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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 described as the resistance of chemicals to transformation and degradation processes. 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*". Degradation may be biotic or abiotic and may take place in both aerobic and anaerobic conditions.

Bioaccumulation (B) 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.(e)). Annex I specifies that 'bioconcentration' means the net result of uptake, transformation and elimination of a substance in an organism due to waterborne exposure (CLP Annex I, 4.1.1.1.(f)).

Mobility (M) refers to the potential of a substance once emitted to the environment to reach water bodies, including drinking water resources 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 remain in the environment, they may be toxic and they tend to accumulate in living organisms. Additionally, exposure to the environment (including pristine/remote regions and humans, Commission Delegated Regulation (EU) 2023/707, recital (7)) is difficult to reverse. Between 1994 and 2007, 141 risk assessments have been performed and concluded by the different Member States[1] under Council Regulation (EEC) No 793/93, Existing Substances Regulation (ESR). Since the entry into force of Regulation (EC) No 1907/2006, the "REACH" Regulation, the identification of substances with PBT and/or vPvB properties entailed the comparison with the 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)) and the Regulation (EC) No 1107/2009 (Plant Protection Products Regulation, "PPP" or "PPPR")[2].

The experience and accumulated scientific knowledge in PBT/vPvB assessment and the need of protection for the environment regarding Substances of Very High Concern

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

[2] For the explicit Regulatory context of approval/renewal under Reg. 1107/2009, European Commission (2012) outlines the POP, PBT and vPvB assessment elements of new/existing active substances and the initial establishment of a list of Candidates for Substitution (CFS). These principles differ to the respective hazard assessment under CLP.

(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 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 persistence 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 and drinking water treatment processes, they can spread over long distances and also cause environmental exposures that are difficult to reverse (Commission Delegated Regulation (EU) 2023/707, recital (8), Neumann and Schliebner, 2019). 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, focussing on waters, including drinking water.

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 provide guidance on how to compare the available information with the CLP criteria to come to a conclusion on whether classification in either of the related hazard classes may apply. The following apply to single substances (mono-constituent substances under REACH and CLP) and their relevant impurities, constituents and/or degradation products, with further considerations on mixtures described in Section 4.3.6. 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 out of the scope of the PBT/vPvB and PMT/vPvM assessment under CLP[3].

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

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

[3] It is noted that this does not automatically apply to related principles in other Regulations. For example, inorganic substances are subjected to PBT assessment according to Regulation (EC) No 1107/2009.

214 **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.

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216 **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.

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218 **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.

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220 **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.

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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 when assessing the PBT/vPvB and PMT/vPvM properties, as well as other related assessment elements (corresponding to the Section named "Interpretation of data" in 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, or sound scientific principles that are internationally recognised or methods validated according to international procedures (CLP Article 8). The CLP Article further expands on the scientific principles that should be followed by manufacturers, importers or downstream users during the performance of any new tests for the purpose of determining whether a substance or a mixture entails a human health or environmental hazard, provided that all other means of generating information have been exhausted. 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), as well as data from comprehensively reported, peer-reviewed academic studies, 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*". 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 the REACH Regulation (1.1.2). Based on these legal provisions, RAC has previously formed opinions on the harmonised 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; see also part 1 of Annex I to CLP).

Concerning active substances in accordance with the PPP Regulation, the Commission Regulation (EU) No 283/2013 of 1 March 2013 (European Commission, 2013) sets out the data requirements, while the related Commission Communication provides test methods and guidelines for the active substances in plant protection products. Concerning active substances in accordance with the BPR, ECHA (2022c) 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 within the meaning of Directive 86/609/EEC shall only be undertaken where no other alternatives exist that would provide reliable, high quality data. In the absence of adequate experimental information, qualitative or quantitative structure-activity relationships ((Q)SARs), suitable *in vitro* tests, information from the application of the category approach (grouping, read-across) and other types of available information (for example, monitoring data, if appropriate) may be used in a WoE determination (see point below, but also within the Sections for the individual properties).

Furthermore, the European Court of Justice has confirmed that the application of the precautionary principle can be taken into consideration in the context of the classification of a substance under CLP, where the assessment of the risks of that substance to the environment and to human health gives rise to uncertainty[4]. In this context, when more than one reliable experimental study is 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. Section 4.3.4 further outlines some general considerations on the application of the WoE.

There may be exceptional situations where it is appropriate to combine several study results to generate a value for comparison with the CLP criteria. This is discussed in Section 4.3.4, as well as under the respective Sections 4.3.3.1, 4.3.3.2, 4.3.3.3 and 4.3.3.4, where the conditions that need to be met for combining results from reliable studies are detailed.

(ii) relevant conditions

Sections 4.3.2.3 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 and 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, as confirmed by both ECHA's Board of Appeal and the European Court of Justice, the purpose of the PBT/vPvB assessment 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[5]. 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 ((EC) No 440/2008)[6], in line with

[4] See paragraphs 96 to 98 of the judgment of the Court of Justice in *SGL Carbon and others v.* Commission, joined Cases C-65/21 P and C-73/21 P to C-75/21 P, not yet published, EU:C:2022:470 accessible at the following link: 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..

[5] See judgment of the General Court in 3V Sigma v. ECHA, Case T-176/19, not yet published, EU:T:2020:621 (https://curia.europa.eu/juris/liste.jsf?language=en&td=ALL&num=T-176/19) and the summaries of the relevant ECHA Board of Appeal decisions in section 11.4 of the Board of Appeal digest of decisions available at https://echa.europa.eu/documents/10162/2314761/digest_of_decisions_of_boa_en.pdf.

[6] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0440

Article 13(3) of the REACH Regulation and bullet point (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 (Q)SARs and read-across approaches

(Q)SAR predictions can be used together with other information in the WoE determination. When using (Q)SARs to predict a substance property, an assessment of both the model and the prediction is needed. A (Q)SAR model must be scientifically valid (using OECD principles (OECD, 2004; OECD, 2007)) and adequate and reliable documentation must be provided. A valid (Q)SAR model does not necessarily produce an acceptable prediction. For an acceptable (Q)SAR 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 (Q)SAR assessment framework (QAF) (OECD, 2023).

Transparent documentation of the validity of the models, as well as for reporting information relevant for judging the reliability of predictions for individual compounds or other comparable documentation must be provided. A (Q)SAR Model Reporting Format (QMRF) displays a description of the (Q)SAR model relative to the five OECD (Q)SAR validation principles in a systematic and summarised way (OECD 2004, 2007; minor update OECD 2023). The information about the (Q)SAR prediction is reported in the (Q)SAR Prediction Reporting Format (QPRF). An updated QPRF template was published in 2023 and it reflects the newly established OECD (Q)SAR Prediction Principles (OECD, 2023).

More information can be found in OECD (Q)SAR assessment framework documents (OECD, 2023), in the Guidance on (Q)SARs and grouping of chemicals, Chapter R.6[7] and in ECHA Practical Guide "How to use and report (Q)SARs"[8].

There are several recognised methodological challenges for P and B assessments. Despite a general preference on reliable experimental data over predicted data, (Q)SAR predictions may still be useful in the evaluation and interpretation of the laboratory studies.

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). 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

[7] https://echa.europa.eu/documents/10162/17224/information_requirements_r6_en.pdf
[8] https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf

the structural similarity, the results will be either similar, or follow a regular pattern. (Q)SAR predictions may furthermore be applied to support read-across, made both by the analogue and the category approach. They may provide information which can be used in the trend analyses as they often/typically extract trends over a larger span of chemicals, as well as in the establishment of the read-across hypothesis, and to analyse how the differences in source and target structures may change the property under analysis.

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[9]) may help assess and, where necessary, improve the read-across. ECHA 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[10]) 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 constituent, 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 most easily performed on single substances with a well-defined identification. However, as discussed below, a substance may be composed by more than one single substance 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[11]. 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,

[9] https://echa.europa.eu/documents/10162/17221/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a and https://echa.europa.eu/documents/10162/17228/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

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

[11] The limit of ≥ 0.1% (w/w) is indicated in the European Union legislation, where there is no specific reason (e.g., based on toxicity) to establish a concentration limit specific to the case. Examples of this generic concentration limit are, i.a., another category of substances of very high concern according to Article 57 of REACH, where the default concentration of Carcinogenic/Mutagenic (category 1A/1B) ingredients in a mixture requiring a Carcinogen/Mutagen (1A/1B) classification of the mixture under Regulation (EC) No 1272/2008 is ≥0.1% (w/w). Furthermore, Articles 14(2)(b), 31(3)(b) and 56(6)(a) of REACH apply a similar principle and the same concentration limit for PBT/vPvB substances in mixtures regarding some obligations under REACH. Additionally, the Judgments of the General Court (Seventh Chamber, extended composition) of 7 March 2013 in cases T-93/10, T-94/10, T-95/10 and T-96/10 (see in particular paragraphs 117 to 121) confirmed the validity of this approach for PBT/vPvB constituents of a substance.

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. More detailed elaboration on the criteria for grouping or read across, is available 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, characterisation and identification of UVCBs or fractions of impurities needs to take place, as comprehensively as possible. However, full characterisation 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/vPvB and PMT/vPvM assessment for all constituents, impurities and additives present in concentrations ≥ 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 ≥0.1% (w/w). In order for the PBT/vPvB or PMT/vPvM 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. It cannot be concluded that a substance warrants a PBT/vPvB or PMT/vPvM classification when, for example, the assessment of persistence has been concluded for one constituent and the assessment of bioaccumulation, toxicity or mobility 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[12] (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

[12] https://echa.europa.eu/registry-of-svhc-intentions

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/vPvB and PMT/vPvM properties. Same principles in establishing similarity of constituents apply for mono-constituent, multi-constituent and UVCB substances. For such similarity criteria, 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. ECHA Guidance on IR&CSA, Chapter Guidance R.11 (R.11.4.2.2) further details certain circumstances that the whole substance approach can be used for certain endpoint-specific assessments. Regarding such substances containing more than one constituent where data on individual constituents are available, they should be evaluated and classified following the same classification rules as mixtures (Section, 4.3.6).

(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/vPvB and PMT/vPvM assessment should be performed on the substance and each of the relevant transformation/degradation product[13]. 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 for example, when it is detected ≥10% of the applied concentration or radioactivity (dose) 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 remains in the test system post formation indicating persistence during a degradation study (see also Section 4.3.3.1.2.1, simulation tests in water, water-sediment and soil). In addition, lower percentages than 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

[13] Currently, Annex II of the EC No 1107/2009 refers only to the PBT/vPvB assessment of the active substance, safeners and synergists, while the transformation products and metabolites are not subject to a PBT/vPvB assessment. Under PPPR a metabolite is defined "relevant" if there is a reason to assume that it has intrinsic properties comparable to the parent substance in terms of its biological target activity, or that it poses a higher or comparable risk to organisms than the parent substance or that it has certain toxicological properties that are considered unacceptable.

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 and aquatic systems 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.

For active substances in plant protection products, the 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 nanoform 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 form of a substance fulfils the criteria of the nanoform definition, specific considerations apply, with REACH Annex I currently noting that the PBT/vPvB assessment under REACH shall address also all relevant nanoforms.

More recently, Commission recommendation of 10 June 2022 on the definition of nanomaterial (2022/C 229/01) has updated the definition of nanomaterial as the one meaning "*a natural, incidental or manufactured material consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50 % or more of these particles in the number-based size distribution fulfil at least one of the following conditions:*

- *one or more external dimensions of the particle are in the size range 1 nm to 100 nm;*
- *the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;*
- *the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm*".

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 of nanoforms 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/or water, high volatility, high instability in biotic or 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 Aquatic Toxicity Testing of Difficult Substances and Mixtures" (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 4.3.3.3.6 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, ECHA Guidance on IR&CSA, Appendix R.7.10-3.

The balance between ionised and non-ionised forms of some substances varies with the pH of the solution in which a substance is dissolved. Since ionised and non-ionised forms can have different solubility in water, small changes in the pH can significantly alter the bioavailability of a substance in a toxicity test. When reviewing data from toxicity tests, the test design 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 (4.3.3.3.6), 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 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 abiotic or biotic process by which a substance is transformed from one chemical species to another.

A **degradation half-life (DegT50[14])** is the time taken for 50% degradation of a test substance when the degradation can be described by (pseudo) first-order kinetics, i.e where the degradation rate constant (k) is independent of concentration..

[14] DegT50 abbreviation is used only for the purpose of guidance documents published by ECHA to describe the degradation half-life and may differ from abbreviations of half-life used in other guidance documents.

Degradation products are all substances resulting from biotic and abiotic transformation reactions of a substance.

Rate constant is a kinetic parameter describing an aspect of the rate (per time) at which a substance dissipates from the environment or an environmental compartment. Such parameter may be non-specific, simply describing net dissipation due to degradation and transfer processes, or they may be specific, describing dissipation due to degradation, formation, or transfer (FOCUS, 2014).

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, leaching, adsorption/desorption, and plant/organism uptake).

DT50 is a generic term to describe the time required for disappearance/dissipation of 50% of a substance.

First order kinetics: is when the rate of a degradation follows a first-order equation, where the rate of degradation is proportional to the concentration of the substance which declines over time.

Pseudo-first order kinetics: behaves mathematically like a first-order reaction even though it may be mechanistically a higher order reaction. In chemical reaction kinetics, this applies when other reactants are present in large excess compared to the substance. The concentration of the reactants in large excess will not change appreciably during the course of the reaction and does not limit the reaction rate, which will then stay proportional to the concentration of the substance. In biodegradation reactions, the micro-organisms or enzymes catalysing the biodegradation reaction can be considered as one "reactant" and, therefore, biodegradation reactions are mechanistically not first-order reactions as the rate is dependent on the amounts of more than one reactant. Pseudo-first-order kinetics can apply to biodegradation when the amounts of the other reactants, including the relevant microorganisms/enzymes, do not change appreciably (FOCUS, 2014, Schmidt SK *et al.*, 1985 and Alexander M., 1999).

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

Inherent biodegradation describes the potential for biodegradation under optimised aerobic conditions designed to promote biodegradation.

Mineralisation (ultimate degradation) is the complete degradation of an organic compound to CO_2, H_2O,other inorganic compounds and biomass under aerobic conditions, and to CH_4, CO_2, H_2O, d other inorganic compounds and biomass 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 formation of a transformation/degradation product.

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 in a 10-day window within 28 days in standard ready biodegradability tests.

The 10-day window is the 10 days immediately following the attainment of 10% biodegradation (DOC removal, ThOD or $ThCO_2$) in ready biodegradability tests. The 10-day window concept does not apply to OECD TG 301C or if the test is carried out on a substance containing more than one constituent with structurally similar constituents and if it is anticipated that a sequential biodegradation of the individual constituents is taking place.

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 (Q)SAR 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, and OECD TG 307 soil)
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 substances and some modifications of the test system may be warranted. 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 degradation 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 4.3.4 of this Guidance will present the assessment of the weight of evidence determination to reach a conclusion if substance meets the CLP criteria for P/vP.

The following sections provide description of methods to derive degradation half-life and presents short overview of different type of studies most commonly used for determining the degradation potential of substances.

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.

Lag phase of degradation could be occasionally observed in simulation studies. A lag phase describes the phase when microbes are 'adjusting' to the new substrate (food source) and/or new environment conditions and depends on the cell density in tests, the possible pre-adaptation of the inoculum and the total amount of specifically degrading bacteria (Ingerslev *et al.*, 2000). 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). The OECD TG 309 includes a lag phase definition and specific advice on the lag phase length 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 the lag phase length in the DegT50 derivation should be provided. When the lag phase is attributed to experimental artefact the validity of the study needs to be assessed carefully as this might indicate issues related to the test design and performance.

The kinetic model that best fits and/or most appropriately describes the experimental data

should be used for estimating the degradation half-life[15]. 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, χ2 error and t-test statistical metric. Detailed description for the criteria for the acceptability of the fit is included in Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (FOCUS, 2014).

When the kinetic of decline is first-order and no lag phase occurs, the DegT50 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 and acceptable single-first order (SFO) fitting is not possible, the best-fit bi-phasic model (e.g. DFOP, HS)[16] should be selected and used for predicting a DegT50. 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 DegT50 predicted from the slow phase where it is assumed that degradation follows (pseudo) first-order kinetics should be preferred for comparison with the P/vP criteria. The First Order Multi-Compartment (FOMC) model, also mentioned in the FOCUS degradation kinetics guidance, is a bi-phasic mechanistic model based on the soil heterogeneous nature (FOCUS, 2014). Considering the uncertainties associated with DegT50 values derived using the FOMC model, this model is the less preferred one to be used for comparison to the P/vP criteria. The use of the FOMC derived DegT50 can be considered in a WoE approach only if the other models do not fit the data adequately. Furthermore, a pseudo-DegT50[17] (DegT90/3.32) also derived from FOMC should not be used as is considered highly uncertain (Section R.11.4.1.1.3 in *Chapter R.11* of the *Guidance on IR&CSA*). 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.

The extrapolation of DegT50 beyond test duration is common (e.g. for slow phase of bi-phasic degradation) and often also necessary considering the duration of the standard tests and the P/vP criteria. Extrapolation will increase the uncertainty of the derived DegT50 value. This should be acknowledged in the interpretation of the data. In general, when a DegT50 obtained from a properly justified kinetic model fulfils the P or vP criterion, even if the DegT50 is extrapolated over study duration, the substance can be concluded P or vP. 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 the calculated degradation half-lives should be interpreted with care. In such a case it is still possible to reach a conclusion on persistence (P or vP) as demonstrated in the cases of substances included in the candidate list (e.g. Melamine, EC 203-615-4 and 1,4-

[15] In the context of the Plant Protection Products Regulation (EC 1107/2009) and specifically within the FOCUS kinetic 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.

[16] The DFOP and HS biphasic kinetic models are based on first order degradation kinetics. The DFOP model (Double-First-Order in Parallel model) consist of two SFO models in parallel (the sum of two first order equations), and the HS model (Hockey-Stick model) consists of two SFO models in series (two sequential first order curves).

[17] Pseudo-DegT50 should not be confused with the Pseudo first order kinetics and any DegT50 derived from such kinetic analysis.

dioxane EC 204-661-8).

Any deviations from the recommended mass balance/recovery, as they are described in the corresponding testing guidelines (OECD TG 309, OECD TG 308 and OECD TG 307) should be reported and justified. Further guidance on handling mass balance/recovery data is provided in ECHA Guidance on IR&CSA, Chapters R.11, Section R.11.4.1.1.3 and Table R.11-6. Furthermore, when dissipation through volatilisation is observed, correction procedures can be applied and for this purpose, correction procedures are available for the parent substance for the SFO kinetics model which could be also applied in the case of HS and DFOP kinetic model (ECHA Guidance on IR&CSA, Chapters R.11, Section R.11.4.2.1.3 and related Appendix R.11-7).

A good knowledge of the degradation pathway up to the transformation/degradation product is essential for deriving a reliable degradation half-life for a transformation/degradation product. When a study is performed on a parent substance and transformation/degradation products are formed, the pathway model approach as described in the FOCUS degradation kinetic Guidance (2014) should be used as it accounts for both formation and removal (degradation) of transformation/degradation products. In the pathway approach, the parent and transformation/degradation data is assessed together. Evaluation of the transformation/degradation products data individually by using only the decline phase (Decline model) is another available option and it should be used only if the pathway fit does not provide visual and statistically satisfactory representation of the data (FOCUS, 2014).

Further information on the degradation kinetic models, the data handling, assessment of the goodness of fit and general recommendations on the kinetic analysis can be found in ECHA Guidance on IR&CSA, Chapters R.11, Section R.11.4.1.1.3. and the Generic Guidance Document for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (FOCUS, 2014). Furthermore EFSA Guidance provides further advice especially on derivation on DegT50 from field studies (EFSA, 2014)[18].

4.3.3.1.2.1. Simulation tests in water, water-sediment and soil

Simulation degradation tests attempt to assess degradation in a specific environment by use of indigenous biomass, media, and relevant solids (e.g. soil and sediment) in relevant test conditions. As detailed in the Section 4.3.4 of the Guidance, degradation simulation studies performed in relevant environmental media specified in Annex I (4.3.2.1.1. and 4.4.2.1.1.) of CLP and at relevant conditions are the tests considered as the ones with the highest regulatory relevance. These tests provide a definitive degradation half-life that can be compared to the numerical persistence criteria as defined in CLP. Such tests allow both biotic and abiotic degradation processes to operate.

The following tests can be used to simulate the biodegradation of organic substances under relevant conditions in soil, sediment or surface water: Aerobic and Anaerobic Transformation in Soil (OECD TG 307); Aerobic and Anaerobic Transformation in Aquatic

[18] To note that there are some deviations in how abbreviations are used for degradation half-live and dissipation between this Guidance and EFSA Guidance (2014) and FOCUS (2014).

Sediment Systems (OECD TG 308); and Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test (OECD TG 309).

The simulation degradation studies include two types of investigations: a) a degradation pathway study where degradation products (i.e. degradation transformation/degradation products) are identified and quantified, b) a kinetic study where the degradation rate constants (and degradation half-lives) of the parent substance and, if applicable, of the transformation/degradation products, are experimentally determined. In the simulation test, the test concentration is low (close to the expected in the environment) to anticipate that the biodegradation kinetics (first order or pseudo-first order) obtained in the test reflect degradation rates 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 disappearance/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 (as relevant). Determination of non-extractable residues is relevant in soil and water-sediment studies (OECD TG 307, OECD TG 308 and Kästner *et al.*, 2014). Determination of non-extractable residues is also recommended in surface water simulation degradation studies (OECD TG 309) especially when relevant for mass balance calculations and derivation of degradation half-life. 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 give a lower 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 acceptable. 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 depends on the goal of the study, e.g. label in the most recalcitrant part of the molecule ensures the determination of the total mineralisation. 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 can be 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).

If the primary degradation or mineralisation half-life for the whole system is below the respective degradation half-life –value of P/vP criteria, the parent substance can be considered not persistent in the tested environmental compartment (surface water, sediment or soil). However, investigation of degradation pathways/transformation/degradation products would be needed since it cannot be excluded that a second transformation route forms a persistent transformation/degradation product in concentrations relevant for the P assessment.

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. In biodegradation studies, also information gained from sterile controls is useful for interpretation of the study results (see Sections R.11.4.1.1.2 and R.7.9.4.1. in Chapters R.11 and R.7b of the Guidance on IR&CSA and ECHA (2022) for more details).

According to the OECD simulation degradation Test Guidelines (TG) the radiolabelled mass balance should target to 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. An incomplete mass balance will introduce severe uncertainty to the interpretation of data. 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 simulation tests conducted in the relevant conditions and in accordance with the respective test guidelines may be directly compared with the numerical P/vP criteria. When bi-phasic models are considered more appropriate to describe the degradation, the DegT50 from the slow phase should be preferred for comparison 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. Any deviation from the relevant/standard test conditions should be taken into account in weighting of the relevance and reliability of the information as part of the WoE assessment.

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 (≤ 100 µg/L) reflecting that expected in the environment is used; and
- study is considered to be performed under relevant conditions; and
- 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 potentially remobilisable NER (Type 1: strongly sorbed and physically entrapped), irreversibly bound NER (Type 2: covalently bound) and NER incorporated into biomass (Type 3: biogenic) (Löffler *et al.*, 2022, ECHA, 2019, Kästner *et al.*, 2018). 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 of the parent substance together with the Type 1 NER are considered for the DegT50 estimation. If such DegT50 is above the P/vP criterion, it can be further refined by taking into account only the quantity of the parent substance concentration in the Type 1 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 quantify and 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. When biodegradation rates or half-lives have been determined in simulation tests, it should be considered to recalculate the degradation rates obtained to reflect an average EU outdoor temperature (European Commission, 2003). Standard environmental characteristics for Europe, including average environmental temperature of 12° (9°C for marine environment), have been established for example in van de Meent (1993), Schoorl et el. (2014), and European Commission (2003).

Unless other evidence is provided, 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 or in a test conducted in the laboratory at the reference temperature. Therefore, temperature correction to the average environmental temperature of 12°C in Europe (9°C for marine environment) should be applied to the DegT50 obtained in a water, sediment or soil simulation test conducted at other temperatures (note that the General Court of the European Court of Justice in its judgment in Case T-177/19[19] considered that there was no manifest error of assessment by ECHA in applying a temperature correction of 12°C for determining the degradation half-life of a specific substance. In another case (Case T-176/19) the Court accepted ECHA's explanation that a substance's degradation rate, must be obtained through such studies being conducted at an environmentally 'relevant' temperature and that that temperature is 12 °C by default[20]). 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. It is acknowledged that temperature correction may induce uncertainty on the derived degradation rate. The closer to the average European temperature the test is conducted, the less uncertainty due to the temperature correction can be expected.

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

[19] See judgment of 9 June 2021 Exxon Mobil v. ECHA, T-177/19, not yet published, EU:T:2021:336 Link to T-177/19.

[20] See judgment of 16 December 2020 3 V Sigma v. ECHA, T-176/19, not yet published, EU:T:2020:621, at paragraphs 76 and 77, Link to T-176/19

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

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

Where

k = rate constant (day^{-1})
A = factor equal to the rate coefficient at infinite temperature (day^{-1})
E_a = 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

$DegT50_{env}$ = half-lives at environmental temperature *Tenv* (typically 285K = 12°C) and

$DegT50_{test}$ = half-lives at test temperature *Ttest* (typically 293K = 20°C).

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 has been derived by EFSA (2007) for soil degradation studies. It corresponds to the median value of available pesticide E_a data. If Arrhenius equation is used for temperature correction, in the absence of valid substance specific E_a-value, the generic E_a-value should be used.

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

<u>Surface water simulation test (OECD TG 309)</u>

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 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 1 g/L dry weight ("suspended sediment test") to simulate a water body with suspended solids or re-suspended sediment. For the purpose of CLP, the OECD TG 309 with water amended with sediment '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).

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

- performed at concentrations e.g. ≤ 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 particulate matter (SPM) between 10 and 20 mg_{dw}/L 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).

The OECD TG 309 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 TG 308). 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 water-sediment systems. 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; 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 (non-radiolabelled 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 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 exposure of sediment in the test system. 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 methods are used, their 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 total water-sediment system DegT50 derived from an OECD TG 308 is a more robust indicator for persistence and should be used in the context of persistence assessment (Honti and Fenner, 2015). For adsorptive substances (e.g. log K_{OC}>4), that will partition primarily to the sediment phase can reasonably assumed to be equal to the degradation

half-life for the total water-sediment system. This degradation half-life ($DegT50_{Total\ system}$) would compare to relevant marine, fresh or estuarine sediment criteria set in CLP Annex I. 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 substances with moderate or low adsorption potential will tend to partition from the water phase to the sediment phase. Due to the complexity of the phase transfer processes in the water-sediment test system, the specific degradation half-lives calculated for the sediment phase ($DegT50_{sediment}$) and the water phase ($DegT50_{water}$) separately are highly uncertain (Honti and Fenner, 2015). Therefore, such results must be interpreted with caution considering the partitioning of the substance between water and sediment when selecting the most representative value for DegT50. OECD TG 308 test setup does not well reflect the surface water conditions. It states that it is not suitable to simulate conditions in flowing water (e.g. rivers). Also Honti and Fenner (2015) reflect that the OECD TG 308 has limited relevance for the open sea conditions and for water bodies having high water:sediment ratios. Therefore DegT50 from OECD TG 308 is not recommended to be used for comparison with the Annex I CLP criteria for persistence in marine, fresh or estuarine waters.

The fact that the parent substance may degrade to more soluble and less adsorptive degradation products that can be released from the sediment to the water phase 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, biodegradation 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 strictly anaerobic sediment degradation 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). Under anaerobic incubation conditions the test system must allow for measurements of oxygen concentration and redox potential.

As noted in the OECD TG 307, aerobic conditions are dominant in surface soils and even in sub-surface soils. Anaerobic conditions may occur only occasionally during flooding of soils after heavy rainfalls or when paddy conditions are established in rice fields. Thus, it is not recommended to conclude on persistence using only anaerobic soil simulation data..

Nevertheless, if anaerobic soil data is available, it may be used as part of the WoE approach.

Results of OECD TG 307 may be used for classification purposes, when conducted in relevant test conditions maintaining relevant temperature (temperature correction to be applied in accordance with text above) and relevant moisture conditions in accordance with the test guideline. 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 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

Relevance of the test conditions to simulate degradation potential in marine, fresh or estuarine water, marine, fresh or estuarine water sediment and soil should be considered when simulation test data derived from any other compartment e.g. wastewater or treated effluent is used in persistence assessment. Such information should not be used on its own to demonstrate that the substances is or is not P/vP.

The studies described below provide important information on degradation during wastewater treatment process and mixing zone after the release of the effluent. Whilst these studies are more relevant for risk assessment than hazard identification, they can be considered as supporting information in the WoE approach.

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) allows 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 respective environmental compartments i.e. soil, water or sediment. 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). Therefore, as stated above such information is considered as supporting information in the WoE approach.

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[21]. OECD Guidance document 232 (OECD, 2016) considers 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. Therefore, DegT50/DT50 values from field studies are not in many cases 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). For existing field studies (legacy studies), EFSA (2014) recommends conducting inverse modelling using time -step normalisation procedure.

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 degradation half-life is not possible to calculate, the substance could be concluded P/vP.

[21] To note that there are some deviations in how abbreviations are used for degradation half-live and dissipation between this Guidance and EFSA Guidance (2014) and FOCUS (2014).

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 DegT50 derivation.

Temperature correction to relevant temperature (12°C for water, soil and sediment and 9°C for marine environment) should be applied to the DegT50 obtained. Means to perform temperature correction are provided above in this Guidance. FOCUS Kinetics 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, the assessment of the likely impact of loss processes (volatilisation, soil surface photolysis, leaching out of the sampled soil layers and possible uptake into plants) is also described in FOCUS (2014).

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. The derivation of DegT50 from lysimeter and mesocosm studies is challenging. Inverse modelling could allow estimating the DegT50 instead of disappearance DT50 from such studies (SANCO, 2014). The derived DegT50 can be related to reference conditions (e.g. reference temperature at 12°C and reference moisture at field capacity). However, inverse modelling has the problem of non-uniqueness (namely the fact that the same model output can be obtained with different parameter combinations) exists, which makes the estimation of only DegT50 uncertain (Sanco, 2014).

More information on lysimeter studies can be found under Section 4.3.3.3.2 Other experimental information on deriving a K_{oc} value. 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 sources of monitoring data. Information may be found for example from national monitoring programmes of Member States (e.g. Swedish national monitoring data

collection[22]), from European monitoring programmes (e.g. NORMAN Network[23]), Information Platform for Chemical Monitoring (IPCheM)[24] 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 or Alpine lakes may be indicative of high persistence. Also, significant concentrations of the substance in higher levels of the food chain may indicate high persistence, besides the potential to bioaccumulate.

Trends of rising concentrations in environmental media or biota may be observed. Information on volumes, uses and releases along the same period, if available, compared with these trends can provide relevant information. Archived samples from environmental specimen banks, dated sediment 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 studies 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. Monitoring programmes may be designed to cover a large spatial area (high number of stations over a large territory), to achieve a high spatial resolution (high number of stations per area unit), or to assess temporal trends (high sampling frequency). Dependant on the design of the monitoring programme, information on detection (frequency) of a specific substance may be used as part of evidence for the persistence assessment. Several factors such as fate of the substance, potential sources and extent of emissions in relation to sampling site(s), sample size and sampling frequency should be considered when detection frequency is used in the persistence assessment. The lack of detection of a substance in monitoring studies 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. Among other factors, dissipation by transfer processes (e.g. volatilisation or plant/organisms uptake) influence the uneven distribution in the environmental samples.

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

EFSA (2023b) and Gimsing *et al.* (2019) provide guidance on how to evaluate groundwater monitoring studies for regulatory purposes in the context of Regulation (EC) 1107/2009.

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

Use of groundwater monitoring data in the weight of evidence assessment for mobility is further elaborated in Sections 4.3.3.3 and 4.3.5.1 of this Guidance.

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.

An overall assessment of the reliability of any monitoring data set as well as its relevance to the assessment purpose should be conducted and the outcomes of this reliability and relevance assessment included in the weight of evidence reasoning.

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.

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 Annex II Section II.2 of this Guidance describes the use of such information to assess rapid degradation as part of the aquatic hazard classification.

Ready biodegradability tests

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.

Additionally, the origin of the inocula should be examined in order to verify whether or not the inocula is adapted to the tested substances. Differences in the adaptation of the inocula may explain the differences in the results (OECD, 2006). Results from tests based on adapted inocula are generally regarded as inappropriate information to support non-persistence but depending on the reliability and relevance they may be used as a part of a weight-of-evidence in P assessment. For example data derived with inocula from wastewater treatment plants (WWTPs) influenced by point sources must not be used as information supporting non-persistence (e.g. if effluents from an industrial site using the substance are connected to the municipal WWTP).

A lower test substance concentration than is generally recommended by the test guideline/method should only be used for substances toxic to microorganisms and when it is still possible to reliably assess biodegradation through the measurement of carbon dioxide evolution, oxygen demand dissolved organic carbon removal. To improve sensitivity, degradation of the parent compound can also be followed, i.e by using

radiolabelled test material. Reliable assessment of ready biodegradability could be possible if ratio of test substance to biomass is kept to the standard of the respective test guideline. If the biomass-substance ratio deviates from the respective test guideline, , results should be treated with caution. Such results are considered to be more relevant for concluding whether the substance is not readily biodegradable. The following pass levels of biodegradation, obtained within 28 days (fulfilling 10-day window[25]), 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 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, ECHA Guidance on IR&CSA R.7b and R.11). The overall results should be assessed in a WoE approach.

Other screening tests

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 ready biodegradability test are negative, it is not on its own enough to demonstrate that the substance fulfils the P criteria. Consequently, result from enhanced ready biodegradability test can be used as part of weight of evidence.

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.

[25] The 10-day window begins when the degree of biodegradation has reached 10% DOC removal, ThOD or ThCO2 and must end before or at day 28 of the test. It does not apply to OECD TG 301 C.

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 are not ready biodegradability tests 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 indication of ultimate biodegradation in the marine environment. Results can also be regarded as a piece of evidence that the substance is likely to fulfil the criteria for ready biodegradability even if, as described in the TG, they cannot be directly compared with criteria of ready biodegradability described above. A result of >20% ThOD or DOC removal in OECD TG 306 (seawater 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. Even if pre-adaptation is allowed in the TG, it is not allowed for the assessment of P/vP.

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 (non-persistence) provided that ≥70 % mineralisation (DOC removal) is reached within 7 d, lag phase is not longer than 3d, removal before degradation occurs is below 15% and inoculum is not pre-adapted or ≥70 % mineralisation (O_2 uptake) is reached in OECD TG 302C within 14 d, lag phase is not longer than 3d, and inoculum is not pre-adapted. Careful interpretation of the 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 or low mineralisation (<20% degradation) in an inherent biodegradability test (OECD TG 302 series) may provide sufficient experimental 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 described degradation test.

Results obtained from the ready biodegradability, enhanced ready biodegradability, and inherent biodegradability test can be mainly used as indication of persistence or evidence of non- persistence or as supporting information in the persistence assessment with other lines of evidence (note some exceptions based on inherent degradation tests above).

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 constituents 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 (iv) 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 exist to assess hydrolysis:

- OECD TG 111: Hydrolysis as a function of pH

In general, the hydrolysis reactions are relatively sensitive to temperature. According to the OECD TG 111 tier 2 of this 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[26] is required. When hydrolysis kinetics has been assessed at different temperatures the activation energy for the hydrolysis of the specific substance can be estimated using equation given in the Annex 2 of the OECD TG 111. If a substance specific activation energy (E_a) is not available or cannot be derived, hydrolysis temperature

[26] Reference temperature for marine environment is 9°C.

correction may be done by using the Arrhenius equation (see Section 4.3.3.1.2.1) by applying generic estimated E_a of 65.4 kJ/mol.

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. 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 exist 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 phototransformation 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 is given in Section 4.3.3 (iv) of this Guidance.

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 available on ECHA's dissemination website[27] and OECD QSAR Toolbox[28]. Information on Biocidal active substances and Biocidal products is also available via the ECHA website[29]. The Japanese National Institute of Technology and Evaluation (NITE) database[30] 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)[31] 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 classification and labelling schemes or according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

The EU Pesticides database[32] includes information on active substances used in plant protection products, Maximum Residue Levels (MRLs) in food products, and emergency authorisations of plant protection products in Member States. The Pesticides Properties DataBase (PPDB)[33] is a relational database of pesticide chemical identity, physicochemical, human health and ecotoxicological data.

The information in these databases is not necessarily 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 ((Q)SARs)

A variety of models have been developed to predict biodegradation and potential degradation products. (Q)SAR predictions can be used 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; OECD, 2023), and the results are adequately reported.

Models for biodegradation estimation include:

[27] https://echa.europa.eu/
[28] https://www.qsartoolbox.org/home
[29] https://echa.europa.eu/information-on-chemicals
[30] http://www.nite.go.jp/en/chem/qsar/evaluation.html
[31] https://www.echemportal.org/echemportal/
[32] https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en
[33] https://sitem.herts.ac.uk/aeru/iupac/index.htm

- The EPI (Estimation Programs Interface) Suite™ is a Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by US 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. 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[34].
    - BioHCwin: Estimates biodegradation half-life for compounds containing only carbon and hydrogen (i.e. hydrocarbons).
    - 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)[35] predicts degradation pathways using biotransformation rules established from the reactions compiled in the EAWAG-BBD database.
- VEGA HUB[36] 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 (Q)SAR models which have been made available via the QSAR Toolbox, or by building regression based (Q)SAR models based on experimental information available in the QSAR Toolbox. QSAR Toolbox ECHA P screening (BETA) profiler[37] identifies substances with the potential for P/vP properties using experimental data and QSAR models available within the QSAR Toolbox. The results of this screening is based on single threshold values for P/vP according to Annex XIII to REACH Regulation (EC) No 1907/2006 and ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment (June 2017).

The above list of models is not exhaustive, and other valid models may also be used. With more experimental data becoming available, and a better understanding of the relationship

[34] https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
[35] http://eawag-bbd.ethz.ch/predict/
[36] https://www.vegahub.eu/
[37] ECHA P screening (BETA) - Toolbox Repository (qsartoolbox.org)

between structure and endpoint, (Q)SAR models are being updated or new models developed. In every case, it needs to be verified that both, the (Q)SAR model and the prediction are acceptable.

In line with CLP Annex I: 4.3.2.4.2. and 4.4.2.4.2., results obtained from well-developed and reliable biodegradation (Q)SAR models can be used when applying the WoE determination as part of the scientific assessment of the information relevant for the P, vP properties. When EPI Suite™ is used for this purpose, it is recommended to use combined results from three estimation models in the EPI Suite™ (US EPA, 2012; R.11).
The results of the following 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)[38] and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value < 2.25 (to 2.75)[39]), **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 is valid and the prediction is acceptable. The use of (Q)SAR 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).

[38] The probability is low that the substance biodegrades fast.
[39] For substances fulfilling this but BIOWIN 3 indicates a value between 2.25 and 2.75 more degradation relevant information is generally warranted.

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

According to CLP, Annex I, section 4.1.1.1, 'bioaccumulation' means 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). Annex I specifies that 'bioconcentration' means the net result of uptake, transformation and elimination of a substance in an organism due to waterborne exposure.

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 internal concentration of a substance in organisms at succeeding trophic levels in a food chain.

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:

Fish 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)

Note that corrections for growth and/or a standard lipid content are not accounted for in this definition of the BCF.

Fish 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. 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 **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, k_2 and can be determined under non-steady state conditions. In principle, the value should be comparable to the Fish BCF_{SS} but deviations may occur if steady state was uncertain or if corrections for growth have been applied to the kinetic BCF.

$$Fish\ BCFK = \frac{k1}{k2}$$

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 (k_2)** 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 (k_2 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.

Although there has been some discussion on the growth correction recently (Gobas and Lee, 2019), the approach described in Annex 5 of the OECD TG 305 is considered valid. Therefore, growth correction should be applied. Explanations on correction for growth dilution are given in ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-6.

Annexes 1 and 7 of OECD TG 305 contain 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).

$$dietary\ Fish\ BMFK = \frac{I \times \alpha}{k2}$$

$$dietary\ Fish\ BMFKg = \frac{I \times \alpha}{k2g}$$

where: α = assimilation efficiency[40] (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 BMF_K is the kinetic dietary BMF without growth correction

Dietary Fish BMF_{Kg} 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).

[40] 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.

1685 The **lipid- and growth-corrected fish kinetic dietary biomagnification factor (Fish**
1686 **dietary BMF_{kgL})** is the dietary BMF which has been growth corrected and corrected for
1687 lipid content of the fish and its food. For any use of the BMFkgL, it is important that the
1688 dietary lipid content and the feeding rate are reported alongside the value.

1689 *Annex I of OECD TG 315 includes the following definitions for bioaccumulation in sediment-*
1690 *dwelling organisms (OECD, 2008a):*

1691 OECD TG 315 indicates that the main endpoint of this test is the **sediment**
1692 **bioaccumulation factor (sediment BAF).**

1693 The **steady-state sediment bioaccumulation factor (sediment BAFss,**
1694 **[$kg_{sediment} \cdot kg^{-1}_{worm}$])** is the BAF at steady state and does not change significantly over a
1695 prolonged period of time, the concentration of the test substance in the surrounding
1696 medium (Cs as g kg^{-1} of wet or dry weight of sediment) being constant during this period
1697 of time.

1698 $$sediment\ BAFss = \frac{Ca\ at\ steady\ state\ or\ at\ day\ 28\ (mean)}{Cs\ at\ steady\ state\ or\ at\ day\ 28\ (mean)}$$

1699 Where

1700 Ca = concentration in worms in g kg^{-1} wet or dry weight

1701 Cs = concentration in sediment as g kg^{-1} of wet or dry weight of sediment

1702 The **kinetic sediment BAF (sediment BAF_K)** is defined as:

1703

1704 $$sediment\ BAFK = \frac{k1}{k2}$$

1705

1706 where

1707 k_1 = sediment uptake rate constant defining the rate of increase in the concentration of
1708 the test substance in/on the test organism resulting from uptake from the sediment phase
1709 [g sediment kg^{-1} of worm d^{-1}]

1710 k_2 = elimination rate constant defining the rate of reduction in the concentration of the
1711 test substance in/on the test organism, following the transfer of the test organisms from
1712 a medium containing the test substance to a substance-free medium [d^{-1}]

1713 The **biota-sediment accumulation factor (BSAF, [kg sediment OC kg^{-1} worm lipid**
1714 **content])** is the lipid-normalised steady-state concentration of test substance in/on the
1715 test organism divided by the organic carbon-normalised concentration of the substance in
1716 the sediment at steady state.

1717 $$BSAF = BAFk \times \frac{foc}{flip}$$

1718 where

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

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

Annex 1 of OECD TG 317 contains the following definitions for bioaccumulation in soil-dwelling organisms (OECD TG 317, 2010):

OECD TG 317 indicates that the main endpoint of this test is the **soil bioaccumulation factor (soil BAF)**.

The **steady-state soil bioaccumulation factor (soil BAFss, [$kg_{soil} \cdot kg^{-1}_{worm}$])** 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.

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

where

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 BAF_K)** is defined as:

$$soil\ BAFK = \frac{k1}{k2}$$

where

k_1 = soil uptake rate constant defining the rate of increase in the concentration of the test item in/on the test organism resulting from uptake from the soil phase [g soil kg^{-1} of worm d^{-1}]

k_2 = elimination rate constant defining the rate of reduction in the concentration of the test item in/on the test organism, following the transfer of the test organisms from a medium containing the test item to a substance-free medium [d^{-1}]

The **biota-soil accumulation factor ($BSAF_{soil}$, [kg soil OC kg^{-1} worm lipid content])** 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.

$$BSAFsoil = BAFk \times \frac{foc}{flip}$$

where

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

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

It should be noted that the term **biota-soil accumulation factor ($BSAF_{soil}$)** 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·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. (ECHA Guidance on IR&CSA, Chapters R.11, R.7c):

Field BMF = Co/Cd

where field BMF is the field 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 CLP Guidance, Section on Aquatic Hazards, Annex III.2. The aqueous exposure test measures 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 a 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 (Fish BCF_{SS}) and as a kinetic bioconcentration factor (Fish 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. The Fish BCF_{SS} is doubtful if the Fish BCF_K is significantly larger than the BCF_{SS}, as this can be an indication that steady-state has not been reached or growth dilution and loss processes have not been taken into account (OECD, 2012).

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 Fish BCF_K for growth dilution. If no information on growth is available, case by case assessment is needed, e.g. depending on the fish species and lifestage. There is currently no method to correct BCF_{SS} for growth dilution.

Fish BCF_{KgL} is the 5% lipid-normalised, growth-corrected, kinetic bioconcentration factor and is normally the preferred result for comparison with the numerical CLP B/vB criteria for substances accumulating mainly in lipids, since the kinetic BCF can be derived even if

no steady state is reached and it can be corrected for fish growth. However, there may be cases where the Fish BCF_{SSL} is more appropriate. This must be assessed on a case by case basis.

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 CLP 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. 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}, within an environmentally relevant range and at least an order of magnitude above its limit of quantification in water by the analytical method used. The highest permissible test concentration can also be determined by dividing the acute 96h LC_{50} by an appropriate acute:chronic ratio (e.g. appropriate ratios for some chemicals are about three, but a few are above 100) (Paragraph 51, OECD, 2012) . 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. Thus, a Fish BCF based on total radioactivity can be considered as a conservative value for the parent substance. 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 and to report the Fish BCF based on parent and not on total radioactive residues. Further guidance is available in paragraphs 6 and 65 of OECD TG 305 (OECD, 2012). 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 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 for comparison with CLP B/vB criteria.

The kinetic Fish BCF (BCF_K) is normally preferred for regulatory purposes since for bioaccumulative substances a real steady state is often not attained during the uptake phase. The Fish BCF_K should be corrected for growth dilution (since some growth is expected, as the fish are fed to keep them healthy and maintain body weight). 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. 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.

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. The test is recommended for substances where the aqueous exposure methodology is not practicable. The only test guideline available currently is OECD TG 305-III: Dietary Exposure Bioaccumulation Fish Test. Such 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. The dietary fish BMF differs from the field BMF, one reason for this could be that exposure is through a combination of water and food in the field situation, while in the dietary exposure study the exposure through the water phase is excluded under controlled conditions. 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.

In a study by Inoue *et al*. (2012) with carp, only two of the five substances that had a BCF value higher than 5000 L/kg, had a dietary BMF value in excess of 1. In a study by Martin *et al.* (2003 a,b) with perfluorinated compounds, one of the three substances with a BCF > 2000 had a dietary BMF of 1.0, while the two others had substantially lower BMF values. Therefore, a dietary fish BMF below 1 cannot be used to conclude that a substance is not bioaccumulative and it should be first assessed if the bioaccumulation potential can be concluded based on the estimated BCF, which can be directly compared to the CLP criteria. A dietary BMF <1 therefore 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_K can be estimated from fish dietary studies using the Dietary Exposure Test Spreadsheet of OECD 305 TG[41]. Detailed guidance on estimation of the fish BCF from the dietary study is given below under "*Considerations when reviewing fish dietary*

[41] https://www.oecd.org/chemicalsafety/testing/section-3-environmental-fate-behaviour-software-tg-305.htm

exposure bioaccumulation tests". Reliable and relevant fish dietary studies have been used in a regulatory context to conclude if a substance fulfils the CLP criteria for B or vB in a WoE approach, using the estimated BCF from the measured depuration rate constant/half-life (see Example B).

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, k_2), the assimilation efficiency (absorption across the gut; α), the kinetic fish dietary biomagnification factor (Fish BMF_K), the growth-corrected kinetic fish dietary biomagnification factor (Fish BMF_{Kg}), and the lipid-corrected kinetic fish dietary biomagnification factor (Fish BMF_{KL}) (and/or the growth- and lipid-corrected kinetic fish dietary biomagnification factor, Fish BMF_{KgL}) 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, in line with ECHA Guidance on IR&CSA, Chapters R.11, 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), Chapter 4.6.3 should be used and the results assessed in a WoE approach. The estimated BCF can be directly compared to the numerical CLP criteria. In case the derivation of a BCF is not possible, the Fish BMF5%, which is the Fish BMF_{Kg} 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 Fish BMF_{KgL}, it is important that the dietary lipid content and the feeding rate are reported alongside the value. Fish BMF5% and Fish BMF_{KgL} could be used in a benchmarking exercise.

Like in the aqueous exposure method, increases in fish mass during the dietary exposure test will result in dilution of the test substance in growing fish and thus the fish (kinetic) BMF will be underestimated if not corrected for growth (OECD, 2012). Annex 5 of OECD TG 305 (OECD, 2012) 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

For poorly soluble substances, if high concentration is spiked to the feed, e.g. in the upper limit indicated in the OECD TG 305, there could be issues with the bioavailability of the substance due to potential crystallisation of the test substance. This could lead to underestimation of uptake and BMF. 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.

Like in the aqueous fish bioaccumulation test, if radiolabelled test substance is used for the dietary exposure test, 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 numerical 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[42], 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 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

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

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. See OECD (2017), paragraph 288 for pros and cons of the method 3.

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 depuration rate constant. The depuration 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 Crookes (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 Brookes 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 and relevant fish dietary tests provide useful information on bioaccumulation but the results cannot be directly compared 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. Invertebrate (*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 2020). A draft OECD TG for the *Hyalella azteca* bioconcentration test[43] is scheduled to be adopted in 2024[44]. 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 numerical CLP criteria for B/vB (CLP Annex I, Section 4.3.2.1.2. and 4.3.2.2.2.).

Comparison between the metabolic rate of *H. azteca* with fish *in vitro* has shown that fish tend to have higher metabolic activity (Kosfeld *et al*., 2020). Since metabolism rates influence the BCF, this may explain why *H. azteca* tends to have higher BCFs than fish when normalised to a default 5% lipid content (Schlechtriem *et al*. 2019). However, the draft OECD TG recommends that *Hyalella azteca* BCF should be normalised to the species specific lipid content of 3% (based on whole body wet weight), unless there is evidence that the test chemical does not primarily accumulate in lipid. The BCF (normalised to 3% lipid where applicable) can be used for direct comparison with the CLP criteria. The test is discussed further in 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 usually exposed for 2-14 days to one or more sub-lethal concentrations of the test substance dissolved in water until steady state is reached. Only sexually mature males (> 8 weeks but < 6 months 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 a steady-state tissue concentration (or after 14 days of exposure), the remaining *Hyalella azteca* are transferred to clean water and the depuration phase is followed. The steady-state BCF_{SS} and kinetic BCF_K can be derived. If a steady state is not achieved, only the kinetic BCF_K is 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*). For many organic chemicals, there is a relationship between lipophilicity and the potential for bioconcentration. Correspondingly, there is a relationship between the lipid content of the test organism and the observed bioconcentration of such chemicals. The BCF should be normalised to 3% lipid to allow comparison between studies, unless it is known that the substance does not primarily partition to lipids. 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

Experience with use of this test is still limited and therefore, results should be assessed carefully.

[43] Available under: Draft documents - Section 3: Environmental Fate and Behaviour - OECD, last accessed: February 2024

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

If readily biodegradable solvents are used, they can cause problems with biofilm formation, leading to dietary uptake of the test substance which alters the uptake kinetics.

If radiolabelled test substances are used and only total radioactive residues have been 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) or high-performance liquid chromatography (HPLC) may have been used before radio-detection in order to determine a BCF based on the parent substance. When available, the BCF for the parent test substance should normally be used for assessment.

The tested concentration should be below the solubility limit of the test chemical in the test media. The selected test substance concentration for *Hyalella azteca* should be below its chronic effect level or 1% of its acute asymptotic LC_{50} (draft OECD TG).

In conclusion, reliable and relevant *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 and species

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 numerical 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 a 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 was used, it should be checked whether parent compound analysis is also available to assess the contribution of metabolites.

Results should be reported on a whole body wet weight basis. For comparison with other

BCF studies in the same species, lipid normalisation of the BCF to a representative lipid content for the tested invertebrate species should be considered, unless it is known that the substance does not primarily partition to lipids. 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 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.

High-quality data on the BCF value for invertebrate species such as mussel, oyster or scallop can be used.

Further information on the evaluation of aquatic invertebrate studies is available in ECHA Guidance on IR&CSA Section, R.7.10.4.1. In addition to data from standard toxicity tests, data from reliable non-standard tests and non-testing methods may also be used if available.

In conclusion, reliable and relevant aquatic invertebrate bioconcentration tests can provide a BCF which can be directly compared with the numerical CLP B/vB criteria. For comparison with other BCF studies in the same species, lipid normalisation of the BCF to a representative lipid content for the tested invertebrate species should be considered, unless it is known that the substance does not primarily partition to lipids.

Other aquatic species

BCF for algae (single-celled) should not be used. Data on apparent accumulation in small organisms, such as unicellular algae and micro-organisms, can be confounded by adsorption to cell or body surfaces leading to higher estimates of bioconcentration than is in fact the case (e.g. cationic substances may adsorb to negatively charged algal cells). Adsorption may also result in apparent deviation from first order kinetics and may be significant for small organisms because of their considerably larger surface/volume ratio compared with that for larger organisms.

Bioaccumulation data from aquatic plants should not normally be used, because it is currently not clear how observed accumulation in aquatic plants contributes to bioaccumulation for classification and labelling purposes. If such data exist, they could be included in the weight-of-evidence approach on a case-by-case basis.

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 a potentially important 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 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 intrinsic clearance data from OECD TG 319A and B may be used as input to mechanistic (IVIVE) and/or relevant QSAR models to predict BCF, e.g. Laue *et al*. (2023) (see also Section 4.3.3.2.6.3). A range of *in vitro* fish toxicokinetic tests are available in

the scientific literature. Preference is given to results obtained from standard tests OECD TG 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) or used regression model (with reference, e.g. Laue *et al*., 2023).

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 (Laue *et al*., 2020). Although the standard guideline *in vivo* methods remain the most informative for classification and labelling purposes, all available and relevant information on bioaccumulation, including *in vitro* biotransformation data and non-guideline methods, should 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, 2008a). 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 numerical 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. 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 criteria are fulfilled.

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.

Principle of the test

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.

For the uptake phase, worms are exposed to sediment spiked with the test substance which is covered with water and equilibrated. Using spiked sediment simulates a contaminated environment. Groups of control worms are held under identical conditions without the test substance. Worms and sediment are sampled at regular time-intervals and the concentration of test substance measured. After reaching an apparent steady-state tissue concentration (or after 28 days, whichever is sooner), the remaining worms are transferred to clean sediment and the depuration is followed.

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, 2008a).

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 (See above, Bioaccumulation Terminology Section 4.3.3.2.2; OECD, 2008a).

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).

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, 2008a).

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 such as OECD TG 317 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 worms and organic carbon content of soil to derive the $BSAF_{soil}$, biota-soil accumulation factor (OECD TG 317; OECD, 2010) which allows comparability between results from different bioaccumulation tests. These results cannot be directly compared with the numerical CLP B/vB criteria. Soil dwelling species are different in their physiology than fish and may have a lower metabolic capacity than fish species.

The soil $BSAF_{soil}$ 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. Considerations for benthic invertebrates are also applicable to terrestrial invertebrates. 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 $BSAF_{soil}$ values an appropriate weight in the B and vB assessment.
Bioaccumulation data from **terrestrial plants** should not normally be used, because it is currently not clear how observed accumulation in terrestrial plants contributes to bioaccumulation in terrestrial food webs for classification and labelling purposes. If such data exist, they could be included in the weight-of-evidence approach on a case-by-case basis.

4.3.3.2.3.8. Field data - 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. Reliable and relevant field data should be given a high weight in a WoE approach where B /vB is indicated.

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). This will lead to biomagnification from prey to predator (trophic magnification). A reliable field BMF or TMF value higher than 1 can be considered as an indication of very high bioaccumulation (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.6). 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 fulfilling the numerical CLP B/vB criteria, but in this kind of case all available data need to be considered together in a WoE approach. This is discussed further below under "Biomagnification (field BMF)".

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.

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 laboratory tests aim to expose fish either only via water or only 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 (especially for endangered species) can give valuable indication of an increased bioaccumulation potential particularly for difficult to test chemicals. See also Section 4.3.3.2.3.9.

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)

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%. If field BAF values (based on reliable information) are above 2000 or 5000, it might be sufficient to conclude that the substance fulfils the B or vB criteria as part of the Weight-of-Evidence approach..

Biomagnification (field BMF)

BMFs describe the increase in concentrations from prey to predator. For field data, BMF values are related to BAF values as both prey and predator are from the same environment (BMF prey-predator = BAFpredator/BAFprey). 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 fulfils the CLP B or vB criteria (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.6). On the other hand, 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 food chain 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 fulfils the CLP B/vB criteria ; a TMF < 1 indicates that the substance undergoes trophic dilution (Weisbrod *et al*., 2009).

Currently, there is no standard procedure for measuring TMFs. Hence, the study design 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. TMF can show variability related to ecosystem characteristics, organism biology and ecology, study design, and the statistical methods used for TMF calculation (Kidd *et al.* 2019). More reliable TMFs may be derived from data for non-migratory species originating from a confined area and sampled in the same period, or from food chains for which low variability in time and space can be assumed (e.g. for vast remote areas).

Guidance on the assessment of TMF studies is given in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.7 and also Burkhard *et al*., 2013 and Borgå *et al*., 2012.

4.3.3.2.3.9. Detection of substances in wildlife and humans

Monitoring data for humans and biota are available in the open literature and some data can be accessed via the platform IPCHeM[45], the NORMAN network[46] or HBM4EU[47]. It is recommended to perform a literature search and to check these databases to check for available monitoring data on a substance. Guidance documents 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. It is important to consider the reliability and relevance of the data, in addition to the quality, for the WoE assessment.

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 (e.g. for smaller wildlife species). 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

[45] https://ipchem.jrc.ec.europa.eu/
[46] https://www.norman-network.com/apex/
[47] https://www.hbm4eu.eu/

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 in the WoE assessment that the substance fulfils the CLP 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. Guidance on the use of field data for bioaccumulation assessment is given in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.7.

Human data

Information coming from scientific analysis of human body fluids or tissues, such as blood, milk, or fat can be used for bioaccumulation assessment in a WoE approach.

Concentrations of substances in blood, serum and urine of humans (workers or the general population) can be used to determine their elimination half-lives. Section 4.3.3.2.3.11 explains that if the whole-body terminal elimination half-lives are above 20 days in humans, it is an indication that the substance has B properties for consideration in a WoE assessment.
For workers exposed to a chemical in their workplace, a significant positive correlation between the number of years in the profession and the chemical concentration in blood could be used as supporting evidence of bioaccumulation in a WoE assessment. Measured exposure concentrations would support such an assessment.

4.3.3.2.3.10. Chronic toxicity tests on animals

Existing data from chronic toxicity studies with mammals (e.g. repeated dose toxicity studies, prenatal developmental toxicity studies, one/two-generation reproduction toxicity studies, extended one-generation study 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 taken up to a significant extent (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 bioaccumulative (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 4.3.3.2.3.11 below.

4.3.3.2.3.11. 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 and humans. 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[48]) gives details on the scientific background. For assessment of bioaccumulation in terrestrial mammals and other air-breathing organisms, see also R.11.4.1.2.8 "Bioaccumulation in air-breathing organisms and approaches" of the Chapter R.11 of the ECHA Guidance on IR&CSA.

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. Three different study designs are possible (Hofer, 2021): Single (high and low) dose with a duration of normally 7 days; repeated (low) dose studies commonly performed for at least 14 day, and 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).

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),

[48] https://echa.europa.eu/documents/10162/17228/bioaccumulation_assessment_of_air_breathing_mammals_en.pdf/

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). The 2.5 days value was derived using equations as explained in ECHA Working group on Toxicokinetics (2022), with conservative assumptions as input (dietary uptake efficiency of 1, feeding rate of approximately 0.065 kg/kg/d, 0.05 kg lipid/kg rat, and 0.2 kg lipid/kg diet). The 20 days for human reflects the same ratio as for BCF 5000/2000.[48]

In conclusion, 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 for consideration in a WoE assessment.

The use of toxicokinetic data in B-assessment is under scientific development and the recommendations above are based on current knowledge and experience. It is advised to follow-up recent and future developments in the field, e.g. via the ECHA website.

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) compatible with the test species, at which the highest fraction of non-ionised form was present. Further information is provided in ECHA Guidance on IR&CSA, Chapters R.7.10-3, R.7c and in OECD GD 23, as well as Armitage *et al.* (2017).

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.

In the absence of such studies, toxicokinetic information (e.g. human, rat) 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 classification and labelling 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:

'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[49].

A further source of data is ECOTOX Knowledgebase[50]. 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
- 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} (CLP Guidance on aquatic hazards, Section 4.1.3.2.3.3).

For neutral organic substances, bioaccumulation is most often driven by partitioning to storage lipid. In these cases, the log K_{OW} can inform about the potential for bioaccumulation, and can be used together with other evidence in a WoE approach. A log $K_{OW} \geq 4.5$ indicates the potential for a BCF ≥ 2000 in aquatic organisms, while a log K_{OW} greater than 2 together with a log K_{OA} greater than 5 indicates the potential for B/vB for

[49] see also https://cefic-lri.org/toolbox/bcf-database/ (last accessed: November 2023)
[50] available under https://cfpub.epa.gov/ecotox/ (last accessed: November 2023)

air-breathing organisms. If the log K_{OW} is less than 2, the substance can normally be regarded as not fulfilling the CLP 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 reach a BCF of 2000 in aquatic species, nor that it is bioaccumulating in air-breathing mammals, because it can be eliminated rapidly enough by exhalation (Saunders and Wania, 2023; see also Section 4.3.3.2.6.2 on octanol-air partitioning). Guidance on log K_{OW} is given in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.10 and Appendix R.11-5.

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 their use in a WoE approach. Generally, the most conservative valid value should take precedence.

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. (Q)SARs) to estimate the log K_{OW}. These two methods are very close to each other in predictivity. 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).

Examples of freely available (Q)SAR software programs that include models for the prediction of log K_{OW} are EPISuite[51], OECD QSAR Toolbox and VEGA. The data originating from calculations with the commercial quantum-chemical software COSMOconf and COSMOtherm have been shown to be more accurate than the data from many other estimation programs. Glüge and Scheringer (2023) have published COSMOtherm values for ca 4400 substances.

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). This also shows the

[51] https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

importance to not limit the B identification to certain experimental values, but to acknowledge also any other evidence from field and monitoring studies. 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.

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). The numerical cut-off aligned to the screening criteria for prioritising bioaccumulating substances to air-breathing organisms are still subject to scientific review. Recently, Saunders and Wania (2023) evaluated thresholds for air-breathing animals across various species and predicted that animals with lower rates of respiration (e.g., manatees and sloths) and those ingesting high-lipid diets (e.g., polar bears and carnivorous birds) were able to biomagnify persistent chemicals with log $K_{OA} < 5$. This was also observed for several temperate reptiles due to their lower respiration rates and internal temperatures.

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[52] (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). 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-(Q)SARs 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 (Q)SARs, careful attention should be paid to the validity of the models and the acceptability of the predictions, which can be assessed

[52] https://github.com/NIEHS/OPERA

against the established principles for the assessment of QSAR predictions and results presented in the OECD (Q)SAR assessment framework documents (OECD, 2023). Further information can be found in the Guidance on QSARs and grouping of chemicals, Chapter R.6[53] and in ECHA Practical Guide "How to use and report (Q)SARs"[54].

(Q)SAR BCFs derived using experimental input data (e.g., log K_{OW} and intrinsic clearance data from OECD TG 319A and B) (e.g., Laue *et al.* (2023)) 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 QSAR Toolbox and VEGA. OASIS Catalogic is also a valuable model for BCF prediction. The Bioaccumulation Assessment Tool, BAT[55] has built-in models including input of *in vitro* biotransformation rate to predict BCFs.

A reliable BCF prediction should not be used alone to decide whether a substance fulfils 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. They extract only the freely dissolved (i.e. bioavailable) fraction of substances from water samples, in proportion to their partitioning coefficient, which is mainly related to the hydrophobicity of the substance and molecular size. In this way they simulate the potential for aquatic organisms to bioconcentrate organic substances by passive diffusion into storage lipids and cell membranes. 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.

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

[53] https://echa.europa.eu/documents/10162/17224/information_requirements_r6_en.pdf/
[54] https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf/
[55] https://arnotresearch.com/bat-reg/ (last accessed: November 2023)

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.

These parameters should only be used as part of a WoE approach. In order to conclude on limited uptake, these parameters must be accompanied by experimental information from long-term exposure studies (e.g. birds, mammals, fish) confirming the low uptake of the substance to conclude that a substance is not bioaccumulative.

These types of information should be examined in a WoE approach together with the non-testing information on the substance to conclude whether the CLP B/vB criteria are fulfilled.

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 and drinking water purification processes may only partially remove them. CLP relates the criteria for M/vM to the log K_{OC} that reflects the potential of a substance to be adsorbed on the organic fraction of environmental matrices such as soil, sludge, sediment particles and dissolved/particulate organic matter[56], and is therefore inversely related to the substance's potential of entering water bodies (CLP Annex I, ECETOC, 2021, McCall *et al.*, 1981). Within the WoE assessment (see Section 4.3.5.1), when 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.

Sorption describes the retention of a chemical species by a solid environmental compartment (Landrot and Sparks, 2023). Adsorption refers to the adhesion and binding capacity of a substance to a surface, absorption refers to the capacity of an amorphous phase to accommodate a substance within its bulk phase, while desorption refers to the release of a substance from a surface. Adsorption is the dominant process for small time scales (for example, 24 hours as in OECD TG 106) and is usually occurring faster than absorption. However, for the sake of simplicity, adsorption will be considered to refer to both adsorption and absorption. The potential for adsorption/desorption of a chemical is an important environmental fate parameter and an indicator of partitioning of the

[56] Soil/sludge organic matter is any organic material present in soil/sludge in varied stages of decomposition. Soil/sludge organic carbon is the measurable amount of carbon in the soil/sludge organic matter

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 ionisable substances, other matrices (for example, clay particles) may also play a role on the adsorption of a substance (4.3.3.3.6). The K_{OC} value of a substance is known to be inversely related to the mobility in the environment (CLP Annex I, ECETOC, 2021, McCall *et al.*, 1981), 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 (adsorption testing) and non-experimental methods are currently available for obtaining the K_{OC} value of a substance. These approaches for deriving a log K_{OC} include soil leaching studies, lysimeter studies, modelling/ computational approaches, as well as analysis of monitoring data. It must be noted that modelling/computational approaches (for example, 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 the identification and assessment of the M or vM properties.

The following Sections specify the type of information that can be considered for the assessment of M/vM properties (Sections 4.4.2.3.2. and 4.4.2.4.2. of the CLP legal text). Section 4.3.5 of this Guidance describes the WoE approach for concluding on these properties. Special considerations regarding ionisable substances are presented in Section 4.3.3.3.6 of this Guidance. Some of the methods in this Section include both experimental and estimation elements to a derive a K_{OC}.

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

A description and interpretation of the relevant experimental studies to be used for classification purposes is provided below.

<u>OECD TG 106 (Adsorption - Desorption Using a Batch Equilibrium Method)</u>

The OECD TG 106 is designed to evaluate the sorption of a chemical on soils with different properties. 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 and soil texture.

The OECD TG 106 does not differentiate between physical and chemical adsorption and absorption. 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). For such substances, variations to the OECD TG 106 may be needed, such as the use of passive samplers for poorly water soluble substances or devices to sample the headspace for highly volatile substances. For such cases, three phase partitioning, namely between water-soil-passive sampler, or water-soil-air, would need to be accounted to derive Koc values.

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. For non-ionisable substances, selected soils should include soil organic carbon content ranging from low[57] to high organic carbon (e.g. >10%). 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. The methods used to obtain these parameters should be provided. 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 (4.3.3.3.6).

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[58]. 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. Note that this document and in particular the tool developed in relation to it, focusses on Tier 3 results of OECD TG 106.

As described in OECD TG 106, 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 aqueous concentration of the stock solution should preferably be three orders of magnitude higher than the detection limit of the analytical method used and should be also below the water solubility of the test substance (OECD TG 106). The selection of the aqueous concentrations (C_{Water}) for deriving K_d should be carefully considered and justified in accordance with the OECD 106 specifications. The test is performed in five different soil types and the respective distribution/partition 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. After equilibrium is reached in Tier 2 testing, the distribution coefficient (K_d) is determined as the ratio of the concentration in the soil (C_{Soil}) to that in water (C_{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);

[57] OECD TG 106 notes that soils with less than 0.3% organic carbon may disturb the correlation between organic content and adsorption and recommends the use of soils with a minimum organic carbon content of 0.3%.
[58] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2017.EN-1326

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

For non-ionisable substances, it is assumed that the sorption is driven mainly by the soil organic carbon, therefore the K_d values for soils with different organic carbon content will vary. 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:

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

The common logarithm (log_{10}) of the K_{OC} value derived from K_d is then compared with the CLP mobility 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 investigation is performed with the five different soil types used in Tier 2 investigation. The Freundlich adsorption isotherm equation is an empirical model that describes the adsorption isotherm of a substance as:

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

K_F is the Freundlich adsorption coefficient, n is the affinity-capacity coefficient indicating the adsorption capacity of the sorbent. 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, in which case the sorption is assumed to be linear.

The 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). If 1/n < 1, sorption is less favourable at higher solute concentrations resulting in a concave downward isotherm shape. This is indicative of saturation of the adsorption sites available to the chemical resulting a relatively less adsorption. The case of 1/n > 1 is generally rare and only occurs when previously sorbed molecules lead to a modification of the sorbent which favours further sorption. This only starts after a certain loading of the sorbent and occurs rarely under environmental conditions. If 1/n equals unity, sorption is mainly governed by absorption into the organic matrix and therefore does not decline with increasing solute concentration. This is mainly the case for partitioning of non-ionic, rather hydrophobic substances into an amorphous organic matrix (Schwarzenbach *et al.*, 2002).

In the same manner as for K_d, the K_F can be normalised to the organic carbon content of the soil (K_{FOC}). As the K_d and K_F are not equal coefficients, the calculated K_{FOC} is not equivalent to K_{OC} (Tier 2) and cannot be compared with the CLP mobility criteria.

However, the K_F can be used for deriving a specific K_d (K_d*) for a defined aqueous concentration (C_{Water}) using the following equation (Chen *et al.*, 1999, Schwarzenbach *et al.*, 2002 and Mejia-Avendaño *et al.*, 2020):

$$K_d^* = \frac{K_F \times C_{Water}^{\frac{1}{n}}}{C_{Water}} = K_F \times C_{Water}^{\frac{1}{n}-1}$$

An organic carbon normalised K_{OC}* can then be derived from the K_d* as described previously. The selection of the aqueous concentrations (C_{Water}) for deriving K_d* needs to be carefully considered and justified on a case by case basis. In any case, the selected concentrations should fall within the concentrations range used for deriving the respective K_F and 1/n (Tier 3) (Chen *et al.*, 1999). Selection of aqueous concentrations outside the range tested in Tier 3 might lead to uncertain K_d* estimates and, therefore, they are not recommended. Both the K_{OC} (Tier 2) and K_{OC}* (Tier 3) can be used for comparing with the CLP mobility criteria.

Studies on activated sewage sludge

Recital (15) of the Commission Delegated Regulation (EU) 2023/707 states that the organic carbon-water partition coefficient (K_{OC}) reflects the ability of a substance to be adsorbed on the organic fraction of solid environmental compartments such as soil, sludge and sediment. Regarding activated sludge, studies such as the OPPTS 835.1110 (Activated Sludge Sorption Isotherm)[59] and the ISO 18749 standard (Water quality — Adsorption of substances on activated sludge - Batch test using specific analytical method)[60] may be compared to the CLP criteria within the WoE determination to provide useful information on the sorption of substances in sludge. Sorption is a key process in wastewater treatment plants and is most relevant for those substances that have been released to or have ended up in wastewater treatment plants.

Due to the differences in the composition and polarity between the organic matter in the sludge and soil and sediments, caution should be applied when interpreting results from such studies (Kile *et. al.*, 1995). Therefore quantitative and qualitative information on the composition, the organic matter and the organic carbon content of the activated sludge should be reported. Generally sludge is characterised by high organic carbon content and, therefore, high K_d values are expected. The normalisation of the K_d to the organic carbon content (K_{oc}) allows for the comparison with the CLP criteria, taking into account the considerations related to the composition of the organic matter in sludge.

The Activated Sludge Sorption Isotherm method (OPPTS 835.1110), describes a procedure for the determination of the sorption potential of activated sludge solids by calculation of a sorption isotherm for different sorbent concentrations. The method derives a Freundlich sorption coefficient after the equilibrium between aqueous phase and sludge solids has been reached that must then be converted into a K_{OC} value. According to the ECHA Guidance on IR&CSA, Chapter R.7.1.15.3, caution should be exercised when interpreting any such results, as the method does not differentiate between adsorption and other elimination methods (such as complex formation, flocculation, precipitation, sedimentation

[59] https://permanent.fdlp.gov/lps59946/835-1110.pdf
[60] https://www.iso.org/obp/ui/#iso:std:iso:18749:ed-1:v1:en

or biodegradation). The method does not describe the origin of the activated sludge, however differences in the composition of the activated sewage sludge from treatment plants receiving industrial or predominantly domestic sewage is expected. For this reason and for the purpose of the mobility assessment under the CLP regulation, sludge originating from treatment plant receiving predominantly domestic sewage is recommended (von Sperling, M., 2007 and Ranade, V. and Bhandari, 2014).

The Water quality — Adsorption of substances on activated sludge - Batch test using specific analytical method (ISO 18749), derives a distribution coefficient K_d (in L/kg) between the aqueous phase and sludge that can then be normalised to the organic carbon content to generate a K_{OC} value. The method is suitable for substances that are water soluble, or allow for stable suspensions/dispersions/emulsions, are not significantly removed by abiotic processes (e.g. stripping/foaming), do not de-flocculate activated sludge, are not readily biodegradable, and have a sufficiently sensitive analytical method. The test has been used as a screening method to determine the degree of adsorption of substances on activated sludge or primary sludge in wastewater treatment plants. As described in the method sludge originating from treatment plant receiving predominantly domestic sewage should be used.

OECD TG 121 (Estimation of the Adsorption Coefficient (K_{OC}) 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. OECD TG 121 is most applicable for substances that are neutral between pH 4-9, namely that are non-ionisable, or have the same 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. The accuracy of the method is affected by the presence of reference substances used for calibration that are structurally similar to the test substance in order to address the same mechanisms of adsorption. If data on relevant reference substances are not available, relevant alternative calibration substances can be selected and their selection should be justified.

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 volatile, poorly water soluble and substances/mixtures with a high affinity to the surface of incubation systems (OECD TG 121 and ECHA Guidance on IR&CSA, Chapter R.7.1.15.3). As this is an estimation method with a limited set of reference substances, the uncertainty of such estimations should be assessed and considered when assessing the data. (see also European Commission (2002) SCP/KOC/002 Opinion)[61]. 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

[61] https://food.ec.europa.eu/system/files/2020-12/sci-com_scp_out128_ppp_en.pdf

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[62] or based on the conversion-dispersion equation (e.g as applied in Vereecken *et al*., 2011). The choice of estimation technique should be justified As mentioned above for OECD TG 121, the accuracy of the method is also affected by the choice of reference substances.

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 of the test substance (measured as a percentage of the amount initially applied) 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 where 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. Furthermore, the soil column leaching studies might underestimate adsorption due to difficulties in the exact determination of the relative rates of movement of the substance, the handling/packing of the soil and the probable non-equilibrium state of the test system.

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).

[62] https://www.oecd-ilibrary.org/docserver/9789264070561-en.pdf?expires=1691490605&id=id&accname=guest&checksum=F04D799468933A0FFB44B22ABD4AB1BC

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 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. As mentioned above for OECD TG 121, the accuracy of the method is also affected by the choice of 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 state for the test system (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 laboratory 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, mass 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 the test substance and its metabolites to leach to the groundwater from the soil is determined by the derivation of their concentrations in the groundwater/leachate and by comparing with the respective national regulatory criteria. 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 (Leistra *et al*. , 2001).

Inverse modelling techniques utilising the data from the field and leaching studies have been 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 as degraded parent substance. Such double-counting of the loss via the treatment of non-extractable residues data should be avoided in this type of modelling. Such modelling techniques consider the fate processes over longer time periods, but require both *K*oc and DegT50 to describe the solute transport modelling. For either or both of these parameters, already known values are needed either as starting point for the fitting of that parameter or as a fixed parameter while fitting the other parameter. Furthermore, the problem of non-uniqueness which refers to the fact that equally good fitting of the experimental data can be obtained with different parameter combinations exists (Sanco, 2014). This, in turn, limits the relevance of the method for classification purposes.

Both field leaching and lysimeter studies are application scenario-specific and are introducing exposure considerations relevant to a local risk but not to an intrinsic hazard assessment. Lysimeter studies provide location-specific information which cannot alone represent the range of environmental conditions in the European Union. They also exhibit other limitations that currently restrict their use for the purposes of classification and labelling, namely The leaching and lysimeter studies are affected by the local environmental conditions (Hansen *et al*. 2000) and there is unclarity on whether they can sufficiently represent the conditions that need to be covered. 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 in their results needing to be given lower weight within the overall WoE.

However lysimeter studies may be used for regulatory purposes in order to qualitatively 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). The current practice regarding pesticidal-active substance approvals according to Regulation 1107/2009 is that metabolites found in lysimeter studies at annual average concentrations exceeding 0.1 µg/L in the leachate are considered as major transformation products, for which a groundwater risk assessment is performed.

<u>OECD Guidance Document for the Performance of Outdoor Monolith Lysimeter Studies (No.22).</u>

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[63]. With monolith lysimeters, mass fluxes of water and chemicals can be monitored and chemical distribution and transformation products can also be determined. As also for all other studies discussed in this Guidance, this method is applicable to substances for which an analytical method with suitable accuracy and sensitivity is available and resembles field conditions (local climatic conditions) closer than other laboratory studies. OECD Guidance Document No.22, finally,

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

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 similar considerations regarding lysimeter and field studies in an overall WoE assessment.

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

A predicted K_{OC} value of a test substance may be used for the purpose of classification and labelling. The conditions discussed earlier in the Guidance (4.3.3 (ii)) must be fulfilled, namely the ones related to the reliability and applicability domain supported with adequate documentation.

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 non-ionisable, non-surface active organic substances for which their environmental sorption is attributed practically entirely to organic matter, where the sorption mechanism is hydrophobic binding. 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*., 1995, 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[64] (US EPA 2012), the OECD QSAR Toolbox, OPERA[65], QSARINS[66] 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.

Another approach refers to the use of the octanol-water distribution coefficient (D_{OW}) as a measure of the distribution of dissociated and non-dissociated species in octanol and water and as a function of pH. The D_{OW} can be derived using the K_{OW} and the dissociation

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

constant (pK_a) (Neumann and Schliebner, 2019; Arp and Hale, 2023) based on the following equations:

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

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

Only in the absence of any other relevant information, D_{ow} may be used instead of K_{OW} for predicting K_{OC} for ionisable substances by use of other *in silico* approaches ((Q)SARs), if available.

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). The identification of any substance within the context of a monitoring campaign is dependent on a range of parameters, such as the proximity of the sampled area/point to emission sources, its uses and the quantity released in the environment, the route of entry into the environment, spatial and weather conditions (for example, geography/ topography, meteorology), its environmental fate (for example, degradation and volatility), any transport and inter-media distribution processes, as well as on the analytical and sampling methods available. 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 indication for mobility. Additionally, temporal trends highlighting potential increases or decreases over time within the same 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).

Further details on the review of monitoring data and its implementation within the regulatory context of the Water Framework Directive (2000/60/EC) are provided in Guidance document No 7[67]. Concerning the conduct of groundwater monitoring studies for pesticide active substances and their metabolites in the context of Regulation (EC) 1107/2009, Gimsing *et al*. (2019) and EFSA(2023b) further elaborated on aspects such as the selection of the monitoring site, study design, time scales, treatment of positive and negative results, avoiding of contamination, as well as study documentation.

In all cases, an overall assessment of the relevance and reliability of any monitoring dataset should be conducted and included in the WoE reasoning.

[67] https://circabc.europa.eu/sd/a/63f7715f-0f45-4955-b7cb-58ca305e42a8/Guidance%20No%207%20-%20Monitoring%20%28WG%202.7%29.pdf

4.3.3.3.5. 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), 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 equilibrium adsorption/desorption studies, in order to confirm the relevance of aged sorption if, 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 sorption concept. It is clear that this approach, similarly to lysimeter and field studies, relates more to risk and not hazard assessment and incorporates a large number of environmental transport, exposure scenario, use and modelling considerations over large time scales. Thus, K_{OC} values derived from such approaches should not be directly compared with the M/vM criteria. Moreover, any potential influence of aging is expected to be less relevant for low or non-adsorbing substances, due to the longer exposure time scales involved is such processes.

4.3.3.3.6. Considerations for ionisable substances

The following terminology will be used in this Section:

"**Non-ionisable**" substances are substances that are not able to be ionised (able to dissociate, forming ionic compounds) under relevant environmental conditions. Respectively, "**ionisable**" substances are substances that are able to be ionised (able to dissociate, forming ionic compounds) under relevant environmental conditions. **Anionic** substances are those substances that are in the anionic (negatively charged) form (in a percentage above 10%) and **cationic** substances are those substances that are in the cationic (positively charged) 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 on their speciation. As defined in the M/vM criteria in CLP, it is necessary for the purpose of classification and labelling 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 (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 anionic 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). 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% when they are in a solution with a pH greater than pK_a - 2 (i.e. pH 2.5) and in a percentage above 99% at a pH greater than pK_a + 2. For the basic substances, the cationic form will exist in a percentage above 1% when they are in a solution with a pH lower than pK_a + 2 (i.e. pH 11.5) and in a percentage above 99% 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.

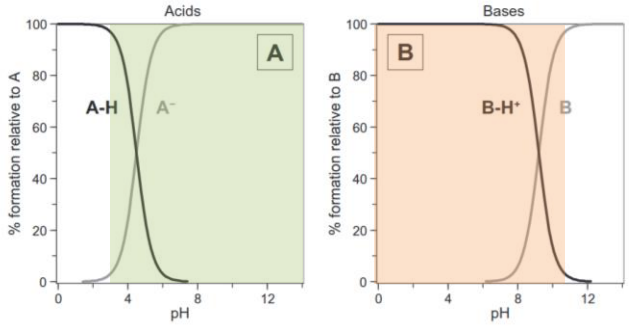

Figure 1. Visualisation of species distribution for monoprotic acidic and basic substances as adapted from Schaffer and Licha (2014). The coloured areas cover the pH range at which the substances are present in the ionic form at > 1%. pK_a acid = 4.5; pK_a base=9.5

Relating to the mechanisms of adsorption/desorption of ionisable substances, extensive published 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) and, therefore, as stated in Section 4.3.3.3.3 adsorption/desorption could be reflected by the estimated K_{oc} value derived from the Kow value. 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 (due to multiple matrix components such as clay and organic carbon), 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[68], dissolved organic matter, soil texture and mineral composition), other phases present (for example, coal,

[68] Ionic strenght should however be representative of values derived from soil solutions in environment. According to Owsianiak *et al.* 2013 (table 1 in supportive document), effective ionic strength in pore water calculated for 760 soils range from 0.0011 to 0.4 M with a median value at 0.0065 M.

black carbon), non-linear sorption mechanisms 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). 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).

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 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. Mechanistic information of the specific sorption mechanism can elucidate further mobility potential of the substance in the environment and whether other than organic carbon normalisation is justified. The application and relevancy of such data to the mobility assessment can be justified on a case by case basis.

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. 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 of anionic substances needs to also include soils of high pH (when feasible, at a pH ≥ 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 (pK_a-2 < pH < pK_a+2), 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 soil pH, the mobility will be minimal due to electrostatic attraction toward negatively charged soil moieties (Sigmund *et al*. 2022). At high pH (for example pH > pK_a +2), 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 lower pH (when feasible, at a soil pH ≤ pK_a - 2). If the value of the soil pH is near the pK_a (pK_a-2 < pH < pK_a+2), 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 (for example, illite, smectite) 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. The derivation of a clay- and/or CEC-normalised partition coefficient as well as their use for comparing with the CLP criteria may be considered in a future guidance update based on the scientific developments in the area.

When interpreting results from batch equilibrium adsorption/desorption studies (OECD TG 106) with ionisable substances, soil selection and characterisation are particularly important information to consider, 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 and ionic strength for assessing the behaviour of such substances in the soil.

Regarding the interpretation of 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), results from two tests should be available: one with the ionised form and one with the non-ionised form. The use of the appropriate buffer solutions and the suitability of the set of data for the reference ionisable substances needs to be carefully considered for assessing the reliability of the adsorption coefficient K_{OC} estimation.

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 results on ionisable substances using the Soil TLC (Thin Layer Chromatography). The Table 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. Dominant species and expected mobility of ionisable substances at soil pH values relative to the pKa of the substance (adapted from Wauchope *et al.*, 2002).

	Dominant species* and mobility	
Soil pH	**Acids**	**Bases**
< pK_a - 2	XH (neutral) Behaves like non-ionisable substance.	$(XH)^+$ or X^+ (cation) Not mobile (clay surface and organic matter 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. For pH >> pKa the neutral species will be the predominant species and an intermediate mobility is expected.
> 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 substances potentially meeting the criteria for classification based on the study results from long-term toxicity testing on terrestrial and sediment organisms 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 and sediment organisms 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 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 class(es)/ 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/vPvB and PMT/vPvM 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 (T) criterion. 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 mutagenicity 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)[69] 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 PPPR, for example the predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil according to OECD TG 226. The Guidance for Biocidal Products Regulation Volume IV Part A: Information requirements (ECHA, 2022c) mentions 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 (for example, OECD TG 213 and 214). Regarding honeybees and other pollinators, relevant tests include also ones performed according to OECD TG 245, 246 and 247 (guidance on the assessment of risks to bees from the use of plant protection products[70] and biocides[71]). These tests are both short- and long-term and are part of the "Additional Data Set" within the BPR context, 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. Possible species of other non-target terrestrial arthropods to be tested in addition to honeybees are reported in the BPR Guidance, Volume IV. Part A, Section 1.1.5.2. (ECHA, 2022c). Similar considerations are also relevant for 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 Section 4.3.3.4.9), 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

[69] As indicated in OECD TGs 216 and 217, if agrochemicals are tested, two concentrations should be used. For non-agrochemicals, a geometric series of at least five concentrations must be used in order to cover the range needed to determine the ECx values.

[70] https://www.efsa.europa.eu/en/efsajournal/pub/7989

[71] Guidance on the assessment of risks to bees from the use of biocides, 2024. https://echa.europa.eu/documents/10162/2324906/guidance_on_assessment_risks_to_bees_from_biocides_en.pdf/1fe886eb-0ba5-6d07-c06d-8a4823931a30?t=1707902780257

terrestrial toxicity data in the framework of PBT/vPvB assessment and classification and labelling have been made by JRC (2014) and, more recently, by the German UBA (2022). Further work on terrestrial toxicity is planned in the context of UN-GHS working groups. Once available, any relevant outcomes will be used for an update of this Guidance.

Until terrestrial hazard class(es) including threshold values are introduced in the regulatory framework, it is hereby proposed that a similar approach is used for soil-living organisms 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 the derived pelagic NOEC or EC_{10} can be compared to the T criterion of 0.01 mg/L of CLP Annex I, 4.3.2.1.3 (a) and 4.4.2.1.3 (a), by use of the following equation:

$$\boldsymbol{NOEC(EC_{10})_{porewater}} = \frac{\boldsymbol{NOEC(EC_{10})_{soil}}}{\boldsymbol{Kd_d}}$$

$NOEC(EC_{10})_{porewater}$: estimated NOEC ($EC_{10}$) for porewater exposure (mg/L)

K_d: adsorption coefficient (L/ kg dw)

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

An EFSA scientific opinion (EFSA Journal 2009; 922, 1-90) 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. However, toxic effects may be also exhibited via other mechanisms than porewater diffusion, whereas the assumption of linear correlation between $NOEC(EC_{10})_{porewater}$ and $NOEC(EC_{10})_{soil}$ may also introduce uncertainties. This could be the case, for example, for highly sorbing neutral organics or for substances with a corresponding adsorption or binding behaviour not predominantly driven by lipophilicity.

Preferably, porewater concentrations are based on well measured experimental porewater concentrations of the soil toxicity test. If these are not available, it might be possible to calculate a porewater concentration with a K_d value specific for the test soil. Otherwise, the K_d can be estimated from the generic K_{oc} as described in the Section on mobility and the organic carbon content of the test soil (Section 4.3.3.3.1). It should be noted that the uncertainty increases in this order of preference, and this has to be taken into account in the WoE.

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. 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 organisms such as *Myriophyllum 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.

$$\boldsymbol{NOEC(EC_{10})_{porewater}} = \frac{\boldsymbol{NOEC(EC_{10})_{sed}}}{\boldsymbol{Kp_{susp}}}$$

$NOEC(EC_{10})_{porewater}$: estimated NOEC (EC_{10}) for porewater exposure (mg/L)

Kp_{susp} : suspended solid-water partition coefficient (L/kg dw)

$NOEC(EC_{10})_{sed}$: measured sediment toxicity NOEC (EC_{10}) (mg/kg dw)

The suspended solid-water partition coefficient can be estimated from the K_{oc} of the

substance as Kp_{susp}= $Foc_{susp} * K_{oc}$ 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 sediment organisms.

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 food chain) and earthworm (terrestrial food chain) (ECHA Guidance on IR&CSA, Chapter 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 (ECHA Guidance on IR&CSA, Chapter 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, if birds are likely to be exposed. 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. One standard study is usually requested, conducted in accordance with OECD TG 206 or USEPA OCSPP 850.2300 (EFSA 2023a).

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 relevant 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 considered as a strong indicator of fulfilment of the (T) criterion (ECHA Guidance on IR&CSA, Chapter R.7.10.16.2). In order to normalise between different bird species and sizes, as well as to account for any avoidance behaviour, a unit conversion of the test concentrations to dietary doses (in mg/kg body weight/day) by use of information on daily food consumption and body weight should also be performed and reported.

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 REACH Annex XI criteria. These quality aspects are further detailed in ECHA Guidance on IR&CSA 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. As an example of a recently validated TG using fish cells, OECD TG 249 is currently available for rainbow trout gill cell lines (Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay).

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), also depending on the availability and quality of all other relevant information. When acute/short-term aquatic toxicity data show that the substance is very acutely toxic (L(E)C_{50} below 0.01 mg/L), a definitive conclusion can be drawn that the substance fulfils the (T) criterion. 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 (Q)SAR 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. For example, ECOSAR[72], KAshinhou Tool for Ecotoxicity (KATE)[73], iSafeRat®[74] may be used for predicting both short- and long-term aquatic effects. PETROTOX (Redman *et al.*, 2017) has been developed to address petroleum substances based on substance composition (see ECHA Guidance on IR&CSA Chapters R.11 and review published in 2012[75] for further details). 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 4.3.4. Similarly, other information from toxicological studies may be relevant (for example, for allergens, neuro/immune toxicants, etc.) and needs to be considered.

[72] https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model
[73] https://kate.nies.go.jp/
[74] https://www.kreatis.eu/isaferat_page
[75] https://echa.europa.eu/documents/10162/17221/review_environmental_physicochemical_methodol_en.pdf (last accessed: November 2023)

4.3.4. Application of the WoE to conclude on PBT/vPvB properties for classification and labelling

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 consider each property, namely persistence, bioaccumulation and toxicity against each respective criterion (P or vP, B or vB, and T) following the provisions and considerations described in Section 4.3.3 of this Guidance. In CLP (green text above), the decision on whether classification in the PBT/vPvB hazard class is warranted is based on a WoE determination using expert judgement. WoE shall be applied in particular where the criteria set out in Sections 4.3.2.1, 4.3.2.2 and 4.3.2.4 cannot be applied directly based on the available information. The following paragraphs will expand on some general principles of the WoE, with property-specific considerations further elaborated on after the current general principles Section.

The following general principles are broadly adapted from OECD report No. 311 on the Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment (OECD, 2019)[76]. Additionally, ECHA has developed a template and background document intended to be used in human health and environmental hazard assessments under REACH and CLP, 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[77]. Similarly, EFSA (2017) has issued a Guidance on the use of the WoE approach in scientific assessments that can also be consulted[78]. Thus, the individual steps in the general scheme are not explained in detail, but only the high-level/ key issues are highlighted. The general scheme can be seen below.

[76] https://www.oecd-ilibrary.org/docserver/f11597f6-en.pdf?expires=1706878428&id=id&accname=guest&checksum=8553021B60DFC988DB5EB50AC48B78C1
[77] https://echa.europa.eu/support/guidance-on-reach-and-clp-implementation/formats
[78] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4971

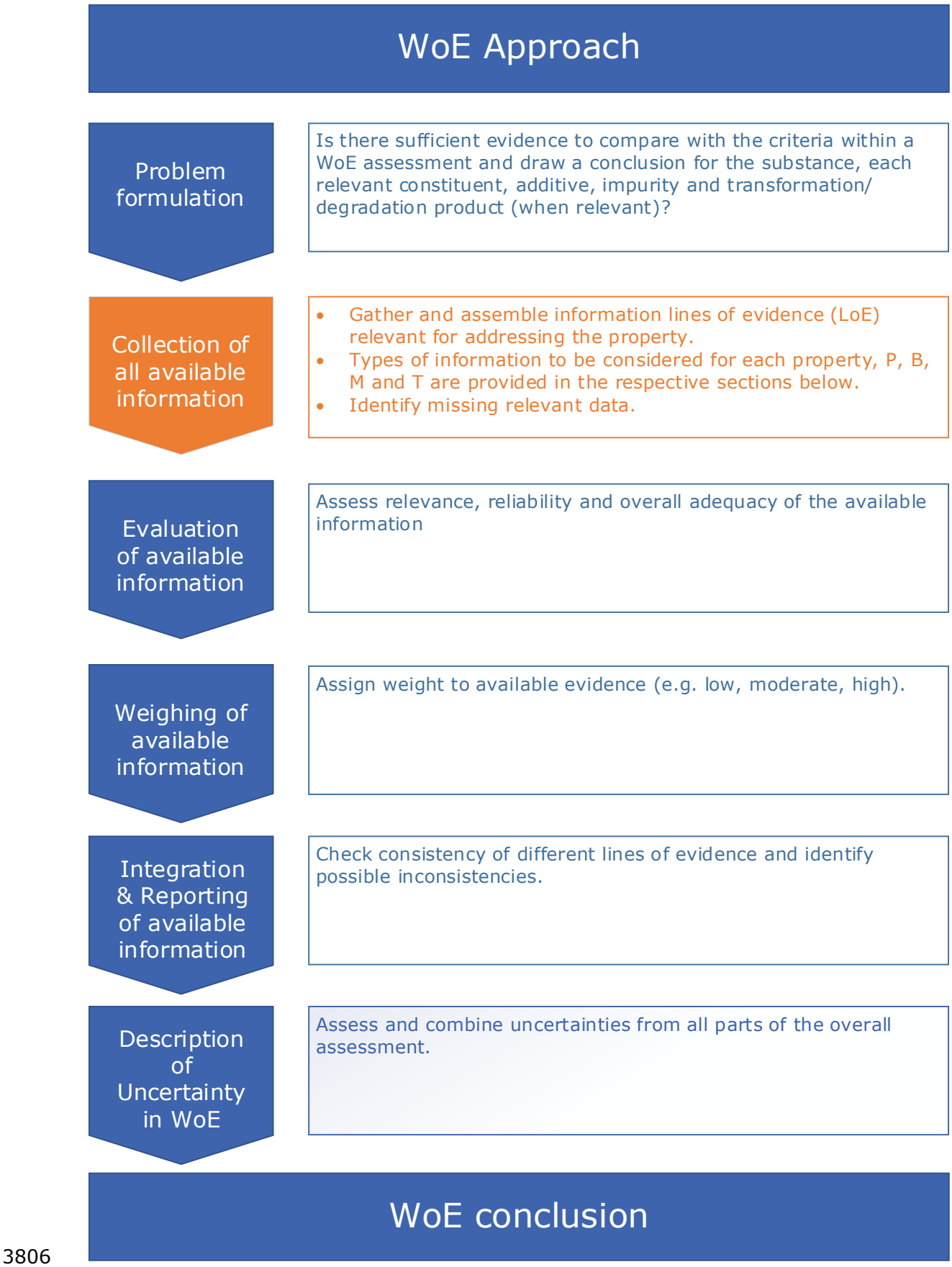

Figure 2: General scheme for the WoE approach (adapted from OECD, 2019)

Problem formulation: aim of the WoE approach

The first step of establishing the WoE approach is by defining the scope and goals of the assessment, namely whether there is sufficient evidence to support the conclusion that the CLP criteria for P or vP, B or vB, and T are or are not met. This refers to the substance, but also each relevant constituent, additive, impurity and transformation/ degradation product.

Collection and documentation of all information

CLP refers to the comparison of all relevant and available information with the criteria, in particular in cases where these criteria cannot be applied directly to the available information (Article 9). The assembled information must be put together into "lines of evidence (LoE)" that can be used to address the question(s) posed in the problem formulation step. It is very important to be transparent on how the information has been assembled and presented, which will also facilitate the easier identification of missing or uncertain evidence. The assembled information will need to cover different types of evidence, both direct and indirect, from different sources.

The collection and documentation refers to all available information (note that new data generation is only permitted as a last resort under CLP Article 8). Available information has 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. Sections 4.3.3.1, 4.3.3.2, 4.3.3.4 have already addressed the use of non-standard tests. Such tests 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).

Evaluation of all available information

Chapter R.4 of the ECHA Guidance on IR&CSA provides guidance on how to evaluate available information under REACH and CLP, including an assessment of the adequacy, relevance and reliability of the available information as defined by Klimisch *et al.* (1997).

The relevance of a piece of information/LoE can be defined as the extent to which it is appropriate for a particular property (see *Chapter R.4* of the ECHA Guidance on IR&CSA). For example, whether the tested substance is representative of the one being assessed, whether the test species and test design are appropriate for the property being investigated, whether the appropriate dose and route of exposure are used, whether critical parameters influencing the property have been considered, etc. The current Guidance elaborates further in detail on several elements to establish the relevance of the provided information, both at a general level (for example, as in bulletpoint (ii) in Section 4.3.3) and at an individual property and study level (see Sections 4.3.3.1-4.3.3.4).

The reliability of test data is the inherent quality of a test relating to test methodology and the way that the performance and results of a test are described (see *Chapter R.4* of the ECHA Guidance on IR&CSA). Klimisch *et al.* (1997) developed a scoring system to assess the reliability of data, particularly from (eco)toxicological studies that may be extended also to physico-chemical and environmental fate studies. More recently, Moermond *et al*. (2016) developed the CRED (Criteria for Reporting and Evaluating Ecotoxicity Data)

evaluation method that includes a set of 20 reliability and 13 relevance criteria, accompanied by extensive guidance. Detailed recommendations of how such studies can be reported have also been developed, to improve transparency and consistency on how ecotoxicological information is presented. In analogy to the Klimisch scoring, 4 reliability categories are also proposed within the CRED framework.

For (Q)SAR predictions and results based on multiple predictions, the Klimisch criteria are not applicable but a scoring system based on the OECD (Q)SAR Assessment Framework (OECD, 2023) has been implemented in OECD harmonised templates (OHT) and consequently IUCLID and is recommended to be used as follows:

- (Q)SAR result with low uncertainty (Reliability 1);
- (Q)SAR result with medium uncertainty (Reliability 2);
- (Q)SAR result with high uncertainty (Reliability 3);
- (Q)SAR results with limited documentation/justification, but validity of model and of prediction considered adequate based on a generally acknowledged source (Reliability 2 or 3);
- (Q)SAR result with limited documentation/justification (Reliability 4).

When more than one relevant and reliable (see text above and Section 4.3.3 (i) for definitions) experimental study results (values) are available for the same property, in most cases the most conservative value is used in order to achieve the regulatory protection goals and to account for any uncertainties of the test method and differing experimental conditions.

However, there may be exceptional situations where it is appropriate to combine a sufficient number of study results from the same study types generated under similar conditions to generate a statistically robust representative value (e.g. suitable mean) for comparison with the CLP criteria. In such cases, statistical combination of the appropriate study results may be possible, provided that it is scientifically robust and properly substantiated. The statistical distribution of the data should be considered when selecting the representative value. A geometric mean is generally recommended when the data follow a log-normal distribution, whereas the arithmetic mean is generally recommended for data that follow a normal distribution. Martinez and Bartholomew, 2017 give detailed guidance on estimation of an appropriate mean value.

As an example, the current CLP Guidance already provides some advice on this concerning aquatic hazards classification (Section 4.1.3.2.4.3 for aquatic toxicity, Annex III. 4.1 for aquatic bioaccumulation). Similarly, the ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.3 provides some guidance on combining experimental study results from simulation degradation tests for the same compartment and conducted under similar test conditions (for example, temperature, pH, organic carbon content, microbial biomass, test design, etc.).

The above refer to the combining of values from experimental studies performed under similar conditions, for example for the same environmental compartment, species, life cycle/stage, test design, etc. It is not recommended that values from different study types (for example, an experimental fish BCF, a mussel BCF and a (Q)SAR prediction for BCF or laboratory simulation and field degradation studies test data) are combined. More detailed, property-specific information on the specific conditions for which combination of study results may be allowed can be found below and in Section 4.3.5.

Expert judgement on the conditions that need to be met in order to be able to combine values for a property will always be needed. The reliability and relevance of all similar study results and their statistical distribution should be considered. An outlier is an observation that appears to deviate markedly from other observations in the sample and may be detected by visual inspection or standard statistical techniques and reasons for any outliers should also be considered. Such considerations should be adequately documented and reported and the scientific assessment will be conducted on a case-by-case basis. In all cases, any such combined representative value should be considered with all other relevant and reliable information available in the overall WoE to compare with the respective CLP criteria.

Weighing of available information

All available relevant information should be considered together and appropriate weight should be given. A more quantitative (for example, by use of numerical scoring systems) or a more qualitative (for example, high, medium, low weighing) approach to the WoE may be taken by the expert. OECD (2019) proposes ways of how available information may be weighed.

Integration and reporting of the available information

Important elements for the integration of the different LoE comprise the quality of the data, the variability and/or consistency of the results, the nature and severity of effects, the relevance of the information, the presence of any biases and outliers. The outcome of this integration will be based on expert judgement to structure the available information in order to come to an overall conclusion that can be used for classification purposes. This outcome will, in turn, be based on the availability and quality of the different LoE, as well as on substance-specific considerations. SCHEER (2018) provides further details on the process of integration of lines of evidence to determine the relative support for hypotheses or answering a question. Again, transparent documentation/articulation of the outcome is of utmost importance.

Description of uncertainty

Uncertainty refers both to any limitations and shortcomings of the available information (e.g. data paucity, study quality and reliability issues, incomplete documentation, question marks on study relevance, use of unvalidated or difficult to reproduce approaches, analytical difficulties based on substance property specific considerations, etc.) and to the level of regulatory acceptance that may impact the conclusion of the WoE.

Limitations of the available information must be identified and, to the degree possible, addressed by use of either more qualitative or more quantitative approaches, for example with the use of probabilistic analysis techniques. Qualitative approaches entail the identification of uncertainties in the hazard assessment and their grouping in sub-categories (high-medium-low, acceptable-unacceptable, etc.). The use of more statistically robust techniques is further detailed in OECD (2019) and ECHA Guidance on IR&CSA, Chapter R.19: Uncertainty analysis. Caution should be taken on the approaches detailed in the ECHA Guidance R.19, as they refer more to risk assessment and not to a solely hazard identification/assessment context as the one in CLP. Their use will also

depend on the availability of adequate data and its statistical distribution patterns. In all cases, the uncertainty of each information source and the overall uncertainty need to be evaluated and transparently documented.

Concerning the level of uncertainty acceptance, a connection with the protection goal must be established, namely the high level of protection of human health and the environment, for CLP. Ultimately, as already reported in footnote 3, the Court of Justice has confirmed that in case of any remaining uncertainty on the existence and extents of risks, appropriate measures should be taken "*in order to prevent certain potential risks for public health, safety and the environment without having to wait until the reality and seriousness of those risks become fully apparent*" (Cases C-65/21 P and C-73/21 P to C-75/21 P quoted earlier above, paragraphs 95-99), in line with the precautionary principle.

WoE conclusion

Separate conclusions are required for both PBT and vPvB, as well as for each of the P, B and T properties, based on the overall WoE. The need for explicit separate conclusions on the individual properties is also due 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 10(1)(d)) and PPPR (Annex II, 4), if applicable. These Regulations also define further the regulatory implications for CfS substances.

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 for all properties) 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 all have to be met in the same test compartment i.e. aquatic, soil or sediment, as the General Court of the European Court of Justice has unequivocally ruled in two judgements[79]. The outcomes of the application of the WoE to conclude on the individual PBT/vPvB properties can be that the substance fulfils the P/vP/B/vB/T criteria or not.

It is very important to also provide clarification/justification why a substance does not meet the P/vP/B/vB/T criteria, in line with the current approach of ECHA's Risk Assessment Committee (RAC) where the opinion documents[80] contain justifications for a substance not meeting the classification criteria. Further elaborations on these are given in CLP Article 40. 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.

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, Chapter

[79] See judgment of 30 June 2021, Global Silicones Council and others v. Commission, T-226/18, not published, EU:T:2021:403, paragraphs 129 to 133 (see https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:62018TJ0226) and judgment of 30 June 2021, Global Silicones Council and others v. ECHA, not published, EU:T:2021:404, paragraphs 107 to 110 (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:62018TJ0519). Both cases have been appealed by Global Silicones Council in Cases C-558/21 P and C-559/21 P respectively. These appeals were dismissed by the Court of Justice on 9 November 2023.

[80] 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/-/

R.11.4.1.1.1 contains more information on specific WoE considerations including the preference for experimental results from reliable studies that can directly be compared to the criteria and their higher relevance over "screening-type" information (for example, Chapters R.11.2.1 and R.11.4.1). This does not mean that all other types of information are 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), as long as all available reliable information is considered within the WoE. This was confirmed in a recent ruling of the General Court of the European Court of Justice (Case T-177/19, see footnote 19).

Benchmarking

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 analysis 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. One of the important aspects highlighted therein refers the reporting of concentrations also based on a molar basis (for example, mmol/l), for a better application of benchmarking to assess toxicity. In this way, comparison of toxicity on molar basis prevents bias from molecular weight differences. This could be especially useful for multiconstituent and halogenated substances.

4.3.4.1. Persistence

The P/vP assessment shall reach one of the following conclusions for each relevant constituent, additive, impurity or transformation/degradation product: P, vP or criteria not met**Error! Reference source not found.**. When a conclusion that the criteria are not met is reached, it should be further justified whether this is based on conclusive data, inconclusive data or 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.

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 decision scheme 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**Error! Reference source not found.**.

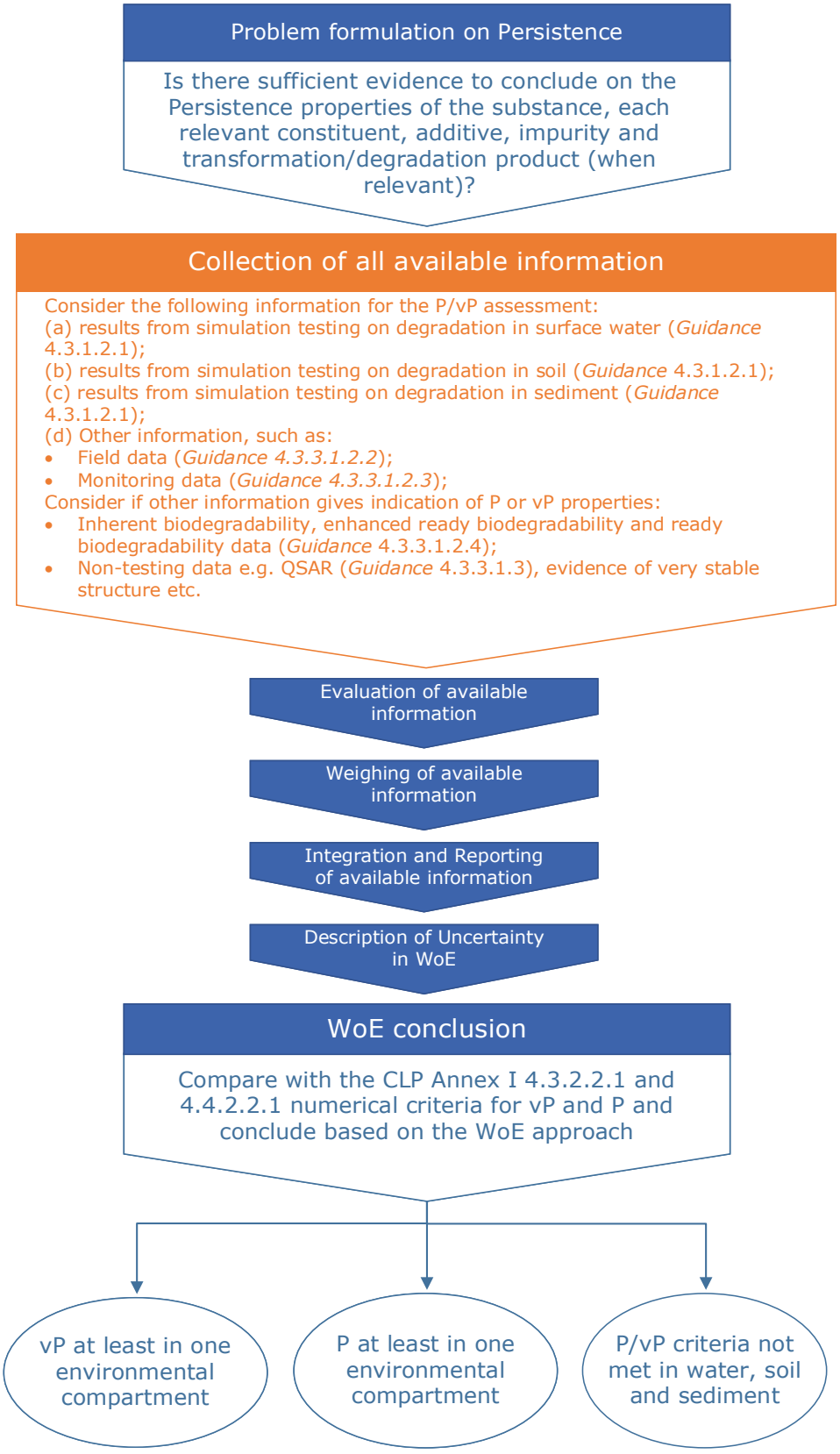

Figure 3: WoE approach for concluding on the persistence properties of the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant). Regarding the available information, the relevant sections of the Guidance are indicated in brackets.

When deciding if a substance fulfils the P or vP criteria, its transformation/degradation potential in the surface water, sediment and soil is to be considered. The WoE approach should address whether there is sufficient evidence to support the conclusion that the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant):

- fulfils the P/vP criteria for degradation half-life in water, sediment or soil;
- in the absence of information to derive numerical degradation half-life values, is there sufficient evidence to support the P or vP conclusion in surface water, sediment or soil?

All available data is to be considered as part of the WoE assessment leading to P or vP conclusion or a conclusion that the criteria are not met. When the vP criterion is met, then also the P criterion is met. Conclusion P or vP reached for one environmental compartment is enough to consider that the substance meets the P or vP criteria. In order to conclude that a substance does not meet the P criteria it must be demonstrated that the criteria are not met in surface water, sediment and soil.

Existing experimental data which can be directly compared with the criteria (see also Sections 4.3.3.1.2.1 and 4.3.3.1.2.2).

The results of the degradation simulation studies are to be given more weight in the WoE assessment than the screening-level studies. 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-lives obtained is preferred for comparison with the P/vP criteria for the sediment compartment. 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 compartments may be possible provided that results/bridging is supported by proper justification.

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. Such DegT50 values can be used in a WoE assessment but their relevance should be considered with care.

Degradation half-lives derived from 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 degradation data are available, they may be used as part of a WoE approach. The difference in degradation rates and pathways between aerobic and anaerobic conditions could sometimes provide important insights into the P assessment. Generally it would be expected that an anaerobic degradation half-life would be greater than an aerobic degradation 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 fast 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[81].

In the presence of a reliable DegT50 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 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 primary degradation is observed, it is necessary to identify the transformation/degradation products and to assess whether they possess PBT/vPvB properties. All relevant transformation/degradation products should be considered in the assessment (See Section 4.3.3(v) of this Guidance).

Field studies provided that their suitability and reliability can be reasonably demonstrated by also taking uncertainties in deriving field DegT50 into account may be used as assessment information (Annex I: 4.3.2.3.1. and 4.4.2.3.1.). However, when DegT50 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 DegT50 must be carefully considered (see also Section 4.3.3.1.2.2 of this Guidance). Influence of dissipation processes in derivation of the field DegT50 is difficult to quantify and thus in many cases lowers the reliability of the estimated degradation half-lives.

If pH dependency of degradation (e.g. ionisable substances) leads to different degradation rates between acidic and alkaline conditions within the environmentally relevant pH range, the most conservative DegT50 value should always be used when compared to the CLP Annex I criteria.

Monitoring studies

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

In addition to the simulation and field test data, existing monitoring data should be carefully examined. Monitoring studies provided that their suitability and reliability can be reasonably demonstrated, may be used as assessment information (Annex I: 4.3.2.3.1. and 4.4.2.3.1. and see Section 4.3.3.1.2.3 of this Guidance). However, mere presence of a substance in a given environmental compartment on its own cannot demonstrate persistence, because presence in the environment is also dependent on a range of factors other than degradation rates, such as emission and distribution rates. Therefore monitoring data should always be considered together with such related factors. For example, 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 may indicate high persistence (beside a potential to bioaccumulate).

Other information on persistence

The conclusion that a substance is not P/vP can be based on screening level information (including enhanced ready biodegradability 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. In general, screening level information (including enhanced ready biodegradability tests) has lower weight than simulation and field studies in the WoE assessment in concluding a substance as P/vP. In some exceptional cases, if scientifically justified and supported by other available information, 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 weight of evidence, lack of or low mineralisation (<20% degradation) in an inherent biodegradability test (OECD TG 302) may provide sufficient information to confirm that the P-criteria are fulfilled for the purpose of persistence assessment. Additional lines of evidence information may for example consist of (Q)SAR predictions, consistency in the lack of degradation in other screening studies, or non-standard degradation studies[82]. 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 as described above). For example, low mineralisation in inherent degradation test supported with (Q)SAR predictions, monitoring data and non-standard studies has been used to conclude substances as vP[83,84]. However, it should be noted that lack of mineralization in

[82] SVHC Support Document - 2,4,6-tri-tert-butylphenol
[83] SVHC Support Document - BIS(2-ETHYLHEXYL) TETRABROMOPHTHALATE COVERING ANY OF THE INDIVIDUAL ISOMERS AND/OR COMBINATIONS THEREOF
[84] SVHC SUPPORT DOCUMENT - BTBPE

inherent degradation test does not equate to lack of primary degradation (see also Section 4.3.3.1.2.4).

The degradation half-lives obtained in a abiotic 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. Abiotic 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.

Results obtained from well-developed and reliable biodegradation (Q)SAR models can be used as part of the scientific assessment of the information relevant for the P, vP properties in WoE determination. For this purpose, it is recommended to use for example combined results from three estimation models in the EPI Suite™ (US EPA, 2012; R.11). Acceptable (Q)SAR predictions can be used furthermore to support 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 based on screening level information and not data obtained in relevant conditions to derive a degradation half-life.

Multiple experimental degradation study results

Where more than one relevant and reliable experimental degradation study resulting in DegT50 is available for the same environmental compartment (either water, soil or sediment), in most cases the most conservative result is used in order to achieve the regulatory protection goals and to account for any uncertainties of the test method and differing experimental conditions.

Combining experimental study results

DegT50 data from different environmental compartments should not be combined for PBT/vPvB classification purposes because each of the environmental compartments, namely water, soil and sediment have compartment-specific DegT50 criteria. However, there may be exceptional situations where it would be appropriate to combine sufficient number of DegT50 results for the same environmental compartment (either water, soil or sediment) to generate statistically robust representative value for comparison with the CLP criteria. However, combining all available DegT50 values is generally not appropriate.

Some existing guidance on generating representative DegT50 values is provided for example in the ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.3. This Guidance provides a possibility to combine experimental study results from more than four simulation degradation tests conducted on the same environmental compartment (water/soil/sediment) under similar test conditions (for example, temperature, pH, organic carbon content, microbial biomass, test design, etc.).

When comparing to CLP Annex I DegT50 criteria, combining DegT50 results from several degradation e.g simulation studies with the same environmental compartment and study type, with similar test conditions, design and degradation kinetics (for example, SFO kinetics or biphasic kinetics), can be considered. DegT50 data from different environmental compartments should not be combined. The statistical distribution of the data should be considered when selecting the approach. Any data outliers should be assessed and removed from the data set if appropriate.

Only test results from the same environmental compartment corresponding to similar test conditions (e.g. laboratory or field, aerobic or anaerobic, marine or fresh water) can be compared. The similarity of the tests results to be combined should be scientifically justified by considering whether the test conditions and/or characteristics of the test media (e.g. temperature, pH, organic carbon content, microbial biomass, source of the test media etc.) significantly influence the degradation potential of the substance. Differences in incubation temperatures can be compensated by normalising the DegT50 values to relevant reference temperature (for example, DegT50 at 12 °C).

In all cases, the approach used to generate a representative DegT50 value should be well justified and documented and should be supported by the WoE analysis. This should include a discussion of any outliers. In particular, the representativeness of the test conditions should be carefully assessed for each value. Particular scrutiny should be given if results from the tests are close to the P or vP threshold.

4.3.4.2. Bioaccumulation

The B/vB assessment shall reach one of the following conclusions for each relevant constituent, additive, impurity, or transformation/degradation product: B, vB, or criteria not met. The latter conclusion is based on conclusive data, inconclusive data or lack of data. Section 4.3.3.2 of the current Guidance document describes the relevant experimental and computational information that may be considered as part of the WoE determination on Bioaccumulation.

The decision scheme 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 4).

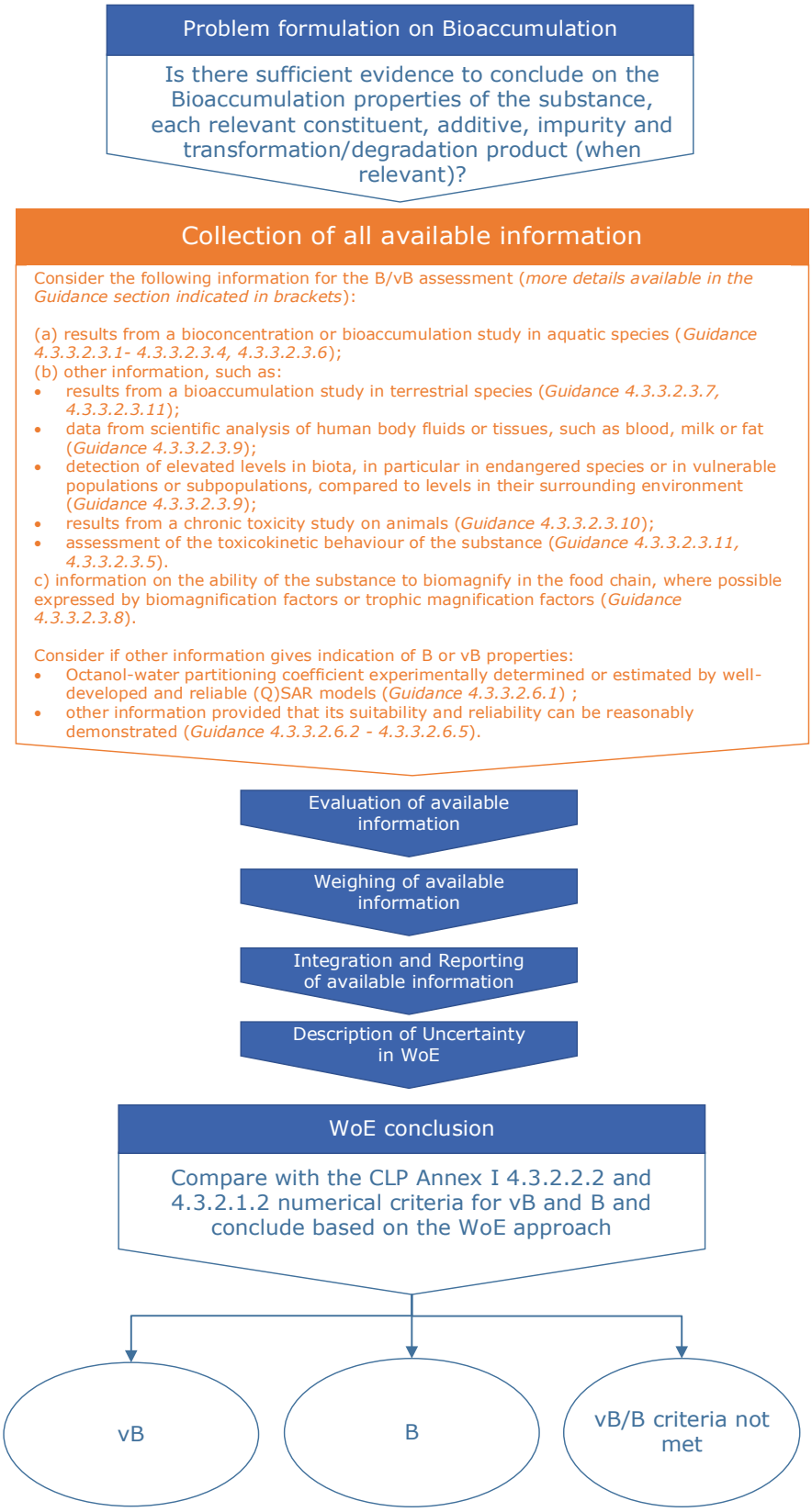

Figure 4: WoE approach for concluding on the bioaccumulation properties of the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant). Regarding the available information, the relevant sections of the Guidance are indicated in brackets.

When deciding if a substance fulfils the B or vB criteria, its bioaccumulation potential in the aquatic environment, the terrestrial environment, wildlife or humans is considered. The WoE approach should address whether there is sufficient evidence to support the conclusion that the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant):

- fulfils the classification criteria in aquatic species
- bioaccumulates/biomagnifies in the aquatic environment, terrestrial environment, wildlife and/or humans.

All available data is to be considered as part of the WoE assessment. As such, a conclusion of B or vB based on either data for aquatic species (CLP Annex I, 4.3.2.3.2. (a)) or other available data (CLP Annex I, 4.3.2.3.2. (b)/(c)) is enough to consider that the substance meets the B or vB criteria. When the vB criterion is met, then also the B criterion is met. Similarly, a conclusion that the criteria are not met must also be based on all available data.

The results of reliable experimental *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.

Existing experimental aquatic *in vivo* data which can be directly compared with the criteria (see also 4.3.3.2.3.1, 4.3.3.2.3.2, 4.3.3.2.3.3, and 4.3.3.2.3.4)

Each BCF study should be assessed in detail for its reliability and relevance considering the test substance, 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 against the respective bioaccumulation criterion of CLP to determine whether the B or vB criteria are met. 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 the 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 k_1 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.

Other *in vivo* data

Field data (see also 4.3.3.2.3.8)

Reliable information from field studies can be used to decide if the CLP B/vB criteria are fulfilled and should be given a high weight in a WoE approach where B/vB is indicated. A reliable 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 fulfils 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. A field TMF represents biomagnification in the specific food web studied. It is possible that biomagnification could still occur in another predator/prey relationship or food web.

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.

Field BAF values (based on reliable information) above 2000 or 5000 may indicate a B or vB concern, respectively, and should be considered as part of the WoE approach. For comparison of a fish field BAF with these thresholds, BAF values should be expressed on wet weight basis for whole body with a lipid content of 5%.

Detection of substances in wildlife and humans (see also 4.3.3.2.3.9)

The detection of a substance in wild biota (concentration or occurrence data) indicates that there had been exposure and that the substance can be taken up by organisms. Such information can be used to support the bioaccumulation assessment on a case by case basis. Together with the exposure level from the surrounding media and/or diet, concentrations in wild biota can be used as evidence for bioaccumulation. Furthermore, data from different time points as well as regions can give indications on temporal and spatial trends. Concentrations in biota increasing with age due to exposure and accumulation over a life-time, particularly in long-lived apex species (top predators), indicate an increased concern for bioaccumulation.

Information coming from scientific analysis of human body fluids or tissues, such as blood, milk, or fat can be used for the bioaccumulation assessment in a WoE approach.

Toxicokinetics data for mammals (see also 4.3.3.2.3.11)

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.

<u>Other available data</u>

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)
- Field data – biomagnification in the food chain (4.3.3.2.3.8)
- Detection of substances in wildlife and humans (4.3.3.2.3.9)
- Chronic toxicity tests on animals (4.3.3.2.3.10)
- Octanol-water partitioning coefficient K_{OW} (4.3.3.2.6.1)
- Octanol-air partitioning coefficient K_{OA} (4.3.3.2.6.2)
- QSAR 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)

Acceptable (Q)SAR predictions for Log K_{OW} and BCF can be used as part of the scientific assessment of the information relevant for the B, vB properties in the WoE determination.

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

Table: Overview of indicative thresholds for WoE assessment

Parameter	**Indicative threshold**	**Guidance Section**
Log K_{OW} (for aquatic organisms)	>4.5	4.3.3.2.6.1
Log K_{OA} *and* Log K_{OW} (for air breathing mammals)	>5 *and* >2	4.3.3.2.6.2
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	4.3.3.2.3.11
Rat whole body terminal elimination half-life/days	2.5 / 4 days	4.3.3.2.3.11

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 between lines of evidence (i.e. same type of test or directed to the same endpoint), relevant and reliable evidence of bioaccumulation cannot be outweighed by information showing no bioaccumulation.

Multiple experimental study results for bioaccumulation

When more than one relevant and reliable experimental study results (values) are available, in most cases the most conservative value is used in order to achieve the regulatory protection goals and to account for any uncertainties of the test method and differing experimental conditions.

Combining experimental study results

BCF test results on different species, life stages or test conditions should not be combined for PBT/vPvB classification purposes. Different species can accumulate substances to a different degree because of differing physiological behaviour such as metabolic activity and ventilation rate (Wassenaar *et al.*, 2020). For BCF studies in fish, the size and age can affect the bioaccumulation potential. Test conditions such as TOC, temperature, pH, feeding rate and test concentration can also influence the measured BCF (Arnot and Gobas, 2006).

However, there may be exceptional situations where it is appropriate to combine a sufficient number of study results to generate a statistically robust representative value for the same species and life stage for comparison with the CLP criteria. For example, for BCF values derived from the same species and life stage, a suitable 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 similar (for example regarding test concentration, pH, temperature, TOC, study design, feeding rate, etc.). The statistical distribution of the BCF data should be considered when selecting the approach. Some guidance on generating representative values for BCF is given in Annex III.4.1. There may be circumstances where a different approach is justified. The reason for any apparent BCF outliers should be assessed and it may be appropriate to remove them from the data set, for example if there was an experimental error.

Fish BCF studies may be performed at two or more exposure concentrations and, thus, one BCF study could give several experimental results (BCF values), one for each tested concentration. It is not recommended to obtain a representative BCF value by averaging BCF values for different exposure concentrations obtained from a single study. This is because the BCF may vary with test concentration, for example due to saturation of metabolic mechanisms (See OECD, 2012 paragraphs 79, 80 and Annex 2 of OECD, 2017).

In all cases, the approach used to generate a representative value should be well justified and documented. This should include a discussion of any outliers. In particular, the representativeness of the test conditions should be carefully assessed for each value. Particular scrutiny should be given if test results are close to the B or vB threshold.

4.3.4.3. Toxicity

The T assessment shall reach one of the following conclusions described in the scheme (Figure 5) for each relevant constituent, additive, impurity, or transformation/degradation product: T, or criteria not met. The latter conclusion is based on conclusive data, inconclusive data or lack of data.

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. The decision scheme needs to be followed on the available information, in order to come to a robust conclusion on whether the CLP criteria for Toxicity are fulfilled (Figure 5).

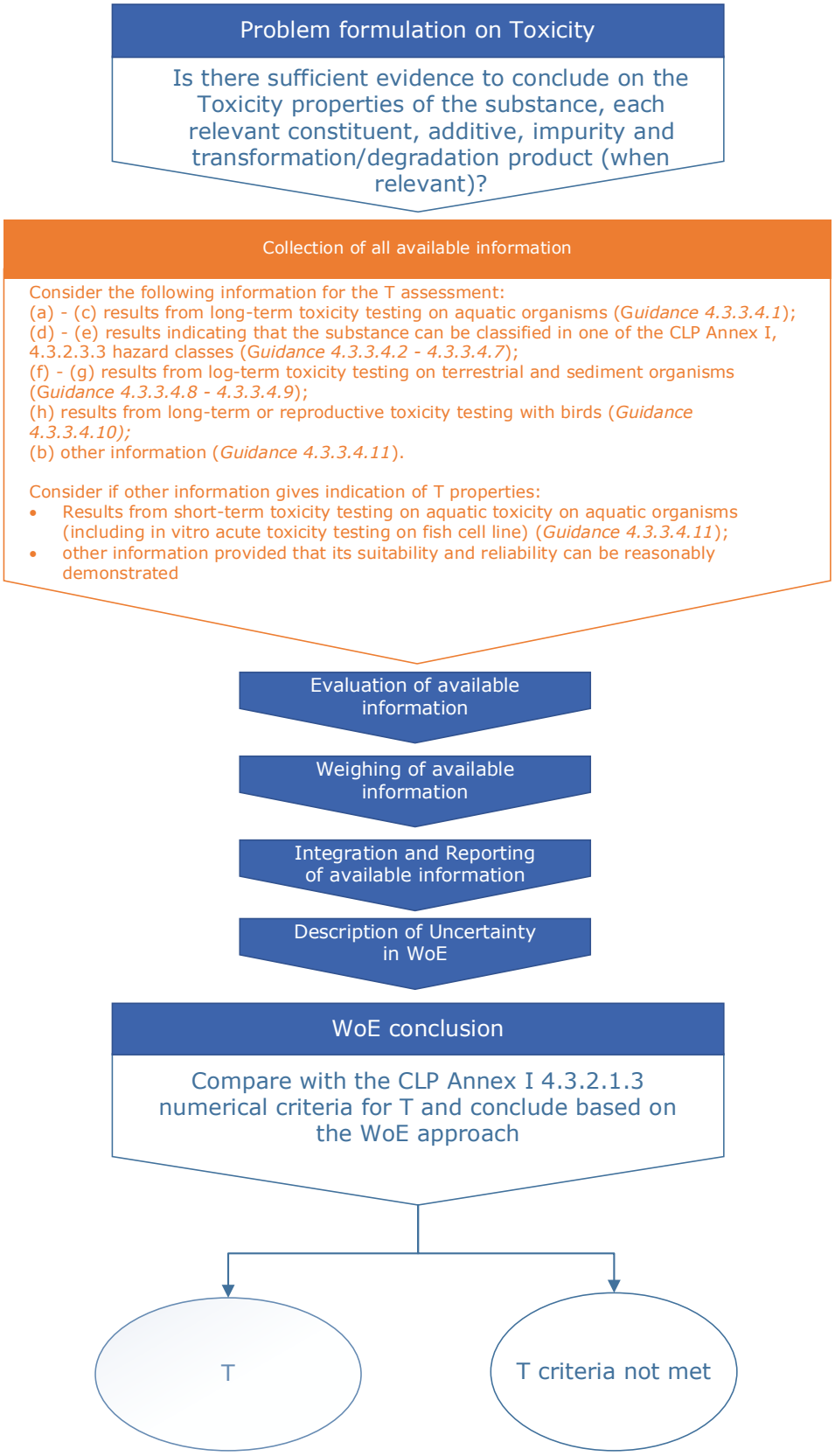

Figure 5: WoE approach for concluding on the toxicity properties of the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant). Regarding the available information, the relevant sections of the Guidance are indicated in brackets.

As discussed in the introduction of Section 4.3.4, 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 (a)-(d) and Sections 4.3.3.4.1 - 4.3.3.4.7 of this Guidance) are to be given higher weight in the WoE assessment**Error! Reference source not found.**. 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. As discussed in Sections 4.3.3.4.8 and 4.3.3.4.9, the equilibrium partitioning method (EPM) may be used to back-calculate a NOEC or EC_{10} value of an existing sediment or soil toxicity test to a corresponding aquatic NOEC or EC_{10}. This approach has uncertainties and the use of any such information needs to be treated with caution, 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 any 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 (Section 4.3.3.4.10), 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, 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 fulfils the Toxicity criteria, 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 and any other data with a suitability and reliability that can reasonably be demonstrated. Some (Q)SAR models predicting chronic and acute aquatic toxicity are currently available and further research on the (Q)SAR prediction of chronic toxicity may increase their predictive capacities. Therefore, at the current state of the art, (Q)SAR models generally seem not to be applicable for an unequivocal assessment of the T criterion (ECHA Guidance on IR&CSA, Chapter R.11.4.1.3.3). However, they may be used when they are applicable, in line with REACH Annex XI. 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, for example, for ionisable substances, as reported in several Sections of the current Guidance.

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/EC_{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. All the general principles on how to combine study results from similar study types must be followed, as reported in the introductory paragraphs of Section 4.3.4 of the Guidance. As one example only, the current CLP Guidance already provides advice on the combination of aquatic toxicity study results in the context of aquatic hazards classification (Section 4.1.3.2.4.3). The similarity of parameters such as species, life stage, pH, test temperature, dissolved oxygen concentration, TOC, test design, duration, etc. must be considered before any such combination is to take place.

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 and data combination 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 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.

4.3.4.4. Overall determination of PBT/vPvB classification

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 4.3.4, 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 fulfilled, the substance is not classified as PBT. If any of the criteria vP or vB are not met, the substace is not classified as vPvB. A conclusion that a substance does not fulfil all PBT/vPvB criteria must be followed by a statement clarifying the reasons for this conclusion.

ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.4 presents further details on the different conclusion types for PBT/vPvB assessment and the use of constituent data. The following

Figure illustrates the decision scheme for concluding on the PBT/vPvB classification, once the assessment and conclusion for the individual properties has been finalised.

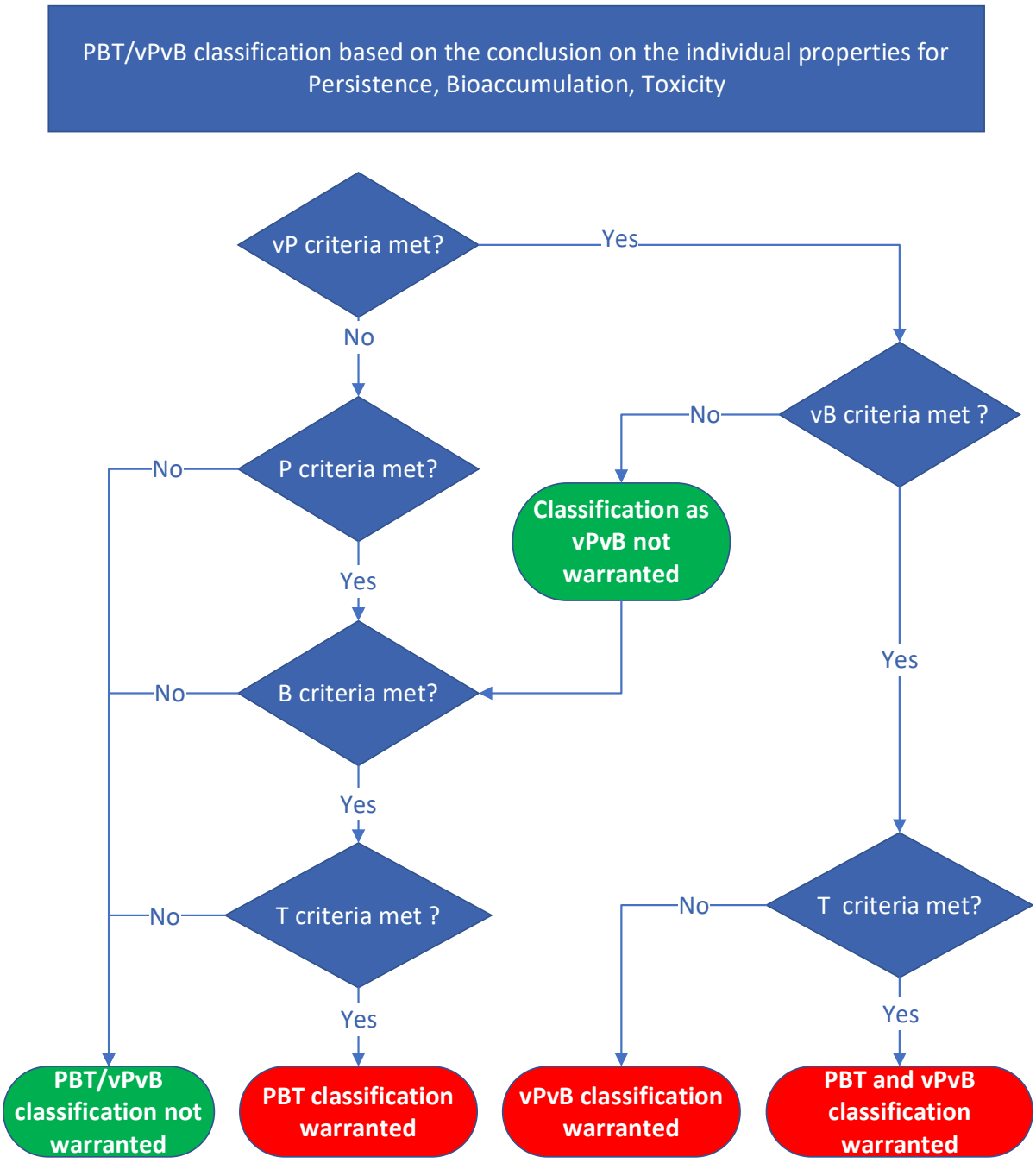

Figure 6. Decision scheme for concluding on PBT/vPvB classification

4.3.5. Application of the WoE to conclude on PMT/vPvM properties for classification and labelling

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 4.3.4 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. Furthermore, in order for the PMT or vPvM 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.

As for PBTs/vPvBs, the conclusion of the application of the WoE on the individual PMT/vPvM properties can be that the substance fulfils the P/vP/M/vM/T criteria or not. The general principles of identification and assessment of hazard information for PMT/vPvM have already been presented in Section 4.3.3.

For **Persistence and Toxicity,** see previous Sections 4.3.4.1 and 4.3.4.3 .

4.3.5.1. Mobility

The M/vM assessment shall reach one of the following conclusions described in the scheme (Figure 7) for each relevant constituent, additive, impurity or transformation/degradation product: M, vM or criteria not met. The latter conclusion is based on conclusive data, inconclusive data or lack of data. When the vM criterion is met, then also the M criterion is met.

Section 4.3.3.3 of this Guidance described the experimental and non-experimental methods that may be provided as part of the WoE determination on mobility. Briefly, test results according to OECD TG 106, sludge tests (ISO 18749 and OPPTS 835.1110), TG 121, TG 312, TLC studies and reliable QSAR methods have been described and important considerations and limitations on their use accounted for. Section 4.3.3.3.6 further presented key considerations for information provided for ionisables including recommendations on testing for K_{OC} derivation.

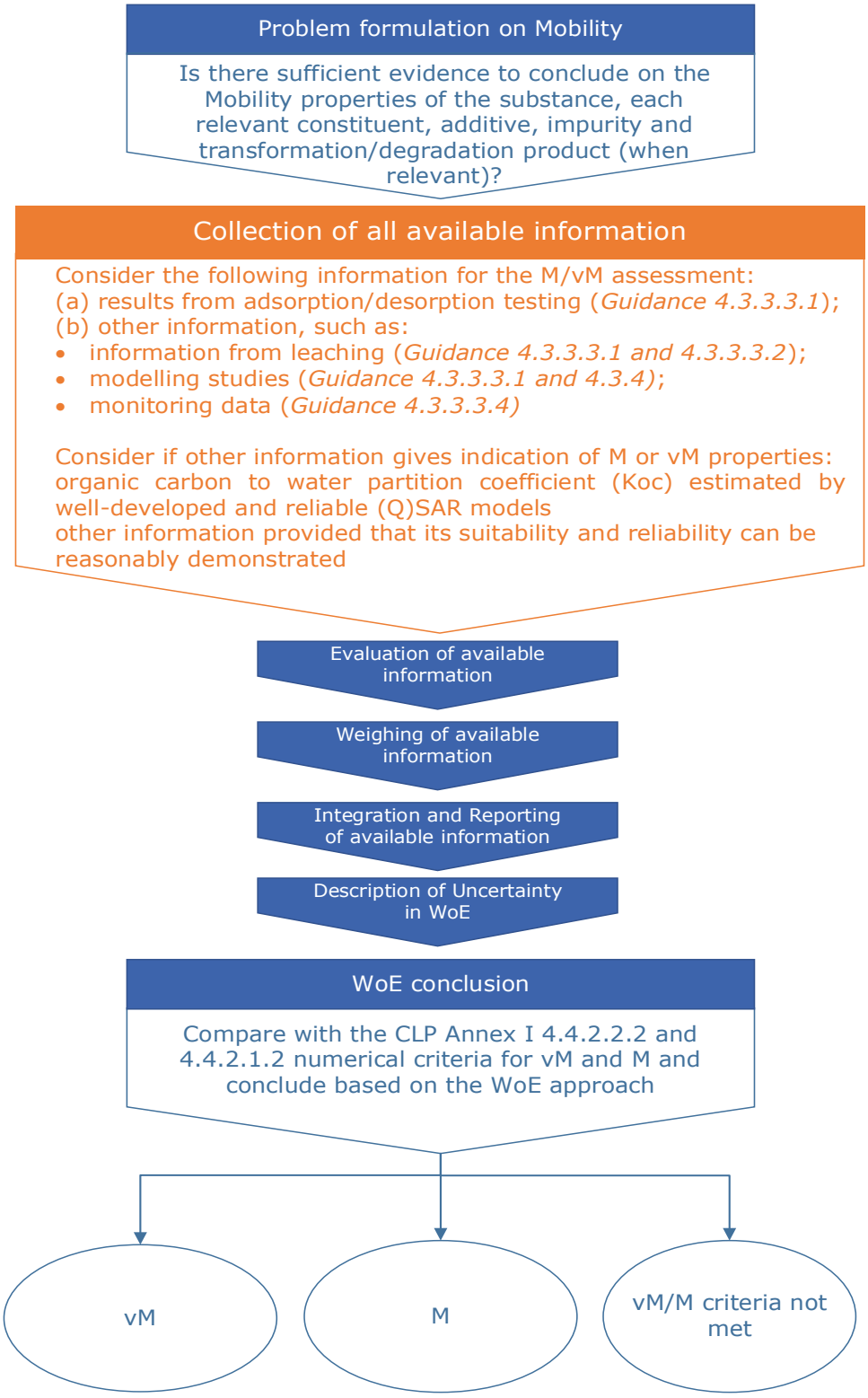

Figure 7. WoE approach for concluding on the mobility properties of the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant). Regarding the available information, the relevant sections of the Guidance are indicated in brackets

Normally results from reliable experimental methods directly deriving a K_{OC} value are given higher weight in the WoE than for other lower weight information. From such methods, preference is placed into the one conducted according to OECD TG 106, as this is a standardised test method which provides information on the intrinsic property of the substance to partition in soils. Furthermore, it is applicable to both non-ionisable and ionisable substances and it includes testing on a wide range of different natural soils with varying soil types to cover the interactions of a given substance with naturally occurring soils.

Activated sludge studies (OPPTS 835.1110 and ISO 18749) are applicable for both non-ionisable and ionisable substances and may be compared to the CLP criteria. More specifically, for substances that can adsorb to the soil only via hydrophobic interactions (non ionisable substances), the derived log K_{OC} can be used for directly comparing with the CLP criteria within the WoE. For substances for which the sorption might also involve processes other than hydrophobic interactions (e.g. ionisable substances), activated sludge studies might be less relevant and, therefore, might be assigned a lower weight within the WoE (see also Section 4.3.3.3.1, Studies on activated sewage sludge).

Test results from studies performed according to OECD TG 121, OECD TG 312 and soil thin and thick layer chromatography (TLC) can also provide relevant information, 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.

(Q)SARs and other estimation methods (Karickhoff equation) deriving a K_{OC} may also provide relevant information particularly for non-ionisable substances, as the sorption of such substances is dominated by the sorption to organic carbon. This is particularly the case for a substance with close structural analogues in the model's training set. However, such approaches are currently not adequately developed and validated for ionisable substances and, therefore, their use for those substances is not recommended. It is expected that (Q)SAR approaches are more broadly used in the future, supported by the projected higher degree of generation of experimental data (for example, by use of OECD TG 106). For more details see also Section 4.3.3.3.3.

Field and lysimeter studies, as well as results from aged sorption studies and modelling can be considered as part of the WoE assessment. However, the relevance of such approaches within a hazard assessment context should be carefully considered due to the uncertainties identified in sections 4.3.3.3.2 and 4.3.3.3.5. Such approaches have been used for the risk assessment of PPP substances and may also provide useful information on the mobility potential of a substance under specific test conditions. Additionally, results from such studies may be used for regulatory purposes in order to qualitatively identify additional transformation products which may be relevant for classification purpose (e.g., PMT/vPvM).

Information from monitoring studies and other approaches not leading to a numerical K_{OC} value may be considered, together with all other available information. 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, shortcomings in analytical methods, soil properties, environmental fate and/or exposure considerations rather than an intrinsic tendency of the chemical not to partition to water. Generally, less weight should be given to monitoring data close to point sources. When monitoring data, used as part of the WoE, show that a substance is present in groundwater or/and other relevant environmental compartments, it may be possible to conclude a substance as M or vM, within the considerations above and in Section 4.3.3.3.4. Examples of supporting lines of evidence based on monitoring data that may be used as part of the WoE to fulfil the M/vM criteria are data on the long range aquatic transport, the chemical presence in effluent water from sewage treatment plants (STPs) and/or the presence in drinking water. More details on monitoring site characterisation and uncertainties related to false positives and negatives when conducting groundwater monitoring studies pursuant to the PPP regulation are given in Gimsing *et al*. (2019) and EFSA (2023b).

Combining multiple studies for mobility assessment

As already discussed in Section 4.3.4, when more than one relevant and reliable experimental study results (values) are available for mobility, in most cases the most conservative of the relevant and reliable values is used, in order to achieve the regulatory protection goals of better protection of human health and the environment. Combining of several relevant and reliable study results from the same test types generated under similar conditions must be justified to generate a statistically robust representative value (e.g. suitable mean) or comparison with the CLP criteria. Approaches for combining study results must be considered in a case-by-case basis by exercise of expert judgement, but should generally follow the principles described in the following paragraphs.

Reliable experimental study results from the same study type may be combined. Study results from different study types (for example, one OECD TG 312 study, a field study, some (Q)SAR predictions and evidence from monitoring data) cannot be combined but will be considered within the WoE, as independent lines of evidence (LoE). The comparability/similarity of the study results to be combined should be scientifically justified by considering whether the test conditions of the study (e.g. temperature, pH, soil texture, soil organic matter content, soil organic carbon content, cation/anion exchange capacity, sludge composition and volume index, etc.) significantly influence the adsorption potential of the substance. Only results from studies in which the test conditions are not significantly influencing the sorption of the substance may be combined.

For substances that can adsorb to the soil only via hydrophobic interactions (e.g. non-ionisable substances) K_{OC} values generated under similar conditions could be combined. A sufficient number of study results is required in order to increase the statistical robustness of the combined value and this number may vary depending on the availability and reliability of the relevant information. The use of arithmetic mean for the different derived Log K_{OC} values (or geometric mean for K_{OC}) is recommended, based on the expected distribution of such data. Regarding Tier 3 test results, K_{OC}* values must first be derived by use of the method detailed in Section 4.3.3.3.1 of this Guidance (one value per test concentration). Tier 3 K_{OC}* values for the same soil may then be combined for the different aqueous concentrations. K_{OC} and K_{OC}* values cannot be considered (combined) together, as they originate from a different set of data (Tier 2 and Tier 3 data sets). Various

approaches can be used for aggregating the data (for example, use of percentiles) and for investigating further the influence of various factors on sorption of the substance.

For substances for which the sorption might also involve processes other than hydrophobic interactions, data combination is generally not recommended. For example, for ionisable substances, the sorption potential of the substance is influenced by the test conditions (for example, pH and soil texture). In such cases, the lowest Log K_{OC} value for pHs between 4 and 9 should be compared with the CLP criteria (CLP Annex I, 4.4.2.1.2 and 4.4.2.2.2).

In all cases, the approach should be well justified and documented and should include a discussion of any outliers. In particular, the relevance of the test conditions should be carefully assessed for each value, with a particular scrutiny given to results from tests that are close to the M or vM threshold.

4.3.5.2. Overall determination of PMT/vPvM classification.

Similar considerations as the ones described at the end of Section 4.3.4 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 8 illustrates the decision scheme for concluding on the PMT/vPvM classification.

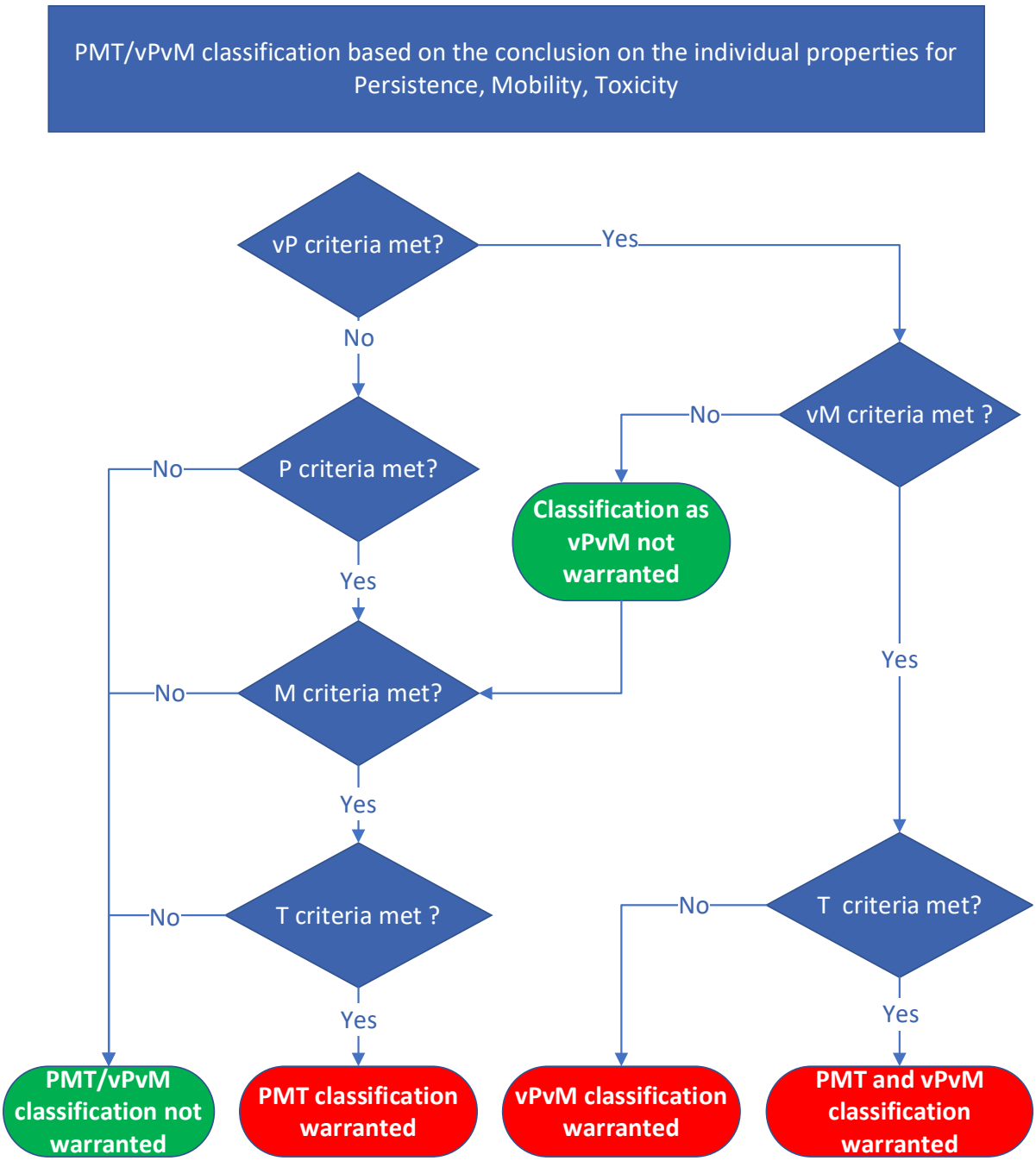

Figure 8. Decision scheme for concluding on PMT/vPvM classification

4.3.6. 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).

Classification of mixtures shall be based on available information for the substances in the mixture and not for the mixture itself. This relates to the persistence, mobility and bioaccumulation properties referred to Sections 4.3.2.3.1, 4.3.2.3.2, 4.4.2.3.1 and 4.4.2.3.2 of CLP Annex I, where the relevant available information for each of the known constituents in the substance shall be assessed[85]. Thus, when at least one of these components is present in the mixture in a concentration equal to or exceeding the generic concentration limit of ≥ 0.1% (w/w), the mixture can be classified as PBT/vPvB or PMT/vPvM.

However, in certain cases, data on the mixture itself may also be relevant. This is the case in particular where that data demonstrates persistent, bioaccumulative and mobile properties, or where it supports data on the individual constituents. Therefore, it is appropriate that data on the whole mixture is used in those cases.

[85] https://data.consilium.europa.eu/doc/document/ST-16721-2023-REV-1/en/pdf. The current text of the provisional agreement from the tripartite negotiations may still change.

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

4.3.7.1. Pictograms, signal words, hazard statements and precautionary statements

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 not applicable for these two new hazard classes and may be introduced at a later stage if adopted in the context of the UN GHS. When included in GHS but not yet implemented in CLP, it is strongly recommended to be used. The wording of the Precautionary Statements is found in CLP, Annex IV, Part 2.

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

4.3.7.2. Additional labelling provisions

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

4.3.8. 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. As, at the time of publication of this Guidance, there is not any experience gained on dealing with such hazard classes under CLP, these examples broadly refer to substances that have already been identified as SVHCs (PBT/vPvB/ELoC) 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[86], part of which comprises 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 targeted hazard assessment conducted by EFSA will not be reproduced in the current document, as the conclusion document on the pesticide peer review is already publicly available[87].

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 basis. If the evaluation shows that the criteria are fulfilled, a classification as PBT/vPvB and/or PMT/vPvM should be assigned. For the labelling elements, see Section 4.3.7 of this Guidance.

List of examples included in this Section:

- 4.3.8.1. Example A: Substance meeting the new CLP classification criteria for PBT and vPvB, based on the overall WoE;
- 4.3.8.2. Example B: Substance meeting the new CLP classification criteria for vPvB, based on constituent data and on the overall WoE;
- 4.3.8.3. Example C: Substance meeting the new CLP classification criteria for PMT and vPvM, 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. This structure is identical to the one followed for aquatic classification (Section 4.1.3.4 of the CLP Guidance) and is not indicative of the order of such information that may be presented in a potential proposal for harmonised classification and labelling.

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

4.3.8.1. Example A: Substance meeting the new CLP classification criteria for 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, Klimisch (1)
Water solubility	0.25 mg/L	WATERNTv1.01; WSKOW v.1.41; (Q)SAR prediction with low uncertainty, Reliability (1)
log octanol/water partition coefficient (log K_{OW})	6.3 (at 23°C)	(Q)SAR prediction with low uncertainty, Reliability (1)
log organic carbon/water partition coefficient (log K_{OC})	4.7	KOCWIN v 2.00 (EPI Suite 4.11)) – QSAR prediction with medium uncertainty, Reliability (2)
Degradation		
Ready biodegradability	2% in 28d	OECD TG 301C, Klimisch (1)
Simulation studies in water-sediment	$DT_{50,wat}$: 4-12d $DegT_{50,sed}$: 30-250d $DegT_{50,whole}$: > 180d	OECD TG 308 (for analogue substance in pond and river systems). Test conducted at 12°C. Reliability (1)
Hydrolysis	$T_{1/2}$ = 350d	OECD TG 111, Klimisch (2)
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 2, 3 and 6 predictions, (Q)SAR predictions with low uncertainty, Reliability (1)
Bioaccumulation		
Bioconcentration in fish (BCF), normalised to 5% lipid [no growth correction reported]	6 000-12 000	OECD TG 305, Klimisch (2)
Aquatic Toxicity		
Crustacea *Daphnia magna*:	3 mg/L (48h EC_{50})	OECD TG 202, Klimisch (1)
Algae/aquatic plants *Desmodesmus subspicatus*:	0.75 mg/L (72h E_rC_{50})	OECD TG 201, Klimisch (1)
Crustacea *Daphnia magna*	0.45 mg/L (21d NOEC)	OECD TG 211, Klimisch (1)
Other Toxicity		
STOT RE2 criteria met		

Hazard assessment elements:

Physico-chemical properties:

- Substance A is poorly water soluble and strongly sorbing to solid matrices (log K_{OW} = 6.3, log K_{OC} = 4.7). No information on dissociation.

Degradation:

- Hydrolysis data indicate long abiotic degradation half-lives;
- During a reliable ready biodegradation study, substance A was shown to be non-readily biodegradable (2% degradation after 28d);
- No simulation study is available for the substance. Water-sediment and soil field studies are available for analogue substances showing very slow degradation in solid matrices. The whole system degradation half-life was above 180 d. Faster dissipation 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.

Mobility:

- One QSAR prediction deriving a log K_{OC} value greater than 4. There is some evidence from the water solubility and other experimental fate information that Substance A may have a preferential partition to solid phases.

Classification (pursuant to CLP Annex I, 4.3 and 4.4):

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**

Persistent, Mobile and Toxic (PMT) properties: CLP Annex I, 4.4 **criteria not met**

Very Persistent, Very Mobile (vPvM) properties: CLP Annex I, 4.4 **criteria not met**

Reasoning:

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

- a water-sediment simulation study on an analogue substance. The read-across

approach has been properly documented and the argumentation for its use (mainly very high structural similarity) is acceptable. The analogue substance 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 (high weight);

- 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 (high weight);
- additional information from several monitoring studies for substance A and other structurally similar substances indicating long-term presence in sediments (medium weight);
- acceptable (Q)SAR predictions appropriate for the structure of substance A indicating slow environmental degradation (medium weight).
- hydrolysis data indicating slow abiotic degradation rates (medium weight);
- a ready biodegradation study that suggests that substance A is not subject to biodegradation (2% after 28 days) (low weight);

Thus, it can be concluded that substance A fulfils the CLP Annex I, 4.3.2.1.1 and 4.4.2.1.1 (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 (high weight). 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 4.3.2.2.2 (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, it can be concluded that substance A fulfils the CLP Annex I, 4.3.2.1.3 and 4.4.2.1.3 (c) (and also REACH Annex XIII 1.1.3.) **T** criteria.

- Mobility:

In the absence of a log K_{OC} value below the regulatory threshold values of 2 and 3, it can be concluded that Substance A does not fulfil the CLP Annex I, 4.4.2.1.2. and 4.4.2.2.2 **M** and **vM** criteria.

Label elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	Danger
Hazard Statement	EUH441[88]
Precautionary statement(s)	P201, P202, P273, P391, P501

[88] In line with Annex III to Regulation (EC) No 1272/2008 Part 1 point (c) "if the hazard statement EUH441 "Strongly accumulates in the environment and living organisms including in humans" is assigned, the statement EUH440 "Accumulates in the environment and living organisms including in humans" may be omitted

4.3.8.2. Example B: Substance meeting the new CLP classification criteria for vPvB, based on constituent data and on the overall WoE

Substance B is a UVCB and its constituent X is present in the substance at ≥ 0.1 % w/w.

Data for Constituent X:

DATA ELEMENTS: Constituent X	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 (EPI Suite v4.11); QSAR prediction with medium uncertainty, Reliability (2) experimental value in Episuite
log octanol/water partition coefficient (log K_{OW})	5.5	KOWWIN v1.68; QSAR prediction with low uncertainty, Reliability (1)
log organic carbon/water partition coefficient (log K_{OC}) pKa	5.3; 4.8 not ionisable	KOCWIN v2.00 (EPI Suite v4.11) MCI method; *K*ow method - QSAR prediction with low uncertainty, Reliability (1) based on chemical structure
Degradation		
Hydrolysis	not expected	based on chemical structure
Phototransformation in air	$DegT_{50}$ 14 hours	AOP v1.92
Phototransformation in water	no significant decrease in concentration after 29 days	Klimisch (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	Klimisch (2)
Simulation study in seawater	Primary $DegT_{50}$ >182 days at 20 °C	Klimisch (4), raw data not available, used as supporting information
BIOWIN 2 & 3 predictions BIOWIN 3 & 6 predictions	slow degradation slow degradation	(Q)SAR result with medium uncertainty, Reliability (2) (Q)SAR result with medium uncertainty Reliability (2)
Bioaccumulation		
Bioconcentration in fish, *O. mykiss* (BCF_{KgL}) BCF_{SSL} (5% lipid), *Cyprinus carpio* BCF_K, *Lepomis macrochirus*	12 993 1900 ± 300; 1100± 200 8148	Klimisch (2), similar to OECD TG 305 Klimisch (4), concentrations in fish not reported Klimisch (4), no information on

Dietary BMFgL (5% lipid), *Oncorhynchus mykiss*	0.2	lipid content or fish growth Klimisch (2), depuration half life 8.1 days; estimated BCF_L 3513-7694 using method 1 of OECD TG 305 (Uptake rate constant estimation method: Use of models to estimate k1, combined with dietary k2 to provide BCF)
BCF (QSAR estimate)	2041; 1146	EPISUITE BCFBAF v3.01 (regression; Arnot-Gobas), (Q)SAR results with medium uncertainty, Reliability (2)
Toxicity		
Crustacea *Daphnia magna*:	48h EC50 0.045 mg/L	Klimisch (4), OECD TG 202
Algae	72h NOECr 1.4 mg/L	Klimisch (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).	Klimisch (4), OECD TG 204 Klimisch (4), OECD TG 203
Fish *Oryzias latipes*	41d NOEC: 11 µg/L	Klimisch (4), OECD TG 210
No relevant human health data	-	-

Data for the whole substance, Substance B:

DATA ELEMENTS: Substance B	Value	Test method / remarks
Physico-chemical properties and environmental fate (Substance B)		
Vapour pressure	0.002 hPa at 20 °C	calculated from experimental data at higher temperature using the Antoine equation, Klimisch (2)
Water solubility	0.061 mg/L at 20 °C	OECD TG 105, Klimisch (2)
log octanol/water partition coefficient (log K_{OW})	5.3 – 6.5 at 20 °C	OECD TG 117, Klimisch (2)
log organic carbon/water partition coefficient (log K_{OC})	4.2 – 6.1 at 20 °C	OECD TG 121, Klimisch (2)
Degradation (Substance B)		
Hydrolysis	not expected	based on structure
Phototransformation in water	-	-
Phototransformation in soil	-	-
Ready biodegradability	14% biodegradation in 35 days (CO_2 evolution)	OECD TG 301B, Klimisch (2)
Simulation studies in water-sediment	-	-
Soil simulation study; similar to OECD TG 307 (temperature corrected to 12°C)	$DegT_{50}$ >218 days	no measurement of NER but study considered reliable and relevant for P assessment, Klimisch (2)
Bioaccumulation (Substance B)		
Bioconcentration in fish (BCF)	-	-

Toxicity (Substance B)		
Crustacea *Daphnia magna*: Crustacea *Daphnia magna* No relevant human health classification	EC_{50} > 0.069 mg/L, NOEC 0.008 mg/L 21 d NOELR for reproduction < 1.0 mg/L -	OECD TG 202. Klimisch (2) OECD TG 211, Klimisch (4)

Hazard assessment elements:

Physico-chemical properties:

- Constituent X is poorly water soluble, lipophilic and is not ionisable based on its chemical structure. It is present in Substance B in the concentration range 0.2-2%.

Degradation:

- Constituent X is not expected to hydrolyse based on its chemical structure. There is no ready biodegradability study on Constituent X but Biowin 2, 3 and 6 predictions indicate slow degradation. A ready biodegradability study (Klimisch 2) on Substance B reached 14% biodegradation in 35 days.
- A reliable (Klimisch 2) simulation test in river water is available for Constituent X showing that it meets the vP 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 9°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 X or Substance B.

Bioaccumulation:

- One reliable (Klimisch 2) fish BCF study (BCF_{KgL} = 12993) and one reliable fish dietary study (Klimisch 2) (DietaryBMFgL (5% lipid) = 0.2, estimated BCF_{L} = 3513-7694) are available for Constituent X performed on *Oncorhynchus mykiss*. BCF QSAR predictions point to a BCF around 2000. The QSAR result is considered to have medium uncertainty, Reliability (2). Two other BCF studies are of unassignable reliability, one pointing to vB and the other pointing to not vB/borderline B. These two studies are given lower weight since their reliability cannot be verified.

Toxicity:

- Substance B is not classified for human health. There is no human health data available for Constituent X. The available aquatic toxicity studies for Constituent X are all of unassignable reliability (Klimisch 4) due to missing information. A long-term *Daphnia* study of unassignable reliability (Klimisch 4) on Substance B gives a NOELR for reproduction of < 1.0 mg/L. It is not clear which constituents contributed to the toxicity. There is insufficient information to conclude that Constituent X fulfils the T criterion.

Mobility:

- (Q)SAR predictions of the Log *Koc* for Constituent X are 5.3 and 4.8. The molecular weight of Constituent X and structural fragments fall within the range of the training set, and similar substances in the training set are predicted well. The QSAR results are considered to have low uncertainty, Reliability (1). Constituent X is not expected to be ionisable based on its chemical structure so influence of pH does not need to be taken into account for the mobility assessment.
- Experimental information for Substance B (OECD TG 121, reliability 4) gives a measured log K_{OC} of 4.2-6.1. None of the constituents of Substance B are expected to be ionisable based on their chemical structures.

Classification (pursuant to CLP Annex I, 4.3 and 4.4):

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**

Persistent, Mobile and Toxic (PMT) properties: CLP Annex I, 4.4 **criteria not met**

Very Persistent, Very Mobile (vPvM) properties: CLP Annex I, 4.4 **criteria not met**

Reasoning:

- Persistence (the lines of evidence are sorted based on their respective weight from high to low weight):
  - A reliable simulation test in river water performed at 12°C is available for Constituent X showing that it meets the vP 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;
  - A simulation study in seawater on Constituent X gave primary DegT50 >182 days at 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);
  - Biowin 2, 3 and 6 QSAR predictions suggest that Constituent X has slow degradation. 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 X, the BIOWIN models recognise the fragments of the constituent X, the training set data included similar substances and the QSAR predictions are considered to have medium uncertainty, Reliability (2). This information is given low weight;
  - A ready biodegradation study on the whole substance 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 X).

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

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

— In a reliable (Klimisch 2) fish bioaccumulation study performed using a method similar to OECD TG 305 a lipid-normalised, growth-corrected kinetic fish BCF of 12 993 was measured in *Oncorhynchus mykiss* for Constituent X (high weight), indicating that the vB classification criterion is fulfilled;

— A reliable Klimisch (2) dietary fish bioaccumulation study in *Oncorhynchus mykiss* gave a dietary BMFgL (5% lipid) of 0.2 and depuration half-life of 8.1 days; the estimated BCF_L using method 1 of OECD TG 305 (Uptake rate constant estimation method: Use of models to estimate k1, combined with dietary k2 to provide BCF) is 3513-7694, which exceeds the B criterion and partly the vB criterion (medium weight);

— Two other experimental fish bioaccumulation studies are given low weight due to missing study information: BCF_{SSL} (5% lipid), *Cyprinus carpio* = 1900 ± 300; 1100± 200 and BCF_K, *Lepomis macrochirus* = 8148

— QSAR predictions using EPISUITE BCFBAF v3.01 (regression; Arnot-Gobas) of medium uncertainty, Reliability (2) give BCFs of 2041 and 1146 with one prediction exceeding the B criterion (low weight).

It can be concluded that Constituent X fulfils the CLP Annex I, 4.3.2.1.2. (and also REACH Annex XIII 1.1.2.) **B**- and **vB**- criteria. Since Constituent X is present in the UVCB Substance at ≥0.1%, Substance B also fulfils the B and vB criteria in accordance with CLP.

- Toxicity:

Neither Substance B nor its Constituent X 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.

- Mobility (the lines of evidence are sorted based on their respective weight from high to low weight):
    - Experimental information for Substance B (OECD TG 121, reliability 2) gives a measured log K_{OC} above 3 (4.2-6.1) indicating that the M and vM criteria are not fulfilled (high weight). None of the constituents of Substance B are expected to be ionisable based on their chemical structures;

    - Two QSAR predictions for Constituent X with low uncertainty, Reliability (1) predict *Log* K_{OC} values above 3 (5.3; 4.8)(medium weight). Based on its chemical structure, Constituent X is not expected to ionise so pH should not influence the K_{OC} value.

In the absence of a log K_{OC} value below the regulatory threshold values of 2 and 3 for any of the constituents of Substance B, including Constituent X, it can be concluded that Substance B and Constituent X do not fulfil the CLP Annex I, 4.4.2.1.2. and 4.4.2.2.2 M and vM criteria.

Label elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	Danger
Hazard Statement	EUH441
Precautionary statement(s)	P201, P202, P273, P391, P501

4995 **4.3.8.3. Example C: Substance meeting the new CLP classification criteria for**
4996 **PMT and vPvM, based on the overall WoE**

4997

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	EU Method A.6, Klimisch (1)
log octanol/water partition coefficient (log K_{OW})	-1.4	ACD/ Labs, QSAR prediction with medium uncertainty, Reliability (2)
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, QSAR prediction with medium uncertainty, Reliability (2)[89] Extrapolation from log K_{OW} OECD TG 106 (pHs 4.5-7.5), Klimisch (1) FOOTPRINT Pesticides Properties Database, experimental information CompTox Chemicals Dashboard Experimental study, non-ionic species
pK_a	7.1	Substance is in the anionic state
Degradation		
Ready biodegradability	3% in 28 days	OECD TG 301C, Klimisch (1)
Simulation studies in surface water	>80 days	OECD TG 309, Klimisch (1)
Biodegradation in soil	> 3 years	ECETOC, non-standard study
Abiotic degradation	Negligible degradation by hydrolysis and photodegradation	Experimental studies
Bioaccumulation		
Bioconcentration in fish (BCF)	<10	OECD TG 305C , Klimisch (1)
Bioconcentration in fish (BCF)	<1	Non-standard study, Klimisch (2)
Bioconcentration in fish (BCF)	<0.5	Non-standard study, Klimisch (4)
Aquatic Toxicity		
Short and long-term fish	> 10 mg/L	Several OECD test protocols, Klimisch (1) and (2)
Short and long-term aquatic invertebrates	> 100 mg/L	Several OECD test protocols, Klimisch (1) and (2)
Algae and aquatic plants	> 100 mg/L	Several OECD test protocols, Klimisch (1) and (2)
Other Toxicity		
STOT RE 1 (H372) criteria met		

[89] The substance is ionisable and, therefore, the Koc prediction should be investigated whether it considers ionisation.

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:

- Substance C is very water soluble, not volatile and with very low adsorption potential. It 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 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 no reporting limitations (indicated that substance C is not bioaccumulative to fish);
- The same conclusion also confirmed by two non-standard studies;
- Non-standard study on terrestrial bioaccumulation is available;
- Indication from the octanol-water partition coefficient (=-1.4) of low biomagnification potential. Caution is advised on the use of such results due to the applicability of such models for ionisables.

Mobility:

- Substance C 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 (caution due to their applicability for ionisables);
- Field evidence that Substance C is present in several different water bodies in high concentrations;

- Modelling evidence that Substance C partitions to water, does not volatilise and is slowly degraded;
- The low calculated Henry's law constant (=4.6 10^{-7} Pa*m^3/ mol, calculated as the ratio of the vapour pressure and water solubility, with a molecular weight of 300 g/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 and 4.4):

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 not met**

Persistent, Mobile and Toxic (PMT) properties: CLP Annex I, 4.4 **criteria met**

Very Persistent, Very Mobile (vPvM) properties: CLP Annex I, **4.4 criteria met**

Reasoning:

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

In the surface water simulation study according to OECD TG 309, the degradation half-life in surface water was higher than 60 days (high weight) Moreover, a degradation half-life of more than 3 years was estimated for soil (medium weight, non-standard study), whilst experimental studies on abiotic degradation (medium weight), ready biodegradability (low weight) and monitoring (low weight) also support the conclusion that Substance C 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.

- Bioaccumulation:

The available data include one experimental BCF study on fish (high weight), a non-standard study on terrestrial bioaccumulation and two non-standard bioconcentration fish studies (medium and low weight), as well as indication from the octanol-water partition coefficient (low weight) support the conclusion that Substance C does not meet the CLP Annex I, 4.3.2.1.2. **B** criteria or 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 (high and medium weight). For the non-ionic species of substance C, a log K_{OC} of 3.2 was derived (medium weight). Furthermore, it has high water solubility and low volatilisation from water potential (H= 2 10^{-7} Pa*m^3/ mol) (low weight). Monitoring data reveal its wide presence in different water bodies with concentrations up to 5 µg/L in groundwater and other surface water bodies (low weight). Distribution modelling computations also confirm its affinity to water bodies and slow environmental degradation (low weight). Finally, there is evidence that Substance C is not likely to be efficiently removed by adsorption to organic materials in sewage treatment plants (WWTP) or in drinking water production (low weight). In summary, Substance C can be concluded to fulfil the CLP Annex I, 4.4.2.1.2 and 4.4.2.2.2 criteria for **M** and **vM**.

- Toxicity:

Substance C fulfils the CLP Annex I, 4.3.2.1.3 and 4.4.2.1.3 **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	EUH451[90]
Precautionary statement(s)	P201, P202, P273, P391, P501

[90] In line with Annex III to Regulation (EC) No 1272/2008 Part 1 point (d) "if the hazard statement EUH451 "Can cause very long-lasting and diffuse contamination of water resources" is assigned, the statement EUH450 "Can cause long-lasting and diffuse contamination of water resources" may be omitted.

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