik, J., Alygizakis, N., Bunke, D., Cincinelli, A., Claßen, D., Dekker, R.W.R.J., Duke, G., Dulio, V., Göckener, B., Gkotsis, G., Hanke, G., Jartun, M., Movalli, P., Nika, M.-C., Rüdel, H. Thomaidis, N.S., Tarazona, J.V., Tornero, V., Treu, G., Vorkamp,

K., Walker, L.A., Koschorreck, J. (2022b). Using environmental monitoring data from apex predators for chemicals management: Towards harmonised sampling and processing of archived wildlife samples to increase the regulatory uptake of monitoring data in chemicals management. Environmental Sciences Europe 34, 81. https://doi.org/10.1186/s12302-022-00664-6.

Ball, A.L., Lavado, R. (2021). Ecotoxicology assessments in avian species using cell-based models: A review. Avian Biol Res. 14(4):105-112.

Barber, M.C. (2008). Dietary uptake models used for modeling the bioaccumulation of organic contaminants in fish. Environ Toxicol Chem 27(4):755-77.

Baskaran, S., Lei, Y.D., Wania, F. (2021a). A database of experimentally derived and estimated octanol-air partition ratios (KOA). J. Phys. Chem. Ref. Data 50, 043101 (2021). https://doi.org/10.1063/5.0059652.

Baskaran, S., Lei, Y.D., Wania, F. (2021b). Reliable prediction of the octanol-air partition ratio. Environmental Toxicology and Chemistry, 40(11), 3166–3180. https://doi.org/10.1002/etc.5201.

Bleeker, E.A.J. Verbruggen, E.M.J. (2009). Bioaccumulation of polycyclic aromatic hydrocarbons in aquatic organisms. RIVM report 601779002/2009. National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

Borgå, K., Kidd, K.A., Muir, D.C.G., Berglund, O., Conder, J.M., Gobas, F.A.P.C., Kucklick, J., Malm, O., Powell, D.E (2012). Trophic magnification factors: Considerations of ecology, ecosystems, and study design. Integr Environ Assess Manag 8:64–84.

Bronner, G., Goss, K.-U. (2011a). Sorption of Organic Chemicals to Soil Organic Matter: Influence of Soil Variability and PH Dependence. Environ. Sci. Technol. 2011, 45 (4), 1307−1312.

Bronner, G., Goss, K.-U. (2011b). Predicting Sorption of Pesticides and Other Multifunctional Organic Chemicals to Soil Organic Carbon. Environ. Sci. Technol. 45 (4), 1313−1319.

Brooke, D., Crookes, M. (2012). Depuration rate constant: growth correction and use as an indicator of bioaccumulation potential. Environment Agency, Bristol, UK. Product code LIT 7371. ISBN: 978-1-84911-283-3.

Burkhard, L.P., Cook, P.M., Lukasewycz, M.T. (2010). Direct application of biota-sediment accumulation factors. Environmental Toxicology and Chemistry 29: 230-236.

Burkhard, L.P., Arnot, J.A., Embry, M.R., Farley, K.J., Hoke, R.A., Kitano, M., *et al* (2012a). Comparing laboratory and field measured bioaccumulation endpoints. Integrated Environmental Assessment and Management 8: 17-31.

Burkhard, L.P., Borgå, K., Powell, D.E., Leonards, P., Muir, D.C.G., Parkerton, T.F., Woodburn, K.B. (2013). Improving the quality and scientific understanding of Trophic magnification factors (TMFs). Env. Sci. technol. 47: 1186-1187.

Burkhard, L..P, Cowan-Ellsberry, C., Embry, M.R., Hoke, R.A., Kidd K.A. (2012b). Bioaccumulation data from laboratory and field studies: Are they comparable? Integrated Environmental Assessment and Management 8: 13-16.

Buser AM, Schenker S, Hungerbühler K (2013). Comparing the Performance of Computational Estimation Methods for Physicochemical Properties of Dimethylsiloxanes and Selected Siloxanols. Journal of Chemical & Engineering Data 58:11, 3170-3178.

Commission Guidance Document (2021). Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. https://food.ec.europa.eu/system/files/2022-02/pesticides_ppp_app-proc_guide_fate_aged-sorption_0.pdf

Corbin, M., Eckel, W., Ruhman, M., Spatz, D., Thurman, N., Gangaraju, R., Kuchnicki, T. Mathew, R. & Nicholson, I. (2006). NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies. US EPA and Health Canada Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/nafta-guidance-document-conducting-terrestrial

Cornelissen, G, Gustafsson, Ö, Bucheli, TD, Jonker, MT, Koelmans, AA and van Noort, PC (2005). Extensive sorption of organic compounds to black carbon, coal, and kerosene in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environ Sci Technol 39:6881-95.

Costanza, J., Lynch, D.G., Boethling, R.S., Arnot J.A. (2012). Use of the bioaccumulation factor to screen chemicals for bioaccumulation potential. Environmental Toxicology and Chemistry 31:2261-2268.

Czub, G., McLachlan M.S. (2004). Bioaccumulation potential of persistent organic chemicals in humans. Environmental Science and Technology 38: 2406-2412.

de Wolf, W., Mast, B., Yedema, E.S.E., Seinen W., Hermens J.L.M. (1994). Kinetics of 4-chloroaniline in guppy *Poecilia reticulata*. *Aquat Toxicol* 28:65-78.

Deneer, J.W., Adriaanse, P.I., van Griethuysen, C., Boesten, J.J.T.I (2015) Estimation of degradation rates in cosm water. Guidance for inverse modelling using TOXSWA. Alterra Wageningen. Alterra report 2679, ISSN 1566-7197. Available at: http://edepot.wur.nl/367261.

Di Toro, D.M., Berry, W.J., Burgess, R.M., Mount, D.R., O'Connor, T.P., Swartz, R.C. (2005). The Predictive Ability of Sediment Quality Guidelines Derived Using Equilibrium Partitioning. In: Wenning RJ and Ingersoll CG (Eds.) Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments, SETAC Press, Pensacola, FL, USA.

Droge, S. T. J., Goss, K.-U. (2013). Sorption of Organic Cations to Phyllosilicate Clay Minerals: CEC-Normalization, Salt Dependency, and the Role of Electrostatic and Hydrophobic Effects. Environmental Science & Technology, 47(24), 14224–14232. https://doi.org/10.1021/es403187w.

Droge, S.T.J., Scherpenisse, P., Arnot, J.A., Armitage, J.M., McLachlan, M.S., von der Ohe, P.C., Hodges, G. (2021). Screening the baseline fish bioconcentration factor of various types of surfactants using phospholipid binding data. Environ. Sci.: Processes Impacts 23, 1930-1948. https://doi.org/10.1039/D1EM00327E.

ECB (2003). Technical Guidance Document on Risk Assessment, Part III, Chapter 4, Use of (Quantitative) Structure Activity Relationships ((Q)SARs).

ECETOC (2014). Information to be considered in a weight-of-evidence-based PBT/vPvB assessment of chemicals (Annex XIII of REACH). Special report no. 18. 158 p. Available at: http://www.ecetoc.org/publications/special-reports/.

ECETOC technical report No. 130 (2021). Persistent chemicals and water resources protection.

ECHA (2008). Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals. https://echa.europa.eu/documents/10162/17224/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9?t=1322594777272.

ECHA (2016a). How to use alternatives to animal testing to fulfil the information requirements for REACH registration. Practical guide. https://op.europa.eu/en/publication-detail/-/publication/e54fc06b-9ffd-11e6-868c-01aa75ed71a1/language-en

ECHA (2016b) Guidance on information requirements and Chemical Safety Assessment, Chapter R.16: Environmental exposure assessment, Version 3.0, February 2016

https://echa.europa.eu/documents/10162/17224/information_requirements_r16_en.pdf/b9f0f406-ff5f-4315-908e-e5f83115d6af?t=1455546505739.

ECHA (2017a). Read-Across Assessment Framework (RAAF). https://echa.europa.eu/documents/10162/17221/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a

ECHA (2017b). Guidance for identification and naming of substances under REACH and CLP. https://echa.europa.eu/view-article/-/journal_content/title/guidance-for-identification-and-naming-of-substances-under-reach-and-clp.

ECHA (2017c). Guidance on the Biocidal Products Regulation Volume IV Environment - Assessment and Evaluation (Parts B + C) Version 2.0.

ECHA (2022b) ECHA note: Options to assess persistence of volatile substances in regulatory PBT assessment. 17 Novenber 2022, p.29. https://echa.europa.eu/documents/10162/17228/note_volatiles_in_simulation_tests_en.pdf/d218ddcb-e5da-7c0a-e5d0-3eae3e1c26dc?t=1669388686441

ECHA Working group on Toxicokinetics (2022) Bioaccumulation assessment of air-breathing mammals. Discussion paper. November 2022. https://echa.europa.eu/documents/10162/17228/bioaccumulation_assessment_of_air_breathing_mammals_en.pdf/56de6276-06e9-9eed-a7dd-a75336fda71b?t=1669388928484

ECHA Working group on Toxicokinetics (2022). Bioaccumulation assessment of air-breathing mammals. Discussion paper. November 2022. https://echa.europa.eu/documents/10162/17228/bioaccumulation_assessment_of_air_breathing_mammals_en.pdf/56de6276-06e9-9eed-a7dd-a75336fda71b?t=1669388928484

EFSA (2007). Opinion on a request from EFSA related to the default Q10 values used to describe the temperature effect on transformation rates of pesticides in soil. EFSA Journal 622:1-32.

EFSA (2013). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA J., 11 (7), p. 3290.

EFSA (2014). EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014; 12(5):3662, 67 pp. Available at: https://www.efsa.europa.eu/fr/efsajournal/pub/3662.

EFSA (2015). Statement on the FERA guidance proposal: 'Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments' (FERA, 2012). EFSA Journal 13(7):4175, 54 pp.

EFSA (2017). Hardy A, Benford D, Halldorsson T, Jeger MJ,Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V,Solecki R, Turck D, Benfenati E, Chaudhry QM, Craig P, Frampton G, Greiner M, Hart A, Hogstrand C,Lambre C, Luttik R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J-L, Fernandez Dumont A,Hempen M, Valtuena Martınez S, Martino L, Smeraldi C, Terron A, Georgiadis N and Younes M, 2017.Scientific Opinion on the guidance on the use of the WoE approach in scientific assessments. Guidance on the use of the WoEapproach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi.org/10.2903/j.efsa.2017.4971).

EFSA (2022). Egsmose M, Fait G, Janzen W, JentzschF, Lava R, Lythgo C, Padovani L, Pickl C and Priegnitz J. Scientific guidance on soil phototransformation products in groundwater–consideration, parameterisation and simulation in the exposure assessment of plant protection products. EFSA Journal 2022;20(3):7119, 48 pp. https://doi.org/10.2903/j.efsa.2022.7119.

EFSA (2023). Alf Aagaard, Philippe Berny, Pierre-Franc ̧ois Chaton, Ana Lopez Antia, Emily McVey,Maria Arena, Gabriella Fait, Alessio Ippolito, Alberto Linguadoca, Rachel Sharp,Anne Theobald and Theodorus Brock. Journal 2023;21(2):7790). https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.7790.

Environment Agency (2023). Lipid normalisation in the OECD 305 dietary test. Environment Agency, Bristol.

Environment Canada (2013). Biological Test Method: Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod Hyalella azteca EPS 1/RM/33 Second Edition.

European Commission (2002). Opinion of the scientific committee on plants on methods for the determination of the organic carbon adsorption coefficient (K_{OC}) for a plant protection product active substance in the context of Council Directive 91/414/EEC. SCP/KOC/002.

European Commission (2008). Pesticides in Air: considerations for exposure assessment. SANCO/10553/2006 Rev 2.

European Commission (2012). https://food.ec.europa.eu/system/files/2016-10/pesticides_ppp_app-proc_guide_fate_evidence_identify-pop-pbt-vpvb-props.pdf.

European Commission (2013). Commission Communication in the framework of the implementation of Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. 2013/c 95/01.

European Commission (2014). Common implementation strategy for the Water framework Directive (2000/60/EC), Guidance document No. 32 on biota monitoring (the implementation of EQSbiota) under the Water Framework Directive. Technical Report - 2014 – 083. ISBN 978-92-79-44634-4 doi: 10.2779/833200.

European Commission (2018). Guidance document No. 27, updated version 2018. Technical Guidance for Deriving Environmental Quality Standards. Document endorsed by EU Water Directors at their meeting in Sofia on 11-12 June 2018.

FOCUS (2001). Generic Guidance for Tier 1 FOCUS Ground Water Assessments. https://esdac.jrc.ec.europa.eu/public_path/projects_data/focus/gw/docs/Generic_guidance_FOCUS_GW_V2-3final.pdf.

FOCUS (2014). Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on Degradation Kinetics. 18 December 2014. Version 1.1, 440 pp. Available at: https://esdac.jrc.ec.europa.eu/public_path/projects_data/focus/dk/docs/FOCUSkineticsvc1.1Dec2014.pdf.

Gustafson, D.I. (1989). Groundwater ubiquity score: A simple method for assessing pesticide leachability. Environmental Toxicology and Chemistry 8:339-357).

Gobas, F.A.P.C., Kelly, B.C., Arnot, J.A. (2003). Quantitative Structure Activity Relationships for Predicting the Bioaccumulation of POPs in Terrestrial Food-Webs. QSAR Comb. Sci. 22: 346-351.

Gobas, F.A.P.C., de Wolf, W., Burkhard L.P., Verbruggen, E., Plotzke, K (2009). Revisiting bioaccumulation criteria for POPs and PBT assessments. Integr Environ Assess Manag 4:624-37.

Gobas, F.A.P.C., Lee, Y.S., Arnot, J.A. (2021). Normalizing the biomagnification factor. Environ Toxicol Chem 40, 1204-1211.

Gomez, C.F., Constantine, L., Huggett, D.B. (2010). The influence of gill and liver metabolism on the predicted bioconcentration of three pharmaceuticals in fish. Chemosphere 81(10):1189-1195 doi:https://doi.org/10.1016/j.chemosphere.2010.09.043

Goss KU, Brown TN and Endo S (2013). Elimination half-life as a metric for the bioaccumulation potential of chemicals in aquatic and terrestrial food chains. Environ Toxicol Chem 32:1663-71.

Goss, KU, Linden L, Ulrich N, and Schlechtriem C (2018). Revisiting elimination half live as an indicator for bioaccumulation in fish and terrestrial mammals. Chemosphere, 210, 341–346. https://doi.org/10.1016/j.chemosphere.2018.07.017

Hansen, J.B., Holm, P.E., Hansen, E.A. & Hjelmar O (2000). Use of lysimeters for characterization of leaching from soil and mainly inorganic waste materials, Nordtest Technical Report 473.

Hashizume N, Inoue Y, Suzuki Y, Murakami H, Sumi S, Ishibashi T, Yoshida T (2018). Comparison of laboratory-derived biomagnification factors for hexachlorobenzene in common carp conducted under 9 test conditions. Environ Toxicol Chem. 37(4):1032-1039. doi: 10.1002/etc.4030.

Henneberger, L, Goss, K-U (2019). Environmental Sorption Behavior of Ionic and Ionizable Organic Chemicals. In Reviews of Environmental Contamination and Toxicology Volume 253, 43-64.

Hennecke D, Bauer A, Shrestha P, Junker T, Fenner, K (2014). Improved test system to determine chemical degradation in laboratory water/sediment systems – experimental results. SETAC Europe 24th Annual Meeting, May 2014, Basel, Switzerland. http://cefic-lri.org/wp-content/uploads/2014/03/SETAC-14-Platform-Hennecke.pdf

Hennecke, D., Kruse, M., Bräutigam, J., Meisterjahn, B., Klein, J., Claßen, D., Trapp, S., Kästner, M., Brock, A. L., and Schäffer, A. (2023). Silylation: a reproducible method for

characterization of non-extractable residues (NER) of organic chemicals in the assessment of persistence. Environmental Science: Advances. https://doi.org/10.1039/D2VA00314G

Hermosin, M. C.; Cornejo, J.; Cox, L. (2000). Calculation and validation of Kclay as predictor for polar or ionic pesticide adsorption by soils. In Pesticide/Soil Interactions. Some Current Research Methods; Cornejo, J., Jamet, P., Eds.; INRA Editions: Paris, France, pp 131−139.

Hodges, G., Eadsforth, C., Bossuyt, B., Bouvy, A., Enrici, M. H., Geurts, M., Kotthoff, M., Michie, E., Miller, D., Müller, J., Oetter, G., Roberts, J., Schowanek, D., Sun, P., & Venzmer, J. (2019). A comparison of log K ow (n-octanol–water partition coefficient) values for non-ionic, anionic, cationic and amphoteric surfactants determined using predictions and experimental methods. *Environmental Sciences Europe*, *31*(1), 1–18. https://doi.org/10.1186/s12302-018-0176-7

Hofer, T., Myhre, O., Peltola-Thies, J. Hirmann, D. (2021). Analysis of elimination half-lives in MamTKDB 1.0 related to bioaccumulation: Requirement of repeated administration and blood plasma values underrepresent tissues. Environment International 155, 106592. DOI: https://doi.org/10.1016/j.envint.2021.106592.

Holmberg, R., Wedebye, E.B., Nikolov, N.G.,Tyle, H. (2021). How many potential vPvM/PMT substances have been registered under REACH? - vPvM/PMT-screening by using the Danish (Q)SAR database. Danmarks Tekniske Universitet: DTU (2021).

Houde, M., Muir, D.C.G., Kidd, K.A., Guildford, S., Drouillard, K., Evans, M.S., Wang, X., Whittle, D.M., Haffner, D., and Kling, H. (2008). Influence of lake characteristics on the biomagnification of persistent organic pollutants in lake trout food webs. Environ Toxicol Chem 27:2169-78.

Huff Hartz K, Weston D, Johanif N, Poynton H, Connon R, Lydy M (2021). Pyrethroid 6988 bioaccumulation in field-collected insecticide-resistant Hyalella azteca. Ecotoxicology 30:514–6989 523.

Ingerslev, F., Nyholm, N. (2000). Shake-Flask Test for Determination of Biodegradation Rates of 14C-Labeled Chemicals at Low Concentrations in Surface Water Systems. Ecotoxicol Environ Saf 45:274-83.

JRC (2014). Hartmann, NB, Gottardo, S, Sokull-Klüttgen, B. Review of available criteria for non-aquatic organisms within PBT/vPvB frameworks. Part II: Toxicity assessment. Report EUR 26737 EN.

Kah, M., Brown, C.D. (2007). Prediction of the Adsorption of Ionizable Pesticides in Soils. J. Agric. Food Chem. 55, 6, 2312–2322.

Karickhoff, S.W., Brown, D.S., Scott, T.A. (1979). Sorption of hydrophobic pollutants on natural 6177 sediments. Water Res 13:241-8.

Kelly, B.C., Gobas, F.A.P.C. (2001). Bioaccumulation of persistent organic pollutants in lichen-caribou-wolf food chains of Canada's Central and Western Arctic. Environ Sci Technol 35:325–334.

Kelly, B.C., Gobas, F.A.P.C. (2003). An arctic terrestrial food-chain bioaccumulation model for persistent organic pollutants. Environ Sci Technol 37:2966–2974.

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Morin, A.E., Gobas, F.A.P.C. (2007). Food web-specific biomagnification of persistent organic pollutants. Science 317: 236-239.

Kidd, K.A., Burkhard, L.P., Babut, M., Borgå, K., Muir, D.C.G., Perceval, O., Ruedel, H.,

Woodburn, K.B., Embry, M.R. (2019). Practical advice for selecting or determining trophic magnification factors for application under the European Union Water Framework Directive. Integrated Environmental Assessment and Management 15: 266-277. DOI: 10.1002/ieam.4102.

Kierkegaard, A., van Egmond, R., McLachlan M.S. (2011). Cyclic volatile methylsiloxane bioaccumulation in flounder and ragworm in the humber estuary. Environmental Science and Technology 45:5936–5942. https://doi.org/10.1021/ES200707R.

Kim, J., Gobas, F.A.P.C., Arnot, J.A., Powell, D.E., Seston, R.M., Woodburn, K.B. (2016). Evaluating the roles of biotransformation, spatial concentration differences, organism home range, and field sampling design on trophic magnification factors. Science of the Total Environment, 551-552 (2016), pp. 438-451. https://doi.org/10.1016/j.scitotenv.2016.02.013.

Klein, J., Skodras, D., Klein, M. (2023). Leachability concept: Leachability as a process-based method to determine the mobility of chemicals in a PMT/vPvM framewor https://www.software.ime.fraunhofer.de/Leaching_Calculator/Leachability_Report.pdf.

Kosfeld, V., Rüdel, H., Schlechtriem, C., Rauert, C., Koschorreck, J. (2021). Food web on ice: a pragmatic approach to investigate the trophic magnification of chemicals of concern. Environmental Sciences Europe 33: 93.

Kumar, V., Upadhyay, N., Singh, S., Singh, J., & Kaur, P. (2013). Thin-Layer Chromatography: Comparative Estimation of Soil's Atrazine. Current World Environment, 8(3), 469–472. https://doi.org/10.12944/CWE.8.3.17.

Laue, H., Gfeller, H., Jenner, K.J., Nichols, J.W., Kern, S., Natsch, A. (2014). Predicting the bioconcentration of fragrance ingredients by rainbow trout using measured rates of in vitro intrinsic clearance. Environmental science & technology 48(16):9486-9495 .

Laue, H., Hostettler, L., Badertscher, R.P., Jenner, K.J., Sanders, G., Arnot, J.A., Natsch, A. (2020). Examining Uncertainty in in Vitro-in Vivo Extrapolation Applied in Fish Bioconcentration Models. Environmental Science & Technology 54(15):9483-9494 doi:10.1021/acs.est.0c01492.

Leistra, M., Van der Linden, A.M.A., Boesten, J.J.T.I., Tiktak, A., Van den Berg, F. (2001). PEARL model for pesticide behaviour and emissions in soil-plant systems. Description of processes. Alterra report 013, Alterra, Wageningen, RIVM report 711401009, Bilthoven, The Netherlands. Available at http://www.pearl.pesticidemodels.eu.

Lo, J.C., Allard, G.N., Otton, S.V., Campbell, D.A., Gobas, F.A.P.C. (2015). Concentration dependence of biotransformation in fish liver S9: Optimizing substrate concentrations to estimate hepatic clearance for bioaccumulation assessment. Environmental Toxicology and Chemistry 34(12):2782-2790 https://doi:10.1002/etc.3117.

Mackay, D. (2000). Multimedia Environmental Models: The Fugacity Approach, second edition, CRC Press.

Mackay, D., Celsie, A.K.D., Powell, D.E., Parnis, J.M. (2018). Bioconcentration, bioaccumulation, biomagnification and trophic magnification: a modelling perspective. Environmental Science: Processes & Impacts 20: 72-85.

McLachlan, M.S., Czub, G., Macleod, M., Arnot, J. (2011). Bioaccumulation of organic contaminants in humans: a multimedia perspective and the importance of biotransformation. Environ Sci Technol 45:197–202.

Mensink, B.J.W.G., Smit, C.E., Montforts, M.H.M.M. (2008). Manual for summarising and evaluating environmental aspects of plant protection products. RIVM report no 601712004. https://www.rivm.nl/bibliotheek/rapporten/601712004.pdf.

Mertens, J., Kahl, G., Gottesbüren, B., Vanderborght, J. (2009). Inverse modeling of pesticide leaching in lysimeters: local versus global and sequential single-objective versus multi-objective approaches.- Vadose zone journal.

Meylan, W.M., Howard, P.H. (2005). Estimating octanol–air partition coefficients with octanol–water partition coefficients and Henry's law constants, Chemosphere 61 (5), 640-644. https://doi.org/10.1016/j.chemosphere.2005.03.029.

Mansouri K, Grulke CM, Judson RS, Williams AJ. (2018). OPERA models for predicting physicochemical properties and environmental fate endpoints. J Cheminform. 2018 Mar 8;10(1):10.

Mount, D.R., Dawson, T.D., Burkhard, L.P. (1999). Implications of gut purging for tissue residues determined in bioaccumulation testing of sediment with *Lumbriculus variegatus.* Environmental Toxicology and Chemistry (18),1244–1249.

Neumann, M., Schwarz, M.A., Sättler, D., Oltmanns, J., Vierke, L., Kalberlah, F. (2015). A proposal for a chemical assessment concept for the protection of raw water resources under REACH. 25th annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC Europe), Barcelona, Spain.

Neumann, M Schliebner, I (2019). Protecting the sources of our drinking water: The criteria for identifying persistent, mobile and toxic (PMT) substances and very persistent and very mobile (vPvM) substances under EU Regulation REACH (EC) No 1907/2006. UBA TEXTE 127/2019. Ger. Environ. Agency (UBA), Dessau-Roßlau, Ger. ISBN 1862-4804.

Nichols, J., Fay, K., Bernhard, M.J., Bischof, I., Davis, J., Halder, M., Hu, J., Johanning, K., Laue, H., Nabb, D., Schlechtriem, C. Segner, H., Swintek, J., Weeks, J., Embry, E. (2018). Reliability of In Vitro Methods used to Measure Intrinsic Clearance of Hydrophobic Organic Chemicals by Rainbow Trout: Results of an International Ring Trial. Toxicological Sciences 164(2):563-575 doi:10.1093/toxsci/kfy113.

OECD (1981). Test No. 302A: Inherent Biodegradability: Modified SCAS Test, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070363-en.

OECD (1992). Test No. 301: Ready Biodegradability, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070349-en.

OECD (1992). Test No. 302B: Inherent Biodegradability: Zahn-Wellens/ EVPA Test, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070387-en.

OECD (1992). Test No. 306: Biodegradability in Seawater, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070486-en.

OECD (2000). OECD Series on Testing and Assessment, Number 22, Guidance Document for the Performance of Out-door Monolith Lysimeter Studies (ENV/JM/MONO(2000)8).

Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282000%298&doclanguage=en

OECD (2001). Guidance Document on the Use of the Harmonised System for the Classification of Chemicals which are Hazardous for the Aquatic Environment. Organisation for Economic Cooperation and Development (OECD), OECD Environmental Health and Safety Publications, Series on Testing and Assessment, No. 27, Paris, France.

OECD (2001). Test No. 303: Simulation Test - Aerobic Sewage Treatment -- A: Activated Sludge Units; B: Biofilms, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070424-en.

OECD (2002). Test No. 307: Aerobic and Anaerobic Transformation in Soil, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070509-en.

OECD (2002). Test No. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070523-en.

OECD (2004). Test No. 309: Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070547-en.

OECD (2004). OECD Principles for the Validation, for Regulatory Purposes, of (Quantitative) Structure-Activity Relationship Models. http://www.oecd.org/document/23/0,2340,en_2649_34379_33957015_1_1_1_1,00.html

OECD (2004). Test No. 111: Hydrolysis as a Function of pH, OECD Guidelines for the Testing of Chemicals, Section 1, OECD Publishing, Paris, https://doi.org/10.1787/9789264069701-en

OECD (2006). OECD Guidelines for the Testing of Chemicals, Revised Introduction To The OECD Guidelines For Testing Of Chemicals, Section 3 Part I: Principles And Strategies Related To The Testing Of Degradation Of Organic Chemicals. Adopted 23 March 2006.

OECD (2006). Series on Testing and Assessment Number 54, Current approaches in the statistical analysis of ecotoxicity data: a guidance to application. ENV/JM/MONO(2006)18.

OECD (2007). Guidance document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models. ENV/JM/MONO(2007)2 . Organisation for Economic Co-operation and Development, Paris, France. Available under https://www.oecd.org/env/guidance-document-on-the-validation-of-quantitative-structure-activity-relationship-q-sar-models-9789264085442-en.htm

OECD (2008). Bioaccumulation in Sediment-dwelling Benthic Oligochaetes. Organisation for Economic Cooperation and Development (OECD), OECD Guideline for the Testing of Chemicals No. 315, Paris, France.

OECD (2008). Test No. 314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater, OECD Guidelines for the Testing of Chemicals, Section 3, OECD

Publishing, Paris, https://doi.org/10.1787/9789264067493-en.

OECD (2009). Test No. 302C: Inherent Biodegradability: Modified MITI Test (II), OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070400-en.

OECD (2009). Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials, Series on the Safety of Manufactured Nanomaterials No. 15, ENV/JM/MONO(2009)21, OECD, Paris.

OECD (2010). OECD Guidelines for the testing of chemicals, TG 317, Bioaccumulation in Terrestrial Oligochaetes. Organisation for Economic Co-operation and Development, Paris, France.

OECD (2012). Bioaccumulation in Fish: Aqueous and Dietary Exposure. Organisation for Economic Cooperation and Development (OECD), OECD Guideline for the Testing of Chemicals No. 305, Paris, France.

OECD (2014). Test No. 310: Ready Biodegradability - CO2 in sealed vessels (Headspace Test), OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264224506-en.

OECD (2016). OECD Series on Testing and Assessment Number 232, Series on Pesticides Number 82. Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies (ENV/JM/MONO(2016)6). Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO%282016%296&doclanguage=en.

OECD (2017). OECD Series on Testing and Assessment Number 264, Guidance document on aspects of OECD TG 305 on Fish bioaccumulation. OECD Environmental Health and Safety Publications, Series on Testing and Assessment, Paris, France. Available at: https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2018a). Guidance document on the determination of *in vitro* intrinsic clearance using cryopreserved hepatocytes (RT-HEP) or liver S9 sub-cellular fractions (RT-S9) from rainbow trout and extrapolation to *in vivo* clearances.

OECD (2018b). Test No. 319A: Determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP). doi:doi:https://doi.org/10.1787/9789264303218-en

OECD (2018c). Test No. 319B: Determination of *in vitro* intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9). doi:doi:https://doi.org/10.1787/9789264303232-en

OECD (2022a). Test No. 117: Partition Coefficient (n-octanol/water), HPLC Method, OECD Guidelines for the Testing of Chemicals, Section 1, OECD Publishing, Paris, https://doi.org/10.1787/9789264069824-en.

OECD (2022b). Test No. 123: Partition Coefficient (1-Octanol/Water): Slow-Stirring Method, OECD Guidelines for the Testing of Chemicals, Section 1, OECD Publishing, Paris, https://doi.org/10.1787/9789264015845-en.

OECD (2023). OECD Series on Testing and Assessment Number 386, Guidance for the

regulatory assessment of (Quantitative) Structure – Activity Relationship models, predictions, and results based on multiple predictions. OECD Environmental Health and Safety Publications, Series on Testing and Assessment, Paris, France. Available at: https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD TG 106 (Adsorption - Desorption Using a Batch Equilibrium Method: https://www.oecd-ilibrary.org/docserver/9789264069602-en.pdf?expires=1674119005&id=id&accname=guest&checksum=08C02624A1091F1206490106A3919FBC).

OECD TG 121 (Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC): https://www.oecd-ilibrary.org/docserver/9789264069909-en.pdf?expires=1674119052&id=id&accname=guest&checksum=526912873A6BC909E714E649F56C8442.

OECD TG 312 – Leaching in soil columns: https://www.oecd-ilibrary.org/docserver/9789264070561-en.pdf?expires=1674118369&id=id&accname=guest&checksum=3267E5B0FC66859FF3916FD76030CDE3

Pignatello, J. J. (2023). Sorption of organic chemicals in soil. Reference Module in Earth Systems and Environmental Sciences, 315–326. https://doi.org/10.1016/B978-0-12-822974-3.00077-X

Regnery, J., Riegraf, C., Jacob, S., Friesen, A. (2022). New insights on in vitro biotransformation of anticoagulant rodenticides in fish. Chemosphere, 294:133727.

Ribbenstedt, A., Armitage, J.M., Gunther, .F, Arnot, J.A., Droge, S.T.J., McLachlan, M.S. (2022). In Vivo Bioconcentration of 10 Anionic Surfactants in Rainbow Trout Explained by In Vitro Data on Partitioning and S9 Clearance. Environ. Sci. Technol. 56(10), 6305-6314. https://doi.org/10.1021/acs.est.1c05543.

Rüdel, H., Kosfeld, V., Fliedner, A., Radermacher, G., Schlechtriem, C., Duffek, A., Rauert, C., Koschorreck, J. (2020). Selection and application of trophic magnification factors for priority substances to normalize freshwater fish monitoring data under the European Water Framework Directive: a case study. Environmental Sciences Europe; 32: 138.

Sabljić, A., Güsten, H., Verhaar, H., Hermens, J. (1995). QSAR modelling of soil sorption. Improvements and systematics of log KOC vs. log KOW correlations. Chemosphere, 31(11–12), 4489–4514. https://doi.org/10.1016/0045-6535(95)00327-5.

Sánchez-Camazano, M., Sánchez-Martín, M. J., Poveda, E., Iglesias-Jiménez, E. (1996). Study of the effect of exogenous organic matter on the mobility of pesticides in soils using soil thin-layer chromatography. Journal of Chromatography A, 754(1–2), 279–284. https://doi.org/10.1016/S0021-9673(96)00546-8.

Sanco/13144/2010, version 3, 10 October 2014. Assessing Potential for Movement of

Active Substances and their Metabolites to Ground Water in the EU. The Final Report of the Ground Water Work Group of FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe). https://esdac.jrc.ec.europa.eu/public_path/projects_data/focus/gw/NewDocs/focusGWReportOct2014.pdf

Sander R (2015). Compilation of Henry's law constants (version 4.0) for water as solvent, Atmos. Chem. Phys., 15, 4399-4981.

Saunders, L.J., Hoffman, A.D., Nichols, J.W., Gobas, F.A.P.C. (2020). Dietary bioaccumulation and biotransformation of hydrophobic organic sunscreen agents in rainbow trout. Environ Toxicol Chem: 39:574-586. https://doi.org/10.1002/etc.4638.

Schaffer, M., Licha, T. (2014). A guideline for the identification of environmentally relevant, ionizable organic molecule species. Chemosphere, 103, 12–25. https://doi.org/10.1016/J.CHEMOSPHERE.2013.12.009.

SCHER (2005). Opinion on RPA´s report "Perfluorooctane Sulphonates Risk reduction strategy and analysis of advantages and drawbacks" (Final report - August 2004). Opinion adopted 4th plenary of 18 March 2005.

Schlechtriem, C., Kampe, S., Bruckert, H.J., Bischof, I., Ebersbach, I., Kosfeld, V., Kotthoff, M., Schäfers, C., L'Haridon, J. (2019.) Bioconcentration studies with the freshwater amphipod Hyalella azteca: are the results predictive of bioconcentration in fish? Environ Sci Pollut Res Int. 26(2):1628-1641.

Schug, H., Begnaud, F., Debonneville, C., Berthaud, F., Gimeno, S., Schrimer, K. (2018). TransFEr: a new device to measure the transfer of volatile and hydrophobic organic chemicals across an in vitro intestinal fish cell barrier. Analytical Methods 10: 4394-4403 doi 10.1039/C8AY01253A.

Schug, H., Maner, J., Begnaud, F., Berthaud, F., Gimeno, S., Schirmer, K., Zupanic, A. (2019). Intestinal fish cell barrier model to assess transfer of organic chemicals in vitro: an experimental and computational study. Environmental Science and Technology 53(20): 12062-12070 doi:10.021/acs.est.9b04281.

SETAC (1993) Guidance Document on Sediment Toxicity Tests and Bioassays for Freshwater and Marine Environments. From the Workshop on Sediment Toxicity Assessment at Renesse, Netherlands on 8-10 November 1993. Hill I, Mathiessen P, Heimbach F (Eds). Society of Envionmental Toxicology and Chemistry – Europe, Brussels.

Shrestha, P., Junker, T., Fenner, K., Hahn, S., Honti, M., Bakkour, R., Diaz, C., Hennecke, D. (2016). Simulation Studies to Explore Biodegradation in Water-Sediment Systems: From OECD 308 to OECD 309. Environ Sci Technol 50:6856-64.

Sigmund, G., Peter, H., Arp, H., Aumeier, B. M., Bucheli, T. D., Chefetz, B., Chen, W., Droge, S. T. J., Endo, S., Escher, B. I., Hale, S. E., Hofmann, T., Pignatello, J., Reemtsma, T., Schmidt, T. C., Schönsee, C. D., & Scheringer, M. (2022). Sorption and Mobility of Charged Organic Compounds: How to Confront and Overcome Limitations in Their Assessment. Cite This: Environ. Sci. Technol, 2022, 4710. https://doi.org/10.1021/acs.est.2c00570.

Tolls, J., Sijm D.T.H.M. (2000). Estimating the properties of surface-active chemicals. *In:* Boethling RS and Mackay D (Eds.) Handbook of property estimation methods for chemicals. Environmental and health sciences, Lewis Publishers, Boca Raton, FL, USA.

Treu, G., Badry, A., Bauer, K., Claßen, D.,Drost, W., Rüdel, H. (2022b). Deliverable B5.5 A draft guideline for assessment of bioaccumulation of relevant pollutants in AP&P samples, LIFE APEX-LIFE17 ENV/SK/000355. https://lifeapex.eu/wp-content/uploads/2022/11/Deliverable_B5.5_1_Draft-guideline_B_assessment_final.pdf

Trowell, JJ, Gobas, FAPC, Moore, MM, Kennedy CJ (2018) Estimating the Bioconcentration Factors of Hydrophobic Organic Compounds from Biotransformation Rates Using Rainbow

Trout Hepatocytes. Archives of Environmental Contamination and Toxicology 75(2):295-305 doi:10.1007/s00244-018-0508-z.

UBA (2022). Scholz-Starke, B, Stibany, F, Hammers-Wirtz, M. PROSOIL – Protection of soil organisms: Development of toxicity criteria for soil organisms in the framework of classification of substances and PBT assessment. https://www.umweltbundesamt.de/sites/default/files/medien/479/publikationen/texte_105-2022_prosoil.pdf.

US EPA (2000). Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, Second Edition.

US EPA (2012). Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA. https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface#citing, lasted accessed: March 2023.

US EPA (2016). Ecological Effects Test Guidelines OCSPP 850.1710: Oyster Bioconcentration Factor (BCF).

Wang Z, MacLeod M, Cousins IT, Scheringer M, Hungerbühler K (2011). Using COSMOtherm to predict physicochemical properties of poly- and perfluorinated alkyl substances (PFASs). Environmental Chemistry 8, 389-398.

Wauchope, R. D., Yeh, S., Linders, J. B. H. J., Kloskowski, R., Tanaka, K., Rubin, B., Katayama, A., Kördel, W., Gerstl, Z., Lane, M., & Unsworth, J. B. (2002). Pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability. Pest Management Science, 58(5), 419–445. https://doi.org/10.1002/PS.489.

Weber, J.B., Wilkerson, G.G., Reinhardt, C.F. (2004). Calculating pesticide sorption coefficients (K_d) using selected soil properties. Chemosphere 55, 157–166.

Weeks J, Guiney P, Johanning K (2020a). In vitro and in vivo metabolic stability of various fragrance materials and insect repellent in rainbow trout (Oncorhynchus mykiss). Journal of Applied Toxicology 40(6):763-779 doi:10.1002/jat.3942.

Weeks J, Li A, Doshi U, Johanning K, Guiney P (2020b). In vitro evaluation of the metabolic stability of nine fragrance chemicals in trout and human hepatocytes. Journal of Applied Toxicology 40(10): 1421-1434 doi:10.1002/jat.3995.

Weisbrod, Annie V, Kent B Woodburn, Albert A Koelmans, Thomas F Parkerton, Anne E McElroy, und Katrine Borgå (2009). Evaluation of Bioaccumulation Using In Vivo Laboratory and Field Studies". Integrated Environmental Assessment and Management 5(4): 598–623. https://doi.org/10.1897/IEAM_2009-004.1.

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

UN (United Nations - Committee of Experts on TDG and GHS) (2006). Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals Twelfth session, 12 (p.m)-14 July 2006, Item 2 (c) of the provisional agenda, Environmental hazards, Classification criteria for the terrestrial environment. Transmitted by the expert from Spain on behalf of the group on terrestrial hazards. UN/SCEGHS/12/INF.5. Available from:

http://www.unece.org/fileadmin/DAM/trans/doc/2006/ac10c4/UN-SCEGHS-12-inf05e.pdf.

Verschoor AJ, Boesten JJTI, Leistra M, Van der Linden AMA, Linders JBHJ, Pol JWW (2001). Evaluation of pesticide leaching in lysimeter and field studies. Parent substances. Bilthoven, The Netherlands: RIVM report no. 601506007. https://www.rivm.nl/bibliotheek/rapporten/601506007.pdf.