ECHA
EUROPEAN CHEMICALS AGENCY

GUIDANCE

Guidance on Information Requirements and Chemical Safety Assessment

Chapter R.11: PBT/vPvB assessment

Version 4.0

December 2023

Legal notice

Guidance on Information Requirements and Chemical Safety Assessment

Chapter R.11: PBT/vPvB Assessment

Reference: ECHA-23-H-10-EN

Cat. Number: ED-02-23-137-EN-N
ISBN: 978-92-9468-326-7

DOI: 10.2823/312974

Publication date: December 2023
Language: EN

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Preface

This document describes the information requirements under the REACH Regulation with regard to substance properties, exposure, use and risk management measures, and the chemical safety assessment. It is part of a series of guidance documents that are aimed to help all stakeholders with their preparation for fulfilling their obligations under the REACH Regulation. These documents cover detailed guidance for a range of essential REACH processes as well as for some specific scientific and/or technical methods that industry or authorities need to make use of under the REACH Regulation.

The original versions of the guidance documents were drafted and discussed within the REACH Implementation Projects (RIPs) led by the European Commission services, involving stakeholders from Member States, industry and non-governmental organisations. After acceptance by the Member States competent authorities the guidance documents had been handed over to ECHA for publication and further maintenance. Any updates of the guidance are drafted by ECHA and are then subject to a consultation procedure, involving stakeholders from Member States, industry and non-governmental organisations. For details of the consultation procedure, please see:

https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach/

Consultation procedure for Guidance [PDF]

The guidance documents can be obtained via the website of the European Chemicals Agency at:

http://echa.europa.eu/web/guest/guidance-documents/guidance-on-reach

Further guidance documents will be published on this website when they are finalised or updated.

This document relates to the REACH Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006[1].

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[1] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396, 30.12.2006, p.1; corrected by OJ L 136, 29.5.2007, p.3).

Document History

Version	Changes	Date
Version 1	First edition	May 2008
Version 1.2	Corrigendum: (i) replacing references to DSD/DPD by references to CLP; (ii) further minor editorial changes/corrections.	November 2012
Version 2.0	Second edition. Full revision of this document was necessary to take into account the amendment of Annex XIII to REACH (according to Commission Regulation (EU) No 253/2011 of 15 March 2011, OJ L 69 7 16.3.2011). Main changes in the guidance document include the following: • Chapter R.11 title has been changed to "PBT/vPvB assessment"; • Chapter R.11 has been re-structured to differentiate more clearly between the obligations of the registrant arising directly from the legal text (Section R.11.3) and the description of the scientific method to assess PBT/vPvB properties (Section R.11.4); • Description of the registrant's obligations in Section R.11.3 has been expanded upon; • The description of the scope of PBT/vPvB assessment regarding relevant constituents/impurities/additives and transformation/degradation products has been expanded upon and divided into two Sections: Section R.11.3.2.1 for legal aspects and Section R.11.4 for the aspects related to assessment; • The different steps of the PBT/vPvB assessment process, in particular the first step of comparison with the PBT and vPvB criteria, and the subsequent conclusions and consequences for the registrant have been refined to take account of the case where the registrant concludes that further information is needed but he decides not to generate additional information by	November 2014

	considering the substance "*as if it is a PBT/vPvB*"; • The number of conclusions deriving from the first Step of the PBT/vPvB assessment process has been reduced from four to three in Section R.11.4.1.4 "*Conclusions on PBT or vPvB properties*"; • Consequences for the registrant of the conclusions deriving from the first Step of the PBT/vPvB assessment process are described in the new Section R.11.3.2. • Section R.11.3.2.2 is new and describes the situation of substances concluded as being PBT/vPvB by ECHA's Member State Committee in relation to the inclusion in the Candidate List of Substances of Very High Concern; • The basic approach to bioaccumulation assessment described in Section R.11.4.1.2 has been slightly extended to reflect in particular the revised OECD test guideline 305 and the possibility to take other bioaccumulation information into account. The molecular length screening threshold value has been removed; • As the screening threshold values for PBT/vPvB assessment are part of the scientific methodology and not part of legal text, they are now presented in relevant parts of Section R.11.4 only. • The document has been re-formatted to ECHA new corporate identity.	
Version 3.0	Revision of this document was necessary in sections related to the scientific assessment approach to take into account recent scientific and technical developments in the field, including recently adopted or revised OECD TGs. Main changes in the guidance document include the following: • Update of Section R.11.4.1.1 on "*Persistence assessment*" and of the corresponding Integrated Testing Strategy described in Section R.11.4.1.1.1 and Figure R.11-3; • Update of Section R.11.4.1.2 on "*Bioaccumulation assessment*" and of the corresponding Integrated Testing Strategy described in Section R.11.4.1.2.1 and Figure R.11-4;	June 2017

	• Update of Section R.11.4.1.4 on "*Conclusions on PBT or vPvB properties*"; • Revision of Section R.11.4.2.2 on "*Assessment of substances containing multiple constituents, impurities and/or additives*"; • Update of Appendix R.11—3 on "*PBT assessment of UVCB petroleum substances*"; • Update of cross references and links to the revised sections of Chapters R.7b and R.7c.	
Version 4.0	Revision of this document was necessary in sections related to the scientific assessment approach to take into account recent scientific and technical developments in the field, including recently adopted or revised OECD TGs. Main changes in the guidance document include the following: • Update of the R.11.3.2.2 to provide clarification on the procedure for the inclusion of substances in the Candidate List of Substances of Very High Concern. • Revision of the R.11.4.1 on "Standard approach" including the Weight-of-Evidence determination, Benchmarking and Reporting of the analytical methods as part of the environmental fate studies. • R.11.4.1.1 on "*Persistence assessment*" and of the corresponding Integrated Testing Strategy described in Section R.11.4.1.1.1 and Figure R.11-3 • Update of the R.11.4.1.1.3 regarding the Degradation half-life (DegT50) derivation • Update of Section R.11.4.1.2 on "*Bioaccumulation assessment*" and of the corresponding Integrated Testing Strategy described in Section R.11.4.1.2.1 and Figure R.11-4; • Introduction in section R.11.4.1.2.2 of text regarding the use of aquatic invertebrate tests (Hyalella azteca bioconcentration test (HYBIT)) • Introduction of new section R.11.4.1.2.4 on In vitro biotransformation data and in vitro-in vivo extrapolation (IVIVE)	December 2023

	• Introduction of new section R.11.4.1.2.8 regarding the assessment of bioaccumulation in air-breathing organisms • Introduction of the new section R.11.4.2.1.3 regarding volatile substances. • Introduction of the new section R.11.4.2.1.4 regarding substances with nanoforms. • Update of the R.11.4.2.2.3 regarding the toxicity assessment for UVCBs. • Deletion of the section of the Appendix R.11—1 regarding Indicators for limited bioconcentration for PBT assessment • Deletion of the Appendix R.11—1 Annex 3 (Examples-Use of the indicators for limited bioaccumulation) • Deletion of the Appendix R.11—2 (Assessment of substances requiring special consideration during Testing) • Introduction of the new Appendix R.11-4 (Approach on NER quantification and characterisation in persistence assessment) • Introduction of the new Appendix R.11- 5 (Comparison of HPLC and KowWIN v1.68 QSAR generated log K_{ow} values) • Introduction of the new Appendix R.11- 6 (Relevance and scientific justification of correction for growth dilution when deriving BCF) • Introduction of the new Appendix R.11-7 (Volatilisation correction approaches for the kinetic analysis of simulation studies) • Update of cross references and links to the revised sections of Chapters R.7b, R.7c and the Appendices for substance in nanoform.	

Convention for citing the REACH Regulation

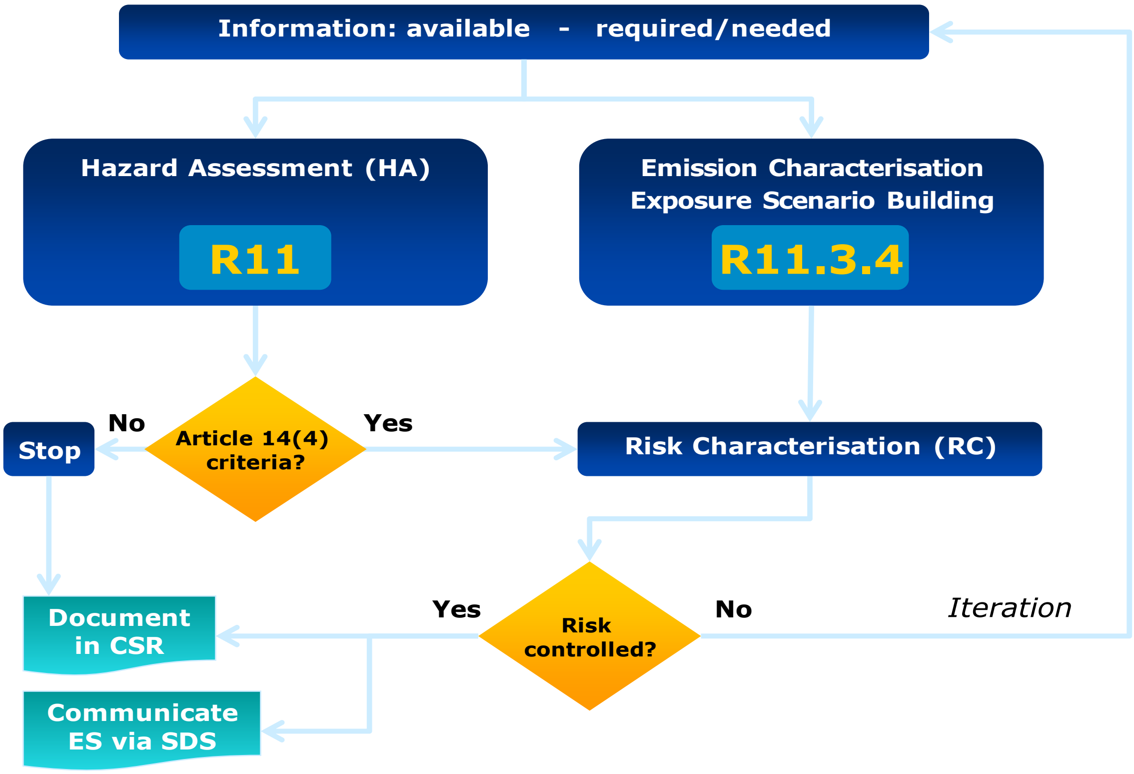
Where the REACH Regulation is cited literally, this is indicated by text in italics between quotes, or text in blue boxes.

Table of Terms and Abbreviations

See Chapter R.20.

Pathfinder

The figure below indicates the location of Chapter R.11 within the Guidance Document:



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R.11 PBT and vPvB Assessment

R.11.1 Introduction

According to Section 4 of Annex I to the REACH Regulation the objective of the persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) assessment is to determine if the substance assessed in Chemical Safety Assessment (CSA) fulfils the criteria set out in Annex XIII. It furthermore states that a conventional hazard assessment of the long-term effects and the estimation of the long-term exposure cannot be carried out with sufficient reliability for the purpose of assessing the safety of substances satisfying the PBT and vPvB criteria in Annex XIII. Therefore a PBT and vPvB assessment is required to be carried out for all substances for which CSA is carried out.

This guidance document contains a description of scientific principles for the PBT and vPvB assessment in accordance with Section 4 of Annex I to the REACH Regulation, and a description of the obligations of the registrant in carrying out a PBT and vPvB assessment as part of CSA.

PBT substances are substances that are persistent, bioaccumulative and toxic, while vPvB substances are characterised by a particular high persistence in combination with a high tendency to bioaccumulate, which may, based on experience from the past with such substances, lead to toxic effects and have an impact in a manner which is difficult to predict and prove by testing, regardless of whether there are specific effects already known or not. These properties are defined by the criteria laid down in Section 1 of Annex XIII to the REACH Regulation (*CRITERIA FOR THE IDENTIFICATION OF PERSISTENT, BIOACCUMULATIVE AND TOXIC SUBSTANCES, AND VERY PERSISTENT AND VERY BIOACCUMULATIVE SUBSTANCES,* henceforth "the PBT and vPvB criteria").

A PBT/vPvB assessment[2] is required for all substances for which a CSA must be conducted and reported in the chemical safety report (CSR). These are, according to Article 14(1) of the REACH Regulation, in general all substances manufactured or imported in amounts of 10 or more tonnes per year that are not exempted from the registration requirement under the Regulation. However, some further exemptions apply as described in Article 14(2), e.g. for substances present in a mixture if the concentration is less than 0.1% weight by weight (w/w), for on-site or transported isolated intermediates, and for substances used for Product and Process Oriented Research and Development (for further information see the *Guidance on Registration*). Therefore, this guidance is mainly targeted at registrants manufacturing or importing a substance in amounts of 10 or more tonnes per year and to downstream users who have an obligation to conduct their own CSA. This guidance is also relevant for ECHA and for Member State competent authorities who carry out PBT/vPvB assessment related tasks under REACH.

Experience with PBT/vPvB substances has shown that they can give rise to specific concerns that may arise due to their potential to accumulate in parts of the environment and

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[2] The term "PBT/vPvB assessment" is applied in this document to denote "PBT and vPvB assessment" and covers both "screening" and "assessment" as described in the following sections.

- that the effects of such accumulation are unpredictable in the long-term;
- such accumulation is in practice difficult to reverse as cessation of emission will not necessarily result in a reduction in substance concentration.

Furthermore, PBT or vPvB substances may have the potential to contaminate remote areas that should be protected from further contamination by hazardous substances resulting from human activity because the intrinsic value of pristine environments should be protected.

These specific concerns occur particularly with substances that can be shown both to persist for long periods and to bioaccumulate in biota and which can give rise to toxic effects after a longer time and over a greater spatial scale than substances without these properties. These effects may be difficult to detect at an early stage because of long-term exposures at normally low concentration levels and long life-cycles of species at the top of the food chain. In the case of vPvB substances, there is concern that even if no toxicity is demonstrated in laboratory testing, long-term effects might be possible since high but unpredictable levels may be reached in man or the environment over extended time periods.

The properties of the PBT/vPvB substances lead to an increased uncertainty in the estimation of risk to human health and the environment when applying quantitative risk assessment methodologies. For PBT and vPvB substances a "safe" concentration in the environment cannot be established using the methods currently available with sufficient reliability for an acceptable risk to be determined in a quantitative way[3]. Therefore, a separate PBT/vPvB assessment is required according to Article 14(3)(d) of the REACH Regulation in order to take these specific concerns into account. Registrants are required to perform this specific PBT/vPvB assessment in the context of their CSA.

According to Section 4 of Annex I to the REACH Regulation, the objective of the PBT/vPvB assessment is to determine if the substance fulfils the criteria given in Annex XIII to the REACH Regulation ("*Step 1: Comparison with the Criteria*"), and if so, to characterise the potential emissions of the substance to the different environmental compartments during all activities carried out by the registrant and all identified uses ("*Step 2: Emission characterisation*"). In addition, in the latter step it is also necessary to identify the likely routes by which humans and the environment are exposed to the substance. According to Section 6.5 of Annex I to the REACH Regulation the registrant then needs to use the information obtained during the emission characterisation step, when implementing on his site, and recommending to downstream users, risk management measures (RMMs) which minimise emissions and subsequent exposures of humans and the environment throughout the life-cycle of the substance that results from manufacture or identified uses. The authorities may further subject substances with PBT or vPvB properties to restrictions or the authorisation requirement, with substitution of the substance as objective in the latter case where economically and technically viable.

The registrant's process for assessing the substance and the registrant's obligations resulting from the conclusions are outlined in detail in Section R.11.3. Guidance on scientific methods

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[3] It should be noted that over the last years a number of methods have been proposed in the scientific literature that could eventually be used to reduce the uncertainty in the risk estimation (on either the exposure or effects side) of PBTs and vPvBs and hence may lead to a better understanding of the level of risk associated with these substances, in particular in a comparative sense.

that can be used for carrying out Step 1 is given in Section R.11.4 of this Chapter. The sub-sections of Section R.11.4 on the assessment of the P, B and T properties of a substance provide guidance on how a registrant or an authority can make best use of the different types of information available in order to conclude with least efforts on the PBT/vPvB–properties of the substance. These sub-sections also contain guidance on specific assessment and testing strategies for substances that are difficult to test, including adaptation of tests, specific rules for interpretation of results, consideration of monitoring data and cut-off criteria.

The guidance explains how all available evidence can be considered in order to decide with sufficient certainty whether the PBT/vPvB criteria are fulfilled or not without always requiring the generation of such types of data that numerically match with the Annex XIII criteria. Generating such data may for instance not be possible because the properties of the substance do not permit the respective tests to be conducted. In these cases a conclusion may need to be drawn on the basis of screening information and all further evidence available. In many cases further information may need to be generated before it can be judged whether the substance fulfils the Annex XIII criteria, and the guidance provides detailed testing strategies that the registrant should use for each endpoint in Section R.11.4.

Substances are considered as PBT or vPvB substances when they fulfil the criteria for all three inherent properties P, B and T or both of the inherent properties vP and vB, respectively. It is the task of the registrant to assess if the information that is available and/or produced is sufficient to assess whether the substance is a PBT or a vPvB substance or not.

It is to be noted that this guidance is not meant to guide authorities directly in identifying substances fulfilling the criteria of Article 57(f) of the REACH Regulation (substances of equivalent level of concern). However, this guidance may in such cases be used as one reference for understanding what indications may be needed to identify a substance to be of equivalent level of concern to PBT or vPvB substances.

R.11.2 Overview of Annex XIII to the REACH Regulation

The purpose of this section is to introduce the content and terminology of Annex XIII to the REACH Regulation. The interpretation of the content is presented mainly from Section R.11.3 onwards. Only some key clarifications of the legal text are included in this section.

R.11.2.1 Elements and terminology of Annex XIII to the REACH Regulation

The introductory section of Annex XIII to the REACH Regulation defines the PBT/vPvB assessment scope regarding substance groups:

Introductory Section of Annex XIII to REACH

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

Annex XIII to the REACH Regulation 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.

The PBT/vPvB criteria as set out in Annex XIII to the REACH Regulation are presented in Section R.11.2.2, Table R.11—1.

Annex XIII defines two levels of assessment within the PBT/vPvB assessment ("**screening**" and "**assessment**") and two sets of information ("**screening information**" and "**assessment information**"). The two sets of information are presented in Table R.11—2 and Table R.11—3, respectively. The differentiation of the two assessment levels within the PBT/vPvB assessment is mainly designed to help the registrant identify his obligations specifically with respect to the PBT/vPvB assessment.

The combination of several passages of extracts of the text of Annex XIII, as cited below, stipulate that **all relevant and available** "assessment information" and "screening information" must be used in the PBT/vPvB assessment:

Introductory Section of Annex XIII to REACH

[…] For the identification of PBT substances and vPvB substances a weight-of-evidence determination using expert judgement shall be applied, by comparing all relevant and available information listed in Section 3.2 with the criteria set out in Section 1. […]

Section 2.1 of Annex XIII to REACH

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

Section 2.2 of Annex XIII to REACH

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

Recital 5 of Commission Regulation (EU) No 253/2011

Experience shows that, for the adequate identification of PBT and vPvB substances, all relevant information should be used in an integrated manner and applying a weight-of-evidence approach by comparing the information to the criteria set out in Section 1 of Annex XIII.

Screening information cannot be directly (numerically) compared with the PBT/vPvB criteria, i.e. the screening information does not contain measured degradation half-life values or BCF values, which could be directly compared with the criteria. Screening information can inform on whether a substance does not have or potentially has a particular property. Screening information involves simple data, typically information from Annex VII to VIII endpoints, that must be used to assess whether further information is needed. Relevant and available assessment information involves data, typically from Annex IX and X endpoints, of higher weight in the Weight-of Evidence. Sections 3.2.1.(d), 3.2.2.(b) and 3.2.3(f) of Annex XIII to the REACH Regulation allow also "other information" to be used as assessment information, provided that its suitability and reliability can be reasonably demonstrated.

A ***Weight-of-Evidence* determination by expert judgment** must be used in the PBT/vPvB assessment (see the blue boxes below). It is defined as follows:

Introductory Section of Annex XIII to REACH

[…]

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

The *Weight-of-Evidence* determination by expert judgement enables the use of all (screening and assessment) information types listed in Section 3 of Annex XIII to the REACH Regulation in the PBT/vPvB assessment for comparing with the criteria, although not all of these information types can be directly (numerically) compared with the criteria.

Examples and principles of *Weight-of-Evidence* determination for the PBT/vPvB assessment further applying the introductory section of Annex XIII to the REACH Regulation are provided in Section R.11.4. In addition, the *Practical Guide* on "*How to use alternatives to animal testing to fulfil your information requirements for REACH registration"* provides a general scheme for building a *Weight-of-Evidence* approach.

As regards the registrants' **specific duties for the PBT/vPvB assessment**, the following provision of Annex XIII to the REACH Regulation must be considered further to Annex I:

Section 2.1 of Annex XIII to REACH

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

When fulfilling the data requirements of Annexes IX and X to the REACH Regulation, adaptations according to Column 2 and Annex XI should be applied wherever possible to minimise testing on animals, which must be only as a last resort under REACH (see REACH recital 47 and Articles 13(1) and 25 and Step 4 of REACH Annex VI).

In addition, the following **principles** must be applied while performing a PBT/vPvB assessment:

Introductory Section of Annex XIII to REACH

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

By "relevant conditions", relevant environmental conditions and relevant testing conditions are generally meant. These are further discussed in Section R.11.4.

Introductory Section of Annex XIII to REACH

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

The term "**constituent**" refers to the main constituents, impurities and additives of substances of well-defined composition and constituents of UVCB substances as defined in the *Guidance for identification and naming of substances under REACH and CLP*. The implication in terms of PBT/vPvB assessment requirement for the registrant is described in Section R.11.3.2.1 and further guidance on what should be considered as **relevant constituents** is provided in Section R.11.4.1.

R.11.2.2 PBT and vPvB criteria and information listed in Annex XIII to the REACH Regulation

The following tables (Table R.11—1, Table R.11—2, and Table R.11—3) summarise the PBT and vPvB criteria given in accordance with Section 1 of Annex XIII to REACH and the relevant information to be used for the PBT/vPvB assessment as provided in Sections 3.1 and 3.2 of Annex XIII to the REACH Regulation.

Table R.11—1: PBT and vPvB criteria according to Section 1 of Annex XIII to the REACH Regulation.

Property	PBT criteria	vPvB criteria
Persistence	A substance fulfils the persistence criterion (P) in any of the following situations: (a) the degradation half-life in marine water is higher than 60 days; (b) the degradation half-life in fresh or estuarine water is higher than 40 days; (c) the degradation half-life in marine sediment is higher than 180 days; (d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days; (e) the degradation half-life in soil is higher than 120 days.	A substance fulfils the "very persistent" criterion (vP) in any of the following situations: (a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days; (b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days; (c) the degradation half-life in soil is higher than 180 days.
Bioaccumulation	A substance fulfils the bioaccumulation criterion (B) when the bioconcentration factor in aquatic species is higher than 2000.	A substance fulfils the "very bioaccumulative" criterion (vB) when the bioconcentration factor in aquatic species is higher than 5000.
Toxicity*	A substance fulfils the toxicity criterion (T) in any of the following situations**: (a) the long-term no-observed effect concentration (NOEC) or EC10 for marine or freshwater organisms is less than 0.01 mg/L; (b) the substance meets the criteria for classification as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2) according to Regulation EC No 1272/2008; (c) there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Regulation EC No 1272/2008.	

* EC_{10} preferred over NOEC (see further explanation in Section R.11.4.1.3).

** Annex I (Part 4) to CLP Regulation (EC) No 1272/2008 was amended to include Classification criteria for PBT. These criteria include a possibility to conclude a substance as T based on classification as endocrine disruptor (category 1) for humans or the environment (19 Dec 2022).

Table R.11—2: Screening information as listed in Section 3.1 of Annex XIII to the REACH Regulation.

Indication of P and vP properties	(a) Results from tests on ready biodegradation in accordance with Section 9.2.1.1 of Annex VII; (b) Results from other screening tests (e.g. enhanced ready test, tests on inherent biodegradability); (c) Results obtained from biodegradation (Q)SAR models in accordance with Section 1.3 of Annex XI; (d) Other information provided that its suitability and reliability can be reasonable demonstrated.
Indication of B and vB properties	(a) Octanol-water partitioning coefficient experimentally determined in accordance with Section 7.8 of Annex VII to REACH or estimated by (Q)SAR models in accordance with Section 1.3 of Annex XI; (b) Other information provided that its suitability or reliability can be reasonably demonstrated.
Indication of T properties*	(a) Short-term aquatic toxicity in accordance with Section 9.1 of Annex VII to REACH and Section 9.1.13 of Annex VIII; (b) Other information provided that its suitability or reliability can be reasonably demonstrated.

* Acute or short-term aquatic toxicity data are considered to be screening information (Annex XIII, Section 3.1) and may be used as an indication that the substance may fulfil the T criterion. However, when acute/short-term aquatic toxicity data show that the substance is very toxic (L(E)C50 < 0.01 mg/L), a definitive conclusion can be drawn that the substance fulfils the T criterion and no further testing is necessary. Acute data cannot be used for concluding definitively “not T”. If long-term or chronic aquatic toxicity data are available, a definitive assessment can be made.

Table R.11—3: Assessment information according to Section 3.2 of Annex XIII to the REACH Regulation.

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.
Assessment of B or vB properties*	(a) Results from a bioconcentration or bioaccumulation study in aquatic species; (b) Other information on the bioaccumulation potential provided that its suitability and reliability can be reasonably demonstrated, such as: - Results from a bioaccumulation study in terrestrial species; - Data from scientific analysis of human body fluids or tissues, such as blood, milk, or fat; - Detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment; - Results from a chronic toxicity study on animals; - Assessment of the toxicokinetic behaviour of the substance; (c) Information on the ability of the substance to biomagnify in the food chain, where possible expressed by biomagnification factors or trophic magnification factors.
Assessment of T properties	(a) Results from long-term toxicity testing on invertebrates as set out in Section 9.1.5 of Annex IX; (b) Results from long-term toxicity testing on fish as set out in Section 9.1.6 of Annex IX; (c) Results from growth inhibition study on aquatic plants as set out in Section 9.1.2 of Annex VII; (d) The substance meeting the criteria for classification as carcinogenic in Category 1A and 1B (assigned hazard phrases: H350 or H350i), germ cell mutagenic in Category 1A or 1B (assigned hazard phrase: H340), toxic for reproduction in Category 1A, 1B and/or 2 (assigned hazard phrases: H360,H360F, H360D, H360FD, H360Fd, H360 fD, H361, H361f, H361d or H361fd), specific target organ toxic after repeated dose in Category 1 or 2 (assigned hazard phrase: H372 or H373), according to Regulation EC No 1272/2008; (e) Results from long-term or reproductive toxicity testing with birds as set out in Section 9.6.1 of Annex X; (f) Other information provided that its suitability and reliability can be reasonably demonstrated.

* At present, there is no guidance on how to apply in the PBT/vPvB assessment the information coming from:

- data from scientific analysis of human body fluids or tissues, such as blood, milk, or fat; or
- the detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment.

Such guidance needs to be developed in the future.

R.11.3 Duties of the registrant

The purpose of this section is to delineate the obligations of the registrant within the PBT/vPvB assessment workflow. For further details, the registrant may refer to the recommendations provided in Section R.11.4.

R.11.3.1 Objective and overview of the PBT/vPvB assessment process

Section 4.0.1 of Annex I to the REACH Regulation defines the objective of the PBT/vPvB assessment:

> **Annex I to REACH**
>
> […]
>
> 4. PBT AND VPVB ASSESSMENT
>
> 4.0. Introduction
>
> 4.0.1. The objective of the PBT/vPvB assessment shall be to determine if the substance fulfils the criteria given in Annex XIII and if so, to characterise the potential emissions of the substance. […]

It furthermore states that a hazard assessment and exposure assessment for CSA cannot be carried out with sufficient reliability for substances satisfying the PBT or vPvB criteria and that therefore a separate PBT/vPvB assessment is required.

According to Section 4.0.2 of Annex I to the REACH Regulation, the process of the PBT/vPvB assessment consists of the following two steps: **Step 1: "*Comparison with the criteria*"** and **Step 2: "*Emission characterisation*"**. Section 6.5 of Annex I to the REACH Regulation requires the registrant to implement for PBT/vPvB substances risk management measures which minimise exposures and emission to humans and the environment, throughout the lifecycle of the substance that result from manufacture and identified uses. The obligations of the registrant for carrying out the PBT/vPvB assessment are defined more in detail in Section 2.1 of Annex XIII to the REACH Regulation. In the following paragraphs the main assessment steps are described.

Step 1 comprises a scientific PBT/vPvB assessment where the relevant available information must be compared with the PBT/vPvB criteria (for detailed guidance on this step, see Section R.11.4). In Step 1 the registrant must come to one of the conclusions presented in Figure R.11—1. Each conclusion leads to specific consequences, which the registrant must comply with. The conclusions are described in more detail in Section R.11.4.1.4 and consequences in Section R.11.3.3.

Conclusion (i): The substance does not fulfil the PBT and vPvB criteria. For screening assessment: there is no indication of P or B properties.

- No consequences for the registrant. The PBT/vPvB assessment stops.

Conclusion (ii): The substance fulfils the PBT or vPvB criteria.

- The registrant must carry out emission characterisation and ensure minimisation of exposures and emissions throughout the life-cycle of the substance that results from manufacture and identified uses.

Conclusion (iii): The available information does not allow to conclude (i) or (ii). The substance may have PBT or vPvB properties. Further information for the PBT/vPvB assessment is needed.

- The registrant must generate relevant additional information (including, where necessary, submission of a testing proposal) and carry out Step 1 again, OR
- The registrant must treat the substance *as if it is a PBT or vPvB*.

Figure R.11—1: Overview of the conclusions from Step 1 ("Comparison with the criteria") and their consequences.

The registrant is only allowed to finalise Step 1 of the assessment process if he is able to reach an unequivocal conclusion on the PBT or vPvB properties (conclusion (i) or conclusion (ii)[4]).

Conclusion (iii) is an interim conclusion in Step 1. This conclusion triggers the requirement for the registrant to generate all necessary additional information and to continue in Step 1 until the available information allows a definitive conclusion. Section 2.1 of Annex XIII to the REACH Regulation requires information to be generated by the registrant irrespective of the standard information requirements of the registrant. This may require several iterative steps of acquisition of further information, testing and assessment. Alternatively, the registrant can decide after conclusion (iii) to apply an exemption from the requirement to generate additional data by considering the substance "*as if it is a PBT or vPvB*". This is only allowed if the registrant applies specific exposure based adaptation conditions as specified in Section 3.2(b) or (c) of Annex XI to the REACH Regulation.

The consequences of each conclusion for the registrant are described in more detail in Section R.11.3.3. Figure R.11—2 provides an overview of the PBT/vPvB assessment process of the registrant as a flowchart. Relevant constituents, impurities, additives, transformation/degradation products must also be encompassed in this process.

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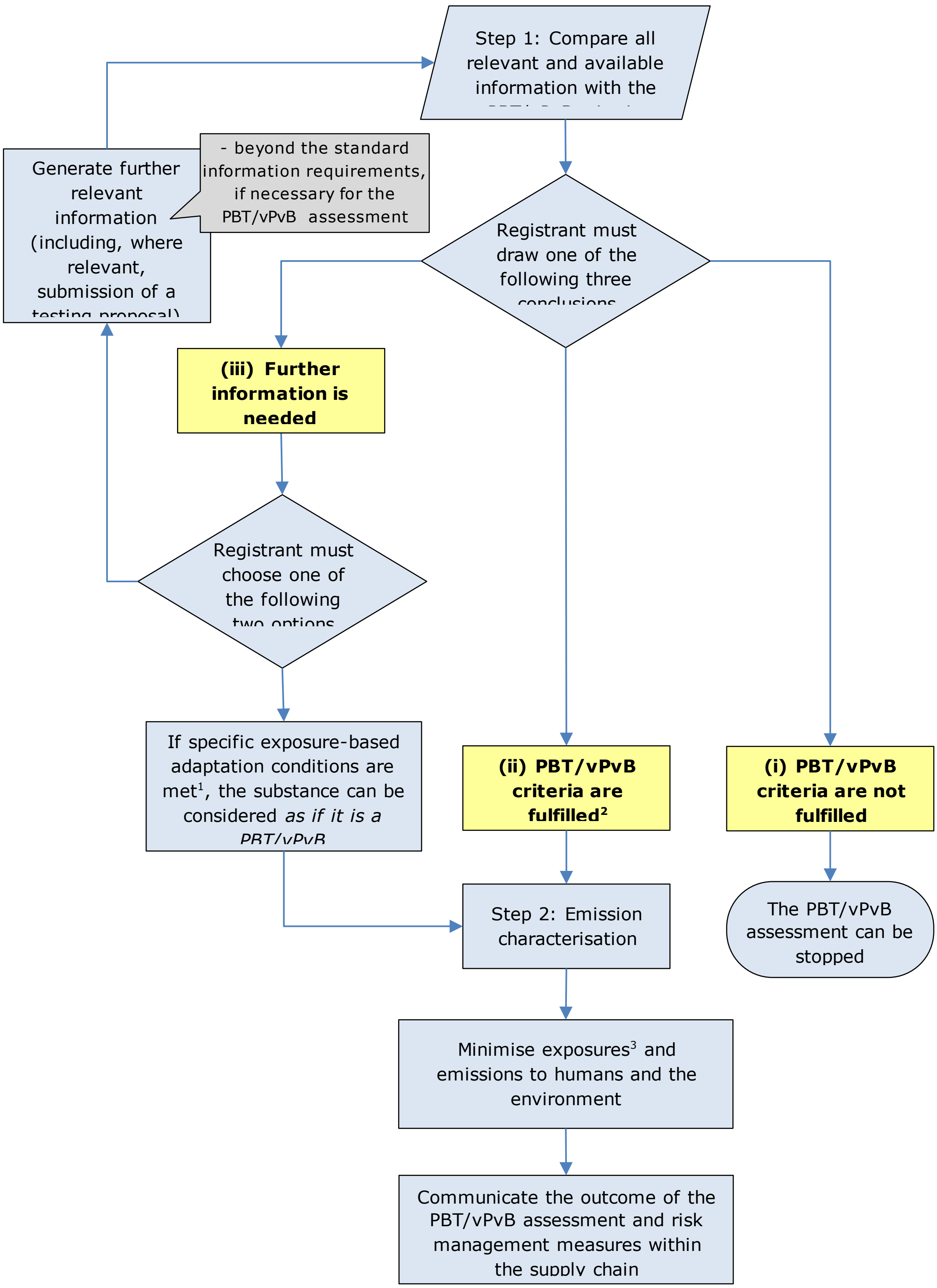
[4] Conclusion (i) and (ii) are either based on a) data directly comparable with the PBT/vPvB criteria or b) based on Weight-of-Evidence expert judgement of information which is not directly (numerically) comparable with the PBT/vPvB criteria or c) a combination of both situations a) and b).



[1] Please refer to the conditions as specified in Section 3.2(b) or (c) of Annex XI to the REACH Regulation.

[2] Normally not applicable if only screening information is available.

[3] For further information on exposure minimisation please refer to Section R.11.3.4.2.

Figure R.11—2: Overview of the PBT/vPvB assessment process for the registrant.

R.11.3.2 Comparison with the criteria (Step 1)

In the following Sections the formal obligations for Step 1 ("Comparison with the criteria") of the PBT/vPvB assessment are described.

In Step 1 of the PBT/vPvB assessment, the standard information requirements are first applied by the registrant as described in the *Guidance on Information Requirements & Chemical Safety Assessment (IR&CSA)*. It should be noted that any data adaptations according to Column 2 of Annexes VII to X or Annex XI to the REACH Regulation should be justified according to the relevant ECHA documents (e.g. *Practical Guides on "How to use and report (Q)SARs"* and on "*How to use alternatives to animal testing to fulfil your information requirements for REACH registration",* and *Chapter 5* and *Chapter 6* of the *Guidance on IR&CSA,*). The information included in the registration dossier as a result of adaptations of standard information requirements and their justifications are part of the available information for the PBT/vPvB assessment, where relevant. The PBT and vPvB assessment must initially be based on all the relevant information available which is as a minimum the information as listed in Annexes VII and VIII to the REACH Regulation. This information normally corresponds to PBT/vPvB screening information as listed in Section R.11.2.2.

The registrant must conclude Step 1 by selecting one of the three conclusions presented in Figure R.11—1 and Figure R.11—2. If conclusion (iii) "The available data information does not allow to conclude (i) or (ii)" applies, Step 1 continues after the necessary new information has been generated (see more details in Section R.11.3.3).

In cases where only screening information as listed in Section R.11.2.2 is available for one or more endpoints, Step 1 of the PBT/vPvB assessment implies first that the registrant is not able to compare the information directly (numerically) with the PBT/vPvB criteria. Although it might be theoretically possible to calculate degradation half-life values or BCF values from screening information, such values must not be directly compared with the criteria. At this stage, the registrant is required to analyse whether the information indicates that the substance may meet the PBT/vPvB criteria, in which case the registrant must draw conclusion (iii) "The available data information does not allow to conclude (i) or (ii)", or whether the information shows that there is no indication on P or B properties, in which case the conclusion (i) "The substance does not fulfil the PBT and vPvB criteria" applies. In Section R.11.4 several screening threshold values and conditions for applying them are described, which the registrant should consider while drawing a conclusion for screening. The screening threshold values are indicative and the registrant must use all relevant pieces of information on his substance to justify his conclusion. Also, where only screening information is available, the choice of the conclusion should be based on a *Weight-of-Evidence* consideration by expert judgement where all relevant and available data for all endpoints are considered in conjunction.

If only screening information is available, it is normally not possible to conclude (ii) ("The substance fulfils the PBT or vPvB criteria") due to the uncertainties related to screening information. However, if scientifically justified, it is in principle possible to draw conclusion (ii) based on screening information. In Section R.11.4 few such exceptional cases are described, where the registrant may make use of screening information for concluding (ii).

The conclusion of Step 1 should be derived by the registrant taking into account also all aspects as described in Section R.11.4.1.4.

The consequences of the individual conclusions to the registrant are described in more detail in Section R.11.3.3.

R.11.3.2.1 Scope of the PBT and vPvB assessment (relevant constituents, transformation/degradation products)

For the purpose of this Guidance it should be noted that the term "constituent" as mentioned in Annex XIII to the REACH Regulation refers to constituents and impurities of well-defined substances, constituents of UVCB substances, and additives to all substances.

The PBT/vPvB assessment must, according to Annex XIII to the REACH Regulation, take account of the PBT/vPvB properties of relevant constituents and relevant transformation and/or degradation products of organic substances (including organo-metals).

Generally, the PBT/vPvB assessment obligations as described in Sections R.11.3.1 and R.11.3.2 have to be applied for relevant constituents, impurities, additives and transformation/degradation products. The registrant cannot stop the PBT/vPvB assessment if there is not enough information available to take into account the PBT/vPvB properties of relevant constituents, impurities, additives and transformation/degradation products. This means that if there is not enough information available on the PBT/vPvB properties of relevant constituents, impurities, additives and transformation/degradation products to derive for the registrant's substance either conclusion (i) ("The substance does not fulfil the PBT and vPvB criteria") or conclusion (ii) ("The substance fulfils the PBT or vPvB criteria"), the registrant must generate the necessary further information on the PBT/vPvB properties of the relevant constituents, impurities, additives and transformation/degradation products until one of these two definitive conclusions can be achieved. The other option, as provided in Sections R.11.3.1 and R.11.3.3 is to treat the substance "*as if it is a PBT or vPvB*".

If the registrant deems as a result of the PBT/vPvB assessment an uncharacterized constituent, impurity, additive or transformation/degradation product relevant for the PBT/vPvB assessment, the registrant must characterize its substance identity as required in the *Guidance for identification and naming of substances under REACH and CLP*.

The interpretation of the term "relevant" constituent, impurity, additive, transformation/degradation product, is described in Section R.11.4.1. It is recommended that the registrant follows this interpretation in the PBT/vPvB assessment, in defining which constituents, impurities, additives, transformation or degradation products are relevant.

The registrant must show in the PBT/vPvB assessment that he has taken into account the relevant constituents, impurities and additives. This is normally possible only if he includes in the PBT/vPvB assessment appropriate justifications for all constituents, impurities and additives or for all fractions/blocks of the substance composition on why these are considered to be relevant or judged to be not relevant for the PBT/vPvB assessment, regardless of whether the substance identity of these could be ultimately determined or not[5]. The registrant may derive such reasoning quantitatively or qualitatively, by using the PBT/vPvB assessment

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[5] The PBT/vPvB assessment of short-chain chlorinated paraffins (EC 287-476-5) used for the identification of the substance to the Candidate List is one of the examples where the constituents were not characterized ultimately. See related Member State Committee SVHC Support Document at http://echa.europa.eu/documents/10162/414fa327-56a1-4b0c-bb0f-a6c40e74ece2.

principles as described in Section R.11.4. This also applies to the transformation/degradation products. It should be noted that also Section 9.2.3 of Annex IX to the REACH Regulation requires identification of degradation products.

R.11.3.2.2 Specific cases: substances fulfilling the PBT/vPvB criteria in relation to the inclusion of substances in the Candidate List of Substances of Very High Concern

According to REACH Article 59, ECHA includes substances into the Candidate List of Substances of Very High Concern (SVHC) if they fulfil the PBT and/or vPvB criteria. Where ECHA takes the decision that the substance fulfils the PBT/ vPvB criteria, the justification for such decision is found in the support document. The support document is published on ECHA's website. When comments are received, unanimous agreement from ECHA's Member State Committee (MSC) is needed before a decision can be taken whether a substance fulfils the PBT/vPvB criteria. Where, due to lack of unanimous agreement at the MSC, the European Commission takes the decision that a substance fulfils the PBT/ vPvB criteria, the justification is found in the European Commission decision published in the Official Journal of the EU. Such Commission decision may refer to the reasoning contained in the majority opinion of the MSC. So far no such Commission decision has been taken for the identification of a substance as a PBT/ vPvB. These decisions and the justifications why these substances meet the PBT/vPvB criteria are published on ECHA's website. If a registrant's substance has been included in the Candidate List as a PBT/vPvB substance, the registrant must align his PBT/vPvB assessment and conclusion with the PBT/vPvB assessment which was the basis of the relevant identification decision. This PBT/vPvB assessment is usually reported in a support document of the decision on inclusion of the substance in the Candidate List and is available on ECHA's website. In such cases, it is appropriate to replace in the CSR the documentation of Step (1) of the PBT/vPvB assessment with a reference to the relevant ECHA or European Commission decision. If the registrant has new information available which was not referred to in the support document of the relevant ECHA or European Commission decision, the registrant must include the new information in the registration dossier and may reflect his opinion of the relevance of the new information to the conclusion in the CSR. If the registrant would in this case present in the CSR the opinion that the new information would trigger another conclusion than the one drawn by ECHA or the European Commission, the registrant is obliged to apply the conclusion of ECHA or the European Commission in his CSR. In case ECHA's Committee for Risk Assessment provides an opinion to the European Commission recommending the restriction of a substance because it meets PBT/vPvB criteria, it is strongly recommended that the registrant(s) recognise and implement the PBT/vPvB status of the substance in their dossiers, minimise releases and exposures in their activities and inform their downstream users about the PBT/vPVB status, even before the European Commission has taken the restriction decision.

If a registered substance contains a relevant constituent, impurity or additive or transforms/degrades to a substance on the Candidate List that meets the PBT and/or vPvB criteria, the registrant must conclude that its substance meets the PBT or vPvB criteria accordingly. To help the registrant, Section R.11.4 provides definitions on what are relevant constituents, impurities, additives and relevant transformation and degradation products.

There are several substances on the Candidate List which have been identified as fulfilling PBT or vPvB criteria because their constituents or transformation/degradation products fulfil PBT or vPvB criteria[6]. The support documents of ECHA decisions on the Candidate List inclusion identify in these cases the constituents or transformation/degradation products of concern and contain a PBT/vPvB assessment of them. If a registered substance contains one of these as constituent, impurity, additive, or transforms/degrades into one of these substances, the registrant should reflect the conclusion presented in such support documents in his own PBT/vPvB assessment. This applies by analogy also to any future cases where inclusion to the Candidate List was due to PBT/vPvB properties of impurities or additives.

R.11.3.3 Consequences of Step 1

The three conclusions from Step 1: "Comparison with the criteria" trigger four different consequences for the registrant (see Figure R.11—1 and Figure R.11—2). These are:

- No consequences: after **conclusion (i)**
- Conduct emission characterisation and risk characterisation: after **conclusion (ii)**
- Generate relevant additional information (including, where relevant, submission of testing proposal) and continue under Step 1: after **conclusion (iii)** **or** Treat the substance "as if it is a PBT or vPvB": after **conclusion (iii)**

In the following sections the consequences are described more in detail.

R.11.3.3.1 No consequences

If the registrant concludes (i): **The substance does not fulfil the PBT and vPvB criteria**, this is the end of the PBT/vPvB assessment process. In this case, the general obligation of REACH Article 22 to take into account relevant new information or relevant changes in the substance composition applies for triggering the need to revise the PBT/vPvB assessment.

R.11.3.3.2 Conduct emission characterisation and risk characterisation

If the registrant concludes (ii): **The substance fulfils the PBT or vPvB criteria**, he must carry out an emission characterisation and implement and recommend such risk management measures which minimise emissions and subsequent exposures of humans and the environment from manufacture and identified uses (see Section R.11.3.4).

Also substances concluded according to the principles described in Section R.11.4.1.4 as fulfilling PBT or vPvB criteria because their constituents, impurities, additives or transformation/degradation products fulfil the PBT or vPvB criteria must be subjected to emission characterisation and minimisation of releases for their whole life-cycle.

It should be noted that if the registrant draws this conclusion within his CSA, it does not automatically lead to initiation of the REACH Article 59 process for inclusion of the substance in the Candidate List but the registrant has the primary responsibility to implement the necessary risk management measures for minimisation of the exposure and emissions.

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[6] Such substances are for example: Coal tar pitch, high temperature (EINECS No: 266-028-2) and Bis(pentabromophenyl) ether (EC 214-604-9).

R.11.3.3.3 Generate relevant additional information (including, where relevant, submission of a testing proposal)

If the registrant concludes (iii): **The available information does not allow to conclude (i) or (ii),** the registrant must generate relevant additional information and continue the PBT/vPvB assessment Step 1 until the comparison with the criteria can be reliably done and a final conclusion (i) "The substance does not fulfil the PBT and vPvB criteria" or (ii) "The substance fulfils the PBT or vPvB criteria" can be unequivocally drawn (see Figure R.11—1 in Section R.11.3.1). The obligation of the registrant to generate relevant additional information for the PBT/vPvB assessment concerns also relevant constituents, impurities, additives and transformation/degradation products. This means that if there is not enough information available on the PBT/vPvB properties of relevant constituents, impurities, additives and transformation/degradation products to derive for the registrant's substance either conclusion (i) or conclusion (ii), the registrant must generate the necessary further information on the PBT/vPvB properties of the relevant constituents, impurities, additives and transformation/degradation products until one of these two definitive conclusions can be arrived at.

This obligation to generate relevant additional information is valid regardless of whether the registrant's dossier contains experimental information on the registered substance for all standard information requirements or whether he has made use of the data adaptation possibilities of Annex XI and Column 2 of Annexes VII to X to the REACH Regulation. In certain cases this may mean that the adaptation the registrant originally made (or planned to make) in the registration needs to be replaced by results from a study which needs to be carried out for the purpose of the PBT/vPvB assessment as required in Section 2.1 of Annex XIII to the REACH Regulation. Especially for such Column 2 waivers of Annexes VII to X to the REACH Regulation which are based on limited or unlikely exposure, it is important to note that the registrant, if not able to conclude (i) ("The substance does not fulfil the PBT or vPvB criteria"), may need to carry out the tests he originally wished to waive in order to be able to conclude the PBT/vPvB assessment ultimately either by conclusion (i) or (ii), unless he decides to treat the substance "as if it is a PBT or vPvB" (see next Section). For example, a registrant may apply the Column 2 adaptation rule "The study need not be conducted if direct and indirect exposure of the aquatic compartment is unlikely" for the testing requirement (bioaccumulation in aquatic species) of Section 9.3.2 of Annex IX to the REACH Regulation. If he concludes the PBT/vPvB assessment with the conclusion (iii) ("The available data information does not allow to conclude (i) or (ii)") because the substance fulfils the P or vP criteria and due to a Log K_{ow} > 4.5 potentially fulfils the B/vB criteria, he must either carry out the bioaccumulation test he originally wished to waive or he must treat the substance "as if it is a PBT or vPvB" (see next Section).

The additional relevant information needed to be generated by the registrant must be identified by the registrant in the technical dossier and CSR. This additional information can relate to one or several tests as listed in Annexes IX or X to the REACH Regulation. The additional relevant information can also be an "other type" of information, which the registrant considers to be optimal for the PBT/vPvB assessment, as Section 3.2 or Annex XIII to the REACH Regulation allows the use of such other information. The other type of information can be experimental information not falling under Annex IX or X, but it may also be a combination of experimental research information and monitoring research or solely research based on monitoring/measured field data. Section R.11.4 provides guidance to the registrant for deciding which information could be necessary in pursuing an unequivocal conclusion (i) or (ii).

The additional information can be generated by the registrant in a tiered way by means of a testing strategy, if this is deemed necessary. Elements of such testing strategies include avoiding unnecessary animal or other testing and ensuring efficient use of resources while optimising the generation of data that can be used to reach definitive conclusion (i) or (ii).

If the registrant, based on the PBT/vPvB assessment, identifies that information listed in Annex IX or X to the REACH Regulation is needed, he must submit appropriate testing proposal(s). Such testing proposals are subject to the normal testing proposal evaluation process of REACH.

If the registrant is using his right to generate for the purpose of the PBT/vPvB assessment an "other type" of information as described above, testing proposals cannot be submitted. The registrant should, however, inform ECHA about his plans to generate any such other information by specifying in the CSR to the degree of detail possible an appropriate information gathering or testing strategy and an estimated time needed to update the PBT/vPvB assessment and the registration dossier. This is the only way the registrant can inform ECHA that he is using this possibility for complying with the data generation obligation in his PBT/vPvB assessment.

The registrant should strive to plan generation of further relevant information in a way that leads to submission of a minimum number of updates of the PBT assessment and technical dossier. However, it is recognized that PBT assessment can be challenging and the information generated may sometimes provide results which indicate that further information not initially foreseen by the registrant needs to be generated to come to final conclusion (i) or (ii). In such cases the registrant is obliged to update the registration dossier (including the CSR) without delay each time new information becomes available. Hence, the registration dossier may in the most complex cases need to be updated several times before the PBT assessment Step 1 can be concluded.

Section 0.5 of Annex I to the REACH Regulation, requires of the registrant that: "[…] While waiting for results of further testing, he shall record in his chemical safety report, and include in the exposure scenario developed, the interim risk management measures that he has put in place and those he recommends to downstream users intended to manage the risks being explored." It is thus the duty of the registrant to identify appropriate interim risk management measures.

Section 2.1 of Annex XIII to the REACH Regulation requires relevant further information to be generated regardless of the tonnage band for the substance of the registrant conducting the PBT/vPvB assessment. This obligation is illustrated by the following example: a registrant with a tonnage band for a substance of 10-100 t/y identifies that more information is needed and that (a) degradation simulation test(s) would be the first test(s) needed, followed by a fish bioaccumulation test if the substance is deemed persistent after simulation testing. He must submit a testing strategy and testing proposals, even though the degradation simulation test and the fish bioaccumulation test are not listed as standard information requirements for 10-100 t/y registrations.

R.11.3.3.4 Treat the substance "as if it is a PBT or vPvB"

If the registrant arrives at the conclusion (iii): **The available information does not allow to conclude (i) or (ii)**, he can also decide - based on REACH Annex XIII, Section 2.1 - not to generate further information, if he fulfils the conditions of exposure based adaptation of Annex XI, Section 3.2(b) and (c). Uniquely to the PBT assessment, the registrant must additionally consider the substance **"as if it is a PBT or vPvB",** i.e. state that he wishes to regard the substance as a PBT/vPvB without having all necessary information for finalising the PBT/vPvB assessment. This option has exactly the same consequences for the registrant and his supply chain, as if the substance had been identified as PBT or vPvB based on a completed PBT/vPvB assessment. This includes the obligation that if a substance is considered "as if it is a PBT or vPvB", the registrant must compile and provide recipients with a Safety Data Sheet (SDS) in accordance with REACH Article 31 even if the substance does not already meet the criteria in Article 31(1)(b) for supply of an SDS. It is important that the registrant clearly flags in the registration dossier and in the supply chain communication that the substance is considered "as if it is a PBT or vPvB".

R.11.3.4 Emission characterisation, risk characterisation and risk management measures

The registrant must develop for a "*PBT or vPvB substance*"[7] exposure assessments including the generation of Exposure Scenario(s) (ES(s)) for manufacturing and all identified uses as for any other substance meeting the criteria for classification for any of the hazard classes or categories of Article 14(4) of the REACH Regulation[8].

Whereas for substances meeting the classification criteria for Article 14(4) hazard classes or categories the objective of an exposure assessment is to make qualitative or quantitative estimates of the dose/concentration of the substance to which humans and the environment are or may be exposed, the main objective of the emission characterisation for "*a PBT or vPvB substance*" is to estimate the amounts of the substance released to the different environmental compartments during all activities carried out by the registrant and during all identified uses.

Additionally, for a substance to be considered "as if it is a PBT/vPvB" (i.e., the substance is regarded as a PBT/vPvB without finalising the PBT/vPvB assessment), appropriate parts of the CSR and the technical dossier must clearly demonstrate that the registrant fulfils the conditions for exposure based adaptation. This is the prerequisite as defined by Section 2.1 of Annex XIII to the REACH Regulation for avoiding the further information needed to finalise the PBT assessment Step 1. All use and exposure related information of the registration dossier must in this case be in line with the specific conditions for exposure based adaptation as stipulated in Section 3.2(b) and (c) of Annex XI to to the REACH Regulation. For a description

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[7] For the purpose of this section including the sub-sections, it is noted, that when reference to a "PBT or vPvB substance(s)" in italics is made, this covers both the case that the substance has been concluded to fulfil the PBT/vPvB criteria and the case that the registrant considers the substance "as if it is a PBT/vPvB" (for when these terms apply, see Section R.11.3.2.1). However, it is noted, that the registrant needs to clearly flag in the technical dossier, CSR and Safety Data Sheet which of the two cases applies to his substance.

[8] i.e.:

- hazard classes 2.1 to 2.4, 2.6 and 2.7, 2.8 types A and B, 2.9, 2.10, 2.12, 2.13 categories 1 and 2, 2.14 categories 1 and 2, 2.15 types A to F
- hazard classes 3.1 to 3.6, 3.7 adverse effects on sexual function and fertility or on development, 3.8 effects other than narcotic effects, 3.9 and 3.10
- hazard class 4.1
- hazard class 5.1

of the required conditions please refer to the *Guidance on intermediates* and *Chapter R.5: Adaptation of information requirements* of the *Guidance on IR&CSA*.

The subsequent risk characterisation for "*PBT or vPvB substances"* requires a registrant to use the information obtained in the emission characterisation step to implement on his site, or to recommend to his downstream users, Risk Management Measures (RMM) and Operational Conditions (OC) which minimise emissions and subsequent exposure of humans and the environment throughout the life-cycle of the substance that results from manufacture or identified uses (Section 6.5 of Annex I to the REACH Regulation). RMMs and OCs are documented in an ES(s).

R.11.3.4.1 Emission characterisation

The objective of the emission characterisation is:

- to identify and estimate the amount of releases of a "*PBT or vPvB-substance"* to the environment; and
- to identify exposure routes by which humans and the environment are exposed to a " *PBT or vPvB-substance"*.

The principal tool to achieve this objective is exposure scenarios. *Part D* and *Chapters R.12 to R.18* of the *Guidance on IR&CSA* provide guidance on how to develop exposure scenarios for substances in general. Parts of the exposure assessment guidance are relevant also for "*PBT or vPvB substances*" (i.e. emission estimation and assessment of chemical fate and pathways). However, since the objectives are not the same, the general scheme for exposure assessment needs to be adapted to the requirements of emission characterisation for "*PBT or vPvB substances*". Guidance is given below on some issues where special considerations are needed for "*PBT or vPvB substances*".

Throughout the development of an ES for a particular use, the objective of the risk characterisation for "*PBT or vPvB substances*", namely the minimisation of emissions and (subsequent) exposures of humans and the environment that results from that use, needs to be considered. Hence the need or a potential to (further) minimise emissions may be recognised at any point in the development of the ES. In this case, the appropriate RMMs or OCs must be included in the risk management framework and their effectiveness be assessed. In particular, for a substance to be considered "as if it is a PBT or vPvB", the exposure scenarios must be in line with the fact that the adaptation criteria of REACH Annex XI Section 3.2(b) and/or (c) are fulfilled. The final ES, or ES(s) in case of different uses, must be presented under the relevant heading of the chemical safety report, and included in an annex to the SDS. It must describe the required OCs and RMMs in a way that downstream users can check which measures they have to implement in order to minimise emissions or exposures of humans and the environment.

It should be noted that a registrant has to take care of his own tonnage (manufactured and imported). In co-operation with his downstream users the registrant has to cover, where relevant, his own uses and all identified uses including all resulting life-cycle stages. However, it can be useful to consider on a voluntary basis exposure resulting from emissions of the same substance manufactured or imported by other registrants (i.e. the overall estimated market volume), c.f. Part A.2.1.

As "*PBTs or vPvB substances*" are substances of very high concern, the registrant must pay attention to the level of detail of his assessment as well as to whether its accuracy and reliability is sufficient for a "*PBT or vPvB substance*". Where generic scenarios and assumptions may be sufficient for exposure assessment of non PBT/vPvB-substances, specific scenarios and data will be needed throughout an emission characterisation for "*PBT or vPvB substances*". The emission characterisation must, in particular be specific in the use description and concerning RMMs, and must furthermore contain an estimation of the release rate (e.g. kg/year) to the different environmental compartments during all activities carried out during manufacture or identified uses. Emissions and losses may e.g. be addressed by performing mass balances. The total amount of a substance going to each identified use must be accounted for and the whole use-specific life-cycles be covered. This can, for instance, be done by performing a substance flow analysis covering manufacture, all identified uses, emissions, recovery, disposal, etc. of the substance. If the total amount of the substance cannot be accounted for, the identification of emission sources should be refined. All effort necessary should be made to acquire for manufacture and any identified use throughout the life-cycle, site- and product-specific information on emissions and likely routes by which humans and the environment are exposed to the substance. However, information on environmental concentrations is normally not needed because minimisation of emissions and exposure is required for "*PBT or vPvB substances*" (data on environmental concentrations, if available, may however be useful in the assessment and should be considered). Gathering of the mentioned information is not required for uses that are advised against as mentioned under heading 2.3 of the CSR and in Section 1.2 of the SDS.

R.11.3.4.2 Risk characterisation and risk management measures for "PBT or vPvB Substances"

According to REACH, the objective of a risk characterisation for PBTs or vPvBs is to minimise emissions and subsequent exposure to these substances. Section 6.5 of Annex I to the REACH Regulation further requires that: "*For substances satisfying the PBT and vPvB criteria the manufacturer or importer shall use the information as obtained in Section 5, Step 2 when implementing on its site, and recommending for downstream users, RMM which minimise exposures and emissions to humans and the environment, throughout the life-cycle of the substance that results from manufacture or identified uses.*"

Risk characterisation for PBT/vPvB substances includes, as for other hazardous substances, the consideration of different risks. These are:

- Risks for the environment
- Risks for different human populations (exposed as workers, consumers or indirectly via the environment and if relevant a combination thereof)
- Risks due to the physico-chemical properties of a substance.

For the assessment of the likelihood and severity of an event occurring due to the physico-chemical properties of a PBT/vPvB substance, the same approach for risk characterisation applies as for any other substance (see Section R.7.1 of *Chapter R.7a of the* Guidance on IR&CSA*).*

The estimation of emissions to the environment and exposure of humans performed in the emission characterisation provides the basis for risk characterisation and risk management of PBT/vPvB substances.

R.11.3.4.2.1 Options and measures to minimise emissions and exposure

A registrant has to generate ES(s) which describe how emissions and exposures to *PBT/vPvB* substances are controlled. These ES(s) have to cover manufacturing, registrants' own uses, all other identified uses and life-cycle stages resulting from manufacturing and identified uses. Life-cycle stages resulting from the manufacture and identified uses include, where relevant, service-life of articles and waste. The registrants are advised to consider at an early stage which uses they wish to cover in their CSR. Obviously, if the registrant substitutes a *PBT/vPvB* substance in his own uses or he decides to stop supplying for certain downstream uses, he does not need to cover these uses in his CSR. Supply chain communication is of high relevance for such cases.

For the uses the registrant decides to include in his CSA and therefore develops ES(s) for, supply chain communication can be crucial for getting detailed enough information on conditions of use applied in practice. The registrant can conclude on the basis of the ES(s) he develops that he is not able to demonstrate that emissions can be minimised from a specific use. He must list any such uses as 'uses advised against' under heading 2.3 of the CSR. Furthermore, this information has also be documented under heading 3.7 of the technical dossier and communicated to the downstream users in Section 1.2 of the SDS.

The registrant has to implement the risk management measures and operational conditions described in the final ES(s) for manufacture and his own uses. He has to communicate as an annex to the SDS the relevant ES(s) for his downstream users. The downstream users have to implement the recommended ES(s) or alternatively prepare a downstream user CSR.

One possibility to develop ES(s) that minimise emissions and exposure is to use a similar approach as for isolated intermediates (outlined below, for further details see the *Guidance on intermediates*).

Rigorous containment of the substance

The "*PBT or vPvB substance*" must be rigorously contained by technical means during its whole life-cycle. This covers all steps in the manufacturing of the substance itself as well as all its identified uses. It further includes cleaning and maintenance, sampling, analysis, loading and unloading of equipment/vessels, waste disposal, packaging, storage and transport. This containment may only become unnecessary from a step in the life-cycle on for which it can be demonstrated that the substance is being transformed to (an)other substance(s) without PBT/vPvB properties or that the substance is included into a matrix from which it or any of its breakdown products with PBT/vPvB properties will not be released during the entire life-cycle of the matrix including the waste life stage. Note however that residues of the original "*PBT or vPvB substance"* in the matrix or impurities with PBT/vPvB properties resulting from side-reactions must additionally be considered (see Section R.11.3.2.1).

Application of procedural and control technologies

Efficient procedural and/or control technologies must on the one hand be used to control and minimise emissions and resulting exposure when emissions have been identified. For example, in case of emissions to wastewater (including during cleaning and maintenance processes), it will be considered that the substance is rigorously contained if the registrant can prove that techniques are used that give virtually no emissions. The same applies to emissions to air or disposal of wastes where technologies are used to minimise potential exposure of humans and the environment. It is important to consider that RMM which protect humans, for instance from direct exposure at the workplace, can in some cases lead to emissions to the environment (e.g. ventilation without filtration of exhaust air). For a "*PBT or vPvB substance*", such a measure is insufficient as exposure of both humans and the environment must be minimised (ventilation plus filtration of exhaust air may thus be an option in the case of the example).

On the other hand, procedural and/or control technologies must also be implemented to guarantee safe use, i.e. to prevent accidents or to mitigate their consequences. Regarding this, the clarifications according to the Directive 2012/18/EU on the control of major-accident hazards involving dangerous substances and the Directive 2014/34/EU concerning equipment and protective systems intended for use in potentially explosive atmospheres might be consulted.

Handling of the substance by trained personnel

In order to minimise emissions and any resulting exposure, it is important that only trained personnel handle "*PBT or vPvB substances*" or mixtures. From this perspective any consumer use of these substances on their own or in mixtures is probably inappropriate, because in these cases sufficient control of the emissions is in practice difficult to ensure.

R.11.3.4.2.2 Risk Characterisation for humans in cases of direct exposure to "PBT or vPvB substances"

Although quantitative risk assessment methodologies can, due to the associated high uncertainties regarding the extent of long-term exposure and effects, generally not be used for estimating the risk posed by "*PBT or vPvB substances*" to the environment or to humans via the environment (indirect exposure of humans), it may be possible to use the quantitative approach for assessing the risk for workers caused by direct exposure to the substance at the workplace, because in this case exposure under the controlled conditions of the working environment is predictable. A quantitative approach can only be applied to characterise the risk for workers resulting from direct exposure.

In case of assessing exposure at the workplace the quantitative approach (i.e. Exposure / DNEL) must be used, wherever possible, to demonstrate that workplace exposure does not result in health risks. If a DNEL cannot be derived (e.g. for substances for which effect thresholds cannot be established), the respective approach for assessing the health risk posed by non-threshold substances must be applied[9]. The overall risk for workers (resulting from all types and routes of exposure) can normally only be assessed in qualitative terms and in doing so the increased uncertainty in estimating the risk via indirect exposure through the environment must be taken into due consideration. As a consequence, the application of a higher margin of safety (i.e. a risk quotient Workplace Exposure / DNEL << 1) than usually applied to non-"*PBT or vPvB substances*" may be required to account for this increased

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[9] Note that, apart from predictable exposure, a further prerequisite for quantitative assessment of risk is the possibility to derive the no-effect level for humans with an appropriate level of certainty.

uncertainty and to consider workplace exposure as safe. Guidance on risk assessment for human health is given in *Chapter R.8 of the Guidance on IR&CSA*.

It should further be noted that even if a quantitative assessment of health risks at the workplace would indicate low risks, this does not imply that the RMM and the OC at the workplace can be considered sufficient where it is technically and practically possible to further minimise emissions and exposure at the workplace.

R.11.3.5 Documentation of the PBT/vPvB assessment

The documentation of the PBT/vPvB assessment in the registration dossier consists of several elements depending on the outcome. Section 8 of the CSR and Section 2.3 "PBT assessment" of the technical dossier generated in IUCLID[10] should be provided by all registrants who need to conduct a CSA. Furthermore, for substances with conclusion (iii) "The available data information does not allow to conclude (i) or (ii)", the registrant must identify the additional information needed in the CSA and in the technical dossier. These elements are described further in the following.

When the registrant conducts a CSA and submits a CSR he needs to conduct the PBT/vPvB assessment based on the relevant and available data (Step 1). This should be reported in detail in Section 8.1 *"Assessment of PBT/vPvB properties"* of the CSR. One of the three conclusion options described in Section R.11.4.1.4 must be recorded in this chapter as well. Furthermore, if the registrant as the result of conclusion (iii) "The available data information does not allow to conclude (i) or (ii)" considers his substance "as if it is a PBT or vPvB", this must be recorded in Section 8.1 as well.

If the registrant concludes that the substance fulfils the PBT/vPvB criteria or considers the substance "as if it is a PBT or vPvB", emission characterisation and risk characterisation shall be conducted and the CSR must contain also a section "Emission characterisation", reported as Section 8.2 of the CSR. It is noted, that the CSR-plugin of IUCLID automatically creates these two section titles. It is recommended that the registrant lists in Section 8.2 all relevant sections of the CSR (Sections 9 and 10), including the details of the emission characterisation elements.

All available relevant data must be recorded in the technical dossier in relevant endpoint study records and those relevant to the PBT/vPvB assessment must be reflected in the CSR, Section 8.1. Furthermore, the conclusions of the PBT/vPvB assessment including brief justification should be recorded in IUCLID Section 2.3. Support on how to fill in the information in Section 2.3 "PBT assessment" of IUCLID in practice is given in the IUCLID End-User Manual. In this section, it is possible to create one endpoint summary and several endpoint records. Note that the objective of the PBT Section 2.3 in IUCLID is not to repeat information already provided in other IUCLID sections. A reference to other IUCLID sections can be made.

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[10] The IUCLID software is downloadable from the IUCLID website at http://iuclid.eu for free by all parties, if used for non-commercial purposes.

If the conclusion (iii): "The available data information does not allow to conclude (i) or (ii)" is drawn in the PBT assessment Step 1 the registrant must as part of the technical dossier submit testing proposals, if the information needed is listed in Annex IX or X to the REACH Regulation. Instructions for recording the testing proposals in the technical dossier are provided in Data Submission Manual 5. If the additional information needed to finalise the PBT assessment Step 1 is not listed in Annex IX or X, the registrant cannot submit a testing proposal as testing proposals on other items than those listed in Annex IX or X will be rejected by ECHA. If the additional information is not listed in Annex IX or X, the registrant should describe in his CSR, Section 8.1 what information is envisaged to be generated. In this case the CSR should also contain the estimated timeline.

After relevant studies have been conducted, the PBT/vPvB assessment must be updated. The same applies to the CSR and the technical dossier including endpoint study records for newly generated information. The tasks of generation of further information and subsequent updating of the CSR and the technical dossier should ideally be carried out in one step. However, it is recognised that PBT/vPvB assessment sometimes may be a challenging task where several updates and cycles of generation of additional information may be needed until the PBT/vPvB assessment can be finalised by the registrant.

Furthermore, the registrant must differentiate in the registration dossier, CSR and Safety Data Sheet between the status of a substance fulfilling the PBT/vPvB criteria and a substance considered "as if it is a PBT or vPvB". This ensures that the downstream user receives enough information to be able to make use of his rights and obligations under Article 37 of REACH. Furthermore, this requirement is consistent with the purpose of the SDS, as stated in Section 0.2.1 of Annex II to to the REACH Regulation: '*The safety data sheet shall enable users to take the necessary measures relating to protection of human health and safety at the workplace, and protection of the environment (…) a safety data sheet must inform its audience of the hazards of a substance or a mixture and provide information on the safe storage, handling and disposal of the substance or mixture*'. Correct information on the hazard is provided when there is a differentiation between substances which meet the PBT/vPvB criteria based on data and those which are treated "as if it is a PBT or vPvB".

If a registrant's substance is included in the Candidate List as a PBT or vPvB substance, please, see also Section R.11.3.2.2.

R.11.3.6 Documentation of the risk characterisation and communication of measures

Given the potential risk exerted by "*PBT or vPvB substances*"[11], the descriptions of the implemented or recommended RMMs and OCs in an ES need to be sufficiently detailed to demonstrate rigorous control of the substance and to allow examination and assessment of their efficiency by authorities. The level of detail communicated in the ES attached to the Safety Data Sheet must further permit downstream users to check that their use(s) are

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[11] "PBT or vPvB substance(s)" covers both the case that the substance has been concluded to fulfil the PBT/vPvB criteria and the case that the registrant considers the substance "as if it is a PBT/vPvB" (for when these terms apply, see Section R.11.3.2.1).

covered by the ES developed by their supplier and that they have implemented the recommended RMMs and OCs correctly.

The risk characterisation for all ESs developed for the identified uses of the "*PBT or vPvB substance*" have to be documented under heading 10 of the CSR. The registrant is obliged according to REACH Article 14 to keep his CSR available and up to date. It should be further noted that any update or amendment of the CSR will require an update of the registration by the registrant without undue delay.

If the registrant concludes based on available information (ii) "The substance fulfils the PBT or vPvB criteria" **or** he considers the substance "as if it is a PBT or vPvB", this triggers the obligation to generate a Safety Data Sheet according to REACH Article 31. For both cases, the general obligations of Article 31 apply. Furthermore, the registrant must differentiate in the Safety Data Sheet which of the two cases applies for his substance. This differentiation is necessary in order to provide the downstream users the possibility to take own action for assessing further the PBT/vPvB properties of the substance.

R.11.4 Assessment of PBT/vPvB properties – the scientific method

This section describes the method for comparison of the available information with the criteria, which for the registrant is Step 1 of the PBT/vPvB assessment process. It should be noted that this section is not meant to set obligations/requirements for the registrant, but the registrant should nonetheless use this part of the guidance for pursuing the overall requirement to clarify unequivocally whether a substance fulfils the PBT or vPvB criteria or not. The method is the same as used by authorities for PBT/vPvB assessments, e.g., for identifying a substance as "Substance of Very High Concern" for the ECHA Candidate List according to REACH Article 59. The method has been developed on a scientific basis and as such lays out the rules of convention.

As in several areas of PBT/vPvB assessment scientific development activities are on-going, it is underlined that the assessor has the responsibility to critically scrutinize and apply in the PBT/vPvB assessment any relevant new scientific developments.

Sections R.11.4.1.1, R.11.4.1.2 and R.11.4.1.3 contain an assessment and testing strategy at the beginning of those sections. It should be noted that there is a high number of different combinations of property–specific conclusions, which a registrant may reach after the assessment. Due to the high number of the possible outcomes, they are not presented in this section. However, Section R.11.4.1.4 (conclusion (iii)) provides an overview of the different situations that may arise for which further information is needed.

Before starting the assessment at the level of individual properties, it is recommended to become familiarised with Section R.11.4.2.2. Any substance containing multiple constituents, impurities and/or additives should be assessed according to that section.

R.11.4.1 Standard approach

The PBT/vPvB assessment must cover a consideration of each property persistence, bioaccumulation and toxicity against each respective criterion (P or vP, B or vB, and T) in order to arrive at an informed decision on the properties of a substance or of its relevant individual constituents, impurities, additives or transformation/degradation products. In principle, substances are considered as fulfilling the PBT or vPvB criteria when they are deemed to fulfil the criteria P, B and T or vP and vB, respectively.

The assessment strategies set out in this section and Section R.11.4.2 should normally be followed and further information be searched for or generated, if necessary. In deciding which information is required on persistence, bioaccumulation or toxicity in order to arrive at an unequivocal conclusion, care must be taken to avoid vertebrate animal testing when possible. This implies that, when for several properties further information is needed, the assessment should normally focus on clarifying the potential for persistence first. When it is clear that the P criterion is fulfilled, a stepwise approach should be followed to elucidate whether the B criterion is fulfilled, eventually followed by toxicity testing to clarify the T criterion.

It should be noted that for some elements of the PBT/vPvB assessment there may be, for the purpose of a particular PBT/vPvB assessment, a need to take the recent scientific developments into account although they have not yet been implemented in this guidance. In such a case the assessor should duly justify the reasons for deviation from, or extension of, the approach presented in this document.

Weight-of-Evidence determination

As described in Section R.11.2.1, a *Weight-of-Evidence* determination using expert judgement is to be applied in the PBT/vPvB assessment. This applies for all assessment situations employing screening and/or assessment information. In order to decide whether the substance must be considered as a potential PBT/vPvB substance based on screening information or as a substance meeting the PBT or vPvB criteria, all relevant available information must be taken into account.

The requirement to use a *Weight-of-Evidence* approach using expert judgement implies, according to the introductory section of Annex XIII to the REACH Regulation, that "The available results regardless of their individual conclusions shall be assembled together in a single *Weight-of-Evidence* determination". This normally means that the individual pieces of data available do not need to be compared individually to each of the P, B, T or vP, vB criteria but all information are assembled together for each of the properties, respectively, for the purpose of a single comparison with the respective criteria. This does not exclude the option to compare information directly with each of the P, B, T or vP, vB criteria to support the assessment, where appropriate. It should be noted that *Weight-of-Evidence* determination is not a mechanism to justify disregarding valid, standard test data. The quality and consistency of the data should be given appropriate weight.

In the *Weight-of-Evidence* assessment the derivation of a conclusion property by property needs expert judgement, especially when very different types of information are available and when the information cannot be directly (numerically) compared with the criteria[12].

The template and related background document for *Weight-of-Evidence* /Uncertainty[13] in hazard assessment contains instructions on problem formulation, collection and documentation of information, reporting of evidence, assessment of the quality of individual evidence, integration and weighing of evidence, and uncertainty analysis. The *Weight-of-Evidence* approach does not mean averaging results. It should rather help to structure the evaluation and integration of all relevant and available information. The *Practical Guide* on "*How to use alternatives to animal testing to fulfil your information requirements for REACH registration"* provides a general scheme for building a *Weight-of-Evidence* approach.

An essential prerequisite for applying a *Weight-of-Evidence* approach is that the reliability and relevance of experimental studies and non-experimental data are evaluated according to *Chapters R.4, R.7b* and *R.7c* of the *Guidance on IR&CSA*. The reliability and relevance of information to the PBT/vPvB assessment is further described in the following sub-sections. The available information should be adequate for purpose and evaluated in relation to the level of certainty and accuracy needed to meet the regulatory requirement and this evaluation must be well documented in the assessment report.

For particular cases, further described in Section R.11.4.1.4, the *Weight-of-Evidence* determination should consider all three properties (i.e. persistence, bioaccumulation and toxicity) in conjunction. In particular, if for one or more of these properties only screening

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[12] In particular, it should be noted that although it might be theoretically possible to calculate degradation half-life values or BCF values from screening information, such values must not be directly compared with the criteria.

[13] ECHA *Weight-of-Evidence*/Uncertainty Template: https://echa.europa.eu/support/guidance-on-reach-and-clp-implementation/formats

information is available and screening threshold values as provided in the following sub-sections are applied to draw a conclusion, all three properties must be considered in conjunction.

Some examples of *Weight-of-Evidence* approaches used in the PBT/vPvB assessments for concluded assessment cases are listed at ECHA's website

Relevant constituents, impurities, additives and transformation/degradation products

The PBT/vPvB assessment should be performed on each relevant constituent, impurity, additive and transformation/degradation product. It is not possible to draw an overall conclusion if, e.g., the assessment of persistence has been concluded for one constituent and the assessment of bioaccumulation or toxicity for another constituent.

Constituents, impurities and additives should normally be considered relevant for the PBT/vPvB 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[14]. Individual concentrations < 0.1% (w/w) normally need not be considered.

In practice, this means that the registrant should carry out a comparison of the available data with the criteria for all constituents, impurities and additives present in concentration of ≥ 0.1% (w/w). Alternatively, the registrant should provide a justification in the CSR for why he considers certain constituents, impurities or additives present in concentration of ≥ 0.1% (w/w) or certain constituent fractions/blocks[15] as not relevant for the PBT/vPvB assessment.

It may not always be possible or even necessary to fully characterize and identify for the purpose of the PBT/vPvB assessment **UVCBs** (substances of Unknown or Variable composition, Complex reaction products or Biological materials) or **fractions of impurities** based on the information given in Section 2 of Annex VI to the REACH Regulation for substance identification. This is because (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, the registrant should make efforts for carrying out a PBT/vPvB assessment for all constituents, impurities and additives present in concentrations ≥ 0.1% (w/w). Section R.11.4.2.2**

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[14] 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.

[15] The terms "constituent fractions" refer to a situation where for a UVCB substance not all its constituents can be identified individually and the substance identity needs then to be based on its fractions/groups of constituents. "Block" is a term analogous to fraction/group and is used in the hydrocarbon block–approach (see Section R.11.4.2.2).

provides further insight into how to carry out PBT/vPvB assessment for fractions of the substance that cannot be fully identified by the registrant. For an example of a application of this recommendation in a specific industry sector, please see the *Environmental assessment guidance on essential oils*[16].

In specific cases it may be considered, for the sake of proportionality of assessment efforts and the level of risk being considered, to elevate or reduce the threshold value above or below 0.1% (w/w) for the PBT/vPvB assessment. Account could be taken of, e.g. the use pattern of the substance and the potential emissions of the constituents, impurities or additives having PBT or vPvB properties. Careful consideration should be given especially when uses are known or anticipated to cause significant emissions.

An elevated threshold value should not exceed 10% (w/w) for the total amount of all constituents, impurities and additives for which conclusion on PBT/vPvB properties can not be reached, and the total amount of these within the manufactured/imported substance should in no case exceed 1 t/year. A reduced threshold might be necessary to derive information relevant for PBT/vPvB assessment, e.g. for very toxic substances, and the information on the toxicity derived for the classification and labelling purposes could be used for defining such a lower concentration limit for PBT/vPvB assessment.

Especially for very complex **UVCBs** it is possible that individual constituents are present in concentrations <0.1% (w/w) and that these have not been characterised by chemical analysis individually. For UVCBs even the whole substance may consist of individual constituents only present in such low concentrations. The fact that all individual constituents of a UVCB-substance are present in concentration <0.1% (w/w) does not automatically exempt the registrant from the obligation to carry out the PBT/vPvB assessment. A close structural similarity of individual constituents within a fraction of a UVCB substance, i.e. constituents with the same carbon number, chain lengths, degree and/or site of branching or stereoisomers, triggers the need to sum up the concentrations of these constituents and to compare the total concentration with the limit of 0.1% (w/w) in order to determine whether these constituents need to be covered in the PBT/vPvB assessment. Criteria for grouping or read across, as mentioned in the Advice on using read-across for UVCB substances, *Practical Guide* on "*How to use alternatives to animal testing to fulfil your information requirements for REACH registration*" and the "*Introductory note to the illustrative example of a grouping of substances and read-across approach*", should be applied to the determination and justification of such fraction and (an) appropriate approach(es) as provided in Section R.11.4.2.2 should be applied for the PBT/vPvB assessment.

Similarly, a **UVCB substance** which contains constituents in concentrations well above 0.1% (w/w) each, but also (a) large fraction(s) where constituents are individually <0.1% (w/w), cannot be concluded as "not PBT/vPvB" unless it can be justified with sufficient reliability that none of the constituents and fractions of minor constituents would cause a concern. For example, a UVCB-substance may contain ten constituents, present in a total concentration of 60% (w/w) and the remaining 40% of the composition consists of not fully identified constituents. All latter minor constituents are individually present in concentration of <0.1%

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[16] Environmental assessment guidance on essential oils: http://echa.europa.eu/support/substance-identification/sector-specific-support-for-substance-identification/essential-oils

(w/w) but are expected to be similar to each other structurally and hence expected to have similar degradation, bioaccumulation and toxicity-properties. Not only the ten constituents making the largest part of the substance, but also the remaining 40% of the composition would need to be assessed using the appropriate approach provided in Section R.11.4.2.2 and testing, where necessary.

The same principles, as described in the two previous paragraphs above for UVCB-substances, apply also to the constituents of **well-defined substances** and their impurity fractions.

It should be noted in this connection that in cases where large fractions of unidentified constituents are present at <0.1% w/w, the assessment efforts need to remain proportionate.

A close structural similarity of individual constituents within a fraction, determined by criteria of grouping or read across as mentioned above, means that the concentrations of constituents with P, B and T (or vP and vB) properties should normally be summed up in order to compare with the threshold of 0.1% (w/w). Structural similarity of the constituents justify assessing the constituents as if they were one substance in terms of their physico-chemical, degradation and bioaccumulative properties and effects. This recommendation relies on the assumption that the mode of action of similar constituents is the same and the fate properties are very similar, hence causing an exposure which triggers effects in humans and the environment as if the exposure were to one substance. This understanding of aggregated exposure (aggregated concentration) leading to corresponding aggregated effects draws from the same scientific basis as the concept of additivity ("joint action", "dose additivity", "concentration additivity", "additivity of toxicity"), used in many regulatory activities, e.g. in the CLP-Regulation (EC, 2012; ECB, 2003; Feron *et al.*, 2002). However, it should be noted, that if the criteria for read across are not fulfilled for degradation, bioaccumulation and (eco)toxicity in PBT-assessment and for the first two properties in the vPvB-assessment, such summing up is not applicable and the normal 0.1% (w/w) threshold should be applied.

Similar arguments apply to **relevant transformation/degradation products**. However, there is no set w/w threshold concentration for transformation/degradation products. A transformation/degradation product can be considered relevant for example if the concentration is continuously increasing or it seems to be stable during a degradation study. The PBT/vPvB assessment should normally be carried out for each relevant transformation or degradation product (see also "Assessment of relevant transformation/degradation products" under Section R.11.4.1.1.3).

It is not possible to draw an overall conclusion for the substance if the assessment of persistence has been concluded for one transformation/degradation product and the assessment of bioaccumulation or toxicity for another transformation/degradation product.

The registrant should endeavour to carry out a comparison of the relevant available data with the PBT/vPvB criteria for each relevant transformation/degradation product (or in case those cannot be ultimately identified: for each group or block of transformation or degradation products), respectively. If the registrant considers transformation/degradation products that are formed (or groups/blocks of them) as not relevant for the PBT/vPvB assessment, he should also clearly explain in the PBT/vPvB assessment the reasons why they are not relevant.

If the available and relevant screening and other information allows the registrant to conclude that the substance is not persistent using the screening threshold values as provided in Table R.11—2, then it may normally be assumed that the substance is mineralized quickly and is not likely to form transformation/degradation products relevant for the PBT/vPvB assessment. However, the available relevant screening or other information (including information from hydrolysis tests and field data) may indicate that transformation or degradation products relevant for the PBT/vPvB assessment are indeed formed. These indications should be addressed in the registrant's PBT/vPvB assessment either qualitatively or quantitatively.

Following the obligation of the registrant under Article 13(3) of REACH in the situation where new degradation simulation testing is necessary, the transformation and degradation products relevant for the registrant's own PBT/vPvB assessment are those products, which must be identified in tests C.23, C.24 and C.25 carried out in accordance with Council Regulation No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation No 1907/2006 (REACH) ("Test Methods Regulation"). It should be mentioned in particular that guideline C.24 requires that "*…in general transformation products detected at ≥ 10% of the applied radioactivity in the total water-sediment system at any sampling time should be identified unless reasonably justified otherwise. 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 limits given above, as this may indicate persistence. The latter should be considered on a case by case basis…*". Identification of continuously increasing transformation/degradation products with accompanying justification always applies when the registrant is in the situation of generating new degradation simulation data for the purpose of the PBT/vPvB assessment because he will have previously concluded that the substance may have PBT/vPvB properties.

For the situation where information from tests comparable to the standard degradation simulation tests mentioned above are already available to the registrant or the registrant considers it more appropriate to generate new degradation information in accordance with Section 2.1 of Annex XIII to the REACH Regulation other than degradation simulation test data (see Section R.11.4.1.1 for the other possibilities), the principles of the standard test guidelines mentioned above for identifying relevant transformation and degradation products should be applied by analogy.

It should be noted that authorities are not bound under the REACH Substance Evaluation and SVHC-identification processes to the stipulations of the Test Methods Regulation or other standards for defining what is a relevant transformation/degradation product but have the possibility to use other types of justified (concentration or formation rate) limits to define on a case-by-case basis which transformation/degradation products are relevant for their PBT/vPvB assessment (e.g, see the Support Document of the Decision to identify Bis(pentabromophenyl) ether as Substance of Very High Concern[17]). Guidance is given in Section R.11.4.2 on the assessment and testing strategy for substances with specific substance properties such as UVCBs or multi-constituent substances with several constituents, in relation to transformation/degradation products, and for substances with low water solubility, high adsorption or volatility requiring deviations from the standard PBT/vPvB assessment.

Benchmarking as a part of *Weight-of-Evidence*

Benchmarking can be used as a part of *Weight-of-Evidence* approach. The benchmarking approach is defined as the approach where the fate or behaviour of a substance is measured

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[17] https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807dd2e6

relative to the fate or the behaviour of a well-described substance(s), the so called benchmark chemical(s) (Adolfsson-Erici, *et al.* 2012). Benchmarking relies on the similarity between the fate or the behaviour of the benchmark chemical and the substance of interest in the environmental system being studied (McLachlan, *et al.*, 2017).

The benchmarking approach is a comparative approach and as such the available data on the benchmark substance and the substance of interest determine the reliability of the conclusion drawn from the benchmarking approach. The available data need to be comparable in terms of (1) test conditions and test set up, (2) tested organisms (species, age, size) and (3) data analysis and interpretation. Similarity of the benchmark chemical in aspects other than the one investigated, e.g., similar physico-chemical partitioning behavior in a degradation study, can lead to higher confidence in the benchmark approach. It is however noted that the benchmark approach is different from read-across, where structural similarity of the substances is important. The benchmark approach is relatively new, and it is recommended to follow developments in the field, e.g. via the ECHA website.

The benchmark chemical can be chosen to represent the worst-case benchmark, an equivalent case benchmark or a safe case benchmark in regard to the fate or behaviour of a chemical. For example, the benchmarking approach can be considered as a worst-case for bioaccumulation when the "well-characterised substance" is a benchmark substance that will not be eliminated by excretion, biotransformation, or respiration in the duration of a BCF experiment (Adolfsson-Erici, 2012). Respectively, an "equivalent case benchmark" or a "safe case benchmark" would be a benchmark substance that will have similar behaviour or a behaviour below a well-defined safe level (bioaccumulation criteria not met).

In the case of persistence, there is currently little experience of the approach. McLachlan, *et al.* (2017) provide some examples, e.g. measurement of the degradation rate of the chemical of interest relative to the degradation rate of another (benchmark) chemical with an already well characterized degradation behaviour.

If benchmarking is used to assess toxicity, it is recommended to report concentrations based on mass (e.g. mg/l), but also on molar basis (e.g. mmol/l). Comparison of toxicity on molar basis prevents bias from molecular weight differences (e.g. a low molecular weight substance would appear more toxic compared to a high molecular weight substance and toxicity of larger molecules may be underestimated, when compared only on mass per litre basis, even if the same amount of molecules are present per litre). This could be especially useful for MOCS and halogenated substances.

In the case of bioaccumulation, Inoue *et al.*, (2012) conducted a benchmarking exercise to compare the fish dietary BMF values measured for nine substances with their measured fish BCF values.

Co-exposure to a benchmark chemical with known bioaccumulation behaviour could provide additional indication of the bioaccumulation potential of the test substance. For example, if the derived BCF is well below or above that derived for a benchmark chemical with known bioaccumulation behaviour (e.g., PCBs) of similar hydrophobicity, this may further indicate that the test substance is less bioaccumulative (or non-bioaccumulative) or more bioaccumulative, respectively. The use of non-metabolisable benchmark chemicals in laboratory bioaccumulation tests can also facilitate the derivation of the other toxicokinetic parameters, including whole-body biotransformation rate constants (Lo *et al.* 2015; Saunders *et al.* 2020). DiMauro, 2018 conducted OECD TG 305 BCF tests using the minimised test design with four substances. Each substance was tested both alone and in the presence of four non-biotransformed reference substances. Comparing the treatments with the test chemicals on their own and the test chemicals in the presence of reference substances, the author concluded that the presence of conservative reference substances did not affect the biotransformation rate constant and BCF of the test chemicals.

When testing more than one substance in a bioaccumulation test, test substances should not interact with one another. The combined dose should be below the combined concentration which might cause toxicity and the potential for interactive effects, such as effects on metabolism should be taken into account (OECD, 2012a).

Furthermore, multimedia bioaccumulation factors (mmBAFs) have been used to support benchmarking for cyclic volatile methylsiloxanes when compared to a known strongly bioaccumulative substance, PCB 180 (Kierkegaard, 2011). The benchmarking approach has been used as part of a *Weight-of-Evidence* for comparing the depuration rate constant (k_2) and BMF derived from an experimental dietary BMF with other substances with known bioaccumulation potential. Such an exercise provides useful evidence for the bioaccumulation assessment.

For some examples for benchmarking in regulatory context see e.g. benchmarking to support bioaccumulation assessment in the Support Document of the Decision to identify Medium-Chain Chlorinated Paraffins (MCCP)[18], Dodecamethylcyclohexasiloxane (D6)[19] and "Dechlorane Plus"™[20] as Substances of Very High Concern. More endpoint specific benchmarking examples are provided under the endpoint specific assessment sections.

Reporting of the analytical methods as part of the environmental fate studies

OECD Test Guidelines (TGs) for simulation and bioaccumulation testing require the use of appropriate methods to sample, extract, and analyse the test substance and its transformation/degradation products (as well as metabolites) in environmental matrices (water, soil and sediments, suspended matter, animal tissues).

Respective OECD TGs specify the information that is required to be reported on the applied analytical methods. Information provided must be detailed enough to allow the independent assessment of the methods used and be reproducible by the description provided. Analytical method description shall include adequate details regarding each step necessary to perform the analyses such as sampling, pre-treatment, extraction, clean-up procedures, instrumental, quantification and data processing methods (e.g. peak smoothing, area reject, integration parameters). In general, the most appropriate method shall be selected and applied, and reasonable effort should be put in development and validation of such a method.

In cases where an appropriate method cannot be developed for the target substance, there must be justification with supporting evidence to show why it is technically not possible to do so. All the efforts made to develop a substance specific method should be reported considering appropriate fit-for-purpose approaches and state of the art techniques and methods,

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[18] https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e185f78852

[19] https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1826466a3

[20] https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e181f392bf

emphasising which specific issues do not allow to conduct the study and achieve its objectives. Such justification should at least include descriptions of test system, sample stability, the applied instrumental techniques and extraction- and clean-up methods with the results achieved. Absence of an existing standard method for a specific substance cannot be used as a justification for the study being technically not feasible.

Further information on analytical methods can be found for example from Vogel's Textbook of Quantitative Chemical Analysis (Vogel *et al.*, 2006) that gives an overview about available quantitative methods. Critical literature review of analytical methods applicable to environmental fate studies published on ECHA website[21] provides an overview of available techniques (chapter 5), guidance on validation criteria concerning analytical methods, such as LOD and LOQ (chapter 6.6) and a detailed description of commonly used extraction techniques (chapter 7). In addition, ECETOC (2013) provides information on relationships between extraction techniques and bioavailability. However, please note that development of analytical methods is a continuously evolving area of science, thus no recommendation on a most suitable analytical method(s) for a specific substance can be provided in the present guidance document.

Development of workflows for the quantification of non-target data from High-Resolution Mass Spectrometry (HRMS) is ongoing. Quantification of non-target data was demonstrated in a study using LC-HRMS based on the ionisation efficiencies of different substances (Liigand *et al.* 2020). However, data analysis workflows are not yet standardised (Hollender *et al.* 2019), but may potentially be considered as supporting analysis of environmental fate studies for example in prioritisation and environmental monitoring (e.g. McCord *et al.*, 2022).

To ensure availability of adequate information of the analytical methods used, the reporting requirements described in the respective OECD TGs must be followed and the relevant information must be provided. Information to be included in the registration dossier can be found in the ECHA's Practical guide 3 (How to report robust study summaries) (Version 3.0, June 2023), sections 5.2.3 and 5.2.4 for biodegradation and bioaccumulation tests, respectively. A review of current analytical methods applicable to environmental fate studies [21] provides an overview of the requirements to be fulfilled for the specific simulation studies in Chapter 3 (Tables 3.5-3.7) (Peter Fisk Associates, 2021).

Key information (not exhaustive list) to be reported where applicable includes details about the analytical methods used and information regarding the validity of the method:

- Sample preparation procedure, type of the extraction, clean-up method, pre-concentration step used, including solvents/reagents, sample storage stability and instrumentation used (e.g. solid phase extraction (SPE), pressurized solvent extraction (PLE), solvent extraction with extraction kits (QuEChERS))

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[21] This consultancy service report provides a critical literature review of feasibility of available analytical methods applicable to environmental fate studies in the context of the PBT/vPvB assessment. It summarises recent scientific developments, describes pros and cons of available analytical tools and provides guidance to improve the quality of the data obtained from environmental fate studies. Available online: https://echa.europa.eu/documents/10162/17228/pfab_750_06_wp4_echa_final_report_en.pdf/b3a7e562-bf9c-ef02-948f-eaf1b8f89e3f

- Materials, reference standards, methods, instrumentation, calculations
- Test material chemical and radiochemical purity and stability
- Efficiency of the extraction method (the efficiency of the extraction should be tested in a pre-study before performing the fate study); total recovery of the method (%).
- Demonstration of the stability of the analyte (and transformation/degradation products, if applicable) in relation to the extraction method used (also to be assessed as part of a preliminary study)
- Instrumentation used (e.g. HPLC-UV, LC-MS/MS, GC-MS, HRMS, NMR, LSC etc.)
- Representative chromatograms/spectra, quantitative reports (controls, blanks, spikes, standards, samples)
- Repeatability and sensitivity including the limit of detection -and quantification (LOD/LOQ) and working range
- Conclusions (applicability)

R.11.4.1.1 Persistence assessment (P and vP)

R.11.4.1.1.1 Integrated assessment and testing strategy (ITS) for persistence assessment

A strategy for degradation assessment and testing in the context of PBT/vPvB assessment is proposed in Figure R.11—3. A tiered approach to assessment and testing is necessary until a definitive conclusion on persistence can be drawn.

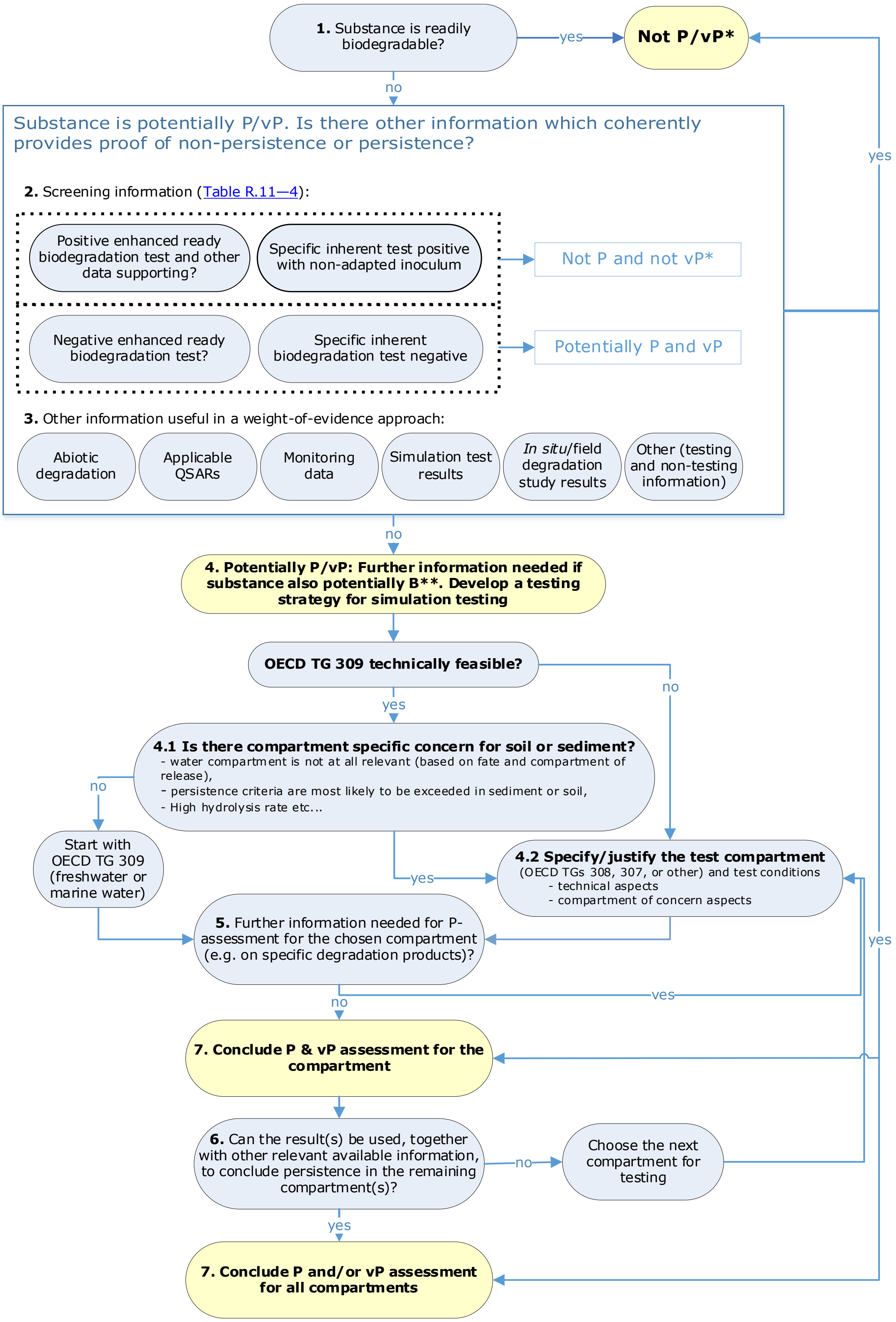
Available data consisting solely of screening information can be employed to derive a conclusion mainly for "not P and not vP" or "may fulfil the P or vP criteria". If screening information indicates that the substance "may fulfil the P or vP criteria", higher tier information generally needs to be made available.

Appropriate data need to be available to conclude the P/vP-assessment with a conclusion "not P/vP" on all three compartments (or five, with marine compartments): water (marine water), sediment (marine sediment) and soil. Either the available data, including in normal case simulation test data from one or two compartments, can be interpreted so that a conclusion can be derived on the remaining compartment(s) for which no higher tier data are available, or data need to be available directly on all compartments, or there is another justification for why a conclusion does not need to be drawn for all three (five) compartments.

In the opposite situation, if a conclusion "P" or "vP" is reached for one compartment, no further testing or assessment of persistence of other environmental compartments is normally necessary. In certain cases it may be possible to draw a conclusion "P" or "vP" based on screening information (e.g. tests on inherent biodegradation) combined with other useful information in a *Weight-of-Evidence* approach, as described later in this section and indicated in the ITS in Figure R.11—3.

Screening test(s) indicating "not P and not vP" may not be sufficient to conclude that a substance is not persistent, if there is contradicting information on persistence. In this case, additional information (e.g. QSAR, literature studies, read across) and quality of the screening tests should be considered in a *Weight-of-Evidence* assessment (see also Section R.7.9.4.1 in Chapter R.7b of the *Guidance on IR&CSA)*. The results of the higher tier degradation simulation studies (Annex XIII, Section 3.2: assessment information) are to be given more weight in the *Weight-of-Evidence* assessment than the screening studies (Annex XIII, section 3.1: screening information). In the presence of a reliable higher tier 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 higher tier study, supersede the screening tests.

For substances containing multiple constituents, impurities and/or additives, the guidance provided below apply to that/those "part(s)" of the substance, which is/are the target(s) of the assessment and testing. The criteria for selecting an appropriate assessment approach is provided in Section R.11.4.2.2.



*This conclusion (not P/vP) can not be drawn if the Weight-of-Evidence assessment indicates a P concern.

** In the context of the Biocidal Product Regulation (BPR), it is worth noting that the P-criteria has to be assessed also when the T-criterion is (potentially) fulfilled.

Figure R.11—3: Integrated Assessment and Testing Strategy for persistence assessment – maximising data use and targeting testing.

Integrated assessment and testing of Persistence - Explanatory Notes to Figure R.11—3.

1. *Evidence of ready biodegradation*

If the substance is readily biodegradable, or if the criteria for ready biodegradability are fulfilled with the exception of the 10-day window, there is normally no reason to perform further biodegradation tests for the PBT/vPvB assessment. The conclusion is that the substance is generally not regarded as fulfilling the criteria for Persistence (P or vP) (see Sections R.7.9.4 and R.7.9.5 in Chapter R.7b of the *Guidance on IR&CSA*, and for multi-constituent substances see Section R.11.4.2.2). However, based on a *Weight-of-Evidence* assessment, screening information indicating "not P and not vP" may not always exclude the substance from being persistent or even very persistent. If assessment information as listed in Section 3.2 of Annex XIII is available, then this information should be compared in a *Weight-of-Evidence approach* against the Annex XIII criteria rather than only relying on screening information[22]. Additionally, it should be ensured that the respective information is relevant and reliable.

2. *Other screening information (Table R.11—4)*

Following the ITS, and based on the screening information, the substance can be concluded as potentially P/vP or not P/vP according to the criteria and conditions described in Table R.11—4 and Sections R.7.9.4 and R.7.9.5 of Chapter R.7b of the *Guidance on IR&CSA*. After consideration of the Explanatory Notes bulleted below, and before concluding that a substance is "not P" or "not vP", it should be carefully examined if counter-evidence to that conclusion exists, e.g. from monitoring data or other available information (see Points 3-7 below for more information). When combined with all available information on persistence in a *Weight-of-Evidence*, the conclusion on persistence may cover one or multiple environmental compartments.

If the substance is confirmed to degrade in other biodegradation screening tests than the tests for ready biodegradability, the results may be used to indicate that the substance will not persist in the environment. Specific enhancement conditions described in Sections R.7.9.4 and R.7.9.5 of Chapter R.7b of the *Guidance on IR&CSA* can be used for this purpose. For example, a result of more than 60% ultimate biodegradability (ThOD, CO_2 evolution) or 70% ultimate biodegradability (DOC removal) obtained under the conditions specified in Chapter R.7b in an enhanced ready biodegradability test may be used to indicate that the criteria for P are not fulfilled (see Sections R.7.9.4 and R.7.9.5 in Chapter R.7b of the *Guidance on IR&CSA*. The enhancements may also be applied to standardised marine biodegradability tests (OECD TG 306, Marine CO_2 Evolution test, Marine BODIS test, and the Marine CO_2 Headspace test).

- **Assessment of inherent biodegradation test data** - Results of a Zahn-Wellens test (OECD TG 302B) or MITI II test (OECD TG 302C) only (not SCAS-test) may be used to confirm that the substance does not fulfil the criteria for P provided that certain additional conditions are fulfilled. In the Zahn-Wellens test, a level of 70% mineralisation (DOC

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[22] Judgment of the General Court of 9 June 2021, Exxonmobil v. ECHA, T-177/19, not published in European Court Reports, EU:T:2021:336, paragraphs 167-174 at paragraph 167.

removal) must be reached within 7 days, the log phase should be no longer than 3 days, and the percentage removal in the test before degradation occurs should be below 15% (pre-adaptation of the inoculum is not allowed). In the MITI II test, a level of 70% mineralization (O_2 uptake) must be reached within 14 days, and the log phase should be no longer than 3 days (pre-adaptation of the inoculum is not allowed). A lack of degradation in an inherent biodegradation test (≤20%) can provide evidence that degradation in the environment would be slow (see further consideration under "Tests on inherent biodegradation in the main text). It should however be noted that the very low solubility of many PBT/vPvB substances may reduce their availability and hence their degradability in the test. The lack of degradation in an inherent test does not always imply that the substance is intrinsically persistent and in some cases further testing might be needed.

- **Enhanced ready biodegradability tests (screening tests)–** Positive results from enhanced biodegradability tests may be used together with other supporting information to conclude that the substance is not P/vP. Enhanced ready biodegradability tests are allowed when there is a need to compensate the poor bioavailability i.e. the substance is poorly soluble and/or adsorptive. Only one enhancement is allowed at a time. The prolongation of the test duration up to 60 days should only be considered if some initial, slow but steady, biodegradation was observed not reaching a plateau by the end of the ready biodegradability test, i.e. after 28 days. However, it is important that the following conditions are met: 1) the enhancements should only be about an extended test duration or an increased test vessel size, 2) the test should be performed with non-pre-adapted/non pre-exposed inocula, 3) the test duration should never be extended beyond 60 days, and 4) the test criteria set for ready biodegradability tests should be applied, i.e. 60% or 70% degradation, depending on analyte, without the 10-day window. If the results are negative, then it is generally not possible to definitively conclude on the absence of persistence of the substance and further testing will be needed. More information on enhanced screening tests can be found in Sections R.7.9.4 and R.7.9.5 of *Chapter R.7b* of the *Guidance on IR&CSA*.

3. *Other information useful for a Weight-of-Evidence approach (not exhaustive)*

All available information on (bio)degradation, including testing, non-testing and monitoring data, should be considered. The overall evaluation could either show that the information available coherently provides proof of (non-)persistence and is sufficient to allow concluding the P/vP assessment, or indicate that further testing is needed. If further testing is needed a testing strategy should be developed following the ITS starting from step 4 below.

- **Use of (Q)SAR (both QSARs and SARs) estimates** – Refer to Section R.11.4.1.1.4 below on "Assessment based on estimation models (QSAR, SAR)", which describes QSARs appropriate for specific P/vP screening.
- **Use of pure culture data** – The data derived from studies with pure culture(s), single species or mixture of species, cannot be used on their own within persistence assessment but should be considered as part of a *Weight-of-Evidence* approach.
- **Use of information on anaerobic degradation** – The data derived from anaerobic degradation studies cannot be used on their own within persistence assessment but should be considered as a part of a *Weight-of-Evidence* approach.
- **Use of information on any other degradation studies** – The data derived from degradation studies other than those described above cannot be used on their own within

persistence assessment but should be considered as a part of a *Weight-of-Evidence* approach (e.g. OECD TG 314).

- **Abiotic degradation** – Concern for P/vP screening cannot be removed by significant and substantial loss of the parent substance by hydrolysis alone. Careful consideration of the hydrolysis test is required (for example mass balance is needed to address concerns for losses by volatilisation or adsorption to glassware). Rapid hydrolysis also needs to be shown across all environmentally relevant pH. Additional evidence is also needed to examine whether the fate properties of the substance would cause attenuation of the hydrolysis rate in sediment or soil, or whether DOC would similarly affect the rate in aquatic media such as river or sea water. Additional studies, e.g. examining the influence of dissolved organic carbon / adsorption processes on hydrolysis rates, may be necessary for this. In biodegradation studies, including sterile controls as a part of study design is useful to indicate abiotic degradation in relevant conditions (see Sections R.11.4.1.1.2 and R.7.9.4.1. in Chapter R.7b of the *Guidance on IR&CSA* for more details). The degradation half-lives obtained in a hydrolysis test cannot be directly compared to the persistence criteria of Annex XIII. As abiotic degradation is primary degradation, careful consideration will need to be given to the potential formation of stable degradation products with PBT/vPvB properties. Hydrolysis products should be identified in accordance with the recommendations contained in the test guidelines (e.g. OECD TG 111).
- **Use of other abiotic data** – Data derived from other abiotic studies (e.g. photodegradation, oxidation, reduction) cannot be used on their own within persistence assessment, but may be used as part of a *Weight-of-Evidence* approach. 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 Chapter R.7b of the *Guidance on IR&CSA*.
- **Field studies –** Data derived from field studies (e.g. mesocosm) may be used as part of a *Weight-of-Evidence* approach. This is discussed in more detail in Section R.11.4.1.1.5 below named "Field studies for persistence".
- **Monitoring data** – If monitoring data, used as part of a *Weight-of-Evidence* analysis, show that a substance is present in remote areas (i.e. long distance from populated areas and known point sources, e.g. Arctic sea or sub-Arctic/Arctic lakes in Scandinavia), it may be possible to conclude a substance as P or vP. 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(in both directions: P/vP or not P/vP). Use of monitoring data in P/vP-assessment encompasses several uncertainties and conclusions should be drawn on the basis of monitoring data only when there is sufficient understanding of the substance distribution and transport behaviour and under the condition that the uncertainties in the monitoring data presented are adequately addressed. The lack of detection of a substance in monitoring data should be considered carefully as it does not necessarily mean that a substance is not persistent (e.g. shortcomings in analytical methods may affect monitoring of substances in the environment). If monitoring data show that the substance levels in environmental media or biota are rising, the reasons for such a time trend should be assessed very carefully against the information on the time trends of volumes, uses and releases. Archived samples from environmental specimen banks, dated sediments cores and ice cores can be used to gain understanding on temporal changes. Where monitoring data clearly indicate that the substance fulfils the vP-criterion or, depending on the case, that the P criterion is fulfilled in addition to other supporting information (and without any conflicting

data), it may not be necessary to generate simulation degradation data. In the latter case, conclusions on the fulfilment of the P/vP criteria may be drawn based on the monitoring data, the information on the substance distribution/transport behaviour, in addition to other supporting information used as part of a *Weight-of-Evidence analysis*.

4. Further information needed to conclude on P/vP – Testing strategy to be developed as described below

If further degradation testing is needed based on steps 1 to 3 of the ITS, a testing strategy on persistence should be developed. The testing strategy should aim to conclude on persistence with the least possible efforts in testing and at the same time cover the assessment of persistence in all environmental compartments (marine water, fresh or estuarine water, marine sediment, fresh or estuarine sediment and soil).

4.1. Identification of any specific environmental compartment(s) of concern

This paragraph describes the part of the ITS where the need for further testing has been identified and there is a need to make a decision on the test compartment(s).

In general, it is recommended to start testing with the OECD TG 309 if it is technically feasible. However, if there is evidence that the OECD TG 309 does not provide means to reflect the persistence of the substance in the environment, other environmental compartments may be considered as first test environment. For example, in case a P/vP criterion is expected to be exceeded in (a) compartment(s) other than water or if the substance hydrolyses fast in environmentally relevant conditions, this should be taken into account in the testing strategy. If, based on the fate and release(s) of the substance, it is considered that water compartment is not a relevant environmental compartment at all, this should also be taken into account in the testing strategy.

If the OECD TG 309 is not technically feasible, selection of the most relevant environmental compartment to test first should be justified (Step 4.2).

OECD TG 309 should be preferred for the following reasons:

- Firstly the aquatic compartment is considered to be a relevant environmental compartment due to the large global volume of water: by default water compartment receives significant amount of emissions directly or indirectly, and transports/distributes the substance through e.g. deposition and run-off (unless based on the fate and release(s) of the substance, it is considered that the water compartment is not a relevant environmental compartment at all). Once entering water, a substance may stay there for very long time and be spread over long distances before it reaches other environmental compartments (*via* environmental transport, partitioning and distribution processes) such as sediments or (*via* air) the soil compartment;
- Particularly for lower water solubility substances which tend to be adsorptive, the OECD TG 309 (with a default concentration of suspended solids of 15 mg_{dw}/L, see section below on OECD TG 309) minimizes potential NER formation. If NER is formed at significant levels in the OECD TGs 307 and 308 studies, this can be difficult to interpret and compare with degradation half-lives criteria of Annex XIII to the REACH Regulation;
- OECD TG 309 is conducted under aerobic condition (there is no "anaerobic" option). This is considered as a relevant test condition as P assessment should first consider aerobic degradation. In general, a test using exclusively anaerobic conditions is not required as a

first step. For further information, see Section R.11.4.1.1.3 below, under "aerobic and anaerobic conditions".

It should be noted that, at this step, considerations of complete absence of uses/releases, and thereby exclusion of the need to test a certain environmental compartment, is not discussed under this section. Further information on exposure-based exclusion of testing may be found in this Guidance under Section R.11.3 and Figure R.11—2.

Information on degradation and from environmental monitoring data, emissions estimated in the CSR, distribution modelling data (e.g. Mackay Level III and SimpleTreat v4.0) and physico-chemical information should be assessed to determine whether there is an environmental compartment (pelagic surface water, pelagic marine, sediment, marine sediment or soil) of specific concern for persistence. The driving factor for the assessment is that a conclusion needs to be derived for all three (five) environmental compartments with the least possible testing efforts. The specific concern for persistence is normally present for the environmental compartment for which the P/vP criteria are most likely to be exceeded or where the expected degradation half-life is the closest to the criteria. Consideration of the environmental compartment(s) of most relevant exposure may also play a role in the identification of the specific environmental compartment for testing. Absence of exposure in a specific environmental compartment may, in exceptional cases, be acceptable to exclude certain compartments from the P/vP assessment.

The following pieces of evidence may help in the identification of the potential environmental compartment of specific concern:

- Any available information suggests that (abiotic and bio-) degradation rates/half-lives are expected to meet the P/vP criteria for a specific environmental compartment;
- Environmental monitoring data suggesting persistence is likely in a particular environmental compartment for a substance;
- Direct discharge to an environmental compartment is expected to occur;
- The life-cycle is well characterised and the environmental emission and exposure assessment (including environmental fate, modelling and/or monitoring data) show that a specific environmental compartment is exposed.

If any environmental compartment other than surface water is chosen for simulation degradation testing, a justification should be provided (see step 4.2 below).

4.2. Specify/justify the test compartment

As explained above (step 4.1) the OECD TG 309 is the preferred test. If another test is selected for further testing, this should be justified. Possible reasons are listed below:

- OECD TG 309 is typically performed at concentrations between 1 and 100 µg/L and preferably in the range of <1-10 µg/L (to ensure that biodegradation follows first order kinetics).
- Generally, when water solubility of a substance is very low (typically below 1 µg/L), testing on sediment (OECD TG 308) and/or soil (OECD TG 307) may be needed instead of a pelagic test (OECD TG 309). The detection limit(s) of analytical methods of quantification needs to be taken into account when designing the test setup.
- Aquatic testing is not technically feasible. Technically feasible means that it has been impossible, with allocation of reasonable efforts, to develop suitable analytical methods and

other test procedures to accomplish testing in surface water so that reliable results can be generated. Appropriate analytical methods should have a suitable sensitivity and be able to detect relevant changes in concentration (including that of transformation/degradation products).

- Indications from available data (e.g. literature) suggest that persistence is likely to occur in a different environmental compartment (i.e. in soil or sediment), including evidence of direct or indirect exposure.
- The substance is a multi-constituent / UVCB which affects the test substance concentration at which the test can be performed (i.e. due to different multiple water solubilities of the individual constituents).

Please see also further considerations on the simulation testing strategy in Section R.11.4.1.1.3 below.

5. *Is there further information needed to conclude on persistence for the tested environmental compartment?*

The information obtained from the performed tests should be assessed and the results compared with the REACH Annex XIII criteria for P/vP:

- If the substance or its degradation products are concluded to be persistent or very persistent, there is no need for further testing for persistence assessment.
- If the substance and its degradation products are concluded to be non-persistent in the tested environmental compartment it should be verified that there is no concern in remaining compartments (see step 6).

6. *Remaining concern in untested environmental compartments*

It should be considered whether the available information is adequate to conclude persistence assessment for all or some of the remaining environmental compartments for which there are no testing data. If it can be concluded that the P and/or vP criteria are fulfilled in one environmental compartment, then no further information is needed for the other compartments (see above step 5).

In general, results of a single simulation degradation study demonstrating non persistence in one compartment cannot be directly extrapolated to other non-tested environmental compartments. However, the results could be sufficient to conclude on persistence or very persistence in other compartments, provided that the relevant test conditions have been applied and the interpretation of the results/bridging is backed by proper justifications. Availability or generation of multiple simulation test data may allow more *Weight-of-Evidence* based conclusions to be drawn by expert judgement regarding environmental degradation half-lives for one or more environmental compartments. At this point of the flow chart, a decision on whether the data cover one, two or all five environmental compartments should be made on a case-by-case basis.

It should be highlighted that the requirement is to draw a conclusion for all three (five) environmental compartments (see REACH Annex I, Section 3.0.2). If for the first tested compartment a conclusion “not P” could be derived, but the available data are not sufficient for drawing conclusions in (an)other compartment(s), further data generation is necessary to complete the assessment for the compartments for which a conclusion could not be drawn. Exclusion of (a) certain environmental compartment(s) from the P/vP assessment based on absence of exposure may be acceptable only in very exceptional cases and upon justification. A justification of absence of exposure in (a) certain environmental compartment(s) is different from a justification for the purpose of normal quantitative risk assessment, because for (potential) PBT/vPvB substances, and hence for the PBT/vPvB assessment, distribution over a very long timespan would need to be considered as well.

7. Evaluation versus the P and vP criteria

The half-life (lives) obtained from the simulation data is evaluated against the criteria of Annex XIII to the REACH Regulation for the three (five) environmental compartments to determine whether the P or vP criteria are met or not. Before finally concluding that a substance is "not P" or "not vP", it should be carefully examined if there exists conflicting evidence from monitoring data, either from national monitoring programmes of Member States (e.g. Swedish national monitoring data collection[23]), from European monitoring programmes (e.g. NORMAN Network[24]) or internationally acknowledged organisations (such as OSPAR or the Danube Convention). For example, findings of significant concentrations of the substance under consideration in remote and pristine environments such as the Arctic sea or Alpine lakes need to be scrutinized carefully as there may be evidence of high persistence. Also, significant concentrations of the substance in higher levels of the food chain in unpolluted areas may indicate high persistence (beside a potential to bioaccumulate). If such evidence indicates that the substance may be persistent, further investigations are required.

R.11.4.1.1.2 Introduction to persistence assessment

When assessing data concerning the persistence of a substance and, if necessary, determining the next steps of the assessment, there are a number of stages to go through. The first part of the assessment should address the extent to which available data enable an unequivocal assessment to be made. These data may comprise simple screening biodegradation tests (e.g. OECD TG 301C ready biodegradability MITI I test) or complex, high-tier simulation tests (e.g. OECD TG 308 aerobic and anaerobic transformation test in aquatic sediment systems). At this stage, it is only necessary to assess the strength of the data in one direction or another. Thus, for example, when an OECD TG 301 study indicates that the substance is readily biodegradable, the decision that a substance is not P could normally be taken. However, , a positive (meeting the set criteria) ready biodegradation test does not exclude that, in some cases, further studies may be required[25]. This is the case when based on the *Weight-of Evidence* there are indications of potential persistence. For example, if a simulation test is available, this data should be used in the assessment rather than only relying on screening information. If a reliable simulation test with a degradation half-life fulfillling the P/vP criteria is available, this would be sufficient to decide that the substance meets the P and vP criteria.. However, as described in Section R.7.9 of *Chapter R.7b* of the Guidance on IR&CSA, a negative

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[23] http://dvsb.ivl.se/dvss/DataSelect.aspx

[24] http://www.norman-network.net/

[25] Judgment of the General Court of 9 June 2021, *Exxonmobil v. ECHA*, T-177/19, not published in European Court Reports, EU:T:2021:336, paragraphs 167-174.

result in a test for ready biodegradability does not necessarily mean that the substance will not be degraded under relevant environmental conditions and persist in the environment. Indeed, there are some cases where ready biodegradation tests have been reported to underestimate the potential for degradation in real environmental conditions (Guhl and Steber, 2006). A failed ready biodegradability test may indicate the need for further testing under less stringent test conditions (e.g. enhanced biodegradation tests, simulation tests…). In addition, all relevant degradation pathways (biotic, abiotic, aerobic, anaerobic conditions) need to be considered with regard to the relevant route of exposure before concluding on persistence.

Often, biodegradation data are not so clear-cut, and frequently they are different and/or contradictory. Therefore careful consideration is needed before a decision is taken in order to avoid a false negative or false positive conclusion. The strategy outlined in this section is a recommendation and is not intended to be an explicit prescriptive description of the sequence of steps to be taken. Ultimately the actual route taken will depend upon the data available and the physico-chemical properties of the substance being assessed. As a minimum, and where possible and technically feasible, information on vapour pressure, water solubility, octanol/water partition coefficient (K_{ow}), other partition coefficients (such as the octanol-air partition coefficient (K_{oa}) and organic carbon normalised adsorption coefficient (K_{oc})), basic dissociation behaviour (if relevant), surface active properties (if relevant) and Henry's law constant must be available. The impact of these data on the test design and data interpretation should be considered.

With regard to persistence, it is insufficient to consider removal alone where this may simply represent the transfer of a substance from one environmental compartment to another (e.g. from the water phase to the sediment). Degradation may be biotic and/or abiotic (e.g. hydrolysis) and result in complete mineralisation, or simply in the transformation of the parent substance (primary degradation). Where only primary degradation is observed, it is necessary to identify the degradation products and to assess whether they possess PBT/vPvB properties. Other properties of transformation/degradation products of specific concern (e.g. ED properties[26]) may also be relevant to be further considered. In addition to the substance intrinsic properties, its transformation and/or degradation is dependent on the surrounding environment.

The following sections give guidance on how to address data from biodegradation studies, abiotic degradation studies and information available from estimation models (QSARs/SARs). A subsequent section addresses information generation and particularly how to choose the correct compartment for further testing. As mentioned above, the sequence in which the subjects of these sections are addressed will depend upon the data available. Furthermore, most of the information reported in this guidance is further developed under the endpoint-specific guidance on degradation, which should also be consulted (see Section R.7.9 in *Chapter R.7b* of the *Guidance on IR&CSA*).

In case only screening information is available, screening threshold values listed in Table R.11—4 can be used to judge whether an ultimate conclusion on the persistence of a

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[26] Annex I (Part 4) to CLP Regulation (EC) No 1272/2008 was amended to include Classification criteria for PBT/vPvB. These criteria include that a substance classified under the CLP as endocrine disuptor for human health (Cat. 1) or endocrine disruptor for the environment (Cat. 1) fulfills the T criterion under the CLP hazard class 'Persistent, Bioaccumulative and Toxic or Very Persistent, Very Bioaccumulative properties' (19 Dec 2022).

substance can be made or whether further information is needed. It should be noted that screening criteria can only be applied as provided. The triggers were originally derived for drawing only those conclusions indicated in Table R.11—4 and are not recommended to be used to draw other conclusions. (However, it should be noted that these criteria are indicative and the assessor should consider the relevance of any other indications e.g. other experimental data than standard simulation and screening tests, other QSARs, monitoring data and expert judgement, before drawing a conclusion.)

Table R.11—4: Screening information for P and vP.

	Screening information	Conclusion
Persistence		
Biowin 2 (non-linear model prediction) and Biowin 3 (ultimate biodegradation time)	Does not biodegrade fast (probability < 0.5)* and ultimate biodegradation timeframe prediction: ≥ months (value < 2.25 (to 2.75)**)	Potentially P or vP
or Biowin 6 (MITI non-linear model prediction) and Biowin 3 (ultimate biodegradation time)	**or** Does not biodegrade fast (probability < 0.5)* and ultimate biodegradation timeframe prediction: ≥ months (value < 2.25 (to 2.75)**)	Potentially P or vP
or other models *	**or** Model specific values	Potentially P or vP
Ready biodegradability test (including modifications allowed in the respective TGs)	≥70% biodegradation measured as DOC removal (OECD TGs 301A, 301E and 306) or ≥60% biodegradation measured as ThCO$_2$ (OECD TG 301B) or ThOD (OECD TGs 301C, 301D, 301F, 306 and 310)***	Not P and not vP
	<70% biodegradation measured as DOC removal (OECD TGs 301A, 301E and 306) or <60% biodegradation measured as ThCO$_2$ (OECD TG 301 B) or ThOD (OECD TGs 301C, 301D, 301F,306 and 310)	Potentially P or vP
Enhanced ready biodegradability tests****	Biodegradable (pass levels as for ready biodegradability tests above) ****	Not P and not vP
	Not biodegradable****	Potentially P or vP
Specified tests on inherent biodegradability:		
- Zahn-Wellens (OECD TG 302B)	≥70 % mineralisation (DOC removal) within 7 d; log phase no longer than 3d; removal before degradation occurs below 15%; no pre-adapted inoculum	Not P and not vP
	Any other result*****	Potentially P or vP
- MITI II test (OECD TG 302C)	≥70% mineralisation (O2 uptake) within 14 days; log phase no longer than 3d; no pre-adapted inoculum	Not P and not vP
	Any other result*****	Potentially P or vP

* The probability is low that it biodegrades fast (see Section R.7.9.4.1 in Chapter R.7b of the *Guidance on IR&CSA*). Other models are described in Section R.7.9.3.1 of Chapter R.7b of the *Guidance on IR&CSA* and in this section below.

** For substances fulfilling this but BIOWIN 3 indicates a value between 2.25 and 2.75 more degradation relevant information is generally warranted.

*** These pass levels have to be reached within the 28-day period of the test. The conclusions on the P or vP properties can be based on these pass levels only (not necessarily achieved within the 10-d window) for monoconstituent substances. For multi-constituents substances and UVCBs these data have to be used with care as detailed in Section R.11.4.2.2 of this Guidance. "Not P/vP" conclusion may not be possible if WoE assessment indicates potential for persistence.

**** See Sections R.7.9.4 and R.7.9.5 in *Chapter R.7b* of the *Guidance on IR&CSA*. Expert judgement and or use of *Weight-of-Evidence* also employing other information may be required to reach a conclusion (i.e. concerning « biodegradable/ not biodegradable »). "Not P/vP" conclusion may not be possible if WoE assessment indicates potential for persistence.

*****see section R.11.4.1.1.3 for concluding ultimately on persistence in particular cases (in particular "Tests on inherent biodegradation"). "Not P/vP" conclusion may not be possible if WoE assessment indicates potential for persistence.

In the ITS for persistence assessment (Figure R.11—3), the types of simulation degradation tests that should be considered is indicated. The information in Table R.11—5 below presents the criteria for the assessment of persistence (P/vP) and identifies relevant test systems for determining environmental degradation half-lives.

Table R.11—5: Persistence (P/vP) criteria according to Annex XIII to the REACH Regulation and related simulation tests.

According to REACH, Annex XIII, a substance fulfils the P criterion when:	According to REACH, Annex XIII, a substance fulfils the vP criterion when:	Biodegradation simulation tests from which relevant data may be obtained include:
The degradation half-life in marine water is higher than 60 days, or The degradation half-life in fresh- or estuarine water is higher than 40 days, or	The degradation half-life in marine, fresh- or estuarine water is higher than 60 days, or	OECD TG 309: Simulation test – aerobic mineralisation in surface water
The degradation half-life in marine sediment is higher than 180 days, or The degradation half-life in fresh- or estuarine water sediment is higher than 120 days, or	The degradation half-life in marine, fresh- or estuarine sediment is higher than 180 days, or	OECD TG 308: Aerobic and anaerobic transformation in aquatic sediment systems
The degradation half-life in soil is higher than 120 days	The degradation half-life in soil is higher than 180 days	OECD TG 307: Aerobic and anaerobic transformation in soil

R.11.4.1.1.3 Test data on biodegradation

In principle, there are three types of tests that measure biological degradation:

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

Tests on ready and inherent biodegradability contribute information at a screening level whilst simulation tests are adequate to assess degradation kinetics, degradation half-lives, information about mineralisation, non-extractable residues (NERs) and transformation/degradation products (extracted residues).

In order to select the appropriate test type, careful consideration of the physico-chemical properties and the environmental behaviour of a substance is required, which is discussed later on in this section.

For further information on test descriptions refer to the degradation guidance (see Sections R.7.9.3 and R.7.9.4 in *Chapter R.7b* of the *Guidance on IR&CSA*).

Tests on ready biodegradation

Tests on ready biodegradation are described in OECD TG 301 A-F and OECD TG 310. Biodegradability in Seawater test (OECD TG 306) can also be used to describe the ready biodegradability in sea water. Degradation is followed by determination of parameters such as dissolved organic carbon (DOC), CO_2 production or oxygen uptake. The parameter measures the mineralisation and the pass level is set to 60% (ThOD or $ThCO_2$) or 70% for DOC removal assuming that the yield for growth of the microbial biomass is 30-40%. In the context of ready biodegradability, test substance-specific analysis can also be used and primary degradation and formation of any transformation/degradation products can be assessed. Measurement of primary degradation is however a requirement only in the MITI I test (OECD TG 301C).

Due to the fact that the test methodology for the screening tests on ready biodegradability is stringent, a negative result does not necessarily mean that the substance will not be degraded relatively fast under environmental conditions. A lack of biodegradability may for example be caused by toxicity of the substance towards microorganisms due to the very high concentration employed in ready biodegradability tests compared with lower, environmentally relevant concentrations. Another reason for negative outcomes in ready biodegradability tests can be low water solubility of the test substance. A low solubility could constitute the rate limiting step for degradation at the environmentally unrealistic high test substance concentrations and not the intrinsic recalcitrance towards microbial transformation. ISO method 10634 and Annex III of OECD TG 301 also describe options to address poorly soluble substances.

Given the time, costs and, in some cases, practical difficulties associated with conducting and interpreting a simulation degradation test, an enhanced ready biodegradation test design offers a cost-effective intermediate screening test in those cases where persistence in the environment is not expected although (a) standard ready biodegradation test(s) give(s) the result "not readily biodegradable". The purpose of enhancements should only be to compensate the poor bioavailability to the degrading microorganisms due to poor solubility and/or adsorptive properties of the substance, but should not be used to induce additional adaptation of the inoculum. If sufficient degradation is shown in an enhanced ready biodegradation test, i.e. the pass level as given in the test guidelines for ready biodegradation is reached, the substance can be considered as "not P". It should be noted that, in this case, the 10-day window indicated in the corresponding test guideline does not need to be fulfilled. According to Gartiser *et al.* (2022), similar to inherent biodegradation tests, degradation below 20% in enhanced ready biodegradability tests could be used as part of *Weight-of-Evidence* to indicate persistence. In general, under PBT assessment the conclusion that a substance is P/vP cannot only be based on screening level information (including enhanced tests). However, 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 higher tier studies indicate non-persistence. Furthermore, as explained in the section below, under some conditions results of inherent biodegradability test may provide sufficient information to confirm that the P-criteria are fulfilled without the need for further simulation testing.

More information on modificationns of screening tests such as ready biodegradability tests or enhanced ready biodegradability tests is contained in Sections R.7.9.4 and R.7.9.5 of *Chapter R.7b* of the *Guidance on IR&CSA*. Please note that these tests are referred to as "enhanced ready biodegradability tests". Allowed enhancements are prolongation of the test duration and testing in larger vessels. Only one enhancement is allowed at a time. The prolongation of the test duration up to 60 days should only be considered if some initial, slow but steady, biodegradation was observed not reaching a plateau by the end of the ready biodegradability test, i.e. after 28 days.

The variation in the degradation potential of different constituents must be considered when conducting screening tests with multi-constituent or UVCB substances (see further information in Section R.11.4.2.2). In general, feasibility of testing the whole substance to demonstrate degradation of all of its constituents must be evaluated.

Tests on inherent biodegradation

Tests on inherent biodegradability are useful to give an indication of biological degradability on a screening level. Inherent tests are similar to ready biodegradability tests as they usually measure sum 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.

Due to the more favourable conditions of an inherent test, results need to meet specific criteria (specified in Table R.11—4 above and Section R.7.9.4.1 "Data on degradation/biodegradation" of *Chapter* R.7b of the *Guidance on IR&CSA*) in order for a substance to be considered as not P/vP.

Lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD TG 302 series may provide sufficient information to confirm that the P-criteria are fulfilled without the need for further simulation testing for the purpose of PBT/vPvB assessment. 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). The tests provide optimum conditions to stimulate adaptation of the micro-organisms thus increasing the biodegradation potential, compared to natural environments. A lack of degradation therefore provides evidence that degradation in the environment would be slow. Care should be taken in the interpretation of such tests, however, 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 Sections R.7.9.4 and R.7.9.5 of *Chapter R.7b* of the *Guidance on IR&CSA*.

Tests on simulation of biodegradation

In principle, degradation simulation studies performed in appropriate environmental media and at environmentally relevant conditions are the only tests that can provide a definitive degradation half-life that can be compared directly to the persistence criteria as defined in REACH Annex XIII. Such tests allow both biotic and abiotic degradation processes to operate. The simulation tests as described in OECD TGs 307, 308 and 309 address the fate and behaviour of a substance as it may be expected in the environment including information about partitioning in the test system, primary or complete degradation, adsorption behaviour and route(s) of transformation/degradation (transformation/degradation products). The endpoints that need to be addressed are primary or ultimate degradation rate and degradation half-life (DegT50) or dissipation half-life (DT50) for the compartments included in the test system as well as the route of degradation, transformation/degradation products and non-extractable residues. In addition, a mass balance is included in these tests and therefore possible losses from the test system during the test period can also be quantified. An incomplete mass balance will introduce severe uncertainty to interpretation of data. This in turn can ultimately impede that a substance can be assessed with sufficient certainty and possibly cause the need to repeat the respective test and/or to give a low weight to the test and its results in the P/vP assessment as part of a weight-of-evidence approach. A simulation study should be performed using a radio-labelled molecule, whenever feasible.

In order to evaluate the outcome of a simulation test, the reporting of the results should follow the respective test guideline(s). Tests should report the degradation rate (or degradation half-life) in each medium determined through mineralisation, e.g. volatile $^{14}C\text{-}CO_2$, and/or direct substance analysis. An option, if measuring mineralisation, is to measure the mineralisation rate for the whole system: if the mineralisation half-life for the whole system is below the respective half-life –value of P/vP criteria, it has been shown that the substance is 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. When the mineralisation half-life for the whole system is above the P criterion, further analysis of the data is needed. A full mass balance of the substance and any transformation/degradation products should be determined (or justification provided if this is not technically feasible), and a determination of the level of non-extractable residues should be included. In general, determination of non-extractable residues is recommended 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 all cases, the extraction method for NER and the choice of extraction solvents should be justified (see more detailed information on NER below in this section). Where primary degradation is observed, the identity of possible relevant transformation/degradation products must also be determined and/or evaluated as regards their possible PBT/vPvB-properties. Where only degradation of the parent substance is monitored, this does not address all the concerns and further assessment of the transformation/degradation products may be required in order to complete the PBT/vPvB assessment (see Sections R.7.9.4 and R.7.9.5 in C*hapter R.7b* of the *Guidance on IR&CSA*).

Degradation half-life (DegT50[27]) derivation

For direct comparison to the P/vP criteria only estimates of degradation half-life (DegT50) are appropriate. Such estimates are mostly based on data derived from simulation biodegradation

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[27] 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.

tests. Degradation half-life (DegT50), 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 is independent of concentration and time. The methods for estimating degradation half-life are described in the paragraphs below. 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). Dissipation comprises two main types of processes: degradation processes (such as microbial degradation, hydrolysis and/or photolysis transforming substances into degradation products) and transfer processes (such as volatilisation, and adsorption). Both processes may affect the disappearance of the test substance from the system. If transfer processes have occurred simultaneously with degradation, the DT50 value is not representative of the DegT50 value.

In the following paragraphs advice is provided on the degradation half-life estimation, the volatilisation correction of the data as well as normalisation approaches in the cases when an incomplete mass balance is observed. 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 the Generic Guidance Document for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (FOCUS, 2014).

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). In the OECD 309, lag phase is defined as the time from the start of a test until adaptation of the degrading micro-organisms is achieved and the biodegradation degree of a chemical substance has increased to a detectable level. OECD TG 309 includes also more specific advice indicating that the lag phase duration is estimated from the degradation curve (semi-logarithmic plot) by extrapolating its linear part to zero degradation or alternatively by determining the time for approximately 10% degradation.

In simulation studies (OECD TG 309, OECD TG 308 and OECD TG 307), the possibility to detect a lag phase and to accurately define its duration depends on the lowest degradation (e.g. a decrease in test substance concentration, or amount of CO2 produced) that is detectable by the corresponding test method (influenced e.g by the test substance concentration and the analytical method used, on the number and frequency of measurements and the degradation rate). Therefore, in simulation testing, even not detectable biodegradation of the test substance might occur already during the lag phase. 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). 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[28]. Prior to degradation kinetic analysis, a qualitative assessment should be made 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. In general, efforts to identify experimental artefacts in laboratory studies must be made. In the case experimental artefacts (e.g. adsorption to test vessels) are observed the acceptability of the study must be carefully investigated. For DegT50 derivation model fits should initially always include all the data (for data sets with a lag phase this does not always apply; see FOCUS 2014 and OECD TG 309 paragraph 44 for further information). After the initial fit, any outlier data points could then be excluded, and the fit should be repeated. The decision to exclude any data point as an outlier is usually based on a clear deviation from the values (or the trend of the values) of the other data points in the same data series and/or from the fitted curve, or information on issues related to the performance of the test. The use of statistical methods to identify outliers is desirable, to remove ambiguity. In any case a justification should be provided for excluding any data point.

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, χ^2 error and t-test statistical metric. Detailed description for the criteria for the acceptability of the fit is included in FOCUS guidance (2014). Regardless of the kinetic model selected for describing the data, a justification should be provided with adequate and reliable documentation on the applied method. Estimated DegT50 can depend on the model used to fit the experimental data.

When the kinetic of decline is first-order and no lag phase occurs, the degradation half-life predicted by single-first order kinetic model (SFO) can be used for direct comparison with the P/vP criteria. Degradation can follow different kinetic patterns which are influenced by the test conditions (e.g. matrix properties, temperature, microbial composition) and the test substance. The use of the SFO model for all types of kinetic pattern might lead to under- or overestimation of the derived DegT50 and thus introduce bias in the persistence assessment.

When the kinetics of decline are bi-phasic (fast initial decrease in test substance concentration followed by a slower decline), the best-fit model (e.g. DFOP, HS)[29] should be selected and used for predicting a degradation half-life DegT50. Bi-phasic kinetics (fast phase followed by slow phase) are predominantly the result of the limited bioavailability of the substance in the system due to its sorption to the solid matrices (soil and sediment or suspended matter) which might lead to non-extractable residues (NERs) formation. When DFOP or the HS kinetic model (both models allow deriving slow phase DegT50) is selected as the best fitting model, the degradation half-life (DegT50) predicted from the slow phase should be preferred for

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[28] In the context of the Plant Protection Products legislation (EC 1107/2009) and specifically within the FOCUS guidance (2014) a distinction is made between trigger and modelling endpoints, for the purpose of the REACH legislation and the PBT assessment according to Annex XIII this distinction does not apply and as described above the kinetic model that most appropriately describes the observed data should be used.

[29] 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).

comparison with the P/vP criteria. Use of bi-phasic kinetic models is recommended to cases where an acceptable single-first order (SFO) fitting is not possible. In any case, a justification for the selection of a 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.

DegT50 based on the slow phase represents a conservative approach compared to overall DegT50. If the slow phase DegT50 is above the P and/or vP it can be expected that the concentration of the substance in the environment would increase over time and that the substance would persist in the environment in the long term. When such substances are released to the environment, their accumulation in the environment is in practice difficult to reverse as cessation of emission will not necessarily result in a reduction in substance concentration. The slow degradation phase may represent a small fraction of the degradation of the substance under the degradation simulation test conditions. However, it is considered that the degradation rate at the slow phase better describes the degradation rate in the environment over time due to the concentration build up and partitioning properties of PBT and/or vPvB substances. For these reasons, when the kinetics of decline are bi-phasic and the DFOP or HS model is used, the more conservative DegT50 slow phase should be preferred (over the fast phase DegT50 or overall study period DegT50) and compared to the P/vP criteria of REACH Annex XIII.

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 as demostrated in the cases of substances included in the candidate list (e.g. Melamine, EC 203-615-4 and 1,4-dioxane EC 204-661-8).

The First Order Multi-Compartment (FOMC) model, also mentioned in the FOCUS guidance is a bi-phasic mechanistic model based on the soil heterogeneous nature (FOCUS, 2014). The model divides the soil into large number of sub-compartments each with different first order degradation rate constants. The use of the DegT50 derived from the FOMC model can be considered in a *Weight-of-evidence* approach only if the other models do not fit the data adequately. Furthermore, when the FOMC model is the best fit model, the derivation of a pseudo-DegT50 from a DegT90/3.32 is used for specific purposes in the FOCUS guidance (FOCUS, 2014). The denominator value of 3.32 is the mathematical ratio between DegT90 and DegT50 derived from single first order kinetics modelling. The recalculated pseudo-DegT50 is longer than the overall DegT50 and might be highly uncertain. The uncertainties associated with the pseudo-DegT50 are associated with the fact that the mathematical ratio of 3.32 is derived from empirical model (SFO) while the FOMC, as stated above, is a mechanistic model and that it is not representative to the actual mathematical ratio between DegT90 and DegT50 of the FOMC model. Furthermore, the FOMC model was developed for the soil compartment and thus its application in the context of the OECD 309 and OECD 308 should be considered carefully (Gustafson and Holden, 1990). Considering the uncertainties around the DegT50 values derived using the FOMC model, this model is the less preferred one to be used for the PBT assessment.

The mass balance/recovery for a substance should be provided and 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.

In case the mass balance decreases over the study, it could suggest possible losses of the test substance or transformation/degradation products from the test system by volatilisation. Other possible reasons for an incomplete mass balance could be e.g. losses due to adsorption or during sampling/sample treatment. In the absence of information on the cause of incomplete mass balance, the unknown fraction is considered non-degraded parent substance for deriving the DegT50. If incomplete mass balance is due to experimental errors (e.g. failed sampling/measurement) and it is observed for few data points, these data points should be excluded from the kinetic analysis and the DegT50[30] derivation. In any case a justification should be provided for excluding any data point.

In case of incomplete mass balance the test should be treated with caution, nevertheless the following three scenarios exist for deriving the DegT50 (Table R.11—6). If none of these scenarios applies the study should be considered inconclusive.

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[30] A robust DegT50 derivation is only possible if the number of observations is appreciably larger than the number of model parameters. However, the number of data points should not be a sole reason for rejecting a study prior to kinetic analysis.

Table R.11—6: Scenarios for deriving DegT50 for substances with incomplete mass balance.

Scenarios/method of calculations	Description	Conclusion
1/ DegT50 without normalisation	This scenario assumes that the mass balance loss is degraded parent substance. It is therefore the less conservative approach. As this is the less conservative option, the DegT50 obtained without normalisation can be used to conclude P or vP. As this option is not conservative enough, it must not be used to conclude a substance as not P or as not vP.	P vP *If you cannot conclude P or vP run scenarios 2 and/or 3*
2/DegT50 with normalisation[31]	This scenario can be used under the following two circumstances: i. The substance concentration/radioactivity measured at the 1st time point (zero time point) differs from the initial applied concentration/applied radioactivity of the substance and the mass balance remains constant over the testing period. ii. The decline rate of the mass balance is constant over the testing period. As this option is not conservative enough, it must not be used to conclude a substance as not P or as not vP.	P vP *If you cannot conclude P or vP, or the substance is P but it is unclear if vP (in particular for substances having vB properties) run scenario 3*
3/ DegT50 with unknown fraction considered as non-degraded parent[32]	In the absence of information on the cause of incomplete mass balance, the unknown fraction is considered non-degraded parent substance for deriving the DegT50 (representing a worst-case scenario). This option can be used to conclude a substance as not P or not vP as this is the most conservative approach.	Not P Not vP P/vP as part of a WoE only

When dissipation through volatilisation is observed, correction procedures can be applied (see also paragraph on Volatile substances below in this Section, Section R.11.4.2.1.3 and related Appendix R.11-7). For this purpose, correction procedures are described for the parent substance for the SFO kinetics model which could be also applied in the case of HS and DFOP kinetic model. The correction procedures assume that the volatile losses of parent substance were adequately identified and quantified in the volatile trapping systems. Each time these correction procedures are used, a justification should be made for their use. These approaches

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[31] **DegT50 with normalisation:** for each data point the residual parent substance can be calculated either as:

(i) a percentage of the initial measured concentration (or the sum of parent substance and transformation products) or as a percentage of the initial measured applied radioactivity

(ii) as a percentage of the total recovered measured applied radioactivity at each time point.

[32] **DegT50 with unknown fraction:** for each data point the unknown fraction is considered as non-degraded parent and is expressed as a percentage of the initial nominal radioactivity applied.

may also apply for transformation/degradation products, though a justification for its use must be made which accounts for when the transformation/degradation products are formed in an experiment. Further advice on how to handle data on non-extractable residues in simulation studies is provided in Appendix R.11-4. Advice on sterile controls, which are also relevant for the estimation of DegT50, is provided Section R.7.9.4.1 in Chapter R.7b of the Guidance on IR&CSA.

Specific advice on DegT50 derivation for transformation/degradation products

When a degradation study is performed on a transformation/degradation product of a substance to be assessed, the data should be analysed as described for the parent substance above. When a study is performed on a parent substance and transformation/degradation products are formed, the pathway model approach should be used as described below. For deriving a reliable degradation half-life for a transformation/degradation product, a good knowledge of the degradation pathway up to this transformation/degradation product is essential.

The initial approach for determining the half-lives for transformation/degradation products is fitting the data with the stepwise pathway approach model, in which parent and transformation/degradation data are assessed together. In a sequential and stepwise manner, each transformation/degradation product is added to the model and the parameters for the newly added transformation/degradation product are fitted while the parameters for the other previously fitted parent or transformation/degradation product are fixed to their estimated value. The kinetic model used for fitting the parent substance data should be considered together with the number of the fitting parameters of the transformation/degradation product kinetic model. In addition, the kinetic model should cover both, the formation phase, which accounts for when the transformation/degradation product is formed, and the decline phase. The stepwise approach may be the preferred way in cases where the degradation pathway is very complex and includes many transformation/degradation products.

Alternatively, one can proceed straight to the complete pathway model approach (cf. simultaneously fitting the parent and all transformation/degradation product data), especially when the pathway is well understood and/or less complex (e.g., only few transformation/degradation products are to be considered) and when the complete pathway model fit generates a good representation of the experimental data, a good visual fit, and reasonable parameters for all transformation/degradation products.

Another option is to evaluate the transformation/degradation products data individually by using only the decline phase (Decline model). This option should be used only if the pathway fit does not appropriately describe the data. It is important to note that the degradation half-lives from a transformation/degradation product decline model fit are more conservative than the degradation half-lives from a pathway model. This is because in a pathway approach model the transformation/degradation product is still being formed after the peak is reached.

Considerations for simulation testing strategy

Annex IX to the REACH Regulation lists three simulation degradation tests as standard endpoints for the CSA (which, according to Annex I to the REACH Regulation, includes the quantitative risk assessment and the PBT/vPvB assessment).

The P/vP assessment should cover all three (five) environmental compartments (water, marine water, sediment, marine sediment, soil). However, a substance can already be concluded as P or vP if the criteria are fulfilled for one compartment only. For the purpose of reducing efforts of testing, the test should be selected in such a way that it reflects the worst case of persistence potential (for which the degradation half-life is close or is more likely to exceed the P/vP criteria). This would also ideally be the environmental compartment with the best possibility to use the results for concluding the P/vP-assessment (as being "worst case").

The influence of the relevant environmental compartment(s) in terms of exposure potential based on fate properties, the identified uses and release patterns to the order of testing also need to be considered. In some cases, it may be necessary, and hence acceptable, to choose an environmental compartment for simulation degradation testing other than the one normally considered as the first preference (see discussion below).

The further elements to be considered when choosing the environmental compartment(s) for testing are described in the context of the ITS (Figure R.11—3).

Before testing, the simulation test(s) that is(are) the most appropriate for addressing degradation should be identified. This is further discussed below.

Simulation studies on ultimate degradation in surface water are warranted unless the substance is highly insoluble in water. If a substance is highly insoluble in water it may not be technically possible to conduct a simulation study that provides reliable results, and at very low concentrations technical issues may make it very difficult to establish a reliable degradation curve in the study. Therefore, depending on the substance physico-chemical properties and the availability of good quality analytical methods for identification and quantification, it may not be possible to conduct this study if the water solubility of the substance is very low (typically <1 µg/L). The surface water transformation test (OECD TG 309) recommends using a test substance concentration for the kinetic part of the study in a range which is environmentally realistic, i.e. in a range of "less than 1 to 100 µg/L". The part of the study performed to identify degradation pathways may usea a higher test substance concentration to ease the analytical identification and characterisation of the transformation/degradation products. Further considerations on the OECD TG 309 study are provided below.

Testing in the aquatic compartment (OECD TG 309) is the preferred first step when there is a need for further information on persistence in the environment, considering the following reasons:

- Firstly, the aquatic compartment is considered to be a relevant environmental compartment for persistence assessment because the criteria for B/vB and T are mainly based on tests performed in this compartment. In addition, by default, water compartment receives a significant amount of emissions, directly or indirectly, and transports/distributes the substance through e.g. deposition and run-off (unless evidence from substance emission data suggests otherwise). Once entering water, a substance may reside there for very long time and be spread over long distances before it reaches other environmental compartments (*via* environmental transport, partitioning and distribution processes) such as sediments or (*via* air) the soil compartment.
- The OECD TG 309 minimises potential NER formation. If NER is formed at significant levels in OECD TGs 307 and 308 tests, this can be difficult to interpret and compare with the degradation half-life criteria of Annex XIII to the REACH Regulation.

Reasons to deviate from this general approach can be that:

- The substance is a multi-constituent / UVCB substance, which affects the concentration at which the test can be performed (due to different multiple water solubilities of the individual components).
- Indications from available data (e.g. literature) suggest that persistence is likely to occur in a different environmental compartment (i.e. in soil or sediment), including evidence of strong sorption potential and direct or indirect emission to specific compartment.
- Aquatic testing is not technically feasible, i.e. it has been impossible, with allocation of reasonable efforts, to develop suitable analytical methods and other test procedures to accomplish testing in surface water so that reliable results can be generated. This may be the case in particular if the water solubility of the test substance is very low. Appropriate analytical methods should have suitable sensitivity to detect relevant changes in concentration (including transformation/degradation products). Please see also substance type specific considerations in Section R.11.4.2.1.
- OECD TG 309 should be performed at concentrations between 1 and 100 µg/L and preferably in the range of <1-10 µg/L (to ensure that biodegradation follows first order kinetics). Generally, when water solubility of a substance is very low (typically <1 µg/L), testing on sediment and/or soil will be preferred, if aquatic simulation degradation testing is not technically feasible due to analytical limitations and low solubility of the test substance. Pre-test is recommended to assess technical feasibility of the study if substance properties indicate the need.
- High sorption affinity of cationic substances to particles may lead to reduced degradation rate in sediment and soil. Therefore, OECD TG 309, in which particles are sparse, may not be the worst-case test for cationic substances and OECD TG 308 or OECD TG 307 could be a better option as a first simulation test to be conducted (Claßen *et al.* 2021 and Holzmann *et al.* 2022).

Soil/sediment simulation degradation testing may be warranted as a first test in the above-listed cases. In addition, as described in the ITS (Figure R.11—3), the soil and sediment degradation simulation tests may be needed when results from simulation tests in water do not exceed the P/vP criteria but there are indications that the substance or its degradation products could persist in soil and sediment, meeting the respective P criteria.

Before performing a soil or a sediment simulation degradation test, it is worth noting that for the purpose of quantitative risk assessment and for adsorptive substances, a simulation test in soil (OECD TG 307) could be more relevant than a simulation test in sediment (OECD TG 308)[33]. Degradation rates/half-lives from simulation tests in soil can be used instead of generic values for the assessment of PECsoil. While degradation rates/half-lives from simulation tests in sediment can be taken into account for the calculation of the PECregional, in practice this would have only a negligible influence on risk assessment.

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[33] Removal of the substance during the WWTP process may be taken into account when considering the emissions through WWTP in relation to the relevance of the simulation test compartment (e.g. incineration of the sludge and removal or degradation during the water treatment process).

Once the appropriate simulation test(s) have been identified and conducted, data need to be interpreted to determine environmental degradation half-lives. A prerequisite for data interpretation is that exhaustive extraction methods are used to ensure that suitable data are generated. Guidance on how to conduct the test and interpret data from a simulation test is available in the present Guidance document and in Section R.7.9.4 of *Chapter R.7b* of the *Guidance on IR&CSA*.

OECD TG 309

OECD TG 309 should be performed at concentrations between 1 and 100 µg/L and preferably in the range of <1-10 µg/L (to ensure that biodegradation follows first order kinetics). However, for low solubility substances, even if their water solubility is within this range, 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).

According to OECD TG 309, the test is applicable for non-volatile and slightly volatile substances. The feasibility of the surface water simulation test for volatile substances depends on the possibility to minimise volatilisation and assure that the test substance is maintained in the water phase, and thus, is available for microorganisms to the extent that a reliable degradation half-life can be determined. It is also important to ensure that the volatilised fraction is adequately trapped and quantified in order to be able to interpret the results reliably. Further information on how to address volatilisation in simulation testing can be found in the paragraph of “Volatile substances” below and in section R.11.4.2.1.3.

Also hydrophobic substances with low water solubility are challenging to add to the test system. OECD TG 309 allows using an organic co-solvent for adding poorly water-soluble substances but requests that steps are taken to minimize actual co-solvent concentrations in the test. Passive dosing and microvolume spiking have recently been proposed as approaches to avoid or at least minimize co-solvent addition (Birch et al, 2023). However, currently there is still limited experience in using such methods in regulatory persistence assessment and further developments and discussions are thus foreseen. OECD TG 309 uses as a default one matrix sample, which is in contrast to the soil (4 soils) and sediment (2 sediments) simulation studies. Nothing prevents registrants from employing or authorities from requesting simulation degradation testing in more than one surface water. It is generally recommended to consider performing the test with more than one water source.

In OECD TG 309, there are options to perform the test as a ‘pelagic test’ or as a ‘suspended sediment test’. In both cases, the coarse particles are removed from the water sample, for example by filtration through a filter with 100 µm mesh size or with a coarse paper filter, or by sedimentation. For the ’suspended sediment test’, surface sediment is added afterwards to obtain a suspension.

Use of sterile controls is required in the OECD TG 309. More guidance on the sterile controls and suitable sterilisation methods are provided in the Section R.7.9.4.1. in *Chapter R.7b* of the *Guidance on IR&CSA* and in *ECHA Note on Sterile controls* available on ECHA Website.

For the PBT/vPvB assessment, the amount of suspended matter in the pelagic test should be representative of the level of suspended solids in EU surface water. For large rivers, the concentration of suspended matter (SPM) is reasonably constant and an EU default of 15 mg_{dw}/L has been proposed, e.g. for the implementation of the Water Framework Directive

(European Commission, 2018) or in EUSES. For marine waters, a default SPM concentration of 3 mg_{dw}/L has been proposed for the Water Framework Directive. Similarly an SPM concentration of 5 mg_{dw}/L has been implemented in EUSES for marine waters. For REACH, using natural surface water containing between 10 and 20 mg_{dw}/L SPM for simulation tests in freshwater and ca. 5 mg_{dw}/L for simulation tests in marine water is considered acceptable. Further details are available in Section R.7.9.4.1 of C*hapter R.7b* of the *Guidance on IR&CSA*.

Even if more than one water source is used in the assessment, it is recommended that the amount of suspended solids still reflects realistic concentrations for the EU surface waters (e.g. samples from different water bodies or to reflect seasonal variations in the concentration of the suspended solids). When the test concentration is well under the water solubility limit of the substance, one might consider testing several water sources instead of testing two concentrations of the test substance. In any case, a reference substance should be used to demonstrate the viability of the system.

According to the OECD TG the 'suspended sediment test' can be used to *simulate surface water free of coarse particles or turbid surface water (which might exist near the water-sediment interface)*. Ingerslev and Nyholm (2000) further indicate that conducting the tests with added suspended sediment significantly enhance the biodegradability of some of the test substances. However, this test design is generally not recommended for P testing purposes as such highly sediment particle loaded surface water systems are not the most prevailing ones. There is also a high probability that increasing the suspended solids concentration will increase the potential for NER formation and to avoid this the pelagic test without artificially added particular material/sediment particles is preferred. In specific cases where there is a need to address the influence of the suspended solids to the abiotic degradation rate in the surface waters, the addition of suspended solids may be justified. If suspended solids are added, it is recommended that a magnetic stirrer bar should not be used for agitation as it may grind the solids/sediment and result in increased levels of NER. Other methods are recommended instead, e.g. shaking of test vessels (Shrestha *et al.*, 2016). According to OECD TG 309, agitation should be continuous and as gentle as possible, while still maintaining a homogeneous suspension.

Following attempts have been reported to decrease variability in OECD TG 309 test system by increasing the amount suspended solids in the test media. It has been demonstrated that 10-fold higher sediment content (10 g/L) compared to allowed in standard suspended sediment OECD TG 309 test reduced interreplicate variability (Seller et at. 2020). Tian *et al.* (2023) reported that in modified OECD TG 309 with sediment and water samples from wastewater impacted rivers having even higher suspended solids concentration (50 g of wet solids/L) and mixture of substances, low spiking concentrations (0.5 µg/L) produced degradation rate constants comparable to those derived from the non-spiked test. However, half-lives obtained from studies with suspended solids concentration much higher than allowed in the respective TG or studies with water and sediment with previous exposure of the substance cannot be directly compared with Annex XIII P criteria.

In order to minimise the formation of NERs, it is recommended that the pelagic test be considered first before conducting any other simulation tests. However, it is worth noting that even for the 'pelagic test', the test water will contain suspended matter onto which the test substance and/or its transformation/degradation products can adsorb. Therefore, the formation of NERs may be significant in the 'pelagic test' too. It is thus necessary for this test as well to quantify the NERs and to explain and scientifically justify the extraction procedure and solvent used (see also Section R.7.9.4.1. in *Chapter R.7b* of the Guidance on IR&CSA).

Unless there is a specific concern for the marine compartment, for the REACH PBT assessment, generally the OECD TG 309 would be performed using a freshwater rather than salt water media. However, the degradation in marine compartment should always be considered in PBT assessment. It should therefore be assessed if the information on degradation in freshwater may be used to extrapolate the degradation rate in marine environment.

The OECD TG 309 proposes quite a flexible framework for designing the test. The registrants should provide justifications in the robust study summaries and/or test plan proposals for the different options taken with regard to, for example the type and characteristics of the water used, whether suspended sediments were added, whether shaking or stirring of the test vessels was used, whether the test was performed in the dark or with diffuse light.

The role of NER in P/vP assessment is discussed further in the section on "Non-extractable residues" below.

OECD TG 308 & TG 307

Testing on sediment (OECD TG 308) or soil (OECD TG 307) may be needed instead of a pelagic test (OECD TG 309) if the latter is not technically feasible; i.e. if the water solubility of the test substance is very low (typically below 1 µg/L) or if it is not possible with reasonable efforts to develop a suitable analytical method or other test procedures for conducting the test in water. Besides, in some situations it can be anticipated that the simulation test in water will not be a worst case and that the persistence criteria will possibly be exceeded in sediment and/or soil but not necessarily in water. This may be the case for example for strongly sorbing or hydrolysable substances, as hydrolysis may be hindered by adsorption onto sediment and soil. Another example is cationic substances for which data suggests that degradation generally seems to be faster in aquatic than in sediment or soil systems (Claßen *et al.*, 2021; Holzmann *et al.*, 2022). For volatile substances that have high adsorption potential, adsorption to sediment or soil may lead to less volatilisation from the test matrices in OECD TG 308 and 307 than in OECD TG 309 test where volatilisation from the water phase could be higher. Testing on sediment and/or soil may also have to be conducted in addition to the test in water, to demonstrate that the substance is persistent in none of the compartments relevant for the PBT/vPvB assessment. Testing in the sediment and/or soil compartments should be considered in particular if there is a specific concern for this compartment, for example, if direct or indirect releases to these compartments are likely, or if the substance is predicted to accumulate in these compartments. K_{oc} or K_d values can be used as an indicator of whether the substance is likely to be of concern for the sediment and soil compartments. As a rule of thumb, substances with log $K_{oc} > 4$ are generally regarded as highly adsorptive and likely to distribute in sediment and soil.

Guidance on how to apply sterile controls is provided in the Section R.7.9.4.1. in *Chapter R.7b* of the Guidance on IR&CSA. There are many benefits of the sterile controls in biodegradation test described R.7.9.4.1. Among others, sterile controls inform on maintenance of the test material in the test system and, in particular, indicate potential technical problems due to the physicochemical properties of the substance (adsorption, low solubility, volatility). The use of sterile controls is already described in OECD TG 307 (including in the list of results to be reported). This guidance recommends the use of sterile controls for both OECD TG 307 and OECD TG 308 tests.

For the PBT/vPvB assessment, a half-life in sediment should be estimated. However, from OECD TG 308 simulation tests, the half-lives calculated for the sediment phase and the water phase separately are less reliable than the half-life calculated for the total water-sediment system. Still, because of the low volume and depth of water relative to the volume of sediment and the surface of the water-sediment interface used in OECD TG 308, even moderately adsorptive substances will tend to rapidly partition from the water phase to the sediment phase. Therefore, for adsorptive substances, the half-life in the sediment can reasonably be estimated from the half-life for the total water-sediment system. This approach avoids the need to determine specific half-lives for each phase separately (Honti and Fenner, 2015)[34]. However, the parent substance may degrade to more soluble and less adsorptive degradation products that can be released from the sediment to the water phase. This should be taken into account in the assessment.

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. There is no specification of the vessel size or geometry in the test guideline, but 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). The dimensions of the test vessel should be included in the robust study summary. Fenner *et al.* (2017) recommend several qualitative and quantitative parameters to be considered for interpretation of the OECD TG 308 results including among others K_{oc}, TOC_w, fraction of organic carbon in sediment, test vessel geometry, height of water and sediment layers and sediment porosity.

The OECD 308 TG states on several occasions that "disturbance of *the sediment is* undesirable and should be avoided *as* far *as possible*" e.g. when introducing the test substance, during aeration and sampling. Deviating from the TG recommendation, sediment spiking instead of addition of the test substance *via* water may in some cases be necessary to ensure realistic exposure of sediment in the test. This may be the case e.g. for substances which would transfer significantly quicker to the atmospheric compartment via volatilisation compared to transfer to the sediment compartment. Pre-tests as well as fugacity modelling (see also paragraph on Volatile substances below in this Section, Sections R.11.4.2.1.3 and R.11.4.1.1.4 on Multi-media modelling) can provide useful information to identify the proper test setup for the final test. Currently, however, deviations from the test guideline may only be justified on a case-by-case basis as there are no generic criteria available when spiking would be appropriate. If sediment spiking is undertaken, the overall half-life from such a test should be assumed to be the sediment half-life (unless there is significant desorption, which seems unlikely in the case of most potential PBT substances). Sediment spiking methods have been developed so far for the purpose of sediment toxicity testing. These are mentioned in Section R.7.8.10.1 "Laboratory data on toxicity to sediment organisms" in *Chapter R.7b* of the

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[34] Part of LRI ECO18 – "Improved strategy to assess chemical persistence at the water-sediment interface" http://cefic-lri.org/projects/lri-eco18-eawag-improved-strategy-to-assess-chemical-persistence-at-the-water-sediment-interface/

Guidance on IR&CSA. However, none can currently be directly recommended for the purpose of sediment simulation testing but further approach development would be necessary.

According to the OECD TG 308, the aerobic test simulates an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient. Aeration of the test system is needed in order to maintain aerobic conditions 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 closed, biometer type systems (for slightly volatile substances). When testing volatile substances in closed systems, regular exchange of headspace gas is necessary to maintain aerobic test conditions. It should also be ensured that the oxygen is distributed from the headspace to the water layer. However, any aeration method should disturb as little as possible the sediment layer and its stratification. For example, visual assessment of resuspension/turbidity of the overlying water is one indication of disturbed sediment.

In the OECD TG 308 agitation 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. While, in the OECD TG 309 continuos agitation is required to maintain particles and microorganisms in suspension and facilitate the aerobic conditions, such aeration should not be used in OECD TG 308. Aeration methods recommended in the OECD TG 308 should be applied instead. If any other method is used, it must be demonstrated that sediment stratification is maintained troughout the study.

Multiple simulation test results

A substance can be concluded to be not-P only if it can be demonstrated that it is not persistent in any of the environmental compartments relevant for the PBT/vPvB assessment, i.e. water, sediment and soil.

Generally, for substances registered under REACH, the likelihood of having more than four different results from the same environmental compartment is deemed to be limited. For determining transformation rates, OECD TGs 307, 308 and 309 recommend respectively that at least four different soils and two different sediments and one type of water should be tested.

For the same environmental compartment, when four or less results are available, the most stringent result should be used with respect to the PBT assessment.

Where more than four results are available for the same compartment, the first step is to assess the validity of the data and whether the different tests are equivalent (for example temperature, pH, organic carbon content, microbial biomass, test design etc). Only test results corresponding to equivalent test conditions can be compared and average/mean derived using all available DegT50 values is generally not applicable. In all cases, the approach should be well justified and documented and should be supported by the *Weight-of-Evidence* analysis. This should include a discussion of outlying results. In particular, the representativeness of the test conditions should be carefully assessed for each test result. Particular scrutiny should be given if results from the tests are close to P or vP threshold.

Aerobic and anaerobic conditions

The following options are available in the environmental simulation test guidelines:

- OECD TG 307 – Aerobic and Anaerobic Transformation in Soil: The test is usually conducted under aerobic conditions. The test can be performed also under partial or strict anaerobic conditions.
- OECD TG 308 – Aerobic and Anaerobic Transformation in Aquatic Sediment Systems: The normally employed test includes aerobic and anaerobic sub-compartments. The test can be performed also under strict anaerobic conditions.
- OECD TG 309 – Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test; There is no "anaerobic" option.

In the anaerobic OECD TG 307 study, the anaerobic conditions can be achieved by covering the soil with water, i.e. mimicking a flooded field, in the absence of oxygen (the soil is purged with nitrogen and oxygen excluded for the test duration). A further option is a flooded soil but without the specific exclusion of oxygen (paddy field simulation). Anaerobic degradation in soil may also have influence on the results in some study cases, for example, if water covered soil environments are studied in the field. However, for REACH PBT/vPvB assessments, neither of the solely anaerobic test conditions are considered to be especially relevant scenarios for the P assessment in the EU. Nevertheless, if anaerobic soil data are available, they may be used as part of a *Weight-of-Evidence* approach in the P assessment.

The OECD TG 309 is an aerobic test. There is no anaerobic option in the test guideline - this would effectively be stagnant water. The main discussion on simulation tests aerobic and anaerobic conditions focuses on OECD TG 308. The "aerobic" OECD TG 308 is a mixture of aerobic and anaerobic sediment. The OECD TG states that the "*aerobic test simulates an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient".* By comparison, the anaerobic test "*simulates a completely anaerobic water-sediment system"*.

It is not recommended to judge whether a substance has an environmental half-life exceeding the P and/or vP thresholds using only anaerobic simulation data. Generally it would be expected that an anaerobic half-life would be greater than an aerobic half-life where the main route of degradation is aerobic, i.e. if there is no oxygen, degradation will be hindered[35]. Care should also be taken where the anaerobic data show rapid degradation of a substance. This is because there is generally no immediate discharge of a substance to anaerobic sediment or soil. Instead, the substance will usually need to cross an aerobic zone before reaching the anaerobic zone. This means it is important in persistence assessment to understand the degradation rate across that aerobic zone before the substance reaches the anaerobic zone[36].

Where anaerobic data are already available, these might be useful as part of a *Weight-of-Evidence* of whether the P or vP thresholds are met. For example the presence of oxygen may be less relevant if the primary degradation step is hydrolysis.

Sediment core data might provide some indication of anaerobic degradation capacity. However some caution should be exercised as the initial starting concentration is rarely known.

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[35] For example, some widely degradable materials may take considerably longer to degrade under anaerobic conditions such as newspapers in landfill waste sites.

[36] New information on anaerobic degradation may be needed in specific cases to understand the degradation dynamics in the sediment compartment.

Therefore any derived degradation kinetics estimating a half-life will have uncertainty due to the assumptions required. The history of any local emissions and contamination at the sample site also provides useful information to help interpret the data. It is more likely that core data can be used in an evidence base for anaerobic degradation, as part of a broader *Weight-of-Evidence* in the persistence assessment.

When new sediment simulation testing is assessed to be required for P/vP characterisation, transformation route prediction[37] or prior knowledge[38] should be used to judge whether additional information will be gained from performing the anaerobic-only test. Exploring an anaerobic route of degradation may be useful in specific cases where a transformation/degradation product may be of concern. However, in general a test using exclusively anaerobic conditions is not required. For the OECD TG 308 sediment simulation test the "aerobic"' test will include anaerobic sediment. Therefore, if a substance is expected to degrade under anaerobic conditions faster than under aerobic conditions, an OECD TG 308 may not be the most suitable test to assess the persistence of the substance. Even in the aerobic version of the OECD TG 308 a large part of the sediment is anaerobic. The substances that degrade only anaerobically may degrade in an OECD TG 308 study but not in an OECD TG 309 study. This has been shown for example with nitro-containing substances, like musk xylene. OECD TG 308 might therefore overestimate the degradation rate in the aerobic environment for some substances. If only an OECD TG 308 study is conducted, the conclusion drawn on persistence may be less conservative compared to the situation where testing is conducted in other environmental compartments. In such cases, to exclude potential false negative results in relation to the P/vP assessment, strictly aerobic degradation should also be assessed if technically feasible, i.e. the surface water simulation degradation tests with its strictly aerobic conditions.

Sterile controls in simulation studies

Considerations on the use of sterile controls as part of the degradation tests and sterilisation methods can be found in Section R.7.9.4.1 in *Chapter R.7b* of the *Guidance on IR&CSA* and in *ECHA Note on Sterile controls* available on ECHA Website.

In simulation tests it is important to differentiate degradation and disappearance of the test substance due to other dissipation processes. An important advantage of using sterile controls in degradation studies is that it allows to estimate to what extent abiotic processes (abiotic degradation and non-degradative dissipation) impact the disappearance of the substance. In addition, sterile controls can be helpful in verifying the exposure level of the test material in the test system and for the determination of a mass balance. Sterile controls are always highly recommended to be included to the test setup, even if not required by the respective test guideline.

For the PBT assessment there is normally no need to differentiate the biotic and abiotic degradation occurring in simulation studies.

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[37] E.g. with the EAWAG-BBD Pathway Prediction System (http://eawag-bbd.ethz.ch/predict/index.html).

[38] For example consider the application of substance – an anti-oxidant would be expected to be affected by oxygen and therefore aerobic degradation is likely to be more relevant.

Volatile substances

In simulation tests it is important to differentiate degradation and disappearance of the test substance due to other dissipation processes. Volatilisation of the test substance makes the interpretation of the study more difficult and increase uncertainty of the persistence assessment. Pre-test is always recommended to ensure feasibility of the simulation test. Based on the outcome of that pre-test, necessary modifications to the test design and set-up may be determined in order to minimise volatilisation or technical infeasibility demonstrated. Modifications should not compromise the relevance of the study and therefore, any modification of the test design or set-up should be consistent with the OECD TGs conditions and the validity criteria and recommendations of the guidelines should be fulfilled.

As vapour pressure or Henry´s Law constant do not solely allow predicting the volatilisation rates in simulation tests, a case-by-case assessment of potential volatilisation taking into account vapour pressure, HLC, distribution modelling and additional factors such as water solubility, phase partitioning and adsorption is recommended. Additionally, experience and information from other existing studies, e.g. volatility observed in ecotoxicity tests, can be useful. Properties of both the parent substance and potential transformation/degradation products should be considered when designing the study as the transformation/degradation products may be more volatile than the parent substance.

Further information on options to address volatilisation of test substances in OECD TGs 307, 308 and 309 is available under Section R.11.4.2.1.3 and how to consider volatilisation in the kinetic analyses can be found in the Appendix R.11-7 and in *ECHA note on Volatile substances* available on ECHA Website. See also Section R.7.9.4.1 in Chapter R.7b of the *Guidance on IR&CSA.*

Test temperature

Guidance on test temperature for the simulation test(s) is provided in Section R.7.9.4.1 of *Chapter R.7b* of the *Guidance on IR&CSA*. The reference temperature for providing results on higher tier tests (and carrying out tests, where relevant) is 12°C for surface water, soil and fresh or estuarine sediment environments and 9°C for marine environment.

Non-extractable residues

Non-extractable residues (NER) in soils are defined as species originating from chemicals, that remain un-extracted by methods which do not significantly change the chemical nature of these residues (Roberts, 1984). With regard to evaluation of water, soil or sediment simulation degradation test results (according to OECD TGs 307, 308 or 309), it is important for persistence assessment to consider the amount and type of NER besides the measured degradation half-lives of a substance. The amount of NER is driven by substance properties as well as test conditions. While NER formation is expected mainly in the soil or sediment simulation tests, it cannot be excluded in a surface water simulation test. The formation of NER in a surface water simulation depends on the Suspended Particulate Matter (SPM) concentration (including its OC content). The formation of NER should not be confused with the degradation phenomenon.

Information on the total amount of NERs (Total NER) is needed for the mass balance calculations which, in turn, are essential for interpretability and reliability of data. To complete the mass balance as a part of the simulation test with radio-labelled substances (^{14}C), the amount of Total NER must be determined. Quantification and further characterisation of NERs is highly recommended to refine the persistence assessment, when relevant and feasible. A tiered extraction scheme for quantifying Total NER and characterising the different NER types is described in Appendix R.11-4. For example, if the substance is concluded to be very persistent (vP) by taking into account only the extractable parent, further refinement is not relevant. When the substance is concluded to be persistent (P) or very persistent (vP) based on the amount of extractable parent and the Total NER, refinement the assessment by characterisation of NER is relevant.

In the context of persistence assessment, the Total NER is considered as a non-degraded parent substance unless further characterisation of the Total NER is performed. Determination and characterisation of the Total NER with the methods described in Appendix R.11-4, rely on the use of radio-labelled substance. Further characterisation of the Total NER is considered technically feasible if the Total NER is ≥10 % of the total applied radioactivity (at least on one of the sampling days) and it should be attempted to be performed for all sampling points. When the Total NER is <10 %, the possibility for further characterisation could still be explored as the technical feasibility of the characterisation might also depend on the test methodology (spiking radioactivity) and availability of analytical methods.

The NER should ideally be differentiated in remobilisable (Type I) and irreversibly bound fractions (Type II and III). While the irreversibly (e.g. covalently bound) and the biogenically bound part can be assessed as a potential removal pathway, the remobilisable fraction (strongly sorbed, physically entrapped) pose a potential risk for the environment.

The following types of NER in environmental matrices can be considered (Löffler *et al.* 2022, ECHA, 2019, Kästner *et al.*, 2018):

- **NER Type I (strongly sorbed and physically entrapped)**: strongly sorbed or physically entrapped into the matrix, contain the parent substance, transformation/degradation products or both. NER Type I have the potential to be remobilised, and should be considered for assessment of persistence. If chemical analyses are conducted, it may be possible to distinguish whether NER Type I consist of unmodified parent substance or of transformation/degradation products.

- **NER Type II (covalently bound)**: residues that are covalently bound to the matrix (e.g. to humic matter) in surface water, soils or sediments and that are considered to have low remobilisation rates. Unless there are indications from the available literature or monitoring data regarding their potential remobilisation, covalently bound residues may be regarded as irreversibly bound. .

- **NER Type III (bioNER)**: incorporated into biomass (biogenic NER, also called bioNER), NER Type III result from the anabolic formation of biomolecules (amino acids, phospholipids, and other biomass compounds) from the degradation products of the parent substance. Dead biomass, and therefore biogenic NER, are eventually fixed in organic matter derived from decaying microbial biomass. NER Type III are considered to be of no concern.

A lack of degradation of the parent substance may be assumed if fast NER formation (with extensive NER formation in several days without any degradation observed) is followed by a period of relative constant levels of NER. This might indicate the fact that the parent substance has become non-extractable, and thus is not readily available to degradation.

Information obtained by comparing results from the NER formation in sterile and non-sterile water-sediments/soils can sometimes provide insight into the processes involved in NER formation. If the NER is only formed at high levels in non-sterile soils/sediments, this may indicate degradation of the parent substance and biogenic NER formation (bioNER). In this case, the formed NER in the non-sterile soil/sediment is unlikely to consist of the parent substance. However, when interpreting the results it should be kept in mind that the sterilisation methods may influence the NER formation (see Chapter R.7b of the *Guidance on IR&CSA* and Kästner *et al.*, 2018). More guidance on the sterilisation methods is provided in the Section R.7.9.4.1. in *Chapter R.7b* of the *Guidance on IR&CSA* and in *ECHA Note on Sterile controls* available on ECHA Website.

In the Appendix R.11-4 a detailed description of the test procedures for the extraction and the characterisation of the Total NER is provided. Quantification and characterisation of the Total NER follows a stepwise approach. For refining the DegT50 based on the Total NER quantification and characterisation adequate number of sampling points should be analysed to allow kinetic analysis. As a first step, the Total NER are quantified under a tiered extraction scheme. The Total NER are considered as non-degraded parent substance in degradation half-life derivation unless further characterisation of the Total NER is performed. As a second step, characterisation of the Total NER should be performed when relevant (Total NER ≥10%) and feasible. The aim of this second step is the quantification of the Type I NER in the Total NER. Type I NER must be considered as non-degraded parent in the degradation half-life derivation. As a third step, characterisation of the amount of parent substance in the Type I NER can be performed and subsequently used to derive the degradation half-life.

Assessment of relevant transformation/degradation products

Where a substance is degraded by abiotic means or partly biodegraded, it may be necessary to consider whether there are any transformation/degradation products formed that could be potential PBTs/vPvBs. Where the original substance forms a transformation/degradation product that could be PBT/vPvB, there should be an assessment to compare the amount of this transformation/degradation product with the parent substance. In relation to degradation testing results, including those from simulation degradation tests which also include investigation of degradation pathways (OECD TGs 307, 308 and 309), there are often practical constraints to the analytical identification of transformation/degradation products. Biotransformation/ degradation pathways may be complex and many different degradation products may be formed and some only in small amounts or at slow rates. Practical constraints in relation to analytical methodologies for identification of degradation products may thus limit the possibility for identifying them chemically, when they occur in very small concentrations. In the simulation degradation test guidelines for soil, water-sediment and surface water, transformation/degradation products detected at ≥10% of the applied concentration of the parent substance at any sampling time (principal transformation/degradation products) should at least be identified unless reasonably justified otherwise. The test guidelines furthermore stipulate that transformation/degradation products detected at values even lower than 10% may be may need to be identified depending on the specific case. However, transformation/degradation products for which concentrations are continuously increasing or seem to be stable during the study should also be considered for identification, even if their concentrations do not exceed the general limit given above, as this may indicate persistence. The need for quantification and identification of transformation/degradation products should be considered on a case-by-case basis with justifications. See also the definition of relevant transformation/degradation products in Section R.11.4.1.

It should be noted that neither a readily biodegradable substance (based on ultimate degradation) nor its transformation/degradation products will normally need to be assessed because any transformation/degradation products can be assumed to be minimal and transient. This is the case when based on the Weight-of Evidence there are no indications on potential persistence.

To assess whether the transformation/degradation products may be potential PBT or vPvB substances, the following approaches may be helpful:

- Based on the structure of the parent molecule, predictions of the structures of the transformation/degradation products may be made. These can be based on QSAR models/expert systems e.g. the freely available EAWAG-BBD Pathway Prediction System (available at: http://eawag-bbd.ethz.ch/predict/index.html), KEGG biodegradation database/prediction tool, the OECD QSAR Tool Box (see microbial metabolism functionality) or the commercial CATALOGIC (successor of CATABOL) or Multicase modelling tools, and by use of expert judgement, supported by appropriate substance-relevant scientific documentation.

For further PBT/vPvB assessment of the relevant transformation/degradation products products, the normal PBT/vPvB assessment approach and data generation principles apply, as described in this Guidance document. See also the definition of and discussion on relevant transformation/degradation products products in Section R.11.4.1.

Assessment of abiotic degradation data

Abiotic degradation tests are not required in a P assessment for readily biodegradable substances, or for substances shown to be (ultimately) degraded in "enhanced" biodegradation tests and modified ready biodegradability tests, or for substances with a degradation half-life in a simulation test not fulfilling the P-criterion. If abiotic degradation tests are available, there is a need to assess the properties of abiotic degradation products against the screening P, B and T criteria (see Sections R.7.9.4. and R.7.9.5 in *Chapter R.7b* of the *Guidance on IR&CSA*).

It should be noted that the abiotic degradation processes typically concern only primary degradation. Hence, when assessing such data for PBT/vPvB characterisation, the identification of the transformation product(s) should be performed.

There are several abiotic degradation/transformation processes in the environment to be considered, including e.g. hydrolysis, direct and indirect photodegradation, oxidation/reduction, surface-controlled catalytic reactions, molecular internal conversions etc. The most important of these processes is usually hydrolysis, which is relatively independent from the mode of entry of the substance into the environment. Hydrolysis may proceed effectively in aquatic, sediment and soil compartments but it is however noted that there are substances reaching rapid hydrolysis rates which are well known to be persistent in soil and/or sediment, e.g. endosulfan and Octamethylcyclotetrasiloxane (D4) (ECHA, 2015) Therefore, rapid hydrolysis rates cannot alone lead to concluding that a substance is not persistent. Test results showing rapid hydrolysis rates always need to be evaluated carefully in context with other information on the substance, such as partitioning and ionogenic properties both of which may significantly influence the extent and strength of sorption to soil and sediment. Hydrolysis also needs to be consistently rapid across the range of environmentally relevant pH. To provide confidence in the hydrolysis results, analytical data identifying transformation/degradation products to provide a mass balance are also needed. These both demonstrate that primary degradation has occurred, and allow subsequent PBT assessment of the transformation/degradation products.

There is currently no cut off for hydrolysis rate, which could alone be used as justification to conclude that a substance is not persistent. 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.

Due to the number of factors that affect photodegradation rates, this process is not generally considered in the persistence assessment for substances registered under REACH. Nevertheless, recent research has investigated the possibility to derive photodegradation half-lives taking into account many of those factors (latitude, season, depth, water DOC) (Lin and Emberger, 2017; Lin *et al.* 2019, 2022). Further discussion on photodegradation is provided in *Chapter R.7b* of the Guidance on IR&CSA.

According to Castro-Jiménez and Van de Meent (2011), light absorption in natural water is significantly slower than measured in laboratory water with photo degradation occurring around 30 times more slowly for typical fresh water, 400 times more slowly for typical coastal sea water, and 500 times more slowly for ocean water. These authors also conclude that the "*contribution of photodegradation in water to overall degradation is significant only for substances that reside in water to a considerable extent*". They highlight that many substances reside in sediment and soil, rather than in water. They give as an example bromophenyl ethers, which are "photochemically labile in water" but only slowly photodegrade in the environment. The relative importance of direct photolysis versus the indirect process varies and is dependent both on the composition of the substance as the prevailing conditions of the media. Indirect photodegradation is stimulated in natural environmental waters by the presence of dissolved organic matter (which is not present in pure laboratory water).

The tests used and their interpretation are discussed in Sections R.7.9.4 and R.7.9.5 of *Chapter R.7b* of the Guidance on IR&CSA.

R.11.4.1.1.4 Assessment based on estimation models (QSAR, SAR)

The use of QSAR and SAR predictions for identifying substances for persistence (P and vP) might be used at the screening level, as described below and in detail in Sections R.7.9.4 and R.7.9.5 of *Chapter R.7b* of the Guidance on IR&CSA.

Biodegradation QSAR models – screening

Generally, it is recommended to consider both the validation status of any QSAR model and whether the substance for which predictions are made may be regarded as being within the applicability domain of the model (see Section R.6.1 in *Chapter R.6* of the Guidance on IR&CSA).

(Q)SAR estimates may be used for a preliminary identification of substances with a potential for persistence. For this purpose, it is recommended to use combined results from three estimation models in the EPI Suite™ (US EPA, 2012, as described above in the Table R.11—4).

Degradation half-lives based on QSAR models using data from ready biodegradation tests should not be used for comparison with the P/vP criteria.

Other QSAR approaches

Pavan and Worth (2006) describe a number of models and approaches that specifically address the issue of identifying structures that meet or do not meet the P criteria.

In the same way, Nendza *et al.* (2013) provide an inventory of *in silico* screening tools that could be used for the assessment of the degradation potential of substances under the REACH Regulation. Such estimates may be used for preliminary identification of substances with a potential for persistence (see also Section above). The combined results of the three freely available estimation models BIOWIN 2, 6 and 3 in the EPI Suite (US EPA, 2012) may be used as follows:

- Non-linear model prediction (BIOWIN 2): does not biodegrade fast (probability < 0.5) and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value < 2.25), **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).

QSAR predictions can be used as part of a *Weight-of-Evidence* approach: predictions that the substance is not rapidly degradable would support the conclusion that the substance is potentially P/vP. In the contrary situation, predictions indicating that the substance could degrade rapidly would support the conclusion that the substance is not persistent. However, QSAR results alone are in most cases not sufficient to conclude on non-persistence but should be supported by additional information. In every case, it should be verified that the QSAR model and predictions are reliable and applicable to the substance. While the QSAR predictions using these models are reliable and the estimation results clearly indicate that the substance is not persistent, all other available information should still be taken into account together with QSAR estimation(s) in order to be able to consider the substance as not fulfilling the criteria for P. 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 *Guidance on IR&CSA*). Note however that, in any case, all other existing and reliable QSAR predictions, read across and test data information should be considered for deriving a conclusion regarding the persistence status of the substance.

The use of QSAR model predictions are 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 (see Section R.11.4.2.2).

Abiotic degradation models

There are very few software models available for predicting hydrolytic degradation, atmospheric and hydrolysis or aquatic photodegradation (e.g. AOPWIN and HYDROWIN modelss are freely available in Estimations Programs Interface for Windows (EPI Suite), and a few published models (Peijnenburg *et al.*, 1992, Stegeman *et al.,* 1993). These are reviewed in Section R.7.9.4 of *Chapter R.7b* of the *Guidance on IR&CSA*.

Other modelling data

Another useful source of information is programmes that predict metabolic pathways for the degradation of a substance. These can be useful for exploring likely routes of degradation as well as for helping identify potential transformation/degradation products (both for analysis and evaluation). One programme is the EAWAG-BBD Pathway Prediction System (formally from the University of Minnesota), which can be found at:http://eawag-bbd.ethz.ch/predict/index.html.

Multi-media modelling

Results from multi-media modelling (e.g. Mackay level III model as this is included in the EPIWIN QSAR package) could also be explored in order to evaluate the environmental exposure and compartment(s) of specific concern. Typically, the results used from such models are the relative (%) mass of the substance (in a steady state situation with continuous environmental release) in each environmental compartment, in a simple "Unit world" consisting of air, surface water, sediment and soil.

Typically, the default situation is assumption of an environmental release pattern with equal release to air, surface water and soil (see the default settings in the Mackay level III part of the EPIWIN). It should be noted that the results of such models should be regarded as qualitative or at most semi-quantitative as they strongly depend on the relative size of the environmental compartments, the emission pattern (see below) and partitioning and transformation parameters employed in the modelling.

Mackay level III modelling includes environmental inputs and provides information on the likely relative importance of intercompartmental transport rates and environmental properties. It is also dependent on the release pattern (fraction of emission between air, water, soil) and thus also on the use of the substance. Mackay level III model can be considered suitable to support the relevance assessment of the compartment(s) for testing.

If a more relevant /realistic release pattern than equal emission rate to air, water and soil can be assumed based on knowledge about use of the substance, the Mackay level III model should be run with an appropriately changed release pattern (for example, this can easily be done in the EPIWIN model package). Typically, but depending on the use profile of the substance, it is relevant to run such models assuming the default environmental risk assessment emission pattern, e.g. release to water only. Alternative and freely available models exist beside that included in EPIWIN, e.g. EQC (Mackay *et al.*, 1996; see also https://www.trentu.ca/cemc/resources-and-models/eqc-equilibrium-criterion-model), SIMPLEBOX (Schoorl *et al.*, 2016; see also www.rivm.nl/en/Topics/S/Soil_and_water/SimpleBox).

Another option is to consider comparing the results of the modelling with the normally employed environmental exposure assessment where emission normally takes place *via* emission to STP, such as **SimpleTreat v4.0** model[39]. SimpleTreat v4.0, which is incorporated in EUSES v2.2, is an assessment tool for the fate and distribution of substances in an STP where the fractions at steady state are presented: volatilisation to air, adsorption to STP-sludge, STP-degradation and the emission fraction to surface water. A process for volatilisation

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[39] Available at: https://www.rivm.nl/en/soil-and-water/simpletreat (last accessed: November 2023)

from the aeration tank (Mikkelsen, 1995) is incorporated in the current version and elimination due to volatilisation from the sewage system is also estimated. Ionisable organics are present as neutral and charged species in fraction according to input substance parameters such as apparent octanol-water partition coefficient (Dow) and acid dissociation constant (pKa) at environmental characteristic pH. Such models also typically employ the fugacity concept. The fraction sorbed to STP sludge is normally assumed to be disposed of on soil and hence indirect exposure of the soil compartment has to be assumed.

For some substances which have distinctive use patterns and pulsed releases into the environment, more specific models could be considered, e.g. the FOCUS models for agrochemicals. The FOCUS modelling framework relies on mechanistic process-based models to predict the exposure from substances, either directly applied in agricultural areas or driven by weather-related compartmental transfer processes such as run-off and drainage. FOCUS models can thus be used to identify the relevant compartment(s) to which agrochemicals will partition, taking into account the specific use and release patterns of those substances.

Multimedia fate modelling, especially the **Mackay fugacity level I** modelling, can be used in screening to assess whether volatilisation of a substance is expected in simulation testing. This model is a steady state calculation with no inflow, outflow, nor intermedia transport. Degradation reactions are not considered either. This type of model predicts the fate and environmental distribution of neutral substances based on calculations considering melting point (MP), VP, HLC, water solubility (WS), log K_{ow} and K_{oc}. The results give an indication on where a substance is likely to partition and in which environmental media the concentrations are likely to be highest (i.e. the fugacity capacity is largest). Hence, Level I models could be suitable for predicting partitioning of substances in a closed system like the closed test vessels used for volatile substances in simulation tests. Therefore, results of Level I models could be used when deciding on the appropriate test system for simulation testing of volatile substances or whether some modifications are needed in the test design to minimise volatilisation (see R.11.4.2.1.3 and in *ECHA note on Volatile substances* available on ECHA Website. Mackay fugacity level 1 modelling can not be used on its own to predict fate and environmental distribution. The results of the SimpleTreat model may also give some insight on the possible volatilisation during simulation testing as it seems to often give relatively similar results as the Level I modelling. However, volatilisation in a simulation test cannot always be excluded even in those cases where distribution to air is predicted to be low by these models. If the model overestimates partitioning to the aquatic and/or sediment compartment, partitioning to air will be underestimated (*Guidance on IR&CSA*, Chapter R.16).

Finally, freely available multi-media models focussing on the potential for long-range environmental (mainly air) transport also exist like the OECD Pov and LRTP Screening Tool (OECD, 2006) (for further information on models predicting long-range transport see Section III of the draft document on long-range environmental transport prepared by the POP Review Committee; UNEP, 2022). They could be employed for considering possible relevance of certain environmental compartments of concern for simulation degradation testing, in particular whether or not pristine environmental compartments (e.g. open sea) may be exposed to a significant extent.

With respect to the results of the distribution modelling results, they should only be regarded as qualitative or semi-quantitative and a case-by-case evaluation[40] of the results is needed.

R.11.4.1.1.5 Field studies for persistence

If field studies are available, they are an option to additionally assess the persistence of substances under realistic outdoor conditions. In contrast to laboratory studies that often include artificial elements such as drying and sieving of soils (e.g. OECD TG 307 study) it is possible to study the degradation of a substance under natural conditions in the undisturbed environment. One of the most important advantages of field studies over laboratory studies is the option to run them over long periods up to several years. There is no risk that the system gets exhausted as what happens with longer-lasting laboratory studies where the microbiological activity might significantly decrease if the study period needs to be extended to derive reliable half-lives. With field studies, it is also possible to study the accumulation potential of substances over several years. However, compared to laboratory studies, field studies are semi-controlled with a range of varying environmental factors. These factors and uncertainties derived therein should be taken into account in the assessment.

Reliable field studies can be used in a *Weight-of-Evidence* approach. PBT assessment is normally not bound to local conditions whereas field studies are particularly dependent on local conditions. Therefore, results from field studies are not directly comparable with one another, laboratory tests or P/vP criteria.

When including field studies in the *Weight-of-Evidence*, the varying temperature conditions should be taken into account (if available). Consideration should be given to whether temperature correction should be applied. Guidance on test temperature is provided in Section R.7.9.4.1 of *Chapter R.7b* of the *Guidance on IR&CSA*.

In general, field studies can be carried out for the different compartments of interest. For the soil compartment several guidance documents exist on how to conduct terrestrial field dissipation studies. These guidance documents were mainly developed for Plant Protection Products (PPPs) but can also be used for any other chemical substance. The NAFTA guidance (2006) is based on the degradation behaviour of substances under realistic exposure conditions considering all possible dissipation and degradation pathways. The use of a conceptual model of the substance behaviour that would depend on results from laboratory studies should be supported and the results confirmed by different modules of the field study. In addition to the environmental conditions, the mass balance and potential NER formation are to be taken into account to determine in which extent the DegT50 of field studies would be comparable to the half-life criteria.

EFSA developed a guidance (EFSA, 2014) focused on biodegradation in the soil matrix. It describes how surface processes such as volatilization and photolysis as well as dissipation by

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[40] This should include consideration of the values of water solubility, octanol-water and organic-carbon partitioning coefficients, vapour pressure and half-life coefficients used in the modelling, since these values may be predicted by the model, even if measured values have been used as input values in the model. A robust study summary should be provided giving sufficient information on the modelling. (i.e. default assumptions and input parameters of the model). Finally, consideration on how the substance is likely to be released to the environment should be made. This is important to understand which fugacity model may be most appropriate – for example 100% release to water, soil etc. A sense check should also be made to review whether the predictions seem reasonable.

leaching to deeper soil layers are taken into account in order to get a DegT50 value that can be used in exposure modelling. In order to avoid surface processes, it is recommended for instance to mix the substance with the topsoil layer of the field or to cover the field after substance application with a sand layer. For mobile substances that can be leached down to deeper soil layers during the course of the study, the EFSA guidance requires sampling down to a depth were no substance can be found anymore to account for all residues.

The OECD Guidance document 232 (OECD, 2016) considers aspects from both the NAFTA (2006) and the EFSA guidance (2014) and is the most recent guidance document. It should be used for the conduct of field degradation studies.

Lysimeter studies, which are often carried out with radiolabelled substances (OECD, 2000a), can also provide useful information about the degradation behaviour of a substance to be used in the context of the P-assessment. Guidance for deriving DegT50 values from lysimeter studies is provided in FOCUS (2014).

For studying the behaviour of a substance in water or sediment, less guidance is available. However, meso- or macrocosm studies, which are sometimes used in ecotoxicology, can in general be used to provide valuable information on the fate of the substance, e.g. on the partition behaviour of the substances. 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 DegT50 (EFSA, 2014). Guidance on how to derive DegT50 values from meso- or macrocosm studies is provided in Deneer *et al.* (2015).

For further references, please see Section R.7.9.4.2 in *Chapter R.7b* of the *Guidance on IR&CSA.*

R.11.4.1.1.6 Monitoring data

Monitoring data in themselves cannot demonstrate persistence because the presence of a substance in the environment is dependent on a range of factors other than degradation rates, namely emission and distribution rates. Potential sources, trends of volume, uses and releases should be considered when evaluating the suitability of monitoring data in the P/vP assessment. Nevertheless, if monitoring data as a part of a *Weight-of-Evidence* analysis show that a substance is present in remote areas (i.e. long distance from populated areas and known point sources, e.g. arctic sea or Alpine lakes), it may be possible to conclude a substance as P or vP. 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 (in both directions: P/vP or not P/vP). Use of monitoring data in P/vP-assessment encompasses several uncertainties and conclusions should be drawn on the basis of monitoring data only when there is sufficient understanding of the substance distribution and transport behaviour and under the condition that the uncertainties in the monitoring data presented are adequately addressed. The lack of detection of a substance in monitoring data should be considered carefully as it does not necessarily mean that a substance is not persistent (e.g. shortcomings in analytical methods may affect monitoring of substances in the environment). If monitoring data show that the substance levels in environmental media or biota are rising, the reasons for such a time trend should be assessed very carefully against the information on the time trends of volumes, uses and releases. Where monitoring data clearly indicate that the substance fulfils the vP-criterion or, depending on the case, that the P criterion is fulfilled in addition to other supporting information (and without conflicting data), it may not be necessary to generate simulation degradation data. In the latter case, conclusions on the fulfilment of the P/vP criteria may be drawn based on the monitoring data, the information on the substance distribution/transport behaviour, in addition to other supporting information used as part of a *Weight-of-Evidence* analysis.

R.11.4.1.2 Bioaccumulation assessment (B and vB)

This section deals with assessment of bioaccumulation data accepted for use in the PBT and vPvB assessment and further provides guidance on how to evaluate whether a substance meets the B or the vB criteria. For this puspose, the section comprises a decision scheme on how to use data of different experimental tests as well as non-testing information. For a B and vB assessment all available relevant information should be taken into account. In accordance with Annex XIII all available information/evidence on bioaccumulation must be considered in a *Weight-of-Evidence* approach. This comprises results from bioaccumulation experiments, monitoring data from the field and toxicokinetic information from toxicity studies on accumulation as well as other testing and non-testing indications of bioaccumulation. The order of data types presented in the below ITS and in the following subsections are not meant to define the order of importance or weight of individual data types. The data types are presented so that the experimental data providing information on bioaccumulation directly comparable with the B/vB criteria are described first and other data relevant for the assessment as last.

Guidance on the evaluation and validation of both testing data and non-testing information can be found in Section R.7.10 of *Chapter R.7c* of the *Guidance on IR&CSA*.

For substances containing multiple constituents, impurities and/or additives, the guidance provided below applies to that/those "part(s)" of the substance, which is/are the target of assessment and testing. The criteria for selecting an appropriate assessment approach are provided in Section R.11.4.2.2.

R.11.4.1.2.1 Integrated Assessment and Testing Strategy (ITS)[41]

If a substance is imported or produced in an amount of more than 100 t/y, information to fulfil REACH Annex IX, 9.3.2. standard information requirement is mandatory. The option of waiving the bioaccumulation test according to Column 2 of REACH Annex IX can only be taken if the information from the experimental test is not required for the conclusion on the PBT/vPvB-properties (see also Section R.11.3.3). Similarly, the standard aquatic bioaccumulation test requirement cannot be adapted according to REACH Annex XI, if the PBT/vPvB assessment shows that a bioaccumulation test in aquatic species is necessary (and it is technically feasible). However, it is noted that the possibility to use information referred to in REACH Annex XI should be investigated in the frame of the PBT/vPvB assessment first before proposing a bioaccumulation test. In that case the evaluation of the B and vB criteria for the PBT and vPvB assessment should be performed simultaneously with the assessment of the BCF

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[41] The mitigating factors that are listed below only refer to the assessment of the B and vB criteria in the context of the PBT and vPvB assessment. If bioaccumulation appears to be a critical parameter in the risk assessment process, it could still be necessary to perform a bioaccumulation test, although this may not be needed from the perspective of the PBT and vPvB assessment.

value. Detailed guidance regarding an ITS for BCF assessment is presented in Section R.7.10 of *Chapter R.7c* of the *Guidance on IR&CSA*.

Figure R.11—4 in this section should be seen as a detailed scheme of the B-assessment block within the ITS.

If the tonnage produced or imported is below 100 t/y, normally a bioaccumulation test is not required and therefore a BCF value may not be available. In that case it should be first considered if the available testing and non-testing data are sufficient to conclude on the B-properties for those substances produced or imported at <100 t/y or if bioaccumulation testing is needed and hence required to draw a reliable conclusion.

A substance meets the B or vB criterion if it is considered bioaccumulative or very bioaccumulative in one or more of the relevant food chains or receptors, e.g. the aquatic environment, the terrestrial environment or wildlife or humans. To determine these classifications, all reliable and relevant information on the bioaccumulation potential of a substance has to be gathered by the registrant and considered in the CSA, including the PBT/vPvB assessment. If available, such information might be sufficient to conclude whether the substance is vB, B, or not B.

- If the substance has a log K_{ow} lower than 4.5 and no specific mechanism of uptake apart from hydrophobic partitioning is known and the possibility for accumulation in other food chains than the aquatic food chain can be ruled out, then the substance can be considered as not B and not vB. In such a case further evaluation of the B and vB criteria is not necessary. A partitioning process other than lipophilic partitioning could for example be the binding to proteins. The possibility of a substance to accumulate in air-breathing organisms instead of aquatic organisms is indicated by the combination of a log $K_{oa} > 5$ with a log $K_{ow} > 2$. A high metabolism rate for the substance could mitigate such a potential for bioaccumulation in air-breathing organisms.

- If the substance has very limited potential to be taken up by biota, this might be indicated by several factors based on substance properties listed below. These indicators should be confirmed by other information to exclude the possibility of a high bioaccumulation potential. If such a lack of significant uptake is proven, the substance can be considered as not B and not vB. In such a case, further evaluation of the B and vB criteria is not necessary. It should be noted that the only conclusion drawn based on this information is that the substance is not (very) bioaccumulative, and not that the substance can't be taken up at all. A substance is unlikely to meet the B criterion (i.e. unlikely to have a BCF > 2,000) if some or all of the following indicators are met:

  1. ***an average maximum diameter ($D_{max\ aver}$) of greater than 1.7 nm (Further information is provided in section R.11.4.1.2.10)***[53]
  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} (Indicator 2.) is a general indicator for uptake, distribution and excretion whereas the octanol solubility (Indicator 3.) reflects the potential for mass storage, which might further prevent uptake in significant amounts in the organism.

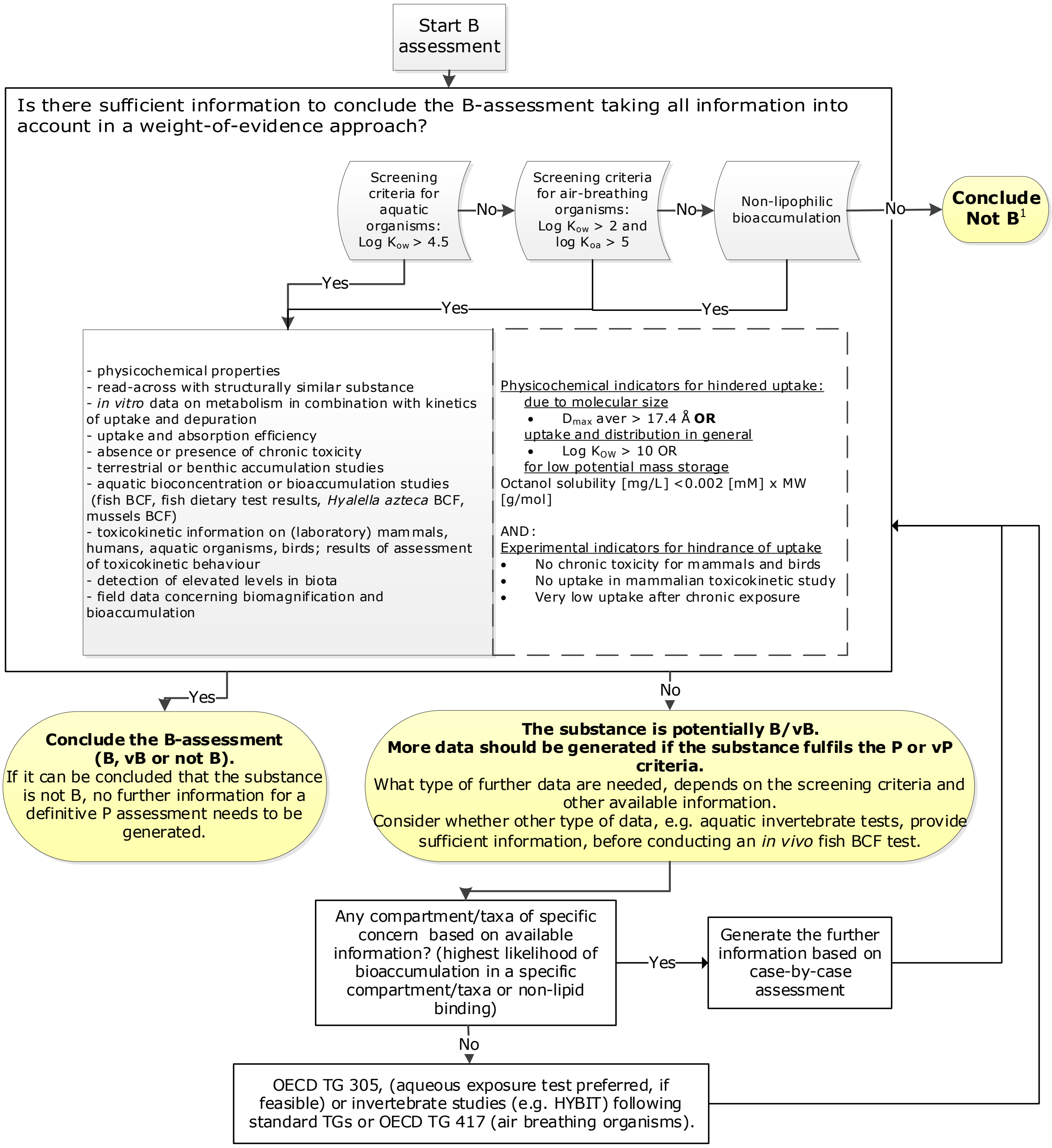
The supplementary information to confirm this limited uptake may comprise data from a chronic toxicity study with mammals (≥ 90 days, showing no toxicity), a toxicokinetic study with mammals or birds, a bioconcentration study with invertebrates, or reliable read-across from a structurally similar substance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

- If there is a reliable aqueous bioaccumulation study available, such as an aqueous exposure OECD TG 305 study, or a bioaccumulation study with *Hyalella azteca* (OECD draft test guideline under revision; OECD, 2023), or a reliable standard bioaccumulation study with another aquatic invertebrate such as mussels, the result can be directly related to the criteria for B and vB. If the BCF is higher than 2000 or 5000 the substance can be assigned to be B or vB. If a reliable BCF is lower than the B criterion (BCF < 2000), this is an indication of reduced uptake or metabolism for hydrophobic substances with a Log K_{ow} > 4.5. Rapid metabolisation of a substance may lead to a lower BCF value. In vitro methods according to OECD TG 319A (Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP)) and OECD TG 319B (Determination of in vitro intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)) provide information on biotransformation in the organism, and thus have potential to support the assessment of bioaccumulation. Further research in future may increase the predictive capacities of *in vitro* methods. Reduced uptake and metabolism will most likely also mitigate the bioaccumulation potential in general. If there are no other indications for accumulation outside the pelagic food chain, such as elevated concentrations in terrestrial and air-breathing organisms, the substance can be considered as not B and not vB. Such a conclusion could also be drawn for substances having log K_{ow} < 4.5. However, in that case additional consideration should be given to the possibility of accumulation in food chains containing air-breathing organisms or humans.
- The results of a dietary bioaccumulation study with fish, such as an OECD TG 305 dietary exposure study, can be used in a similar way to that described above to conclude on the B and vB criterion. 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 REACH Annex XIII criteria, unless it can be demonstrated that the uptake rate constant cannot be reliably estimated with the available methods. In that case other methods (direct application of k_2, or using a correlation of dietary BMF and BCF results to interpolate other BMF results) as describe in the Guidance document on aspects of OECD TG 305 (OECD, 2017) shall be explored. For very hydrophobic substances, k1 estimates may become increasingly uncertain.
- In some cases, a conclusion can be drawn from additional information only. This could be information from field studies showing clear accumulation in a food chain, or long half-lifes from monitoring studies in humans or wildlife. If this type of information is

assessed as reliable, this could be considered as sufficient information to draw a conclusion on the bioaccumulation potential as part of a *Weight-of-Evidence* approach.

In any other case, no conclusion on the bioaccumulation potential can be drawn and the B and vB properties should be evaluated in more detail. Based on the above described information, this refers to the following cases:

- No direct information on bioaccumulation (e.g. BCF, BAF or BMF data) are available and the substance has a Log K_{ow} higher than 4.5, or the partitioning process into aquatic organisms is not driven by lipophilicity.
- Information on bioaccumulation is available for aquatic compartment indicating that substance is not B, but the screening information indicates potential bioaccumulation in air-breathing organisms and no conclusion could be derived for them based on available data. In this case new information may need to be generated on bioaccumulation potential in air-breathing organisms (mammals), e.g. by appropriate testing or by generating suitable biomonitoring data, based on a case-by-case assessment of the needs.
- Direct data on bioaccumulation are available but these data are not reliable and/or consistent to a degree sufficient to conclude whether the B or vB criteria are met.



Start B assessment

Is there sufficient information to conclude the B-assessment taking all information into account in a weight-of-evidence approach?

Screening criteria for aquatic organisms: Log $K_{ow} > 4.5$

Screening criteria for air-breathing organisms: Log $K_{ow} > 2$ and log $K_{oa} > 5$

Non-lipophilic bioaccumulation

Conclude Not B[1]

- physicochemical properties
- read-across with structurally similar substance
- *in vitro* data on metabolism in combination with kinetics of uptake and depuration
- uptake and absorption efficiency
- absence or presence of chronic toxicity
- terrestrial or benthic accumulation studies
- aquatic bioconcentration or bioaccumulation studies (fish BCF, fish dietary test results, *Hyalella azteca* BCF, mussels BCF)
- toxicokinetic information on (laboratory) mammals, humans, aquatic organisms, birds; results of assessment of toxicokinetic behaviour
- detection of elevated levels in biota
- field data concerning biomagnification and bioaccumulation

Physicochemical indicators for hindered uptake:
due to molecular size
- D_{max} aver > 17.4 Å **OR**

uptake and distribution in general
- Log K_{OW} > 10 OR

for low potential mass storage
Octanol solubility [mg/L] <0.002 [mM] x MW [g/mol]

AND:
Experimental indicators for hindrance of uptake
- No chronic toxicity for mammals and birds
- No uptake in mammalian toxicokinetic study
- Very low uptake after chronic exposure

Conclude the B-assessment (B, vB or not B).
If it can be concluded that the substance is not B, no further information for a definitive P assessment needs to be generated.

The substance is potentially B/vB.
More data should be generated if the substance fulfils the P or vP criteria.
What type of further data are needed, depends on the screening criteria and other available information.
Consider whether other type of data, e.g. aquatic invertebrate tests, provide sufficient information, before conducting an *in vivo* fish BCF test.

Any compartment/taxa of specific concern based on available information? (highest likelihood of bioaccumulation in a specific compartment/taxa or non-lipid binding)

Generate the further information based on case-by-case assessment

OECD TG 305, (aqueous exposure test preferred, if feasible) or invertebrate studies (e.g. HYBIT) following standard TGs or OECD TG 417 (air breathing organisms).

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[1] This conclusion can not be drawn if there is other relevant information available, e.g. as listed in the subsequent text box, that does not support the 'not B' conclusion.

Figure R.11—4: Integrated assessment and testing strategy for B-assessment.

Integrated assessment and testing of Bioaccumulation - Explanatory Notes to Figure R.11-4.

Bioaccumulation is most often driven by **partitioning to storage lipid**. In these cases, a log K_{ow} greater than 4.5 is used as screening criterion for aquatic organisms, and a log K_{ow} greater than 2 together with a log K_{OA} greater than 5 as screening criteria for air-breathing organisms. If log K_{ow} is less than 2, the substance can normally be regarded as not fulfilling the B/vB criteria. If the substance has a log K_{ow} between 2 and 4.5, but log K_{oa} is below 5, then it can be expected that the substance is neither hydrophobic enough to bioaccumulate in aquatic species, nor that it is bioaccumulating in air-breathing species, because it can be eliminated rapidly enough by exhalation. Because of the importance of log K_{ow} as a screening criterion, its uncertainty needs to be taken into account, especially when close to the threshold. Guidance on the derivation of log K_{ow} is given in chapter R.11.4.1.2.10 and in the Appendix R.11-5

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 *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 (Kelly *et al.*, 2009). Guidance on consideration for bioaccumulation assessment of organic substances that do not partition to lipid is given in Appendix R.7.10 3 of *Guidance on IR&CSA*, Chapter R.7c.

When assessing the bioaccumulation potential, all available relevant information has to be considered in a *Weight-of-Evidence* approach. Information to be considered includes:

- physicochemical properties;

- read-across with structurally similar substance (Further data R.11.4.1.2.10);

- in vitro data on metabolism in combination with kinetics of uptake and depuration (R.11.4.1.2.4);

- uptake and absorption efficiency (R.11.4.1.2.3);

- absence or presence of chronic toxicity (R.11.4.1.2.9);

- terrestrial or benthic accumulation studies (R.11.4.1.2.5);

- aquatic bioconcentration or bioaccumulation studies, such as fish bioaccumulation studies with aqueous or dietary exposure, *Hyalella azteca* bioconcentration test (OECD, 2023), mussel bioconcentration test (ASTM, 2003)) (R.11.4.1.2.2, R.11.4.1.2.3);

- toxicokinetic information from dietary exposure Bioaccumulation Fish test (R.11.4.1.2.3) and toxicokinetic information from studies with mammals (R.11.4.1.2.8);

- detection of elevated levels in biota and field data concerning biomagnification and bioaccumulation (R.11.4.1.2.6);

- indication of hindered uptake by physicochemical parameters, together with observed absence of chronic toxicity in experimental studies or very low uptake after chronic exposure in mammals and birds; physicochemical parameters comprise molecular size, logKow and octanol solubility; molecular weight as parameter to indicate hindered uptake is no longer supported (R.11.4.1.2.10 and Appendix R.11-1.1).

The Bioaccumulation Assessment Tool (BAT), accompanied by guiding principles in the BAT manual (Armitage *et al.*, 2021), promotes standardised recording and evaluation of various lines of evidence related to the endpoint bioaccumulation. When integrating and weighing information, reliable evidence of bioaccumulation cannot be outweighed by information showing no bioaccumulation. (see also *Chapters R.4* of the Guidance on IR&CSA and Section R.11.4.1). See also ECHA *Weight-of-Evidence* templates[13]

If the *Weight-of-Evidence* approach described under "Conclusions on the Endpoint" is not sufficient to draw a conclusion, the performance of an experimental bioaccumulation test or generation of other appropriate bioaccumulation information is required. However, before such a study is conducted for assessing the B and vB criteria, the P criterion should be investigated in order to prevent unnecessary testing of animals. Further generation of information on bioaccumulation is only necessary, if the P criterion has been confirmed to be fulfilled for the substance.

If generation of further bioaccumulation data is necessary, there are several options for the most appropriate strategy. Additional data should always be generated in a tiered way revisiting the B-assessment after each time new data are made available. In normal case it may be possible to conclude on the B/vB properties after one study, but in specific cases several bioaccumulation studies may be needed.

The available data define the choice of the study/test. Hereby, the understanding of in which type of species/compartment the bioaccumulation potential seems highest is crucial for the choice of the test. In very specific cases, the most relevant compartment(s) of exposure may also influence the choice of the study.

R.11.4.1.2.2 Experimental aquatic bioconcentration factor (BCF) data

It should be noted that the greatest weight under PBT assessment for REACH is placed on a valid aquatic BCF test due to the current understanding that BCF is the most representative way of reflecting the bioaccumulation potential of a substance, where aquatic bioaccumulation is relevant. If BCF-values are inconsistent with other data types, it is very important to address the reasons for such inconsistency and discuss carefully about the plausibility of the BCF-values in this context. If a substance has a valid and plausible aquatic BCF > 2000 or 5000 (indicating a significant accumulation in the test organism), the substance is defined as B or vB regardless of whether further indications for biomagnification or trophic magnification exist.

Aqueous Exposure Bioaccumulation Fish Test

In most cases where experimental information on bioaccumulation in aquatic species is needed, a flow-through aqueous exposure bioconcentration fish test according to OECD TG 305-I (or OECD TG 305-II) gives reliable results for comparison with the B/vB criteria. Whenever possible, the freshwater amphipod *Hyalella azteca* bioconcentration test (HYBIT) (OECD draft TG under revision; OECD, 2023) should be considered to avoid vertebrate testing (See section Aquatic invertebrate tests: Hyalella azteca bioconcentration test (HYBIT), page

100). Only in specific cases, described in following subsections, other study/test types may be warranted as the option for generating further information.

In line with Annex 1 of the OECD TG 305, the following definitions are used in this guidance:

- The bioconcentration factor (BCF) at any time during the uptake phase of this accumulation test is the concentration of test substance in/on the fish or specified tissues thereof (C_f as mg/kg) divided by the concentration of the substance in the surrounding medium (C_w as mg/L). BCF is expressed in $L·kg^{-1}$. Please note that corrections for growth and/or a standard lipid content are not accounted for in this definition of the BCF.

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

- The kinetic bioconcentration factor (BCF_K) is the ratio of the uptake rate constant, k_1, to the depuration rate constant, k_2 (i.e. k_1/k_2 – see corresponding definitions in Annex 1 of the OECD TG 305). In principle the value should be comparable to the BCF_{SS} (see definition above), but deviations may occur if steady-state was uncertain or if corrections for growth have been applied to the kinetic BCF.

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

- The lipid normalised, growth corrected kinetic bioconcentration factor (BCF_{KgL}) is a 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 as described in Annex 5 of the OECD TG 305 (see also Appendix R.11-6).

Bioconcentration data from controlled laboratory experiments can be used in assessing the bioaccumulation potential of a substance. For example, OECD TG 305-I: Aqueous Exposure Bioconcentration Fish Test (OECD, 2012a) or an equivalent test protocol for fish can be followed for producing experimental bioconcentration data. Valid BCF results from the Bioconcentration Fish Test can be used directly for comparison with the B and vB criteria. Nevertheless, it is underlined, that in addition to BCF values, other relevant information should be considered. The REACH Annex XIII Introduction requires all other available bioaccumulation data to be taken into account in an integrated manner and applying a *Weight-of-Evidence* approach using expert judgement to derive the conclusion. If BCFs seem not coherent with other data or there are very different BCF-values available, it is important to address the reasons for inconsistency and discuss in which way this inconsistency impacts the overall conclusions on bioaccumulation potential.

The use of other taxonomic groups than fish (e.g. Hyalella azteca, OECD, 2023; mussel bioconcentration test, ASTM, 2003) is possible for measuring bioconcentration in the aquatic environment. Valid BCFs determined in tests with other taxonomic groups can be used in assessing whether or not the B/vB criteria are met. Furthermore, in case a Log K_{ow} as screening information is considered likely to be reliable for estimating the bioaccumulation potential of a substance while still some experimental information is needed to refute or confirm this assumption, the OECD TG 305-II: Minimised Aqueous Exposure Fish Test may also be used to assess B or vB, provided that the final results will most likely not result in borderline cases of meeting either the B or vB criterion. This should be investigated before the test is initiated, e.g. by the use of QSARs, to avoid the results of the test being insufficient for the B assessment after the test has been completed. Conditions for selecting the minimised OECD TG 305-II instead of the OECD TG 305-I are described in the OECD TG 305 and it should be noted that the OECD TG 305-II test conducted within those conditions can be used for the bioaccumulation assessment to minimise animal use. Whether minimised tests should be carried out depends on a range of factors including the required level of precision of the determination of the BCF value for a particular substance. For instance, if it is estimated that the BCF-value may be close to the threshold values of either 2000 for 'B' or 5000 for 'vB', the BCF determination by OECD TG 305-II is not warranted because the result may be associated with too much uncertainty. In such a case an OECD TG 305-I test would be appropriate.

Bioconcentration can be tested experimentally for substances that are water soluble to an extent allowing that the exposure concentration(s) can be maintained constant throughout the uptake phase of the test, as demonstrated by regular analytical verification of the exposure concentrations. A proper analytical method should be available to measure the test substance concentration in water at the used test concentrations that should always be below the water solubility limit of the substance (taking into account the effect that the test media composition may have an effective solubility). In addition, an analytical method is required to measure the substance accumulated in the animal tissues. In bioconcentration tests, the water phase must be the only route of exposure and exposure via feed must be avoided.

The aim of the bioconcentration testing is to produce a reliable estimate of how much substance could concentrate from the aquatic compartment (C_w) to fish (C_f) so that a bioconcentration factor (BCF_{SS}) can be calculated by using ratio C_f/C_w at steady-state. However, a BCF_k value is preferred, which is calculated as the ratio of the uptake rate constant (k_1) and the depuration rate constant (k_2). This approach is especially useful in those cases in which steady-state is not reached during the uptake phase, as BCF_k in these cases can still be estimated. If uptake follows first order kinetics and steady-state was reached, calculation of BCF_k and BCF_{ss} should in principle lead to the same result. However, for bioaccumulative substances a real steady-state is often not attained during the uptake phase, and the conclusion of steady-state from the concentrations in fish at three consecutive time points could be erroneous. If the BCF_k based on first order kinetics is significantly different from the BCF_{SS}, this is a clear indication that steady-state has not been attained in the uptake phase.

Besides that, the BCF_{ss} cannot be corrected for the growth of fish as no agreed method is available to correct BCF_{SS} for growth. The increase in fish mass during the test will result in a decrease of the test substance concentration in the growing fish (= growth dilution) and thus the BCF may be underestimated if no correction is made. To avoid complications of interpretation due to growth dilution, the feeding rate should be selected such that fast growth and large increase of lipid content are avoided. If possible, avoid testing fish species during a (juvenile) life-stage with rapid growth (OECD TG 305). Although there has been some criticism on the growth correction recently (Gobas and Lee, 2019), this is considered insufficient justification to refrain from growth correction. Therefore, growth correction should be applied in the B assessment. Further explanations on correction for growth dilution are given in Appendix R.11-6. To avoid uncertainty caused by growth correction, non-growing adult fish are preferred for testing.

Growth dilution may affect both BCF_{SS} and BCF_K and therefore the BCF_K should be calculated and corrected for growth dilution, BCF_{kg}, if fish growth is significant during the test (this is especially important for fast growing juvenile fish, such as juvenile rainbow trout, bluegill sunfish and carp). OECD TG 305 (Annex 5) contains two different methods for growth dilution correction. For bioaccumulative substances the kinetics of bioaccumulation are slow and growth dilution may have a major impact on the BCF. In conclusion, BCF_{Kg} is preferred for PBT substances due to i) the slow kinetics possibly leading to non-equilibrium within the timeframe of the experimental bioaccumulation test, and especially ii) the correction for growth dilution, which is not included in the BCF_{SS}. More emphasis on BCF_{Kg} is also given in OECD TG 305.

For older fish bioaccumulation studies, information on growth may not be available. In this case, an assessment of the likely significance of growth on the results should be made to determine what weight should be given to the study in the *Weight-of-Evidence* assessment. As noted in the OECD TG 305 (paragraph 32), juvenile fish may be fast growing at the life-stage (and size) they are tested in the OECD TG 305. Small rainbow trout (*O. mykiss*) are an example of this. In contrast, fish such as zebrafish (*D. rerio*) are usually adults and therefore significantly slower growing (for example see an analysis in Brooke and Crookes, 2012). In the absence of growth data, the uncertainty in a BCF value derived from a fast-growing fish will be greater than a slow growing fish, which is important for results near a regulatory threshold. Overall, any approach to using fish bioaccumulation data where growth data are not available needs to be considered on a case-by-case basis with justification for the conclusion drawn. It should be noted that apart from growth dilution, several other factors have been suggested to potentially influence test results, for example water-to-fish-ratios, temperature, sex differences, feeding procedure and slight variances in water chemistry and dissolved oxygen concentrations (Wassenaar *et al.*, 2019). Most of these, and other variables can influence the metabolic capacity of the test animals and/or are directly related to changes in activity or oxygen consumption.

The preferred way to derive k_1 and k_2 is, in most cases, to fit both parameters simultaneously by non-linear regression to the data for both, the uptake phase and the depuration phase (see Annex 5 of the OECD TG 305 (OECD, 2012a), and Guidance document on aspects of OECD TG 305 (OECD, 2017)). This procedure represents the best way to fit both parameters to all available data and yields a consistent fit for the uptake and depuration phase. Another way to derive k_1 and k_2 is to use a sequential fitting procedure and find values of k_1 and k_2 independently. This may sometimes lead to a gap in the fit between the uptake and depuration phase. However, a benefit of sequential fitting is that k_2 is fitted first, and is therefore unaffected by the uptake phase, which might be relevant if kinetics of both phases are significantly different due to the increased fish size during the test duration. The depuration rate constant, k_2, is the parameter of most interest in a bioaccumulation test given that the uncertainties in its derivation are understood and can be addressed. As recommended in Annex 5 of OECD TG 305, visual inspection of the modelled uptake and depuration curves when plotted against the measured sample data can be used to assess and compare the goodness of fit of both methods. This is a reporting requirement of OECD TG 305.

The data could be transformed by taking the natural logarithms, if this transformation reduces the variation in the replicates and/or leads to a better fit of the data. However, care must be taken as such a transformation could give too much weight to very low concentrations observed at the end of the depuration phase, leading to a worse fit towards the end of the uptake phase and beginning of the depuration phase. If fish concentrations are log normal-transformed, a geometric mean for the water concentration should be used instead of an arithmetic mean. The fitting routine bcmfR R-package (OECD-TG305 R-Package)[42], provided by the OECD to support the OECD TG 305, allows also box-cox transformation next to untransformed and ln-transformed data. This box-cox transformation can be considered as an intermediate between the untransformed and ln-transformed data. Next to the fitting itself, it also determines which transformation fits the best from a statistical point of view.

Normally, the concentration of the test substance in fish tissues should be lipid normalised. Suitable methods should be used for determination of lipid content (Schlechtriem *et al.* 2012). A lipid normalisation to a fish with a 5% lipid content as recommended in OECD TG 305 should be performed unless it is evident that the substance does not primarily accumulate in lipid tissues. The resulting BCF that is preferred for a comparison with the bioaccumulation criteria is the kinetic growth corrected and subsequently lipid normalised (to 5% lipids) BCF value (BCF_{KgL}). A justification is needed in case no normalisation is carried out.

Aquatic invertebrate tests: Hyalella azteca bioconcentration test (HYBIT)

Bioaccumulation of aquatic species, preferably fish, is a standard information requirement under REACH Annex IX. The criteria for (very) bioaccumulative substances under REACH Annex XIII are based on bioconcentration factors in aquatic species. Fish as well as other aquatic species, including aquatic invertebrates, may be used for bioaccumulation assessment (Section R.7.10.3.1 of Chapter R.7c of the *Guidance on IR&CSA*). Information on bioaccumulation in aquatic invertebrates has been used as part of the weight-of-evidence in B/vB identification[43].

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 2000a; ASTM International 2020). The freshwater amphipods can be easily cultured in the laboratory and are available during the entire year. Due to their high reproduction rate and fast growth, experimental organisms can be raised within a few weeks to adult size to meet the need for a high number of large organisms required for bioaccumulation testing (Schlechtriem *et al.* 2019).

A draft OECD TG for the *Hyalella azteca* bioconcentration test (HYBIT) is under revision (OECD draft TG under revision; OECD, 2023). Current regulation on bioaccumulation focuses on the bioconcentration factor (BCF) for fish. The draft OECD TG provides a non-vertebrate test to derive BCF values, which allow for the assessment of the bioconcentration potential of substances. BCF values for lipophilic substances determined with *H. azteca* show a strong correlation with BCFs that have been determined according to the OECD TG 305 when BCF values are normalised with a default 5 % lipid content (based on whole body wet weight), which is equivalent to the lipid content commonly used for the normalisation of fish BCF values (Schlechtriem *et al.* 2019). *H. azteca* can reach total lipid concentrations of up to 20 % on a dry weight basis (Cavaletto and Gardner, 1999) which is, assuming a dry to wet weight ratio of

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

[43] MCCPs https://echa.europa.eu/documents/10162/98611952-49d5-b0be-d4b9-3df6579315c9; Methoxychlor https://echa.europa.eu/list-of-substances-proposed-as-pops/-/dislist/details/0b0236e1848bd5fc; Chlorpyrifos https://echa.europa.eu/documents/10162/9fbe060a-cf61-4800-cc44-346068cd2a91

0.25 (Othman and Pascoe, 2001), equivalent to the default 5 % lipid content (w/w). However, the lipid content of lab-grown and field-caught *H. azteca* is usually lower than 5%, mostly ranging from about 1-3 % (w/w) (Schlechtriem *et al.* 2019, Kosfeld *et al.* 2020; Arts *et al.* 1995; Huff Hartz *et al.* 2021). A normalisation of *H. azteca* BCF values to a default value of 3 % lipid content (w/w) typical for *H. azteca* is thus recommended.

H. azteca has the ability to metabolise substances, but the biotransformation reactions can be different from fish species (Fu et al, 2021; Kosfeld *et al.*, 2020). 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).

Bioconcentration factors (BCFs) from *H. azteca* bioconcentration tests can be compared against the REACH Annex XIII criteria on B and vB properties. Consequently, it could become an alternative test to the bioaccumulation test with fish (OECD TG 305). This could help to avoid unnecessary vertebrate testing using fish.

The following definitions are used in the OECD draft TG on HYBIT (OECD draft TG under revision; OECD, 2023).

- BCF: The bioconcentration factor at any time during the uptake phase of this accumulation test is the concentration of test substance in/on *H. azteca* (C_H as mg/kg) divided by the concentration of the chemical in the surrounding medium (Cw as mg/L). BCF is expressed in $L \cdot kg^{-1}$. Please note that correction for a standard lipid content are not accounted for.
- BCF_K: The kinetic bioconcentration factor is the ratio of the uptake rate constant, k1, to the depuration rate constant, k_2 (i.e., k_1/k_2 – *cf*. respective definitions). In principle the value should be comparable to the BCF_{SS} (*cf*. respective definition), but deviations may occur if steady-state was uncertain.
- BCF_{KL}: The lipid normalised kinetic bioconcentration factor is normalised to *H. azteca* tissue with a 3 % lipid content (w/w).
- BCF_{SS}: The steady-state bioconcentration factor 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 of time (*cf*. Definition of steady-state).
- BCF_{SSL}: The lipid normalised steady-state bioconcentration factor is normalised to *H. azteca* tissue with a 3 % lipid content (w/w).
- Bioconcentration: Bioconcentration is the increase in concentration of the test substance in or on an organism (or specified tissues thereof) relative to the concentration of test substance in the surrounding medium.
- Depuration: The depuration or post-exposure (loss) phase is the time, following the transfer of the test organism from a medium containing test substance to a medium free of that substance, during which the depuration (or the net loss) of the substance from the test organism (or specified tissue thereof) is studied.

- k_1: The uptake rate constant is the numerical value defining the rate of increase in the concentration of test substance in/on test organism (or specified tissues thereof) when exposed to that chemical (k_1 is expressed in L kg^{-1} day^{-1})[44].
- $\underline{k_2}$: The depuration (loss) rate constant is the numerical value defining the rate of reduction in the concentration of the test substance in the test organism (or specified tissues thereof) following the transfer of the test organism from a medium containing the test substance to a medium free of that substance (k_2 is expressed in day^{-1}).

The small size of *H. azteca* enables reduction of the size of the test system and can lead to substantial savings of test substance as steady state is reached faster and the test duration is shorter than in fish. Especially for test substances with high log K_{ow}, this can be an advantage. *H. azteca* have a larger surface/volume ratio compared with that for larger organisms such as fish. This can theoretically lead to higher estimates of bioconcentration due to adsorption of chemicals to their body surface. However, an apparent deviation from first order kinetics as a result of potential adsorption processes has not been observed for hydrophobic organic substances. Apart from the established flow-through regime commonly applied in bioconcentration studies, semi-static regimes can be used in studies carried out according to the OECD draft TG for HYBIT (OECD draft TG under revision; OECD, 2023). Both regimes have been validated as part of an international ring trial. For information on the test design, further reference can be made to Kosfeld *et al.* (2020) and Schlechtriem *et al.* (2019). An appropriate analytical method of known accuracy, precision and sensitivity should be available for the quantification of the substance in the test solutions and in biological material. The test is applicable to neutral and ionised organic substances. However, there is no information on its applicability to surface-active substances. Testing of very hydrophobic substances via aqueous exposure may be difficult, e.g. due to challenges in establishing stable exposure concentrations. A preliminary experiment should be conducted to optimise the test conditions of the definitive test, such as selection of test substance concentration(s), duration of the uptake and depuration phases.

As for fish, the appropriate duration of the uptake phase is dependent on the hydrophobicity of the test substance. Schlechtriem *et al.* (2019) recommended that for substances having log K_{OW} <4, an exposure period of 2 days is sufficient for the uptake period. For substances with log K_{OW} of 4-6, exposure of at least 4 days is needed to ensure that steady-state conditions are reached. For substances with log K_{OW} >6, exposure periods of >12 days are required and only the BCF_K rather than BCF_{SS} should be calculated.

H. azteca BCF results converted to 3 % lipid ($BCF_{KL,}$ 3 %) are preferred for a comparison against the REACH Annex XIII criteria on B and vB properties, and deviations should be justified. If a substance has a valid and plausible *H. azteca* BCF (3%, w/w) >2000 or >5000 (indicating a significant accumulation in the test organism), the substance is defined as 'B' or 'vB', respectively. A *H. azteca* BCF (3%, w/w) <1200 and <3000 indicates 'not B' and 'not vB' for the aquatic compartment, because even with a lipid normalisation to 5 % (w/w) as applied for fish, the threshold values of 2000 and 5000 for 'B' and 'vB' would not be passed,

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[44] To note that k1 is the initial rate of uptake, as during the uptake phase the concentration increase already levels off due to elimination.

respectively. A 'not B' and 'not vB' conclusion for the aquatic compartment can only be drawn if there is no other relevant and reliable information indicating the contrary.

For lipid normalised *H. azteca* BCF values between 1200 and 2000 and between 3000 and 5000, it cannot be excluded that due to the lower lipid content of the amphipods (3 %, w/w) the bioaccumulation potential of a substance may be underestimated compared to fish (5 %, w/w). In such a situation, further investigations are required to allow a clear "B" and "vB" conclusion. Further information, such as an *in vitro* test according to OECD TG 319 could help to clarify the bioaccumulation potential of the test substances. Estimated reliable BCF values < 2000 and < 5000 obtained from the OECD TG 319 could be used to confirm the 'not B' and 'not vB' conclusions for the aquatic based on the *H. azteca* BCF, respectively. If no conclusion can be made with the available data, and uncertainties remain about the bioaccumulation potential on aquatic organisms, further testing with fish should be considered as a last resort, if justified.

The use of *H. azteca* to assess bioaccumulation is based on current knowledge and experience. Registrants are advised to follow recent and future developments in the field, e.g. via the PBT expert group website.

R.11.4.1.2.3 Dietary Exposure Bioaccumulation Fish Test

A dietary exposure test, preferably OECD TG 305-III: Dietary Exposure Bioaccumulation Fish Test, should be considered for substances for which it is not possible to maintain and measure aqueous concentrations reliably. For strongly hydrophobic substances (log K_{ow} > 5 and a water solubility below ~ 0.01-0.1 mg/L), testing via aqueous exposure may become increasingly difficult. A high K_{oc} usually indicates a strong dissipation of substances from water to organic matter. However, an aqueous exposure test is still preferred for substances that have a high log K_{ow} as long as they have an appreciable water solubility with respect to the sensitivity of available analytical techniques, and the maintenance of the aqueous concentration as well as the analysis of these concentrations do not pose any constraints.

The use of solvents and dispersants (solubilising agents) may help to produce suitably concentrated stock solutions, but is not generally recommended according to OECD TG 305 and every effort should be made to minimise the use of such materials. The use of technical devices such as solid-phase desorption and passive dosing systems (Schlechtriem *et al.* 2017; Wang *et al.* 2022) should be considered, as they are suitable to provide stable aqueous concentrations of strongly hydrophobic substances and allow the testing of such substances in fish BCF studies, according to OECD 305, without using solubilising agents. A preliminary experiment to optimise the test conditions of the definitive test should be conducted. Improved analytical techniques or the use of a radiolabelled substance should be considered first to improve the detection limit of strongly hydrophobic substances in water before deciding on whether a dietary test is indeed the only feasible option.

Nevertheless, if the expected fish concentration (body burden) following aqueous exposure is expected to be below the detection limit within 60 days, the dietary test may provide an option to achieve body burdens that exceed the detection limits for the substance. The endpoint for a dietary study is a dietary biomagnification factor (dietary BMF), which is the concentration of a substance in predator (i.e. fish) relative to the concentration in the prey (i.e. food) at steady state. The dietary test also provides valuable toxicokinetics data including the dietary chemical absorption efficiency, the whole body elimination rate constant (k_2) and the half-life for substances for which obtaining aquatic BCF data is technically not feasible. From the elimination rate constant, an aquatic BCF can be estimated using the Dietary Exposure Test Spreadsheet of OECD 305 TG[45]. Several methods are described in Annex 8 of OECD TG 305 (OECD, 2012a) and the Guidance Document on Aspects of OECD TG 305 (OECD 2017).

The following definitions are used in this guidance:

- The dietary biomagnification factor (dietary BMF) is the term used in OECD TG 305 to describe the result of a dietary exposure test, in which exposure via the aqueous phase is carefully avoided.
- The BMF value from a field study (field BMF) is the concentration of a substance in a predator relative to the concentration in the predator's prey (or food) originating from the same ecosystem at steady-state and in which both, water and dietary exposure may be combined.

The dietary BMF differs from a field BMF in terms of exposure routes. Furthermore, the laboratory dietary study is usually not performed using environmentally relevant concentrations but uses high concentrations in food to dose the organism quickly to a level sufficient to assess the depuration. However, the test concentration in food should not lead to tissue concentrations/body burden that may cause adverse effects in the test fish. Another important difference that can occur between the dietary BMF and the field BMF for substances with biomagnification potential, is the variability of fish growth rates under laboratory and field conditions, but this difference should be covered by growth correction of the kinetic data from juvenile fish in the dietary exposure test. It is possible to simulate field BMFs from lab BMFs to address these two differences using mass balance toxicokinetics (bioaccumulation) models.

Use of the results from a dietary exposure study in the B assessment

The dietary bioaccumulation approach results in a BMF rather than a BCF, which is commonly used for bioaccumulation assessment. However, Annex 8 of the OECD TG 305 summarises approaches currently available to estimate kinetic BCFs from data collected in the dietary exposure study. Guidance document on aspects of OECD TG 305 (OECD, 2017) gives further details in chapter 4.6.3, and summarises three available approaches that can be used to estimate a BCF from dietary study data: 1) Uptake rate constant estimation method, 2) Relating depuration rate constant directly to BCF and 3) Correlating dietary BMF with BCF.

The calculation for the **uptake rate constant estimation method (Method 1)** is based on a model predicted uptake rate constant (k_1) and the depuration rate constant (k_2) determined from the dietary bioaccumulation study. In this way, it is possible to use the dietary experimental data to estimate BCFs, which allow for a comparison against the BCF criteria for PBT assessment outlined in Annex XIII. It should be noted that these calculated BCFs may be more uncertain than experimental BCFs due to the uncertainty in the k_1 prediction. In particular, k_1 is a function of chemical properties relating to the chemical transfer efficiency from water (e.g., membrane permeation or absorption efficiency), the physiology of the fish (body size, respiration rate), the experimental conditions (e.g., dissolved oxygen concentrations, water temperature, gill water pH for ionic substances) and the interdependence of these parameters. Several models are available to estimate a k_1 value

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

needed to calculate an aqueous BCF from a dietary bioaccumulation study (OECD, 2017). Results for k_1 must be used with reference to the models' assumed applicability domains (e.g., mostly restricted to neutral organic substances with log K_{ow} above 3.5 but including some weakly acidic or basic substances as well). Uptake and elimination processes are different for ions compared to neutral chemicals (Rendal *et al.*, 2011) and ionic substances thus need to be discussed separately. For poorly soluble non-polar organic substances, first order uptake and depuration kinetics is assumed. More complex kinetic models should be used only for substances that do not follow first order kinetics. Generally, estimates of k_1 should be derived according to all the models available to give a range of BCFs. These results should be used in a *Weight-of-Evidence* approach for the assessment of bioaccumulation, possibly together with other information on bioaccumulation. The estimation of k_1 may be less reliable for large or bulky molecules, log Kow above ca 9 and/or low assimilation efficiency (see paragraph 253 of OECD, 2017). For very hydrophobic substances, k1 estimates may become increasingly uncertain.

A field BMF > 1 indicates that biomagnification of a substance occurs. The dietary BMF however differs from the field BMF, because 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. This leads to dietary BMF values that are generally lower than field BMF values. For very bioaccumulative substances (BCF > 5000) such as the often used reference substance hexachlorobenzene, the BMF values sometimes have been even below one (e.g. Hashizume et al 2018). 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 BMF value in excess of 1. In a study by Martin *et al.* (2003 a, b) with perfluorinated substances, one of the three substances with a BCF > 2000 had a BMF of 1.0, while the two others had substantially lower BMF values. Therefore, a laboratory dietary BMF below 1 cannot be used to conclude on no B concern 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 criteria.

The lipid and growth-corrected kinetic BMF (BMF_{kgL}) is the preferred endpoint in the OECD TG 305-III (OECD, 2012a). However, there have been several observations indicating that a lipid normalised dietary BMF is unsuitable as an endpoint for bioaccumulation from a regulatory perspective. For instance, in a study examining biomagnification of hexachlorobenzene in carp using different feeding regimes, it was shown that this BMF_{kgL} did not result in a constant value for different experimental conditions (Hashizume *et al.* 2018). In particular, the BMF_{kg} did not correlate well with dietary lipid content (L_{diet}) but was significantly correlated with the lipid content of the fish (L_{fish}). Normalising the BMF_{kg} to the lipid content of both fish and diet (BMF_{kgL}), yielded increasing BMF_{kgL} values with increasing L_{diet}. The same finding was found in the interlaboratory ring test of the OECD TG 305 dietary study (OECD, 2012b: page 142). Based on this finding, Hashizume *et al.* (2018) recommended to normalise the value of the BMF_{kg} to a fish with a 5% lipid content in an effort to standardise dietary BMFs ($BMF_{5\%}$).

Gobas *et al.* (2021) evaluated these results. The presented modelling of these data showed an increasing lipid normalised dietary BMF_L (i.e. BMF divided by L_{fish} and multiplied by L_{diet}) with increasing lipid content in the diet too, while the $BMF_{5\%}$ was shown to be relatively constant with L_{fish}. Thus, also from a theoretical point of view, normalising the BMF to both, the lipid content of the fish and the lipid content of the diet, makes the resulting BMF_L dependent on feeding conditions, while reducing variability if results are only normalised to the fish lipid content.

Environment Agency (2022) noted that the change in lipid content as the food is digested is important, rather than the dietary lipid content itself, and further, concluded that the dietary BMF deviated in this respect from the field BMF. It was reasoned that a dietary BMF that does not depend on the lipid content of the diet is not inconsistent with field BMFs that are dependent on the lipid content of both predator and its prey. Gobas *et al.* (2021) stated that the lipid normalised BMF_L is most meaningful from the point of view of fugacity increase. Indeed, this is a very relevant metric for field BMFs. However, the observations of low dietary BMF_L for bioaccumulative compounds and the variability with lipid normalisation make a lipid normalised dietary $BMF_L > 1$ unsuitable as an endpoint for bioaccumulation from regulatory perspective.

It is noted that Gobas *et al.* (2021) showed that $BMF_{5\%}$ is on average lower than BMF_L, and highlighted the potential for miscategorising bioaccumulative chemicals as non-bioaccumulative using the $BMF_{5\%}$ metric. Because of the uncertainties of dietary BMFs, it should first be assessed if the bioaccumulation potential can be concluded based on the estimated BCF, which can be directly compared to the criteria. In case this is not possible, the $BMF_{5\%}$ may be useful to compare results from different studies (Environment Agency, 2022). For any use of the BMF_{kgL}, it is important that the dietary lipid content and the feeding rate are reported alongside the value. BMF5% and BMFkgL could be used in a benchmarking exercise.

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 substances tested in this set-up, it was shown that a BCF_L value of 5000 L/kg, normalised to a lipid content of 5%, corresponds to a lipid corrected BMF_{kgL} from the dietary test of 0.31 kg food lipids/kg fish lipids, and a BCF_L of 2000 L/kg corresponds to a BMF_{kgL} of 0.10 kg food lipids/kg fish lipids.

A different benchmarking could be obtained from aqueous and dietary bioaccumulation studies for perfluorinated substances 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 of Annex XIII. If benchmarking is used for comparing dietary BMF values with BMF values for substances with a known bioaccumulation potential, it must be ensured that these BMF values were obtained under similar conditions (i.e. fish species, fish weight/size, diet lipid content, feeding rate, fish lipid content and temperature).

Another endpoint from the dietary OECD 305 test is the elimination rate constant. The elimination rate constant has been proposed as an endpoint for the bioaccumulation assessment (e.g. Brooke and Crookes, 2012; Goss *et al.* 2013, Goss *et al.* 2018). For example, Brooke and 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 Brooke and Crookes (2012) there is considerable scatter around the regression line between log BCF_L and log k_2 (lipid normalised), which may be caused by the variability in fish weight used in the underlying studies, at least partly. This implies that it is not possible to set one value for the depuration rate constant for different organisms. If aqueous bioconcentration is considered, an uptake rate constant of 520 L/kg/d could be estimated for fish with a weight of 1 g (Sijm *et al.*, 1995). The depuration rate constants that lead to bioconcentration factors of 2000 and 5000 could thus be estimated to be 0.26 d^{-1} and 0.10 d^{-1}. For fish weighing ten grams these values would be approximately half of these values (0.12 d^{-1} and 0.05 d^{-1}).

No agreed criterion is available for the dietary BMF to indicate B or vB. Moreover, the dietary BMF seems dependent on both the food characteristics (type of food, lipid content, feeding rate) and on fish characteristics (as also observed for BCF itself). This will hamper the further derivation of a single value of BMF as a criterion. Similarly, for the depuration rate constant, no agreed criterion is available to assess bioaccumulation. The depuration rate constant is also dependent on other factors, for example the fish size, which makes it difficult to set a criterion for this endpoint too. Therefore, in the B assessment, the preferred endpoint from the OECD TG 305 dietary exposure test is the BCF value estimated from an experimentally derived elimination rate constant, which can be directly compared to the criteria, unless it can be demonstrated that the uptake rate constant (k_1) cannot be reliably estimated with the available methods. This would also be consistent with the data treatment of the OECD TG 319A/B *in vitro* tests, in which experimental data are only available for the depuration rate constant. In both cases (OECD 305-III dietary test and OECD TG 319 A/B *in vitro* tests), the estimation of the BCF from the depuration rate constant follows the same principle.

R.11.4.1.2.4 In vitro biotransformation data and in vitro-in vivo extrapolation

In vitro methods such as fish liver S9 and primary hepatocyte assays provide information on biotransformation in the organism (OECD TG 319B and OECD TG 319A, respectively). Because biotransformation is considered to be the dominant mechanism of elimination of hydrophobic substances, such *in vitro* tests have the potential to support the assessment of bioaccumulation in a Weight-of-Evidence approach and may contribute to a reduction in (or refinement of) animal testing. Specifically, *in vitro* biotransformation tests could serve as an interim step between screening based on physico-chemical properties and *in vivo* tests. Kosfeld, *et al.* (2020) have shown that IVIVE (*in vitro–in vivo* extrapolation) BCF estimation via rainbow trout hepatocytes delivers plausible result ranges for lipophilic organic substances, but recommend further investigations with a broader range of substances. Experience with *in vitro data* and IVIVE is still limited, and therefore it is recommended to follow developments in the field, e.g. via the ECHA website.

In vitro biotransformation assays were first developed in the pharmaceutical sector and later on adapted for fish and the use in bioaccumulation assessment (Nichols *et al.* 2006). Over the years, the methods were refined and validated (Fay *et al.* 2014; Nichols *et al.* 2018) and finally adopted in the two OECD test guidelines 319 A/B (OECD 2018b; OECD 2018c), accompanied by a guidance document (OECD 2018a) and excel spreadsheets for IVIVE calculations[46]. For

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[46] https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm; GD No 280: Hepatocytes: https://www.oecd.org/env/ehs/testing/HEPspreadsheet.xlsx; S9-mix: https://www.oecd.org/env/ehs/testing/S9spreadsheet.xlsx

ionisable substances, the OECD TG 319 may apply, however, the currently available *in vitro-in vivo* extrapolation models may not always apply to all (types of) ionisable substances and adaptation may be needed (Regnery *et al.* 2022).

The OECD test guidelines 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 h). From the measured substrate depletion curve, the *in vitro* biotransformation kinetics can be determined. The methods were successfully applied for a variety of chemicals, for example PAHs (Lo *et al.* 2015; Nichols *et al.* 2018; Trowell *et al.* 2018), fragrance chemicals (Laue *et al.* 2014; Laue *et al.* 2020; Weeks *et al.* 2020a,b), sunscreen agents (Saunders *et al.* 2020), pharmaceuticals (Gomez *et al.* 2010; Regnery *et al.* 2022) and ionisable chemicals (Ribbenstedt *et al.* 2022).

For highly hydrophobic or highly volatile chemicals, performance of *in vitro* biotransformation assays becomes increasingly challenging and modifications, for example, passive dosing methods or the use of closed vials, are discussed for these cases (Schug *et al.* 2018, 2019; see Guidance Document (OECD 2018a) for details). Many slowly metabolised substances cannot be accurately assessed with these *in vitro* test systems due to their relatively short working lifetimes (2-4 h) and therefore the depletion of such chemicals can not be confidently quantified. The activity of a trout liver S9 substrate depletion assay has been shown to decline over time, presumably due to proteolytic degradation of biotransformation enzymes. To address this problem, protease inhibitors (i.e., phenylmethylsulfonyl fluoride) have been added to homogenisation buffers and/or reaction mixtures which can increase the working lifetime of these assays and therefore could improve the detection of slow *in vitro* clearance rates (Nichols *et al.* 2021).

To make use of OECD TG 319 data for bioaccumulation assessment, the application of *in vitro–in vivo* extrapolation (IVIVE) bioaccumulation models is needed. Essentially, these models accomplish two tasks:

i) Converting *in vitro* biotransformation data to corresponding *in vivo* biotransformation estimates, taking into account (a) differences in binding *in vitro* and *in vivo* and (b) differences in the amounts of metabolically active components (e.g, hepatocytes or S9) in both cases.

ii) Calculating kinetic BCFs from a combination of rate constants for gill uptake, gill elimination, fecal egestion and biotransformation.

Over the past years, several IVIVE-bioaccumulation models have become available (Krause and Goss 2020; Nichols *et al.* 2013; Trowell *et al.* 2018). Recent refinements concerning the models are the use of the revised *in vitro-in vivo* extrapolation formalism (Krause and Goss 2018) and the use of composition-based binding algorithms (Krause and Goss 2021; Lee *et al.* 2017; Saunders *et al.* 2020), rejecting the assumption that binding *in vitro* and *in vivo* is the same (“f_u = 1”), which is is especially important in case of hydrophobic organic chemicals. These aspects should be considered in all IVIVE-bioaccumulation models. Accordingly, differences between existing models primarily concern the way in which the various rate constants are combined and the BCFs are calculated. In an evaluation by Krause and Goss (2020) it was found that results with multi-compartment models were similar to results with a simple one-compartment model, except in cases with fast biotransformation, in which the BCFs were underestimated with the one-compartment model. Different complexities are possible regarding the models, but in most cases the use of a very simple approach (one-compartment model) may suffice.

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 (EROD, 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)

Conceptually, the *in vitro* methods fall into the class of experimental information on bioaccumulation as follows: the flow-through tests OECD TG 305-I and OECD TG 305-II provide experimental information on uptake and on elimination, the OECD TG 305-III test provides experimental information on elimination while using estimates of uptake for BCF calculation, and the *in vitro* methods provide experimental information on biotransformation as a central part of elimination and use estimated parameter for uptake and other elimination pathways for BCF calculation. The *in vitro* assays hardly require animals compared to the other methods and can also be adapted for use with air-breathing animals (using appropriate *in vitro* material and IVIVE bioaccumulation models), thus also providing a potentially useful method for terrestrial bioaccumulation assessment.

R.11.4.1.2.5 Experimental sediment and soil bioaccumulation data

Experimental sediment bioaccumulation data (experimental Bioaccumulation Factors BAF and BSAF for sediment)

In most cases where experimental information on bioaccumulation is needed, a bioconcentration test with aquatic species is preferred due to the better possibilities of comparing the results from such test with the B/vB criteria. However, there may be some very specific cases, where a fish (or aquatic invertebrate) bioaccumulation test is not expected to reflect sufficiently the bioaccumulation potential but testing of bioaccumulation potential in sediment might provide the necessary information for deriving conclusions on the B/vB-assessment in a *Weight-of-evidence* approach.

In line with Annex 1 of the OECD TG 315, the following definitions are used in this guidance:

- The bioaccumulation factor (BAF) at any time during the uptake phase of this bioaccumulation test is the concentration of test substance in/on the test organism (Ca in g·kg^{-1} wet or dry weight) divided by the concentration of the substance in the surrounding medium (Cs as g·kg^{-1} of wet or dry weight of sediment). In order to refer to the units of Ca and Cs, the BAF has the units of kg$_{\text{sediment}}$·kg^{-1}$_{\text{worm}}$.

- The steady state bioaccumulation factor (BAFss) is the BAF at steady state and does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium (Cs as g·kg^{-1} of wet or dry weight of sediment) being constant during this period of time.
- Bioaccumulation factors calculated directly from the ratio of the sediment uptake rate constant divided by the elimination constant kinetic rate constants (ks and ke, respectively - see Annex 1 of the OECD TG 315) are termed kinetic biota-sediment accumulation factor (BAF$_K$).
- The biota-sediment accumulation factor (BSAF) is the lipid-normalised steady state concentration of test substance in/on the test organism divided by the organic carbon-normalised concentration of the substance in the sediment at steady state. Ca is then expressed as g·kg^{-1} lipid content of the organism, and Cs as g·kg^{-1} organic content of the sediment. BSAF is expressed in kg$_{\text{sediment OC}}$·kg^{-1}$_{\text{worm lipid content}}$. To reduce variability in test results for organic substances with high lipophilicity, the BSAF should be reported (OECD, 2008).

The units of the concentration values used for the calculations must all be related either to dry weight or to wet weight. The unit used should be reported. Optimally, calculations based on both the wet and the dry weights are presented.

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.

Bioaccumulation studies on sediment dwelling organisms can be used both for the screening and as part of the *Weight-of-Evidence* assessment of bioaccumulation properties. It should be considered that (soil or sediment) invertebrate species may have a lower metabolic capacity than fish species, e.g. as is the case for polycyclic aromatic hydrocarbons (Bleeker and Verbruggen, 2009). Bioaccumulation in these invertebrates may therefore be higher than in fish under the same exposure conditions. No systematic analysis on a comparison with fish bioaccumulation data has been conducted, therefore data on sediment dwelling invertebrates should be considered in a *Weight-of-Evidence* approach.

The OECD TG 315 Bioaccumulation in Sediment-dwelling Benthic Oligochaetes is the preferred method for generating additional information. The recommended oligochaeta species are *Tubifex tubifex* (Tubificidae) and *Lumbriculus variegatus* (Lumbriculidae). The species *Branchiura sowerbyi* (Tubificidae) is also indicated but it should be noted that it has not been validated in ring tests at the time of writing. The bioaccumulation factor (expressed in kg wet (or dry) sediment·kg^{-1} wet (or dry) worm) is the main relevant outcome and can be reported as a steady state biota-sediment accumulation factor BAF$_{ss}$ or as the kinetic biota-sediment accumulation factor (BAF$_K$). In both cases the sediment uptake rate constant k$_s$ (expressed in kg wet (or dry) sediment·kg^{-1} of wet (or dry) worm d^{-1}), and elimination rate constant k$_e$ (expressed in d^{-1}) should be reported as well. The normalised biota-sediment accumulation factor (BSAF) should be additionally reported for highly lipophilic substances. It is important to consider the implications of the worm gut contents when interpreting the study results (Mount *et al*, 1999; OECD TG 315).

OECD TG 315 recommends the use of artificial sediment. If natural sediments are used, the sediment characteristics should be specifically reported as described in the test guideline. For lipophilic substances, BSAFs 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, BSAF normalised to organism lipid and sediment total organic carbon content is used. This allows tests on the same substance and tests on different substances to be comparable. The load rate should be as low as possible and well below the expected toxicity, however it should be sufficient for ensuring that the concentrations in the sediment and in the organisms are above the detection limit throughout the test.

The relevance of bioavailability of the substance for the test organism should also be considered and if relevant and possible, bioaccumulation could be expressed as a BCF between organism and dissolved pore water concentrations.

It should be noted that it is not possible to give any threshold values for using sediment BSAF values in PBT assessment. Therefore, this type of test does not directly result in endpoints that can be compared to the REACH Annex XIII bioaccumulation criteria although the BSAF in combination with K_{ow}/K_{oc} can provide evidence of high bioaccumulation potential (Appendix R.11-3). BCF values can be calculated based on measured or estimated pore water concentrations according to Appendix R.11-3. If BCF values are normalised to a lipid content of 5%, they can be considered as a conservative estimate for fish, because metabolism is generally much lower in invertebrates than in fish. A case-by-case assessment based on expert judgement of the reliability and relevance of the available information is required in order to be able to give BSAF values an appropriate weight in the B and vB 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 OECD TG 315 test or a low depuration rate, both representing slow kinetics, are relevant parts of a *Weight-of-Evidence* approach when considering whether B or vB criteria are fulfilled. Substances with background sediment concentrations and potentially adaptable uptake mechanisms need careful consideration because sediment-dwelling organisms may have adapted to such substances, potentially affecting the bioaccumulation process.

Experimental soil bioaccumulation data (experimental Bioaccumulation Factor BAF and BSAF for soil)

The Bioaccumulation in Terrestrial Oligochaetes test according to OECD TG 317 measures the residues of a test substance in earthworms after exposure to the test substance mixed with soil. In those cases where the bioaccumulation concern is for bioaccumulation in air-breathing species and not so much for aquatic organisms, a bioaccumulation test in soil organisms like the earthworm (*Eisenia fetida*) will not be sufficient to disprove concern for bioaccumulation in vertebrates/mammals. The test is useful for aspects of secondary poisoning, and may support the bioaccumulation assessment in a *Weight-of-Evidence* approach. The earthworm may be more in equilibrium with the pore water and its diet is not comparable to a vertebrate diet.

Bioaccumulation studies with terrestrial invertebrates (e.g. according to OECD TG 317) are insufficient to assess the bioaccumulation potential in terrestrial mammals and other air-breathing organisms.

In line with Annex 1 of the OECD TG 317, the following definitions are used in this guidance:

- The bioaccumulation factor (BAF) at any time during the uptake phase of this bioaccumulation test is the concentration of test substance in/on the test organism (C_a in $g \cdot kg^{-1}$ dry (or wet) weight of worm) divided by the concentration of the substance in the surrounding medium (C_s as $g \cdot kg^{-1}$ of dry (or wet) weight of soil); the BAF has the units of kg dry (or wet) soil·kg^{-1} dry (or wet) worm.
- The steady state bioaccumulation factor (BAFss) is the BAF at steady state and does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium (C_s as $g \cdot kg^{-1}$ of dry (or wet) weight of soil) being constant during this period of time.
- Bioaccumulation factors calculated directly from the ratio of the soil uptake rate constant and the elimination rate constant (ks and ke,) are termed kinetic biota-soil accumulation factor (BAF_K).
- The biota-soil accumulation factor (BSAF) is the lipid-normalised concentration of the test substance in/on the test organism divided by the organic carbon-normalised concentration of the test substance in the soil at steady state. C_a is then expressed as $g \cdot kg^{-1}$ lipid content of the organism, and C_s as $g \cdot kg^{-1}$ organic content of the soil; the BSAF has the units of $kg_{soil\ OC} \cdot kg^{-1}{}_{lipid}$.

The units of the concentration values used for the calculations must be all related either to dry weight or to wet weight. The unit used should be reported. Optimally, calculations based on both the wet and the dry weights are presented.

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

Earthworms and enchytraeids are the recommended taxonomic groups to be tested according to OECD TG 317. To ensure comparability of results between different soils, BSAF normalised to organism lipid and soil total organic carbon content is used. The dependence of these values on the concentrations of the substance in soil, and when relevant, the soil characteristics should be specifically reported.

The bioaccumulation often varies with the organic carbon content of the soil. Typically a substance will have greater availability to the organism when the soil organic carbon content is low, compared to a higher OC. To ensure comparability of results between different soils, a BSAF should be derived by normalising the results both to the soil organic carbon content and the lipid content of the organisms employed. The load rate should be as low as possible and well below the expected toxicity, however it should be sufficient to ensure that the concentrations in the soil and in the organisms are above the detection limit throughout the test.

The relevance of bioavailability of test substances potentially containing irreversibly bound fractions in soil should also be considered and, if relevant and possible, the BSAF should be corrected for the bioavailable fraction.

It should be noted that it is not possible to give any threshold values for BSAF in soil. Therefore, this type of test does not directly result in endpoints that can be compared to the REACH Annex XIII bioaccumulation criteria although the BSAF in combination with K_{ow}/K_{oc} can provide evidence of high bioaccumulation potential (Appendix R.11-3). BCF values can be calculated based on measured or estimated pore water concentrations according to Appendix R.11-3. If BCF values are normalised to a lipid content of 5%, they can be considered as a conservative estimate for fish, because metabolism is generally much lower in invertebrates than in fish. A case-by-case assessment based on expert judgement of the reliability and relevance of the available information is required in order to be able to give BSAF values an appropriate weight in the B and vB 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 317 study or a low depuration rate, both representing slow kinetics, are relevant parts of a *Weight-of-Evidence* approach when considering whether the B or vB criteria are fulfilled. It should be noted that organo-metals and other substances with background soil concentrations and potentially adaptable uptake mechanisms require particularly careful consideration, as the soil-dwelling organisms may have adapted to such substances which potentially affects the bioaccumulation process.

Some additional parameters relevant to bioaccumulation that can potentially be used for screening or in a *Weight-of-Evidence* approach, may be derived from other invertebrate studies. For the OECD TG 222 earthworm reproduction test, in which earthworms are exposed for 28 days to a test substance spiked into soil, it has been demonstrated that at test end (provided that the relevant analytical procedures are available) the concentration of the test substance in the adult worms can give an indication of uptake into the organism (Kinney *et al.*, 2012). Care must be taken that the assessment of uptake is performed at a non-toxic test concentration (i.e. at which less than 10% mortality and no significant loss of body weight compared to control occurs over the 28d test period). It must also be noted that only uptake is measured at test termination and that elimination of the substance is not considered. As such, the results of this test should be interpreted with caution, but it can provide valuable screening information on substance accumulation that can help as preliminary information for considering whether more specific testing for bioaccumulation according to OECD TG 317 is needed. The same approach could potentially be useful for other guideline studies on invertebrate species as well, such as the 21 day larval survival test on dung beetles (OECD GD 122), the developmental test with dipteran flies (OECD TG 228) or the collembolan reproduction test (OECD TG 232), depending on the expected route of exposure. However, measuring tissue residues in these studies could be hampered by the small size of the test organisms (Hoke *et al.*, 2015).

R.11.4.1.2.6 Field data and biomagnification

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. Furthermore, the same information may be used to support the assessment of persistence, in particular for possible long-range transport, if significant concentrations are found in biota in remote areas or in locations distant from known point sources. 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 and/or metabolized) by the organism (this principle is also used in the fish feeding test for bioaccumulation), which will lead to biomagnification from prey to predator (trophic magnification). A reliable field BMF or TMF value higher than 1 (see also Section R.7.10.1.1 in *Chapter R.7c of the Guidance on IR&CSA*) can also be considered as an indication of very high bioaccumulation. For aquatic organisms, this value indicates an enhanced accumulation due to additional uptake of a 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 B/vB criteria in Annex XIII to the REACH Regulation, but in this kind of case all available data need to be considered together in a *Weight-of-Evidence* approach.

The quality of field data needs to be assessed and interpreted correctly. Respective guidance documents and recommendations for assessing the quality of biomonitoring data including interpretation of wildlife biomonitoring has 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).

Types of field/biomonitoring data and objectives

Field or biomonitoring data can be used as supporting evidence that substances are taken up by organisms in the environment. Indeed, the substance concentrations in wildlife samples show that uptake is actually taking place from the environment. Care should be taken if gut content and adsorption to skin contribute significantly to the measured concentration.

At present, an increasing number of biota monitoring data of high quality are generated under different European and global initiatives. The European Commission (EC) has made significant efforts to make (biota) monitoring data better accessible and comparable, e.g. via the platform IPCHeM (https://ipchem.jrc.ec.europa.eu/) or the NORMAN network (https://www.norman-network.com/apex/). These open access data bases can be used to screen for presence and concentrations of contaminants in wildlife and humans.

BCFs, BAFs express ratios of substance concentrations in biota to water, while BMFs and TMFs reflect ratios of substance concentrations in predator–prey relationships (Burkhard *et al.*, 2012). Field BAF or field BMF of a substance may be greater than what is estimated based on BCF and BMF from laboratory experiments. This is because in the laboratory tests fish are exposed either via water or via food, while under field conditions organisms are exposed to substances via all exposure routes depending on where they live (terrestrial or aquatic) and which taxa they belong to (air-breathers or water-breathers like fish). Furthermore, apex (top) predators reflect biomagnification over the whole food chain while laboratory tests usually include only one trophic level in the biomagnification process from diet to test organism. This will ultimately lead to higher bioaccumulation in wild organisms feeding at higher trophic levels compared to the laboratory experiments for substances that are not rapidly metabolized and eliminated. Furthermore, 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.*, 2012). Despite this, wildlife monitoring data can give valuable indication of an increased bioaccumulation potential particularly for difficult to test chemicals.

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

Field bioaccumulation metrics

Following types of biota field data can generally be considered in the context of bioaccumulation screening and assessment:

- **Bioaccumulation factors (BAFs/BSAFs)**

Field studies can be used to derive bioaccumulation factors (Field BAFs). They usually have been done with fish or aquatic invertebrates such as mussels and crustaceans and have been used to develop water quality standards (Moermond and Verbruggen, 2013). The bioaccumulation factor (BAF) can be expressed for simplicity as the steady-state (equilibrium) ratio of the substance concentration in an organism to the concentration in the surrounding medium (e.g. water in natural ecosystems). Similarly, field measured biota-sediment or biota-soil accumulation factors (BSAF) are derived by the concentration of a substance in biota divided by the concentration in the sediment or soil (Burkhard *et al.*, 2010).

Substance concentrations in biota that are orders of magnitude higher than those in water and air are important for several reasons. Such large concentrations may adversely affect organisms across food webs, if internal concentrations reach toxic levels (Mackay *et al.*, 2018).

Field BAF values are largely variable due to site-specific environmental conditions affecting their determination and less available than BCF values (Arnot and Gobas 2006; Weisbrod *et al.* 2009; Costanza *et al.* 2012). The basis for the field BAF value is the ratio of the concentration in wet weight (ww) of the organism divided by the water concentration. The unit of the field BAF is $L \cdot kgww^{-1}$. 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).

If field BAF values (based on reliable information) are above the criteria for B or vB it should be considered whether this information is sufficient to conclude that the substance meets the B or vB criteria as part of the Weight-of-Evidence approach. For comparison of a fish field BAF with the Annex XIII criteria, BAF values should be on wet weight basis and for whole body and also lipid normalised to 5%. Care should be taken that the exposures from all relevant routes and compartments are considered when field BAF values are evaluated.

- **Field biomagnification**

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

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

- **Trophic magnification factor (TMF)**

TMF can be used to understand the biomagnification potential of a substance as it represents the average increase or decrease of concentration levels in a food web per trophic level (TL): a TMF higher than 1 indicates that the substance biomagnifies in the food web (i.e. concentration increases with each trophic level) and thus can on its own be considered as a basis to conclude that a substance meets the B or vB criteria; a TMF lower than 1 indicates that the substance undergoes trophic dilution (Weisbrod *et al.* 2009). 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. As such, the TMF represents the average biomagnification per trophic level within that food web. Currently, there is no standard procedure for studying TMFs. Hence, the conductance and sampling may vary considerably between different studies. The validity of the TMF is strongly dependent on the spatial and temporal scales over which the samples were retrieved. 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). See also publications from Borgå *et al.*, 2012; Kidd *et al.*, 2019; Kosfeld *et al.*, 2021; Rüdel *et al.*, 2020, ECETOC (2014), Burkhard *et al.*, 2013, Guidance Document No. 27 for deriving Environmental Quality Standards prepared under the Water Framework Directive 2000/60/EC (European Commission, 2018) and Kidd *et al.* (2019) for discussion on uncertainties.

As for the BMF, for the assessment of substances that partition into lipids the TMF should be derived from lipid-normalised biota concentrations versus TL. For non-lipophilic substances, 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).

The way data, on the basis of which the TMF values are calculated, are treated has a great impact on the outcome of the TMF value. Not only the magnitude of the TMF value can be impacted, but also whether biomagnification or biodilution occurs. In addition, the setup of the field study could have an influence on the resulting TMF values as well. These aspects cover both spatial and temporal variability in sampling, but also the selection of species belonging to the food web (Kosfeld *et al.*, 2021). Spatial variability can lead to different organisms being exposed to different environmental concentrations (Kim *et al.* (2016). Temporal differences could have a strong impact on trophic magnification as well. Such temporal variability further complicates the interpretation of the observed TMF values. If there are large differences in space or time with regard to the samples within a food chain, the results of such a food web study should be considered with care. Further, it appears that TMF values could be strongly dependent on the inclusion or exclusion of certain species and on which part of the food chain is considered, for example pelagic species only or the benthopelagic food web. Apart from that, even from similar food webs widely varying results can be obtained for the TMF (Houde *et al.*, 2008). The Figure R.11—5 illustrates the relevant metrics used in the B/vB assessment.

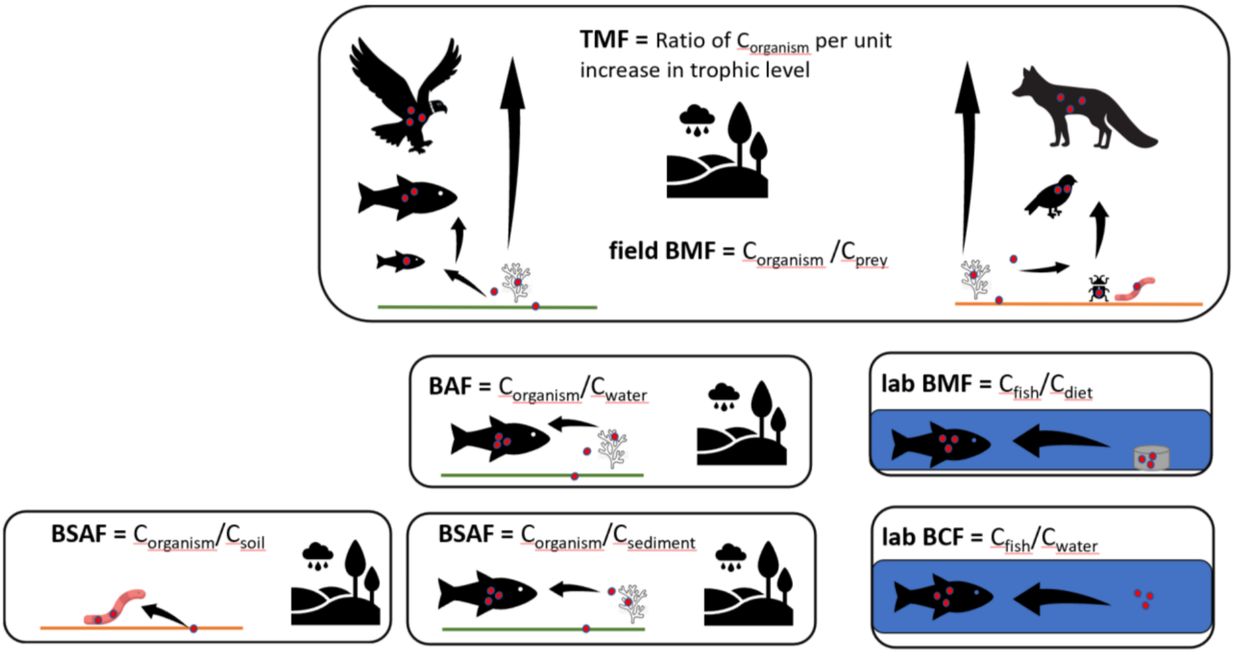

Figure R.11—5: Schematic overview on bioaccumulation metrics: laboratory bioconcentration factor (lab BCF), laboratory biomagnification factor (lab BMF), field biomagnification factor (field BMF), bioaccumulation factor (BAF) referenced to water, biota sediment or soil accumulation factor (BSAF), and trophic magnification factor (TMF). TMF is obtained from the slope of a plot of concentration versus trophic level. Please note that lipid content of the organisms need to be considered in the assessment for substances that partition into lipids. For non-lipophilic substances, another reference parameter than lipid content may be considered for normalisation,

e.g. dry weight or protein content. Red bullets refer to chemicals, green lines refer to sediment and orange lines refer to soil.

- **Detection of substances in wildlife**

The detection of substances in wild biota (concentration or occurrence data), in particular in apex species (top predators), provides a clear indication that it has been taken up by that organism. Care should be taken if gut content and adsorption to skin contribute significantly to the measured concentration. These data could be used at the screening level or within a *Weight-of-Evidence* 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 exposure level from the surrounding media and/or diet would be needed for such an assessment. Thus, concentrations measured in prey species or water in the surrounding media can be helpful to identify cases where bioaccumulation occurred in wild organisms. Furthermore, data from different time points as well as regions can give indications on temporal and spatial trends.

In cases where no data is available on sources and contemporary exposure levels, a high frequency of appearance of a substance in several biota species across different compartments could indicate bioaccumulation potential. In such cases, other available evidence of the substance’s bioaccumulation potential should be thoroughly examined before reaching a conclusion.

Detection of elevated levels of a substance in biota compared to levels in their surrounding environment indicates an increased concern for bioaccumulation. Reliable monitoring data can be used as line of evidence that the substance meets the B/vB criteria.

Concentrations in biota increasing with age due to exposure and accumulation over life-time, particularly in long-lived apex species (top predators), indicate an increased concern for bioaccumulation.

Finally, it is is important that the quality of monitoring data (detection or quantification of a substance in biota) needs to be assessed and interpreted correctly.

R.11.4.1.2.7 Addressing uncertainty of field data in bioaccumulation screening and assessment

The uncertainties related to field data apply to all field metrics described above. If field data are available, these should be considered in the assessment. In particular, if the number of field studies is not very high and do not cover a range of conditions and/or species, a comprehensive discussion on the uncertainties is required. Generally, bioaccumulation and biomagnification are influenced by an interplay between physicochemical properties of a substance, source distribution, trophic interaction, species biology and many other biotic and abiotic factors which are not fully understood yet. This makes the interpretation often difficult. The following elements are essential to be discussed for each study (where relevant) and when compiling the information from the studies together to draw an overall conclusion from the field studies:

Sampling:

- Quality assurance and reporting quality criteria throughout the sampling, sample treatment, storage and analysis (including (field-) blanks and spiked samples) of biota samples; detailed guidance can be found at the Life Apex project dissemination website as well as in Badry *et al.* (2022a and b).
- Sampling design (site selection, spatial resolution, frequency of determination, etc.) and details of the sampling methodology.
- Dividing the concentration of a substance in a predator by that in a prey implies that this prey is the sole food source. However, the food sources may be diverse. Additionally, there is no standard procedure so far how to conduct such field studies, and different study designs may therefore have an influence. The uncertainties of field studies have been addressed and discussed by Borga *et al.* (2012) and Kidd *et al.*, (2019).
- Problems arise with increasing body size of predators because analysis is based on tissue or serum samples. This is especially true for organisms at the higher trophic levels (e.g., polar bears), while it is feasible to measure the whole-body on smaller species at lower trophic levels. Whole-body analysis is not feasible for ethical reasons, and due to the challenging logistics with respect to sampling and laboratory constraints. Therefore, in many cases the derived field BMF-values are restricted to certain tissue samples rather than whole body samples.
- It is worth noting that Jurgens *et al.* (2013) discusses differences in contaminant content in whole fish versus dissected tissues, and points out that "although concentrations of hydrophobic substances tended to be higher in the liver than in the rest of the fish, the difference largely disappeared when the results were lipid-normalised". This suggests that regimes that use (or have used) liver sampling can deliver results that are comparable to whole fish sampling if the data are lipid-normalised.
- Tissue-to-whole body extrapolations of measured concentrations, where this cannot be avoided, introduce additional uncertainties which need to be addressed.
- The number of organisms sampled at each point of the food web.

Species ecology:

- Interspecies differences in gut physiology, diet preference, foraging strategies, environmental interactions, mobility and migration, physiological differences, and other species-specific ecological traits can have important consequences for substance exposure, uptake and metabolism as recently reviewed e.g. for birds (Kuo *et al.*, 2022).
- Therefore, the influence of sampling location(s) and timing(s), concentration gradients and migration behaviour need to be considered. In particular, migratory behaviour might strongly impact exposure levels. Care should be taken that the samples used to derive bioaccumulation and biomagnification/trophic magnification factors are collected at the same time and from the same location, and sufficient details are provided.
- Details of the organisms being analysed, including species, sex, size, weight, lipid content, life history, and ecological traits (e.g. migration, diet, and food web structure

which may be determined using measurements on nitrogen or carbon isotopes, and composition). For resident species, the sample collection should be fairly straightforward. Migratory species may present special challenges in determining which food, sediment, or water sample should be used to calculate the field biomagnification factor.

- Difference between poikilotherms and homeotherms (cold and warm blooded): An investigation of an Arctic food web revealed the unequal magnification behaviour of POPs within both thermal groups (Hop, 2002). These results may be explained by a higher food intake, caused by a higher energy demand, and a longer life span of birds and mammals. Intrinsic differences in gastrointestinal absorption mechanisms have also been suggested as an explanation for these differences between homeotherms and aquatic poikilotherms (Drouillard, 2000). Therefore, when the trophic magnification potential of a substance is determined via a single regression for the overall food web, the magnification in poikilotherms may be overestimated and the magnification in homeotherms, in particular apex predators, may be underestimated (Fisk *et al.*, 2001).

- Influence of species physiological characteristics (e.g. typical lipid content, whether air-breather or water respirer); Influence of digestion rate/diet energy content, size and growth, ability to metabolise, sex, age;The influence of habitat and exposure to potential point pollution (e.g. avoidance of human settlements especially in the case of hemerophile species);Influence of diet (generalist vs specialist), which might be controlled by analysing stable isotope values of predators and potential prey species (d15N, d13C); challenges and alternative methods like Sulphur stable isotope analysis are discussed in Elliott *et al.* (2021).

Uptake routes and analytical considerations:

- Data on biomagnification (TMF, BMF or BAF-values) should be calculated based on lipid-normalised concentrations (unless lipid is not important in the partitioning process, e.g. for protein binding substances). Normalisation of measured data with respect to lipid content is described in Guidance Document No. 32 on Biota Monitoring prepared under the Water Framework Directive 2000/60/EC (European Commission, 2014).

- Opportunistic feeders vary their diet and point sources may influence observed BMFs and TMFs. Additionally, apart from the diet, direct uptake of a substance by an apex predator occurs, e.g. from contaminated air or water. The relative importance of food versus e.g. water exposure therefore influences the magnitude of the TMF. It has to be noted that for typical PBT substances the relative contribution of uptake via air and (drinking) water is negligible compared with food.

- Data on the concentrations in the surrounding environment and on the temporal trend of environmental inputs are often missing, which complicates the bioaccumulation assessment when only using monitoring data.

- Selck *et al.* (2012) showed that at lower trophic levels (mayfly and polychaete), variability in bioaccumulation of benthic organisms is mainly driven by sediment composition and substance partitioning to sediment components, which is in turn dominated by the influence of black carbon[47]. At higher trophic levels (yellow perch and the little owl), food web structure (i.e., diet composition and abundance) and substance concentration in the diet became more important particularly for the most persistent substance, PCB-153. These results suggest that variation in bioaccumulation assessment can most effectively be reduced by improved identification of food sources as well as by accounting for the substance bioavailability within the food web.

- Although tissues of top predators can be used to monitor contaminant levels in the environment, variation in diet can confound the interpretation of the results (Braune *et al.*, 2014a; Braune *et al.*, 2014b; Hebert *et al.*, 2000). Therefore, an accurate B-assessment for higher trophic level predator species such as raptors or marine mammals requires knowledge on the substance concentration in potential prey species.
- Stable isotope (SI) analysis is an integrated approach to improve the overall understanding of feeding behaviour and food web ecology and to unravelling animal diets, as stable isotopes in animal tissues and excreta reflect dietary preferences, and yield insight into the environmental conditions experienced by the animal (e.g. West *et al.*, 2006, review by Kelly, 2011). Thorough elucidation of the food-web structure (feeding ecology; determination of the trophic level) and identification of the position in the food web is important for interpreting BMFs/TMFs. Nitrogen stable isotopes are commonly used as proxies for estimating the trophic position of animals since consumers get enriched with 15N in relation to 14N (ratio expressed as δ15N) by compared to their prey (Vanderklift and Ponsard, 2003), whereas measuring the enrichment of 13C in relation to 12C (ratio expressed as δ13C) can be used to distinguish different carbon sources of terrestrial (depleted δ13C) and aquatic (enriched δ13C) environments (Kelly, 2000). From the literature it seems that an average difference in δ15N of 3.4‰ between adjacent trophic levels has been suggested to be remarkably constant among different types of consumers in aquatic food chains (Post, 2002; Vander Zanden and Fetzer, 2007). For freshwater foodchains, the trophic level is calculated assuming an enrichment of 2 to 5‰ (usually 3.4 or 3.8‰) for δ15N (based on stable nitrogen isotope ratios) per trophic level (European Commission, 2018). The δ13C is a useful marker to distinguish benthic, benthopelagic and pelagic carbon sources. Although all three isotopes are associated with habitat, δ13C also varies systematically with trophic position and δ18O reflects variable contributions from diet. In contrast, δ34S may be a particularly useful dietary tracer of spatial origin as δ34S varies little from source to consumer (Florin *et al.*, 2011). The combined use of multiple isotopes could provide a more nuanced description of food web structure in the context of environmental pollution. In particular, the use of amino acid specific δ15N and δ13C values, including both those that change for predator to prey (‘trophic’ and ‘non-essential’ amino acids, respectively, for δ15N and δ13C) and those that do not (‘source’ and ‘essential’), may additionally refine the dietary estimate (Elliott *et al.*, 2021).
- It is worth noting that the relative abundance of these isotopes and thus the determination of the trophic level and TMF is influenced by the physiology of the organism and its life trait history. Rapid growth with a higher protein demand for new

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[47] Black carbon is formed by the incomplete combustion of fossil fuels, wood and other fuels and has a strong binding capacity for organic chemicals.

tissue leads to lower enrichment factors than those with slower growth rates. Insufficient food supply and fasting and starvation leads to catabolism of body proteins and an increase of 15N in organisms relative to those organisms with adequate food supply.

- An alternative approach to the use of stable isotopes in order to elucidate the diet composition of the animals can be the analysis of the diet with hard component analysis (i.e. bones…) of the stomach content or metabarcoding of the gut content.
- Many food webs are complex, and a single metric (food chain length) is unable to represent all variation in relationships (Elliott *et al.*, 2021). Contaminant levels often vary among habitats due to processes such as long-range transport, point source pollution, microbial degradation of organic substances, and variation in processes at the base of food webs might be as important as biomagnification for understanding contaminant levels in higher trophic level species ('habitat variation hypothesis'; (Elliott and Elliott, 2016; Lavoie *et al.*, 2015). Additional dietary tracers associated with the spatial origin of diet (δ13C, δ18O, δ34S) are recommended to refine diet reconstruction (Elliott *et al.*, 2021; Elliott and Elliott, 2016; Hobson *et al.*, 1993).

Also where a high number of field studies are available, the discussion on uncertainties mentioned above may support the assessment. It should also be noted that field studies often sample vertebrate species. Therefore, as Annex XI to the REACH Regulation requires vertebrate testing to be the last resort, the need for additional field studies requires careful consideration for whether alternative sources (e.g., already existing stored samples from specimen banks) could provide the same information, particularly in the light of uncertainties stated above.

Further considerations on field evaluation of bioaccumulation (with particular focus on terrestrial bioaccumulation) can be found in Van den Brink *et al.* (2016). Furthermore, a recent report on the use of biomonitoring from apex predators to support the screening for bioaccumulative chemicals was developed by the LIFE APEX project (Treu *et. al.,* 2022b, Badry *et al.* (2022a and b)).

R.11.4.1.2.8 Bioaccumulation in air-breathing organisms

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 gastrointestinal tract, metabolism, exhalation and growth (dilution) (Kelly and Gobas, 2003; Kelly *et al.*, 2007). In this context, air-breathing organisms also include marine mammals. The main concern of bioaccumulation is that concentrations in an organism reach levels that lead to adverse effects, especially in apex predators at the top of the food chain.

In a tiered approach, the first tier can be used for de-/prioritisation, while the higher tiers include a testing approach to allow a conclusion upon B and vB properties according to REACH

Annex XIII, 3.2.2 (b), and 3.2.2 (c), where possible expressed by biomagnification factors or trophic magnification factors (REACH Annex XIII). Relevant assessment endpoints are the biomagnification factor (BMF), the whole-body total (or terminal) elimination rate and the biotransformation rate. This guidance refers mainly to neutral organic substances; for ionisable and surface-active substances, it may be possible to apply the same principles, taking into account specificities as described in Appendix R.7.10-3 in *Chapter R.7c* of the *Guidance on IR&CSA*).

The BMF is the steady-state ratio between the chemical concentration in an organism (C_{org}; mg/kg) and its diet (C_{diet}; mg/kg), but it can also be expressed using rate constants describing competing rates of chemical uptake ($k_{dietary\ uptake}$) and elimination ($k_{elimination}$) in an organism:

$$BMF = \frac{C_{organism}}{C_{diet}} = \frac{k_{dietary\ uptake}}{k_{elimination}} = \frac{E_{diet} \times I}{k_{elimination}}$$

The chemical uptake is the product of the absorption efficiency of the chemical from the food (E_{diet}; unitless) and the ingestion rate (I; $kg_{diet}/k_{body\ weight}/d$). The BMF is inversely related to the whole-body total (or terminal) elimination rate constant and is proportionally related to the elimination half-life.

For neutral hydrophobic organic chemicals, the BMF can be expressed as the ratio of the lipid normalised concentrations in the organism and in its diet from the same food web and same time period, i.e., units kg-lipid/kg-lipid (Gobas *et al.*, 2009; Mackay *et al.*, 2013), if referring to steady state conditions.

Normalisation of contaminant concentrations to the lipid content is usually applied to concentrations along trophic levels in a food web, to assess the thermodynamic activity (or fugacity) of a chemical in a consumer organism compared to that in its diet (Debruyn and Gobas 2006). Increase in concentrations along the food chain or food web occurs when a chemical is taken up in the process of food digestion (food components are assimilated, which is leading to a change in composition and reduction in volume, and the capacity of the food for the chemical reduces), and its elimination rate is slower than the uptake rate by food ingestion. The elimination rate is inversely related to the lipid content of the organism apart from confounding factors such as metabolism, so the higher the lipid content, the lower the $k_{elimination}$. Lipid normalisation is relevant for lipophilic organic substances in a food web that partition to fatty tissue. Other substances such as PFAS may partition to other body compartments, thus it has to be carefully considered if lipid normalisation is applicable (Gobas *et al.*, 2015). If lipid normalisation is not considered appropriate, it should be examined whether normalisation to another compartment, e.g. proteins is applicable or if non-normalised data are preferred.

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

Tier 1: Screening assessment:

For the assessment of bioaccumulation in mammals and other air-breathing organisms at screening level, the following properties of a substance are assessed:

- Volatility; chemicals that are sufficiently volatile will be readily eliminated by exhalation.

- Hydrophilicity; chemicals that are sufficiently water soluble will be readily eliminated by urinary excretion.

These properties can be estimated or measured and compared with threshold values, i.e. log K_{ow} > 2 and logK_{oa} > 5, that are meant to separate chemicals that are potentially bioaccumulative from those that clearly will not be able to bioaccumulate.

As screening criteria for substances that might bioaccumulate or biomagnify in air-breathing organisms, a combination of the octanol-water partition coefficient K_{ow} and octanol-air partition coefficient K_{oa} have been identified (Gobas *et al.*, 2003). Baskaran *et al.* (2021a) 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. Furthermore, the ability of five techniques, specifically polyparameter linear free energy relationships (ppLFERs) with either experimental or predicted solute descriptors, EPI Suite's KOAWIN, COSMOtherm, and OPERA, to predict the K_{oa} of organic substances, was assessed by comparison with available measured K_{oa} values. The ppLFER equation using experimental solute descriptors predicted the K_{oa} the best and was described by Baskaran *et al.* (2021b) as the preferred method. The ppLFERs can be obtained from the UFZ-LSER data base (Ulrich *et al.*, 2017), and used for the estimation of K_{oa}. K_{oa} can furthermore be calculated based on the information available in the registration dossier: 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 Chapter R.16 in the *Guidance on IR&CSA*). 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[48].

An efficiently absorbed, non-biotransformed neutral organic substance with a log K_{oa} ≥ 5 in combination with a log K_{ow} ≥ 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 precise values for the K_{ow} and K_{oa} values indicated in the ITS are a function of the modelled organisms, food webs and environments used to obtain these values (e.g., Kelly *et al.*, 2007; Armitage and Gobas, 2007). It should be further noted that these screening criteria are only applicable to neutral organic substances that primarily partition into lipids. For other substances, log Koa and log K_{ow} are not suitable predictors for the distribution coefficients between organisms and the environment. In such cases, it should be investigated whether there is a concern for bioaccumulation. In some cases, e.g., for ionogenic substances, the use of artificial liposome-water partition coefficients could give additional information on the bioaccumulation potential.

Tier 2: Intermediate assessment:

A screening based solely on K_{ow} and K_{oa} would identify a large proportion of substances as potentially bioaccumulative, because the fraction of chemicals that can be judged as

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[48] Available under: https://www.henrys-law.org/henry/ (last accessed: 28 October 2022).

sufficiently volatile and/or sufficiently water soluble for rapid respiratory and urinary elimination based on the partitioning properties predicted for their neutral form is relatively small. On the other hand, substances that are biotransformed rapidly will be readily eliminated and therefore not bioaccumulate (Wania *et al.*, 2021). Note that this assumption is only true for the majority of substances where the metabolites are more water soluble and less bioaccumulative than the parent. For some substances, the metabolite may be more persistent and more bioaccumulative than the parent, e.g., DDT and its metabolite DDE. Thus, the transformation/degradation products should be considered separately in the assessment, in agreement with the general procedure for the whole PBT assessment.

Biotransformation rates can be estimated from *in vivo* studies, *in vitro* bioassays and by quantitative structure activity relationships (QSARs). Before performing animal testing, a search for existing reliable quality measured *in vivo* data should be conducted. If such data are available this could facilitate a definitive assessment (tier 3) to conclude whether a substance is bioaccumulative in air-breathing organisms or not. Relevant databases of measured toxicokinetic (TK) data in air-breathing organisms include the OECD QSAR Toolbox[49], EAS-E Suite[50] and MamTKDB[51]. The OECD QSAR Toolbox presently contains a database with whole body terminal elimination half-life (HL_T) data and will soon be expanded with HL_T data from animals through addition of MamTKDB data. It is also possible to search publications for TK data for structurally similar substances. Existing rat or other mammalian absorption, distribution, metabolism and excretion (ADME) / TK data should be evaluated for relevance and reliability. Empirical in vivo HL_T data have been collected for several hundred chemicals in humans (Arnot *et al.*, 2014, Obach *et al.*, 2008, Lombardo *et al.*, 2018), other mammalian species (Hofer *et al.*, 2021), and birds (Kuo *et al.*, 2022). Databases to obtain in vivo HL_T data parameters are also emerging, e.g., www.eas-e-suite.com and the Mammalian toxicokinetic database (MamTKDB).

QSAR models (need for further development)

QSAR models for the estimation of biotransformation rates of substances in humans have been developed by Arnot *et al.* (2014) and Papa *et al.* (2018), further models are under development. More good quality information on biotransformation rates of substances in terrestrial biota is needed to further develop and improve the predictability of QSAR models (Gobas *et al.*, 2023). Hence, QSAR predictions can be used for prioritisation, but cannot be recommended yet to be used as stand-alone information to justify that there is no bioaccumulation concern for air-breathers. Future improvements of the proposed procedure depend largely on the development and validation of prediction methods for the biotransformation kinetics in air-breathing organisms and for the potential for renal reabsorption (Wania *et al.*, 2021).

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[49] www.qsartoolbox.org, last accessed: September 2022

[50] www.eas-e-suite.com, last accessed: September 2022

[51] Mammalian toxicokinetic database (MamTKDB) 1.0 - Data Europa EU, last accessed: September 2022

In-vitro testing (need for further development)

While an OECD test guideline is available for fish hepatocytes and S9 mix (OECD TG 319A and B), this is not the case for mammalian cells yet, although similar tests have been in use by industry for other purposes. The use of mammalian *in vitro* tests for the estimation of biotransformation rates that could then be extrapolated to an *in vivo* half-life for biotransformation, which can be used in bioaccumulation assessment, is a promising pathway to be developed further (Goss *et al.*, 2018). IVIVE (*in vitro-in vivo* extrapolation) methods are available and may need to be/have been adapted to air-breathing organisms (see discussion paper "Bioaccumulation assessment of air-breathing mammals" (ECHA Working group on Toxicokinetics, 2022) available at the ECHA website and CEFIC LRi ECO41: Enhanced screening methods to determine bioaccumulatin potential of chemicals in air-breathing species). Since current *in vitro* tests for bioaccumulation assessment measure depletion of the parent substance only, metabolites are not usually identified or quantified. Metabolites can be taken into account by additional experimental measurements of their concentrations in the *in vitro* tests. Also, since very hydrophobic substances may sorb to any surfaces in the test vials or volatilise from the test system, it must be ensured that losses due to sorption or volatilisation are not erroneously attributed to biotransformation.

Tier 3: Definitive assessment: In vivo testing

Preferred options for testing

If the screening assessment and/or intermediate tier assessment point to possible bioaccumulation in air-breathing organisms, and the substance cannot be concluded to be B or vB through a bioaccumulation assessment using aquatic organisms, an *in vivo* test may have to be performed. The preferred test guideline would be the OECD 417 on Toxicokinetics, and the preferred organisms the rat. If no *in vivo* test has been performed yet, the following considerations should be taken into account for the test to best serve bioaccumulation assessment:

TG 417 offers quite some flexibility in study design to accommodate for different regulatory needs, but it does not include guidance on how to assess accumulation. Several factors will influence the clearance rate (or the corresponding elimination half-life) thus it is not a fixed value but relates to the test conditions, rat strain, animal age (fat content), etc. In repeated daily administration studies, clearance rates are preferably measured after steady state conditions have been reached, when the administration is stopped. The time to establish a steady state will differ depending on substance and dose. Repeated (compared to single) dosing should better ascertain a high radiolabelled substance load into peripheral organ/tissue compartments and establishment of steady state. This is because some large and/or deep organs or tissues may have slow influx rates due to little blood perfusion, unfavourable partitioning, low 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).

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 (for background on derivation of these thresholds see ECHA Working group on Toxicokinetics (2022)). If the terminal elimination half-lives are assessed to be longer than these, taking into account the considerations described above, 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 whole body terminal elimination half-life and therefore should be interpreted with care. Measurements of declining concentrations in organs/tissues are often more relevant than in blood plasma/serum, which often underrepresents elimination half-lives in organs/tissues (Hofer *et.al*. 2021). Elimination in blood is relevant for substances with a high blood distribution such as PFAS. If whole-body terminal elimination half-lives are between 2.5 and 4 days in rat, and/or 20 and 50 days in human, the assessment of the B property should be accompanied by a T assessment (PBT concern). It is noted that the derived elimination half-life thresholds for rat and human are tentative. 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.

The discussion paper "Bioaccumulation assessment of air-breathing mammals" which is available at the ECHA website was developed by the ECHA Working group on Toxicokinetics (2022), describes and discusses the tiered approach, scientific developments and recommendations for further work, and provides background information to the guidance given in this section.

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

R.11.4.1.2.9 Other testing data

In the following section other testing information which may be relevant for the bioaccumulation assessment is discussed. It should be noted from the outset that this other information does not override valid information on aquatic bioaccumulation of the substance if the aquatic data indicate high bioaccumulation potential.

Chronic toxicity studies with mammals

If chronic toxicity studies with mammals are available, the complete absence of any effects in the long-term is an indication that the substance is either chronically non-toxic and/or that it is not taken up to a significant extent. 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 *Weight-of-Evidence* approach that a substance is likely to be not bioaccumulative.

Particular attention should be drawn to the toxicokinetic studies considered to be included in the PBT/vPvB-assessment. For further information, see Sections R.7.10.14 and R.7.12 in Chapter R.7c of the Guidance on IR&CSA. In future assessments it might be advisable to combine a toxicokinetic assessment with toxicity testing if there is a concern for bioaccumulation.

R.11.4.1.2.10 Further data

In this section, several types of non-animal data are discussed that can be used in a *Weight-of-Evidence* approach for the B and vB assessment. If average molecular size, log K_{ow}, and octanol solubility are above or below certain values (as described below), they may indicate a limited bioaccumulation potential due to the lack of uptake[52]. However, these parameters should never be used on their own to conclude that a substance is not bioaccumulative. The information from these parameters should be accompanied by other information confirming the low uptake of the substance in living organisms, e.g. by read-across with similar substances, absence of toxicity or lack of uptake in toxicokinetic studies with mammals. Evidence of significant uptake in fish or mammals after long-term exposure implies that the indicators above will likely underestimate the real bioaccumulative potential of the substance and thus these indicator values should be considered unreliable for assessing the bioaccumulation potential.

Some studies have proposed a reduced uptake based on experimental bioconcentration studies. The reduced uptake then usually refers to reduced uptake via the fish gills. This does not imply that there will be reduced or no uptake possible via the gut, i.e. from food, where other uptake mechanisms may play a role (Larisch and Goss, 2018; Li *et al.*, 2014). The extent to which those additional uptake mechanisms play a role in bioaccumulation, however, is inadequately quantified for fish and aquatic invertebrates. There is evidence, however, for certain highly persistent and super hydrophobic substances, that significant accumulation via the food chain takes place (e.g. chlorinated paraffins (Ding *et al.*, 2021, Zhou *et al.*, 2020), chlorinated flame retardants (Wu *et al.*, 2010)).

Other methods such as *in vitro* methods or biomimetic extraction procedures may also be useful and are mentioned briefly at the end of the section.

(Q)SAR models

BCF-QSARs and other computer models may be used to address aquatic bioconcentration, provided that the model is appropriate for the chemical class (see Section R.7.10.3.2 in *Chapter R.7c* of the *Guidance on IR&CSA* and in Appendix R.11-1.1 and Appendix R.11-1.2 of this guidance document). For very hydrophobic substances (substances with logK_{ow} above 5-6), fish BCF predictions may not be indicative of low bioaccumulation potential due to a risk of underestimation, e.g. when predicted BCF is not based on freely dissolved chemical concentration in water (Glüge, *et al.* 2022, Böhm *et al.* 2016, Ehrlich *et al.*, 2011).

Read-across with other substances

If a valid and reliable BCF value for a structurally closely-related substance is available, read-across can be applied. When applying read-across data in bioaccumulation assessment, two

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[52] The original document on parameters indicating hindered uptake was drafted as part of an ECETOC report on the use of alternatives in assessing the environmental safety of substances (ECETOC, 2005). A revised document was included in the R.11 guidance published in 2017. For the current version, the molecular weight parameter has been removed as indicator. Relevant information from Appendix R.11—1: Indicators for limited bioconcentration for PBT assessment has been integrated into the current version, and the Appendix 1 as such has been removed.

generally important aspects have to be considered in addition to the normal criteria of read-across applicability: hydrophobicity and the likelihood for metabolisation of both substances. ,

Molecular size

Information on molecular size can be an indicator to strengthen the evidence for a limited bioaccumulation potential of a substance. One parameter for molecular size is the maximum molecular length of a substance, which is defined as the diameter of the smallest sphere into which the molecule would reside, as written, i.e. not accounting for conformers. From a certain minimum length upwards it may be assumed that the substance disturbs the structure of the lipid bilayer of cell membranes and therefore does not accumulate to a significant extent, i.e. has a BCF value lower than 2000. Folding of long linear structures may alter the effective length of the molecule of the substance, which renders it more easily transferable across cell membranes. Therefore, the criterion for molecular length should only be used in a *Weight-of-Evidence* approach together with other information as described under "conclusion on the endpoint". In conclusion, an assessor may justify that, in certain cases when information on the effective length and other information indicating a low bioaccumulation potential is available, the criterion for B and hence also for vB is not met. It is noted that currently there is no agreed cut-off criterion for molecular length and therefore the use of molecular length as one indicator of low bioaccumulation potential needs to be well justified. An earlier threshold of a maximum molecular length (MML) of greater than 4.3 nm as indicator for hindered uptake was based on a small dataset and cannot be recommended in this Guidance as agreed by the Partner Expert Group consulted during the first revision of this Guidance (v2.0 – Nov 2014).

A parameter that directly reflects the molecular size of a substance is the average maximum diameter ($Dmax_{aver}$). $Dmax_{aver}$ is defined as the diameter of the smallest sphere into which the molecule may be placed. Often this will be the same as the maximum molecular length, especially for rigid molecules. However, when flexible molecules are assessed, energetically reasonable conformers could be present for which these parameters might differ substantially. In this document the average value for this Dmax for "energetically stable" conformers is used, i.e. $Dmax_{aver}$. Very bulky molecules will less easily pass through the cell membranes. This results in a reduced BCF of the substance. Consistently with this notion, one study of a diverse set of substances showed that for substances with a $Dmax_{aver}$ larger than 1.7 nm[53] the BCF value will be less than 2000 (see Environment Agency, 2009)). However, the applicability of a numeric cut-off should be considered on a case-by-case basis. Also, it should be noted that the estimate of molecular size depends on the substance conformation as well as the method used. More information on the background of the molecular size indicator is given in ECETOC (2005).

The definitions of maximum molecular length and $Dmax_{aver}$ are shown graphically in Appendix R.11-1.2 together with examples of software that may be used for their calculations.

Log K_{ow}

For the B/vB assessment for aquatic organism, a screening threshold value has been established, which is log K_{ow} greater than 4.5. The assumption behind this is that the uptake of an organic substance in aquatic organisms is driven by its hydrophobicity. For organic

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[53] Please note that the indicator value of 1.7 nm for the average maximum diameter was derived using the descriptor Dmax from OASIS. However, it appears from the Environment Agency (2009) that the use of different software tools could lead to variable results for the same substance.

substances with a log K_{ow} value below 4.5 it is assumed that the B criterion, i.e. a BCF value of 2000 (based on wet weight of the organism, which refers to fish in most cases), is not exceeded.

At log K_{ow} values between 4 and 5, Log BCF increases linearly with log K_{ow}, if the substance is absorbed at the same rate and it is not biotransformed. This linear relationship is the basis for the B screening threshold value of log $K_{ow} > 4.5$. However, at very high log K_{ow} (>6), the relationship between these two parameters becomes progressively non-linear (Nendza, 1991). Apart from experimental errors in the determination of BCF values for these very hydrophobic substances, reduced uptake due to the increasing molecular size may play a role as well. Moreover, the experimental determination of log K_{ow} for very hydrophobic substances is normally also very uncertain due to experimental difficulties. Reverse phase HPLC method enables partition coefficients to be estimated in the log Kow range between 0 and 6, but can be expanded to cover the log Kow range between 6 and 10 in exceptional cases (OECD, 2022a). It is advised to always generate QSAR estimations of log Kow together with HPLC generated estimates, especially if the HPLC generated estimate of log Kow is in the range of one log unit below or above the screening value of log Kow = 4.5. Especially ionising substances, salts, metal complexes and complex mixtures would benefit from additional estimated data (next to HPLC generated data) from a variety of log Kow QSAR models and a Weight-of-Evidence evaluation (Appendix R.11-5). The reliability of measured and modelled log K_{ow} values > about 8 is often lower than the reliability of measured and modelled log K_{ow} values < about 8. Ideally the results of several model predictions for log K_{ow} should be considered.

The aquatic BCF of a substance is probably lower than 2000 if the calculated log K_{ow} is higher than 10. Given that none of the models have experimental information in this range, more than one model should be used to estimate the log K_{ow} value and the results evaluated by expert judgement. If a log K_{ow} value indicates that the substance screens as B/vB, but a registrant concludes it is not B/vB based on other data (see R.11.4.1.2.10 "Further data"), there should be specific reference to the REACH guidance indicating how such a conclusion was drawn. It should be noted that neither a high K_{oc} value nor low water solubility value can be used to argue that a substance lacks significant bioaccumulation potential. Instead these properties may influence the form of PBT testing required.

Octanol solubility

Octanol is often used as a surrogate for fish lipids. With a low solubility in octanol, the Log K_{ow} and hence the BCF can be either high or low, depending on the water solubility of the substance. Therefore, the solubility in *n*-octanol is not a parameter that is directly related to the BCF value. However, if the solubility of a substance in octanol is so low that the maximum concentration levels that can be attained in organisms do not reach levels sufficient to elicit any toxic effects, it can be reasoned that such accumulation would not be of concern. The concentration of a substance at which the occurrence of toxic effects normally can be excluded is 0.002 mmol/L in *n*-octanol (measured octanol solubility (mg/L) < 0.002 mmol/L × MW (g/mol)). Furthermore, octanol solubility is only an indicator for substances accumulating in fatty tissues and certain substances may bind to proteins instead of partition into lipids. Finally, information on octanol solubility should in particular be accompanied and complemented by information on mammalian toxicity or toxicokinetics to confirm the absence of uptake and/or chronic toxicity. More information on the background to this indicator is given in ECETOC (2005).

Biomimetic extraction procedures

Biomimetic extraction procedures with semi-permeable membrane devices (SPMD) and solid phase micro extraction (SPME) are used to mimic the way organisms extract substances from water. These types of methods are at the moment only well described for hydrophobic substances. For more detailed information, see Section R.7.10.3.1 in *Chapter R.7c* of the *Guidance on IR&CSA*.

R.11.4.1.2.11 Use of a fugacity approach for bioaccumulation assessment

The use of fugacity ratios has been proposed as method to convert laboratory and field bioaccumulation metrics into a common fugacity ratio scale to facilitate the interpretation of different sources of bioaccumulation data (Burkhard *et al.*, 2012, Mackay *et al.*,2013, Armitage *et al.*, 2021). The ratio between the fugacity in the organism divided by the fugacity in the diet expresses the increase in fugacity from food to organism. Mackay *et al.* (2013) proposed the BMF as the lipid-normalized ratio of the concentration in the predator to that of the diet with BMFs exceeding 1.0 indicating an increase in lipid concentration and thus also an increase in thermodynamic potential or fugacity with ascending position in the food chain or food web. This approach converts a wet weight based BMF, which can be defined by the concentration in the organism divided by the concentration in the diet, into a fugacity based BMF for lipid sorbing substances.

The calculation of a fugacity ratio is an approximation based on certain assumptions. One of the assumptions often made is that the partitioning to lipids is equal to the octanol-water partitioning and this may not always be the case. The fugacity capacity of an organism can be derived from partition coefficients that are not estimated from K_{ow} (from ppLFERs). Fugacity capacity can also be calculated based on partitioning different tissue components (storage lipid, membrane lipid, albumin, structural protein) if the partition coefficients for these components are known. An example of this approach was recently described by Fremlin *et al.* (2021).

However, there is a lack of agreement on how to interpret fugacity ratios and the method has not yet been validated sufficiently, for example with existing POP and PBT substances. That is why, the fugacity approach in bioaccumulation assessment under REACH cannot be recommended at this stage.

Apart from these considerations, it must be realised that the use of fugacity ratios is only justified in cases of thermodynamic equilibrium between the different compartments that an organism is exposed to. When applied to field studies, this is seldom the case. If for example a ratio between biota and sediment is used as basis for the fugacity ratio the assessment might be strongly hampered by strong sorption to the sediment and consequently very slow depuration of the substance from the sediment into (pore-)water. In such cases, which for example could be expected for many well-known PBT substances, the fugacity ratio between biota and sediment will be low, while the fugacity ratio between biota and the depleted pore-water could be high. However, also in laboratory studies, thermodynamic equilibrium between different exposure media (water and food) is even prevented. In both the aqueous and dietary OECD TG 305 studies, fish are exposed to only one exposure route, either water or diet. The consequence is that the remaining medium to which fish are exposed simultaneously have arbitrarily a very low fugacity compared to fish and the exposure medium. Further, variable results in laboratory settings were obtained when normalising different feeding regimes to the lipid content of the food (Hashizume *et al.*, 2018). It appeared that the fugacity in an organism is rather dependent of the dose delivered with the food than of the fugacity in the food itself. This shows that for the same organism, fugacity increase is not a constant factor.

The fugacity ratio only considers a substance of concern for bioaccumulation if there is an increase in fugacity, i.e. biomagnification occurs. Indeed, if biomagnifications is confirmed this is a clear indication of bioaccumulative properties of a substance (Gobas *et al.*, 2009). Nevertheless, the bioaccumulative properties of substances that do not biomagnify could be considered of concern as well. Polycyclic aromatic hydrocarbons (PAHs) could be considered as an example of this concern. These substances are very efficiently taken up in invertebrates with very high bioaccumulation factors. However, they are not biomagnified in higher trophic levels, such as fish. Still, the additional uptake due to the consumption of high concentrations in invertebrates can lead to significantly higher bioaccumulation factors in the field (Khairy *et al.*, 2014) than would be predicted based on laboratory bioconcentration data. This example illustrates that high bioaccumulation in a part of the food chain may have unpredictable effects throughout other parts of the food chain as well.

Even though the fugacity approach in bioaccumulation assessment under REACH cannot be recommended at this stage, it is noted that the approach allows various lines of evidence to be put into a consistent framework to apply a quantitative *Weight-of-Evidence* determination as to whether or not a substance biomagnifies.

R.11.4.1.3 Toxicity assessment (T)

R.11.4.1.3.1 Integrated testing and assessment strategy (ITS) for T-testing in support of PBT assessment for the aquatic environment

In this section guidance on the recommended testing and assessment strategy is provided as an annotated flow chart (Figure R.11—6). The strategy is based on the T criteria (Table R.11—1), which state that the T criterion is fulfilled if at least one of the data types listed in the criteria is fulfilled. If P and B criteria are fulfilled, information would need to be generated until for each (eco)toxicity data type it is clear whether the criterion is fulfilled or not.

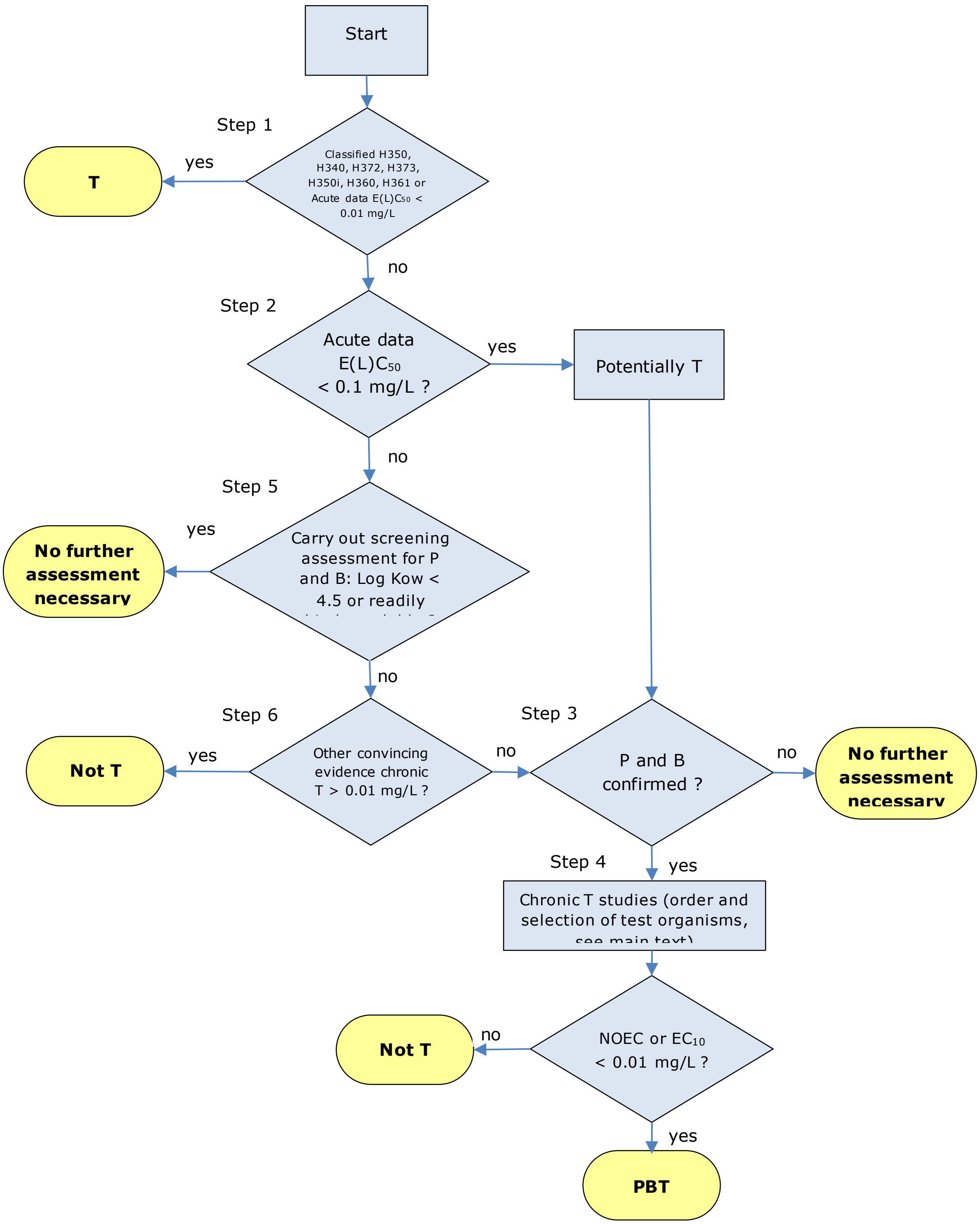

Figure R.11—6: T testing in support of PBT assessment for the aquatic environment.

According to Article 14 of REACH, PBT assessment is required for substances registered at quantities of ≥ 10 t/y (it is assumed that at least acute algae, daphnia and fish data are available):

Step 1: Assessment of mammalian toxicity data and acute aquatic toxicity data;

- IF classified or likely to be classified as carcinogenic (cat. 1A or 1B), germ cell mutagenic (cat. 1 or 1B) or toxic to reproduction (class 1A, 1B or 2) or STOT RE 1, STOT RE 2 or any EC_{50} or LC_{50} < 0.01 mg/L, THEN define the substance as T and stop assessment
- IF not classified or likely to be classified as carcinogenic (cat. 1A or 1B), germ cell mutagenic (cat. 1A or 1B) or toxic to reproduction (cat. 1A, 1B or 2) or STOT RE 1, or STOT RE 2 or any EC_{50} or LC_{50} ≥ 0.01 mg/L, THEN move to step 2.

Step 2: Assessment of acute aquatic toxicity data;

- IF any EC_{50} or LC_{50} < 0.1 mg/L, THEN the substance is a Potential T candidate. Move to step 3.
- IF all EC_{50} or LC_{50} ≥ 0.1 mg/L, THEN it needs to be confirmed that this is not a false negative (i.e. a substance with possibly a high chronic toxicity). Move to step 5.

Step 3: Consider outcome of P and B assessment* (Note.: it is considered good practice to assess P, B and T in that order)

- IF P and B confirmed, THEN proceed to Step 4 (chronic T testing) **
- IF confirmed not P or not B, THEN STOP

Step 4: Chronic T testing (on fish, daphnids, algae). The approach here is that chronic aquatic toxicity testing should be firstly carried out on non-vertebrate species, unless there are indications that fish is the most sensitive group (NB: it is not defined in this ITS how to rank the sensitivities). If the T-criterion is fulfilled by the chronic algae or *Daphnia* data, a chronic fish test is not necessary for PBT assessment, even if it would be the most sensitive species.

- IF NOEC or EC_{10} < 0.01 mg/L, THEN PBT confirmed
- IF NOEC or EC_{10} ≥ 0.01 mg/L, THEN not T, and STOP

Step 5: Screening of the substance for P and B *

- IF Log K_{ow} ≤ 4.5*** or other B-cut-off criteria met, and no other indications are available that the substance might bioaccumulate in other ways than by absorption to lipids, then not B and STOP.
- IF substance is readily biodegradable, then not P and STOP
- IF Log K_{ow} > 4.5 AND not readily biodegradable, THEN move to step 6

Step 6: Other long term T-evidence (e.g. by means of read across and *Weight-of-Evidence* or group approach)

- IF chronic toxicity cannot be excluded, THEN move to step 3 (P & B confirmation)

- IF strong evidence for non-T properties, THEN STOP.

* For specific guidance on the identification of P & B substances, please refer to Section R.11.4.1.1 for persistence and Section R.11.4.1.2 for bioaccumulation

** If B is likely but vB is not and a reliable BCF is not available, consider conducting tests on invertebrates to check the T status for these organisms before considering tests on fish (either for chronic toxicity or for obtaining a BCF).

*** Care must be taken in case a substance is known to bioaccumulate by a mechanism other than passive diffusion driven by hydrophobicity; *e.g.* specific binding to proteins instead of lipids might result in an erroneously low bioaccumulation potential if it is estimated from Log K_{ow}.

Care must also be taken for substances classified as polar non-volatiles (with low Log K_{ow} and high Log K_{oa}). This group of substances has a low bioaccumulation potential in aquatic organisms but a high bioaccumulation potential in air-breathing organisms (unless they are rapidly metabolised).

R.11.4.1.3.2 The toxicity criterion

According to Section 1.1.3 of Annex XIII to REACH, a substance is considered to fulfil the toxicity criterion (T) when[54]:

- the long-term no-observed effect concentration (NOEC) or EC10 for marine or freshwater organisms is less than 0.01 mg/L; or
- 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 the CLP Regulation; or
- there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: STOT RE 1, or STOT RE 2 according to the CLP Regulation.

For the assessment of aquatic toxicity, EC10 values are preferred compared to NOEC values for deriving long-term toxicity to marine or freshwater organisms[55].

The evidence of CMR and chronic toxicity specified above does not only refer to substances that are already classified accordingly (i.e. CLP hazard statements H350, H340, H372, H373, H350i, H360 and H361[56]) but also implies an obligation to check whether the criteria for assigning the respective classifications are fulfilled in accordance with the provisions of Annex I to REACH (Section 1.3 *Step 3: Classification and Labelling*). If any classification criterion leading to the assignment of the mentioned classifications is met, the substance fulfils the T criterion and there is no need to perform any further aquatic studies for T assessment. If data are available for birds these cannot be directly (numerically) compared with the T criterion

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[54] Annex I (Part 4) to CLP Regulation (EC) No 1272/2008 was amended to include Classification criteria for PBT. These criteria include a possibility to conclude a substance as T based on classification as endocrine disruptor (category 1) for humans or the environment (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2023:093:TOC).

[55] An OECD workshop (OECD, 1998) recommended that the NOEC should be phased out from international standard. Indeed, concerns were expressed about deciding to abandon the NOEC since it may not be sufficiently protective because of the danger of false negatives. According to the Report of the OECD Workshop on Statistical Analysis of Aquatic Toxicity Data (OECD, 1998), NOECs are leading to misunderstandings, misinterpretations and NOECs are statistically unfounded.

[56] H360 and H361 here include also all the possible combinations (e.g H360F, H360FD, etc).

(see Section 1.1.3 to Annex XIII). However, reprotoxicity studies or other chronic data on birds, if they exist, should be used in conjunction with other evidence of toxicity as part of a *Weight-of-Evidence* determination to conclude on the substance toxicity (a NOEC of ≤ 30 mg/kg food in a long term bird study should in this context be considered as strong indicator for fulfilling the T criterion).

The rest of this document is limited to testing of the T criterion on the basis of evidence from aquatic tests.

Due to animal welfare concerns, the general scheme of testing is sequentially first P, B and then T if there are no specific reasons for deviation from that sequence. Furthermore, vertebrate animal testing should be generally minimised by first testing non-vertebrate species as data from invertebrates are equivalent to vertebrate data in the context of the PBT/vPvB-assessment. For determination of whether a substance fulfils the criteria for aquatic toxicity, and in the absence of any long-term ecotoxicity data on aquatic species, a 21-d Daphnia reproduction test (OECD TG 211) would normally be the preferred test to perform with the few exceptions described later in this section where the results from short-term tests can already lead to concluding that the criteria are fulfilled. Under most circumstances, the T criterion of 0.01 mg/L (NOEC or EC10) can be compared to results from tests listed in REACH annexes VII to X. Existing data from other equivalent test methods must be assessed on a case by case basis based on the recommendations described in the effects assessment methodology.

As the aquatic T criterion is based on a NOEC or EC10 for pelagic organisms, the standardised chronic tests on fish, daphnids and algae are preferred to assess the NOEC or EC10. However, for poorly water-soluble substances, the feasibility of performing a test via the water phase needs to be considered carefully. Such a study may be technically difficult to perform as the substance will partition out of solution, especially if it is known to partition strongly to sediment and suspended solids. In such cases, it may be both impractical and uninformative to test pelagic species via the water phase. Tests with sediment dwelling species may provide more useful information on the toxicity of the substance in the compartment in which it will be mainly found. However, the T criteria do not include a chronic value for sediment as only NOEC or EC10 values related to pelagic toxicity are accounted for in Annex XIII. A possible way to determine whether a substance has equivalent toxicity in sediment to that in the water column could be to extrapolate the sediment toxicity value (e.g. NOEC) to a pelagic toxicity value by assuming that sediment toxicity occurs mainly through the pore water and using the equilibrium partitioning method (EPM). The EPM method is normally used to calculate a $PNEC_{sediment}$ from a pelagic $PNEC_{water}$ (see Section R.7.8 in *Chapter R.7b* of the *Guidance on IR&CSA*).

However, the EPM method may also be used to back-calculate a NOEC or EC10 value of an existing sediment test to a corresponding pelagic NOEC or EC10. The pelagic NOEC or EC10 derived can then be compared with the T criterion of 0.01 mg/L given in Annex XIII. The sediment concentration equivalent to a pelagic NOEC or EC10 value of 0.01 mg/L increases linearly with the suspended matter-water partitioning coefficient (see Section R.7.8 in *Chapter R.7b* of the *Guidance on IR&CSA*).

To check whether the T criterion of 0.01 mg/L is fulfilled, the equation for the equilibrium partitioning method used in order to calculate the PNEC$_{sediment}$ is slightly revised:

$$NOEC(EC10)_{porewater} = \frac{NOEC(EC10)_{sed,dw}}{Kp_{susp}}$$ **Equation 11-1**

$NOEC(EC10)_{porewater}$ $(mg.L^{-1})$
Kp_{susp} $(L.kg^{-1}\ dw)$
$NOEC(EC10)_{sed\ dw}$ $(mg.kg_{dw}^{-1})$

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

It should be noted that NOEC$_{sed}$ derived from experimental studies are given in dry weight (as mg/kg dw).

As the equilibrium between sediment and water is influenced by the suspended solid-water partition coefficient (Kp$_{susp}$), it is necessary to calculate the T criterion for each substance, using its own partitioning coefficient.

For substances with water solubility below 0.01 mg/L, a chronic limit test ($C_{sed,lim}$) can be performed at the spiked sediment concentration that is calculated to be at equilibrium with the water solubility limit of the test substance.

$$C_{sed,lim} = C_{watersol} . Kp_{susp}$$ **Equation 11-2**

$C_{watersol}$ $(mg.L^{-1})$
Kp_{susp} $(L.kg^{-1}\ dw)$
$C_{sed,lim}$ $(mg.kg^{-1}\ dw)$

If no chronic effects are found from this limit test, the result can be regarded as experimental evidence that the substance does not meet the pelagic T criterion for invertebrates provided that the equilibrium partitioning theory holds in the particular case (for guidance on the limitations of the equilibrium partitioning method, see Section R.7.8.10.1 in *Chapter R.7b* of the *Guidance on IR&CSA*). However no final conclusion on pelagic toxicity can be drawn if no further reliable toxicity data on fish and algae are available. If chronic effects are found then this is an indicator that T could be met in a pelagic test and consideration should be given to further testing (although care has to be taken at high spiking concentrations that the test substance does not cause indirect effects, e.g. by oxygen depletion as a result of biodegradation).

R.11.4.1.3.3 Use of QSAR data

Only a few QSAR models predicting chronic aquatic toxicity are available but further research on the QSAR prediction of chronic toxicity may increase their predictive capacities. Therefore at the current state of the art, QSAR models generally seem not to be applicable for an unequivocal assessment of the T criterion. However, it should be noted that the registrant is, within the frame of Annex XI to REACH, allowed to make use of QSARs when they are applicable.

R.11.4.1.3.4 Screening information and screening threshold values

If only screening information is available for the PBT/vPvB assessment, screening criteria listed in Table R.11—7 can be used for screening. It should be noted that these criteria are indicative and further description on the application of these criteria is provided below.

Table R.11—7: Screening threshold values for toxicity.

Toxicity	**Screening information*** **	**Conclusion**
Short-term aquatic toxicity (algae, daphnia, fish)*	EC50 or LC50 < 0.01 mg/L****	T, criterion considered to be definitely fulfilled
Short-term aquatic toxicity (algae, daphnia, fish)**	EC50 or LC50 < 0.1 mg/L****	Potentially T

* From acute tests.

** From acute tests or valid/applicable QSARs.

*** The screening assignments should always be considered together for P, B and T to decide if the substance may be a potential PBT/ vPvB candidate.

**** These threshold values only apply for the aquatic compartment.

A substance is considered to potentially meet the criteria for T when an acute E(L)C50 value from a standard E(L)C50 toxicity test (REACH Annexes VII to X) is less than 0.1 mg/L. 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 *Guidance on IR&CSA*).

The toxicity criterion (T) for PBT assessment cannot be decided upon the basis of acute studies alone. If the screening threshold value is met, the substance is referred to T testing and chronic studies are needed unless E(L)C50 < 0.01 mg/L. Normally, the testing order for conclusion on T based on chronic data is *Daphnia* and then fish[57]. If the T-criterion is fulfilled by the chronic algae or *Daphnia* data, a chronic fish test is not necessary and should therefore not be carried out as it would be an unnecessary vertebrate animal test.

For certain lipophilic substances (with a Log K_{ow} > 4) acute toxicity may not occur at the limit of the water solubility of the substance tested (or the highest concentration tested). In such situations, chronic toxicity with a NOEC/EC10 < 0.01 mg/L cannot be excluded. Therefore, it may not be possible to draw a screening conclusion for T (see decision tree for aquatic endpoints, steps 2, 5 and 6, and Figure R.11—6).

In the absence of conclusive information on T, for substances with very high lipophilicity, a *Weight-of-Evidence* or grouping approach for long-term toxicity may be used to predict whether long-term effects are likely to occur. If convincing evidence is available that aquatic toxicity is not expected to occur at < 0.01 mg/L, chronic testing may not be required. Such evidence should be based on expert judgement and *Weight-of-Evidence* of data including reliable QSAR predictions/read-across/grouping approaches indicating a narcotic mode of

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[57] Algae are not mentioned here because chronic algae data (i.e. 72h NOEC) normally will be available, as it can be easily obtained from the same 72h standard test from which the acute endpoint (72h EC50) is derived.

action together with measured low chronic fish toxicity from a related substance. Supporting information could be chronic data on aquatic species such as, e.g., daphnids, algae or sediment dwelling species and/or low acute or chronic mammalian and avian toxicity.

If data from this approach provide insufficient evidence that toxicity will not occur in a chronic test a conclusion on the P and B properties should be drawn before further T-testing is considered. If the substance is found to be both P and B, a chronic study is required (testing order see above).

In choosing the appropriate test organism, the data from the available base set of toxicity tests for algae (acute / chronic), *Daphnia* (acute) and fish (acute) should be evaluated under consideration of the possible hydrophobic properties of the test substance, and hence the expected time to steady-state. Any specific mode of action of the test substance also needs to be considered.

If it can be concluded that one taxonomic group is significantly more sensitive than the others, e.g. because there is evidence for a specific mode of action, this sensitive group should be chosen for chronic testing and conclusion on the T-properties[58]. If no conclusive evidence for significant differences in sensitivity between the groups can be found the testing order as mentioned above applies.

If the relevant test species is selected in accordance with the suggested approach in the paragraph above, lack of toxicity at or below the T criterion for the tested species is evidence that further studies on T are not necessary. If however a long-term test on *Daphnia* or algae provides a NOEC close to but above 0.01 mg/L, a long-term fish study is likely to be needed to confirm "not T" unless, taking into consideration the above-mentioned approach, convincing evidence exists that the fish NOEC will be higher than 0.01 mg/L. Supporting evidence in such considerations could be an acute fish value that is a factor of 10 or more greater than that of the other two trophic levels under the provision that the acute daphnid test showed toxicity at least one order of magnitude lower than the limit of solubility.

Certain chemical characteristics (such as high adsorption or extremely low solubility) are likely to make any toxicity testing extremely laborious if not technically impossible. Guidance has been developed by OECD on toxicity testing of difficult substances (OECD, 2019)[59]. Some examples together with recommendations to overcome the technical difficulties are provided in the chapter on assessment of problematic substances (see *Chapter R.7b of the Guidance on IR&CSA*).

R.11.4.1.3.5 Water accommodated fraction (WAF)

For any substance with very low water solubility, all efforts should first be made to produce a reliable and stable test concentration. For a UVCB substance which is only partially soluble in water, it may not be feasible to conduct the test for individual constituents/blocks of constituents due to the properties of the substance or due to disproportionate efforts. In this case the water accommodated fraction (WAF) can be considered as last resort to generate

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[58] This could mean that no further testing is necessary if it is concluded that algae are significantly more sensitive than daphnids or fish and the available chronic algae data are well above a NOEC of 0.01 mg/L.

[59] The OECD "Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals" has introduced additional recommendations for poorly water-soluble chemicals, and in particular with regard to the use of liquid/liquid saturator units and of passive dosing.

exposure in a test (OECD, 2019; Girling *et al.*, 1992, see also Appendix R.7.8-1 in *Chapter R.7b* of the *Guidance on IR&CSA*). Test results are expressed as a lethal or effective loading that causes a given adverse effect after a specified exposure period. For complex multi-constituent substances, the principal advantage of this test procedure is that the observed aquatic toxicity reflects the multi-component dissolution behaviour of the constituents at a given substance to water loading. Expressing aquatic toxicity in terms of lethal loading enables multi-constituent substances comprised primarily of constituents that are not toxic to aquatic organisms at their water solubility limits to be distinguished from substances that are more soluble and which may elicit aquatic toxicity. As a consequence, this test procedure provides a basis for assessing the toxicity of UVCB substances as a whole. Effect concentrations in tests based upon WAFs can be calculated from (1) the loading rates and are identified as either LL_{50} or EL_{50} values and/or (2) the measured mass of test substance in the WAF and are identified as either LC_{50} or EC_{50} values. LL_{50} or EL_{50} values are comparable to LC_{50} or EC_{50} values determined only for pure (i.e. mono-constituent) substances tested within their solubility range. Similarly the NOEC (No Observable Effect Concentration) becomes the NOELR (No Observable Effect Loading Rate). The statistical methods used to determine LL_{50}, EL_{50} and NOELR values are the same as those used to determine LC_{50}, EC_{50} and NOEC values.

The WAF procedure has been adopted for use in environmental hazard classification (for acute and long-term hazard classification) (OECD, 2019; UNECE, 2003). Poorly soluble substances that exhibit no observed chronic toxicity at a substance loading of 1 mg/L indicate that the respective constituents do not pose long term hazards to the aquatic environment and, accordingly, do not require hazard classification (CONCAWE, 2001; UNECE 2003). Using WAF data for PBT assessment may be more complex as PBT assessment is required for all constituents of the substance fulfilling P and B criteria and by its nature the WAF-method is testing several constituents. Where toxicity is exhibited by a test substance containing several constituents, interpretation of the test results can be problematic if the toxicity cannot be allocated to specific constituents directly. In such case, interpretation of the results (given that use of WAF is the last resort) should be supported by use of other data, such as QSAR –values or read-across values from a structurally similar substances. Generally, the loading rate cannot be directly compared to the Annex XIII toxicity criterion as true ECx value may be overestimated. To facilitate the interpretation of such data, analytical verification of the constituents present in the test solution must be provided. Only in the case of analytical verification of the water-soluble fraction(s), this type of tests might be used in the PBT assessment.

R.11.4.1.3.6 Use of non-testing data

At preliminary stages in the assessment, in cases where no acute or chronic toxicity data are available, the assessment of the T criterion at a screening level can be performed using data obtained from quantitative structure activity relationships (QSARs) for acute aquatic toxicity as described in Table R.11—7. In order to be suitable, the QSAR prediction should comply with the general principles described in Chapter R.6.1 of the *Guidance on IR&CSA*. Long-term testing is required if QSAR estimations indicate that the substance fulfils the screening threshold values for T (EC50 or LC50 < 0.1 mg/L). It may, on a case by-case-basis, be decided whether confirmatory chronic testing on fish is necessary if valid QSAR prediction indicates that the acute E(L)C50 is < 0.01 mg/L. Alternatively either first an acute fish toxicity limit test could be performed to check whether the acute toxicity is below 0.1 mg/L or the QSAR-prediction could be accepted as providing sufficient evidence of the T criterion being fulfilled.

If the substance is confirmed to fulfil the P and B criteria, testing on long-term toxicity should be performed to determine whether the substance meets the criteria for T. Alternatively, QSARs for chronic toxicity, if applicable, may be used by the registrant to conclude that the substance fulfils the T criterion, but normally, due to the uncertainties of the present QSAR-models, these cannot be used to conclude "not T".

When considering the use of non-testing data, it is important for substances containing multiple constituents, impurities and/or additives, to consider first the appropriate assessment approach provided in Section R.11.4.2.2.

R.11.4.1.4 Conclusions on PBT or vPvB properties

A detailed analysis of the Persistence, Bioaccumulation and Toxicity should be brought together into a clear overall conclusion. Three conclusions for the comparison of the relevant available information on the PBT properties with the criteria listed in REACH Annex XIII Section 1 are possible.

(i) **The substance does not fulfil the PBT and vPvB criteria**. The available information show that the properties of the substance do not meet the specific criteria provided in REACH Annex XIII Section 1, or if the information does not allow a direct comparison with all the criteria there is no indication of P or B properties based on screening information or other information.

(ii) **The substance fulfils the PBT or vPvB criteria**. The available information show that the properties of the substance meet the specific criteria detailed in REACH Annex XIII Section 1 based on a *Weight-of-Evidence* determination using expert judgement comparing all relevant and available information listed in Section 3.2 of Annex XIII to REACH with the criteria.

(iii) **The available data information does not allow to conclude (i) or (ii).** The substance may have PBT or vPvB properties. Further information for the PBT/vPvB assessment is needed.

The sub-chapters below provide more details on the circumstances that would lead to each of these conclusions. The consequences of each conclusion for the registrants are described in Section R.11.3.

The prerequisite for drawing a correct overall conclusion is that the endpoint –assessments described in Sections R.11.4.1.1, R.11.4.1.2 and R.11.4.1.3 are carried out and concluded correctly. Additionally, the assessment described in Section R.11.4.2.2 for substances containing multiple constituents, impurities and/or additives needs to be carried out in such manner that the principles for choosing an approach are fulfilled (see Section R.11.4.2.2 for details). A very high number (tens) of combinations of end-point conclusions is possible. . If a substance contains multiple relevant constituents, impurities and/or additives, the overall picture may be highly complex. In such cases the overall conclusion(s) can be best presented by providing conclusion tables for all relevant constituents, impurities and/or additives (or fractions, where relevant).

R.11.4.1.4.1 (i) The substance does not fulfil the PBT and vPvB criteria. The available information show that the properties of the substance do not meet the specific criteria provided in REACH Annex XIII Section 1, or if the information does not allow a direct comparison with all the criteria there is no indication of P or B properties based on screening information or other information.

This would be the case if, as a result of an analysis of existing data, or of data generated after conclusion (iii) any one of the parameters, i.e. environmental degradation half-life in an appropriate environmental compartment, the BCF for aquatic species (or a comparable metric) or, in the case of a decision on PBT, long-term aquatic toxicity and the appropriate human health hazard classification do not meet the criteria in Annex XIII.

In many cases, the information available, while not allowing a direct comparison with the criteria in Annex XIII, can be considered sufficient for a decision to be made, by applying *Weight-of-Evidence* based expert judgement, that the substance is not PBT/vPvB. Such would for instance be the case if the screening threshold values as provided in Section R.11.4 were not met for any particular endpoint based on screening information. Furthermore, when the screening threshold values for persistence or bioaccumulation as defined in the following sub-sections are not fulfilled, further PBT/vPvB assessment can stop when there is a well justified lack of counter evidence which would raise concern for the substance to have PBT or vPvB properties. In this case, the registrant can also draw the conclusion (i).

It has to be kept in mind that the fact that a substance does not meet the T criterion is not a sufficient basis on which to stop the evaluation of the remaining endpoints in the PBT/vPvB screening step.

Where supplementary information is available, such as sufficient evidence based on monitoring data, that indicates that a particular property, such as persistence or high bioaccumulation may in fact be present, a cautious approach should be followed and conclusion (iii) may need to be drawn (see below).

When drawing conclusion (i), the registrant should show in the PBT/vPvB assessment that there is no indication that the relevant constituents, impurities, additives or transformation/degradation products have PBT or vPvB properties.

It should be noted that where toxicity is a critical parameter for PBT assessment, i.e. the substance is persistent and bioaccumulative but there are insufficient (only acute valid) toxicity data, it will be necessary to conduct further testing (unless the registrant decides to treat the substance “as if it is a PBT or vPvB”). In such cases, the assessor must choose conclusion (iii) instead of conclusion (i).

R.11.4.1.4.2 *(ii) The substance fulfils the PBT and/or vPvB criteria. The available information show that the properties of the substance meet the specific criteria detailed in REACH Annex XIII Section 1 based on a Weight-of-Evidence determination using expert judgement comparing all relevant and available information listed in Section 3.2 of Annex XIII to REACH with the criteria (for more specific terminology, also used in IUCLID, please, see subsection "Terminology").*

In principle, substances are only considered as PBT or vPvB when they are deemed to fulfil the PBT or vPvB criteria for all inherent properties. This would be the case if, as a result of an analysis of existing data, or of data generated after concluding that further information is needed (conclusion iii), the environmental degradation half-life in an appropriate environmental compartment, the BCF for aquatic species or a comparable metric and, in the case of a decision on PBT, long-term aquatic toxicity or an appropriate human health hazard classification show the criteria to be met. The data must show that all three criteria are met in the case of PBT, or both vP and vB criteria in the case of vPvB. In this context it is important to note that even where one criterion is marginally not fulfilled but the others are exceeded considerably, the assessor may, based on a justification relying on the available evidence and considering weigh-of-evidence, conclude in specific cases that the substance fulfils the Annex XIII criteria.

If a constituent, impurity or additive of a substance fulfils the PBT/vPvB properties (based on the assessment of the registrant or of ECHA), a ≥0.1 % (w/w) threshold applies for concluding the substance as fulfilling the same PBT or vPvB criteria. For substances containing PBT/vPvB constituents, impurities or additives in individual amounts <0.1 % (w/w) of the substance, the same conclusion need not normally be drawn. This is in line with the threshold used for considering PBT and vPvB substances in mixtures (Article 14(2)(f) of REACH).

Furthermore, where a substance contains a high number of constituents, impurities or additives in individual amounts <0.1% (w/w) which are structurally similar and therefore can be considered together as a fraction, the concentration limit is considered to apply for the fraction. This in particular applies to highly complex substances where all or most individual constituents are present in concentration <0.1 % (w/w) but also to other substances containing blocks of similar constituents whereby the assessment efforts should remain proportionate (for further details, please, see Section R.11.4.1 on "Relevant constituents, impurities, additives and transformation/degradation products" and Section R.11.4.2.2).

Additionally, there may be other particular cases for which specification of percentages below 0.1% is required. This requirement is then driven by the toxicological profile of the constituent, impurity or additive (e.g. high potency carcinogenic, mutagenic or reprotoxic (CMR) and the provisions for classification and labelling) and not by the fact that the respective constituent is concomitantly a PBT/vPvB. If a substance (its constituents, impurities or additives) degrades or is transformed into transformation/degradation products which fulfil the PBT or vPvB criteria (based on the assessment of the registrant or of ECHA) and if these are formed in relevant amounts, the substance is concluded to fulfil the PBT or vPvB criteria. The definition of "relevant" transformation/degradation product for the registrant's substance is provided in Section R.11.4.1. Authorities should justify case by case what they consider as relevant transformation/degradation in their PBT/vPvB assessments. Terminology provided at the end of this section must be applied in the registration dossier to the substance subject to PBT/vPvB assessment to distinguish which of the cases above the substance represents.

Overview of case types of conclusion (ii)

The following differentiation is used for substances which have to be concluded to fulfil the PBT and/or vPvB criteria:

- *The substance is PBT/vPvB.* This conclusion is drawn because this is a mono-constituent substance and it has a main constituent present at a concentration of 80% or more with PBT and/or vPvB properties;
- *The substance is PBT/vPvB*. This conclusion is drawn because this is a mono-constituent substance, well-defined multi-constituent substance or UVCB substance. and it contains one or more relevant[60] (group(s) of) constituent(s)[61] which fulfil the PBT and/or vPvB criteria[62];
- *The substance is PBT/vPvB*. This conclusion is drawn because one or more (group(s) of) constituent(s), impurity or additive of the substance degrade(s) or is/are transformed into substance(s) which fulfil the PBT and/or vPvB criteria and these transformation or degradation products are formed in "relevant"[60] amounts.
- Combination of two or all of the above types.

It should be noted that there is no difference in risk management between the different types. The consequences of conclusion (ii) for the registrant are described in Section R.11.3.

R.11.4.1.4.3 *(iii) The available information does not allow to conclude (i) or (ii). The substance may have PBT or vPvB properties. Further information for the PBT/vPvB assessment is needed.*

The consequences of this conclusion for the registrant are described in Section R.11.3.3.

This conclusion is derived when one or more of the following combinations of endpoint–specific conclusions apply:

Potential P/vP + Potential B/vB + any T -conclusion
Potential P/vP + B but not vB + Potential Teco
Potential P/vP + B but not vB + Potential Thh
Potential P/vP + B but not vB + Teco
Potential P/vP + B but not vB + Thh
Potential P/vP + vB + any T -conclusion
Potential P/vP + B/potential vB + any T -conclusion

P/potential vP + Potential B/vB + any T -conclusion

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[60] "Relevant" is defined in section R.11.4.1.

[61] "Constituent" as referred to in Annex XIII of REACH means "constituent", "impurity" or "additive" as described in the *Guidance for identification and naming of substances under REACH and CLP*.

[62] The terminology corresponds with IUCLID 6 section 2.3 terminology. The constituent(s) or constituent group(s) fulfilling the PBT/vPvB criteria should be specified in specific endpoint study records in section 2.3 of IUCLID.

P/potential vP + B but not vB + Potential Teco
P/potential vP + B but not vB + Potential Thh
P/potential vP + vB + any T -conclusion
P/potential vP + B/potential vB + any T -conclusion

P but not vP + Potential B/vB + Potential Teco
P but not vP + Potential B/vB + Potential Thh
P but not vP + Potential B/vB + Teco
P but not vP + Potential B/vB + Thh

P but not vP + B/vB + Potential Teco
P but not vP + B/vB + Potential Thh

vP + Potential B/vB + Any T-conclusion

vP + B + Potential Teco
vP + B + Potential Thh

Where the data on the PBT properties of a substance do not allow a direct (numerical) comparison with the criteria specified in Annex XIII, but there are nevertheless indications from other data such as screening data, that the substance may be PBT/vPvB, then it is necessary to consider which information is needed to draw a final conclusion.

Where it is concluded that further information is needed, consideration should first be given to clarifying the persistence of the substance since persistence is a critical property in determining PBT/vPvB properties and since degradation testing does not involve the use of vertebrate animals[63].

Once the new information is available, comparison with the criteria in Annex XIII should be carried out according to the principles described above and a decision be taken on whether the substance falls under conclusion (i) (is not a PBT/vPvB) or (ii) (i.e. is a PBT/vPvB). In certain cases the revised assessment may again lead to the conclusion that further information still needs to be generated. If for one of the relevant constituents, impurities, additives or transformation/degradation products there is indication that it may have P and B properties, the registrant should draw conclusion (iii) and generate the necessary additional information until the available information allows to draw one of the two ultimate conclusions in relation to the whole composition (see Section R.11.4.1 for description of "relevant" and Section R.11.4.2.2 for the relevant assessment approaches).

There may be cases where a clear decision on the properties of a substance cannot be made, but there are indications from available information that the substance may fulfil the PBT or vPvB criteria. In these cases conclusion (iii) applies. For instance, where there is a reason to

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[63] Depending on the substance properties it may, however, be appropriate to consider bioaccumulation testing first. Guidance on the general approach to P, B and T testing is given in Section R.11.4.

expect that a substance may contain a known PBT constituent , impurity or additive (or fractions thereof) but it is not possible to characterise a substance identity to an extent that will allow the registrant to state with enough confidence that his substance does not contain PBT/vPvB constituents/impurities/additives or that it does not generate transformation/degradation products with PBT/vPvB properties above the relevant threshold levels as specified in Section R.11.4.1.

Finally, there may be cases where it is simply technically not possible to conduct testing, either at screening or at confirmatory level and therefore not possible to derive conclusion (i) or (ii). If there are no indications or justification which would exclude the possibility that the substance could potentially fulfil the criteria, conclusion (iii) should be drawn.

R.11.4.2 Assessment of PBT/vPvB properties – consideration of specific substance properties

R.11.4.2.1 Assessment of substances requiring special considerations with regard to testing

For substances that have exceptional properties (e.g. very high sorptivity, very low water solubility, or high volatility), or which consist of multiple constituents, test guidelines used to determine persistence, bioaccumulation and toxicity in the PBT/vPvB assessment may not be directly applicable. Instead specific testing and assessment strategies may be warranted.

R.11.4.2.1.1 Substances with very high sorptivity

The assessment strategy should be applicable to strongly sorbing substances in general.

General considerations

In R.11.4.1.2.10 "Further data" indicators for limited bioaccumulation are described. For substances with very high calculated Log K_{ow}, e.g. > 10, reduced bioaccumulation is expected. Log K_{ow} values > 8 cannot be measured reliably due to technical issues and need therefore to be calculated by property estimation methods based on the concept of Linear Free Energy Relationship (LFER). Before using a specific LFER method the extent to which the structural elements of the substance under consideration are covered by the applicability domain of the LFER needs to be checked. For example, organometallic substances like tin organics may not be covered whereas the corresponding carbon analogue of the substance is.

It is very important to realise that the calculated Log K_{ow} values > 10 are used simply to indicate a degree of hydrophobicity that is extreme. Such values should not be used in a quantitative manner.

Assessment steps

STEP 1 Calculated / measured Log K_{ow}

Check/generate the calculated / measured Log K_{ow} of the substance of interest.

STEP 2 Assessment type to be applied

If the Log K_{ow} is < 10 an assessment of P, B and T should follow the standard approach as described in Section R.11.4.1.

If the Log K_{ow} is > 10 it should be checked if available ecotoxicity and / or mammalian data do not meet the T criteria. If the T criteria are not met, a specific vPvB assessment might be applicable as described below.

If for a substance with Log K_{ow} > 10 data are available demonstrating toxicity in accordance with the T criteria for PBT substances, then a standard PBT assessment as described in Section R.11.4.1 is warranted.

STEP 3 vPvB Assessment for substances with Log K_{ow} > 10

Step 3a Persistence check

Substances with transformation potential

If the substance can be transformed abiotically or biotically (e.g. when it has structural moieties like ester groups, phosphites or phosphonites) it should be checked if a specific biodegradation test at low concentrations and specific analysis or a specific hydrolysis test (see Section R.7.9.4 in *Chapter R.7b* of the *Guidance on IR&CSA*) could be carried out to demonstrate transformation with a primary half-life of < 40 d. In such circumstances, the transformation/degradation products will need to be checked to ensure they do not have PBT or vPvB properties. If the substance is transformed into substances not having PBT or vPvB properties it can be considered not to fulfil the vPvB criteria. **In this case Step 3b can be omitted.**

Substances with limited transformation potential

If a substance may not be easily transformed based on the structure (e.g. it has no ester functions or the transformation rate is limited by very low (bio)availability) it is nevertheless recommended to estimate the metabolic pattern, using e.g. CATALOGIC, which is the successor of Catabol (Mekenyan, 2006). For all relevant transformation/degradation products it must be checked that they do not fulfil the criteria for PBT or vPvB substances. For these substances Step 3b is mandatory.

Step 3b Bioaccumulation check for substances with limited transformation potential

Claims of a low bioaccumulation potential indicated by the Log K_{ow} > 10 should be supported by additional information indicating limited bioaccumulation as described in R.11.4.1.2.10 “Further data”). This information may comprise results from an animal study (mammalian or fish) confirming no or low bioaccumulation.

Log K_{ow} >10 and at least one additional indicator for limited bioaccumulation

If for a substance with Log K_{ow} > 10 at least one additional indicator (1. or 2.) mentioned above is fulfilled the substance should not be considered as vPvB, provided that potential metabolites are themselves not PBT or vPvB.

Log K_{ow} >10 and no additional indicator for limited bioaccumulation

If none of the additional indicators (1. or 2.) mentioned under Step 3b is met, then an appropriate test as described in Section R.11.4.1.2 is warranted.

STEP 4 Overall conclusions

Log K_{ow} >10 and ready biodegradability in a specific biodegradation confirmed

No further investigation necessary, if transformation/degradation products are neither PBT nor vPvB. In this case the (parent) substance is not vPvB.

Log K_{ow} >10 and <u>no</u> ready biodegradability confirmed

If at least one additional indicator for limited bioaccumulation is fulfilled and potential transformation/degradation products are not PBT or vPvB, then the substance is not vPvB.

If no additional indicator for limited bioaccumulation is fulfilled a standard vPvB assessment as described in Section R.11.4.1 is warranted.

R.11.4.2.1.2 Substances with low solubility in octanol and water

The assessment strategy should be applicable to substances with low solubility in octanol and water and for which lipid is the target compartment for accumulation in organisms. For illustrative purposes certain organic pigments are used as examples (see List of Pigments, in Table R.11—8).

It should be noted that these examples are presented under the assumption that the named pigments would not have specific nanoform -related properties. Whether the assumption is correct or not is not relevant for the purpose of the examples.

General considerations

1) Critical body burden (CBB) concept and octanol solubility

In R.11.4.1.2.10 "Further data" it is described how octanol solubility could be used in the B assessment (Critical Body Burden approach) as well as the limits of the approach.

As octanol is a reasonable surrogate for fish lipid, a low substance concentration in octanol may indicate reduced bioconcentration / bioaccumulation potential. The concept is based on available measurements for substances using a safety factor of 10 for the uncertainty of the available CBB measurements. It is proposed that where a substance shows no specific mode of action and has a

$C_{octanol}$ [mg/L] < 0.002 [mMol/L] x Mol weight (g/Mol) **Equation 11-3**

it can be assumed that the substance has only a limited potential to establish high body burdens and to bioaccumulate. If it does bioaccumulate, it would be unlikely to rise to levels in biota that would cause significant effects.

2) Octanol water partitioning

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.

K_{ow} values derived from fragment based LFER methods like KOWWin (US EPA, 2000b) often overestimate the actual K_{ow} of such substances e.g. organic pigments (Table R.11—8). In order to overcome the difficulties in measuring the K_{ow}, the solubility in octanol (C_o) and water (C_w) may be determined separately. With these solubilities the quotient Log C_o/C_w can be calculated. This quotient is not exactly identical to Log K_{ow}, as the latter is related to the partitioning of the substance in water-saturated octanol and octanol-saturated water. For Pigment Yellow 12, Log C_o/C_w as well as Log K_{ow} (from solubility measurements using water-saturated octanol and octanol-saturated water) have been determined as 2.1 and 1.8, and hence being in the same order of magnitude (see Table R.11—8). This single comparison between Log C_o/C_w and Log K_{ow} needs further verification but the figures available for Pigment Yellow 12 can be interpreted as follows: as water saturation in octanol diminishes the octanol solubility of the substance and octanol saturation in water enhances the water solubility, the Log K_{ow} of the substance should normally be smaller than Log C_o/C_w (see values for Pigment

Yellow 12, Table R.11—8). A measured Log C_o/C_w = 4.5 would mean that the measured Log K_{ow} should be < 4.5.

In Table R.11—8 solubility data are given for some other organic pigments as well. The comparison of the measured quotient Log C_o/C_w with estimated Log K_{ow} using KOWWIN (US EPA, 2000b) shows that the estimated K_{ow} exceeds C_o/C_w by between 1 and 8 orders of magnitude.

Table R.11—8: Solubility of some pigments and comparison of their Co/Cw values with estimated K_{ow}s (US EPA, 2000b)

Colour Index Name	Mol weight (g/Mol)	Co (µg/L) at ambient temperature	Cw (µg/L) at ambient temperature	Log Co/Cw	Log Kow (KOWWin)
Pigment Yellow 12	630	48* 50	0.8 0.4	**1.8*** **2.1**	**7,1**
Pigment Red 122	340	600	19,6	**1,5**	**2,5**
Pigment Red 168	464	124	10,8	**1,1**	**7,1**
Pigment Red 176	573	15	1,9	**0,9**	**7,3**
Pigment Violet 23	589	330	25	**1,1**	**9,4**

* values relating to saturated solvents = water saturated octanol, octanol saturated water, this Log Co/Cw corresponds to Log K_{ow}.

3) Additional Indicators to be used for the 'B' Assessment

As described in R.11.4.1.2.10 "Further data", additional indicators for low bioaccumulation potential, such as results from an animal study (mammalian or fish) confirming no or low uptake into the organism, might also be applicable for substances with low solubility in octanol and water.

Assessment steps

STEP 1 Solubility measurements for Substances with low Octanol & Water Solubility

For the determination of the water solubility the column elution method and the flask method exist (OECD TG 105) but it needs to be checked which one is the most appropriate (Section R.7.1.7 in Chapter R.7a of the *Guidance on IR&CSA*). No OECD Guideline exists for the measurement of the octanol solubility but in principle the OECD TG 105 methods may be used in adapted form.

STEP 2 B and T Assessment

The octanol solubility of the substance is compared with the critical body burden (CBB) according to equation (11-3) given above using the Mol weight of the substance.

Result 2A: C_o < CBB

If the octanol solubility is below the CBB, the maximum uptake of the substance can be expected to be below the CBB and toxicity is not likely.

Animal studies should, in addition, be checked to confirm reduced uptake and low toxicity. In this case the substance has low bioaccumulation potential and low toxicity.

Result 2B: C_o > CBB and Log $C_o/C_w \leq 4.5$

If the octanol solubility is above the CBB a build-up to a critical concentration of the substance in lipid cannot be excluded and additional information on adsorption is required. If the quotient Log Co/Cw of measured solubilities is ≤ 4.5 (if measurable / available) a reduced uptake is expected as well. Animal studies should, in addition, be assessed to confirm reduced uptake and low toxicity. In this case the substance can be considered to have low bioaccumulation potential.

Result 2C: C_o > CBB and Log $C_o/C_w > 4.5$

For this substance a standard approach of P, B and T assessment as described in Section R.11.4.1 must be applied. No conclusion on B and T can be drawn.

In addition indicators like molecular weight and average size of the molecule and reduced uptake in mammalian studies should be checked for further evidence, if necessary, and be used in a *Weight-of-Evidence* approach.

STEP 3 *Weight-of-Evidence* approach for Results 2A & 2B

Based on the results of Step 2 (2A and 2B) a *Weight-of-Evidence* approach with the elements Co, CBB, Log Co/Cw, possibly molecular weight and Dmax (size) as well as ecotoxicity and uptake behaviour in animal studies, is warranted to demonstrate that the substance is not a vPvB or PBT substance.

R.11.4.2.1.3 Volatile substances

This section describes different aspects related to degradation simulation testing of volatile substances and their data treatment.

In simulation tests it is important to differentiate degradation and disappearance of the test substance due to volatilisation. Volatilisation of the test substance makes the interpretation of the study more difficult and increase uncertainty of the persistence assessment. In order to achive acceptable mass balance, use of appropriate traps for the parent and potential transformation/degradation is essential.

If the substance properties or properties of the potential transformation/degradation products indicate potential for volatilisation during the degradation test, this needs to be taken into account as part of the degradation test design. Henry´s Law constant (HLC) >1.0 Pa m3/mol or vapour pressure (VP) above 300 Pa may be used as such indicators for volatility even if these do not solely allow predicting the volatilisation rates in simulation tests. A case-by-case assessment of potential volatilisation is needed, and pre-study is always recommended to ensure feasibility of the simulation test. The ECHA note on Volatile substances[64] provides further considerations on identifying volatile substances and their persistence assessment.

In addition, the **Level I** fugacity models, based on steady-state calculations, may provide information on partitioning in a closed system like the closed bottles of simulation tests. Another suitable model, **SimpleTreat v4.0** predicts the volatilisaton process from the aeration tank (Mikkelsen, 1995) of a wastewater treatment plant. Level I and Simple Treat models seem to often predict relatively similar distributions between air and the other compartments (see *ECHA note on Volatile substances* available on ECHA Website for further details). However, volatilisation in a simulation test cannot always be excluded even in those cases where distribution to air is predicted to be low by the models (i.e. Level I and Simple Treat). For some substances with high WS and/or high K_{oc}, the models may overestimate the partitioning to the aquatic and sediment compartment, respectively, and underestimate the partitioning to air (*ECHA Guidance on IR&CSA Chapter R.16*).

Several options to address volatilization of substances in OECD TGs 307, 308 and 309 are described below. As a general rule, any modification of the test design or set-up should be consistent with the OECD TGs conditions and the validity criteria of the guidelines should be fulfilled. Minimising the losses in surface water simulation test due to volatilisation Birch *et al.* (2023) recommend handling aqueous solutions using gas tight syringes, conduct the studies in gas tight vials and apply automated analysis directly on unopened test vials.

A pre-test or pilot study (OECD TGs 307, 308 and 309) could be necessary in order to determine what modifications to the guideline are necessary and feasible (e.g. whether biometer type or closed test vessels should be used).

Whenever volatility is suspected, **biometer-type-flasks or closed test vessels** should be used to ensure adequately high recovery rates. Following considerations should be taken into accont regarding the biometer-type and closed vessels design:

- Closed vessels with minimised headspace (OECD TGs 307, 308 and 309). For example volume ranging from 100-125 ml have been used (Shrestha *et al*, 2019; OECD TG 309 with EC 202-046-9[65]).
- Maintaining aerobic conditions in closed test systems (OECD TGs 307, 308 and 309) to compensate the oxygen consumption by the biomass. A gentle stirring of the water surface is recommended in the TGs 309 and 308. According to the OECD TG 307 if biometer-type flasks are used, exchange of air is maintained by diffusion. However, the guideline indicates that under aerobic conditions, the soil moisture content should be adjusted to maintain adequate aeration and nutrition of soil microflora. Care should be given to prevent or minimise losses of test substance and/or transformation/degradation products by volatilisation during moisture addition in soil.

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[64] *ECHA note:* Options to assess persistence of volatile substances in regulatory PBT assessment *(2022) available at* https://echa.europa.eu/documents/10162/17228/note_volatiles_in_simulation_tests_en.pdf/d218ddcb-e5da-7c0a-e5d0-3eae3e1c26dc?t=1669388686441

[65] https://echa.europa.eu/en/registration-dossier/-/registered-dossier/10843/5/3/3/?documentUUID=6a872efb-688c-446e-89b2-9d8d33361290

- System geometry (OECD TG 308). In OECD TG 308 a system geometry with a larger diameter to increase surface area at the air-water and water-sediment interfaces has been used to improve oxygenation (Shrestha *et al.*, 2016). Increasing the water-air interface may also lead to more losses due to volatilisation. 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 test design and any modification proposed in the system geometry should be consistent with the range indicated in the standard OECD TGs conditions for water-sediment volume ratio and height and weight of the sediment layer.

- Influence of the organic carbon (OC) content (OECD TGs 307, 308 and 309)**.** In general, higher volatilization is expected for systems with low organic carbon content as adsorption of the substance to organic material may hinder volatilisation. In sediment simulation tests, the OC content of sediments should be within the range indicated in the OECD TG 308. The amount of suspended solids in the water phase of the OECD TG 309 pelagic tests should be representative of the level of suspended solids in the EU surface water, c.a. 15 mg dw/L. Testing natural surface waters containing between 10 and 20 mg SPM dw/L is considered acceptable in the OECD TG 309 (pelagic test). In the case of testing volatile substances in the OECD TG 307 it is recommended to include at least one soil with high organic carbon content, e.g. > 10%, which is the highest organic carbon content range for soils recommended for soil adsorption/desorption studies in the Final Report of the OECD Workshop on Selection of Soils/Sediments (OECD, 1995), which is referenced in the OECD TG 307.

- Absorbing surfaces (all TGs). Use of tubing, plastic and rubber components or other absorbing surfaces in respirometer systems should be minimised. These components have been observed to lead to increased losses of volatile substances from test systems[66] (Brown *et al.*, 2020) resulting in lower bioavailability, lower biodegradation observed and lower mass balance recoveries. Special attention should be paid to components located in the headspace. Stainless steel, plastic-free bottle caps and lock systems (Swagelok connections, Hamlet valves and fitting) have been used to completely close the test setup (Shrestha *et al* 2019, 2020).

Increase number of samplings to monitor substance concentrations in water, sediment, soil and air. Additional sampling points during the initial period of the study may be needed in order to determine the rate of distribution between the different phases of the test system (water, sediment, soil and air) for a successful kinetic modelling.

Adsorption traps. In flow-through systems, the setup should be permanently connected to a series of different trapping solutions in order to passively trap the volatilized fraction, e.g., NaOH and ethylene glycol or tenax flasks to trap mineralized $^{14}CO_2$ and volatile parent/degradation products, respectively. In biometer type flasks, systems for adsorbing volatile parent/degradation products, e.g. coated glass wool or polyurethane foam, and CO_2, e.g. soda lime, can be used. Other methods such as using purge and trap gas

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[66] Biodegradation in water and sediment: simulation tests of Di-tert-butyl 3,3,5-trimethylcyclohexylidene diperoxide (Trigonox 29) (EC number: 229-782-3 | CAS number: 6731-36-8) . https://echa.europa.eu/en/registration-dossier/-/registered-dossier/13187/5/3/3/?documentUUID=8cb36c04-6283-4938-a9f1-70fafd154f4e

chromatography/mass spectrometry with evaluation of mass balance at each time point may also be applicable for volatile substances.

Preparation of test solutions (All TGs). During the preparation of the stock solution and the diluted test solutions for exposure, flasks should be sealed and the headspace kept to a minimum or eliminated (OECD GD 23). The combination of hydrophobicity and volatility makes testing challenging especially to dissolve a substance in water and avoid evaporative losses. The use of a co-solvent could be needed but it can decrease the oxygen concentration in the water phase due to the oxygen consumption by microorganisms degrading the solvent (Shrestha *et al.* 2020). Therefore, the use of co-solvent should be avoided or minimised as much as possible, and, if needed, a solvent with slow degradation rate should be chosen.

Methods for substance application. In the case of volatile substances, the application solution can be spiked directly on the sediment matrix and the biometer-flask immediately closed using an insert cap to avoid losses due to volatilization. Sediment spiking is currently not mentioned as an option in OECD TG 308 but it has been used in water-sediment system studies for highly volatile substances and the results have been found suitable for regulatory persistence assessment, e.g. sediment simulation study with decamethylcyclopentasiloxane[67] (EC 208-764-9)).

Monitoring oxygen levels. It is recommended to monitor the aerobic conditions inside the closed flasks, including the water phase and headspace, preferably by external oxygen measurements, thereby minimizing test substance losses due to opening of the vessel. This is not necessary if it can be reliably demonstrated that O_2 concentration remains sufficiently high until the end of the study. Samples with high ThOD e.g. reference substance and solvent (if applied) may be used to indicate that the O_2 was sufficient for the period of degradation of the reference substance, although it may not inform on O_2 level at the end of the study.

Sterile controls. Sterile controls are always recommended to determine to what extent the decrease of the test substance is due to biotransformation or to potential abiotic losses (e.g. adsorption to caps and leaking out of the test system). Test conditions and operational conditions in sterile controls should be the same as in active vessels (see also Section R.11.4.1.1.3 on "Sterile controls" and Section R.7.9.4.1 in Chapter R.7 of the *Guidance on IR&CSA* for additional information). Abiotic losses can be counted via concentration ratios between the test system and sterile controls (Birch *et al.* 2023).

Use of ^{14}C radiolabelled material. The simulation study must be performed preferably using a radiolabelled test material and a mass balance has to be included in reporting. Use of ^{14}C labelled test substance facilitates the monitoring of the test substance and verifying degradation kinetics, obtaining the complete mass balance and differentiate mineralization, NER formation and volatilization. For highly volatile substances this is especially important because decreasing mass balance during the study can indicate that there may have been leakage of the test substance from the test system. If radiolabelling is not feasible, a systematic and reliable monitoring of the test substance and transformation/degradation

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[67] https://echa.europa.eu/en/registration-dossier/-/registered-dossier/14807/5/3/3/?documentUUID=525345fa-cff1-4b4c-9cca-a2f7e256db6d

products should be performed in all compartments of the test system (water/sediment or soil and air) and reported during the whole simulation study.

Data treatment and interpretation issues

There are several volatilisation correction approaches available for the kinetic analysis of simulation study results. More detailed description of the following procedures is included in Appendix R.11-7 and in *ECHA note on Volatile substances* available on ECHA Website.

A. Separate fitting of data on total dissipation and volatile traps
B. Simultaneous fitting of data on parent substance in water-sediment and volatile traps to SFO kinetics in OECD 308 using suitable software (e.g., CAKE)
C. Simultaneous fitting of data on parent substance in soil and volatile traps to SFO kinetics in OECD 307 using suitable software (e.g., CAKE)

In all correction procedures it is assumed that the volatile losses of parent substance are adequately identified and quantified in the volatile trapping systems. Losses through other processes, such as adsorption, increase uncertainties in the kinetic analyses.

R.11.4.2.1.4 Substances with nanoforms

When a substance fulfils the criteria of the nanoform definition, specific considerations on the testing and assessment apply. The term nanoform refers to a nanoform or a set of similar nanoforms as described in REACH Annex VI. According to REACH Annex I, PBT and vPvB assessment shall address all nanoforms covered in the substance registration.

Definition of a nanoform and a set of similar nanoforms (REACH Annex VI)

On the basis of the Commission Recommendation of 18 October 2011 the definition of nanomaterial, a nanoform is 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.

The term 'nanoform', refers to a nanoform or a set of similar nanoforms, when one has been defined. A 'set of similar nanoforms' is a group of characterised nanoforms where the clearly defined boundaries in the parameters of the individual nanoforms within the set still allow to conclude that the hazard assessment, exposure assessment and risk assessment of these nanoforms can be performed jointly.

Updated Commission Recommendation (10 June 2022)

'Nanomaterial' means 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: (a) one or more external dimensions of the particle are in the size range 1 nm to 100 nm;(b) 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; (c) 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. In the determination of the particle number-based size distribution, particles with at least two orthogonal external dimensions larger than 100 µm need not

According to REACH Annex VI, each nanoform needs to be characterised. Furthermore, Annex VII to X information requirements (*i.e.* fate and (eco)toxicity data) apply individually to each nanoform of a substance. For example, degradation assessment of nanoforms that are not soluble, nor have high dissolution rate, shall consider also morphological transformation (*e.g.* irreversible changes in particle size, shape and surface properties, loss of coating), chemical transformation (e.g. oxidation, reduction) and other abiotic degradation (e.g. photolysis).

Hazard properties of a nanoform may differ between different nanoforms and from its non-nanoform and, in general, testing of these properties require specific attention and advice. Therefore, PBT/vPvB assessment has to be carried out for each relevant organic and organometallic nanoform of a substance. For nanoforms the surface treatment agent must also be considered. Currently, there is no nano-specific Appendix for *ECHA Guidance IR&CSA, Chapter R.11: PBT/vPvB assessment*. *Appendices for nanoforms applicable to ECHA Guidance on IR&CSA Chapters R.7a, R.7b and R.7c* provide advice for hazard identification of nanoforms.

Where it can be demonstrated that a nanoform behaves in the same way as a non-nanoform of the substance, the *ECHA Guidance on IR&CSA Chapters R.7a, b and c and R.11* may in general apply. This is the case when a nanoform is considered to be highly soluble i.e. no detectable particles are present. According to the *Appendix for ECHA Guidance IR&CSA on nanoforms applicable to Chapter R.7a, Section 1.2.1*, nanoform is highly soluble if its water solubility is measured to be above 100 mg/L in less than 24h (according to OECD GD 318). It is expected that such nanoforms will elicit the same response as the soluble non-nanoforms of the substance.

If the water solubility of a nanoform is less than 100 mg/L, within 24h, then PBT assessment has to be performed with specific attention to the specific behaviour of the nanoforms by considering the corresponding appendices for nanoforms (solid or undissolved fraction) applicable to *ECHA Guidance on IR&CSA Chapters R.7a, R.7b and R.7c*. For the dissolved fraction *ECHA Guidance IR&CSA, Chapter R.11* can be followed.

R.11.4.2.2 Assessment of substances containing multiple constituents, impurities and/or additives

Annex XIII to the REACH Regulation requires that relevant constituents are taken into account in the PBT/vPvB assessment. Section R.11.3.2.1 describes registrants' obligations in this matter and Section R.11.4.1 (under "Relevant constituents, impurities, additives and transformation/degradation products") provides ECHA's interpretation of the term "relevant".

This section gives recommendations on how to assess a substance containing several/many constituents, impurities and/or additives. In the following the term "constituent" is used to cover all these, in line with the legal text. A particular emphasis is given to UVCB substances, but the guidance should be applied by analogy for those well-defined substances[68] which contain several/many relevant constituents.

The assessment stages, listed briefly below, are the same as for assessing pure (i.e. mono-constituent) substances but contain some additional features due to the complexity of assessment. **The additional features are highlighted in bold** and discussed in the corresponding subsections. The purpose of these additional features is to enhance the assessment efficiency by showing ways to use the limited information normally available on different constituents and to help in building an effective strategy for generating further information, where needed. Ultimately this helps to avoid the elaborate option of taking into account – i.e. assessing – all relevant constituents individually.

- Gathering of available information: similar requirements as for any substance under REACH apply. However, for substances containing multiple constituents specific attention needs to be paid that all relevant information on identity and properties of the constituents and on the whole substance is gathered. In addition, specific attention needs to be paid that all relevant information on the test item identity/composition is gathered in order to be able to assess to which extent the gathered data actually represents the registered substance.
- Assessment:
  - **Initial profiling of the substance composition** for the purpose of the PBT/vPvB assessment, including profiling of the unidentified constituents/constituent fractions using available information on substance identity
  - Assessment using one or more of the **assessment approaches** described below. If the approaches and principles defined in this section are correctly applied, guidance in sections R.11.4.1.1, R.11.4.1.2 and R.11.4.1.4 can be applied to the target "entities" of assessment and testing but additionally also taking into account **specific aspects** of assessing substances containing multiple constituents.
    - If necessary, generation of further information: For the purpose of further specification of identity of specific constituents or fractions of constituents. It should be noted that the PBT/vPvB assessment may eventually require characterisation of constituents or fractions of constituents to a level beyond what is normally

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[68] For definition of UVCBs, well-defined multi-constituent and mono-constituent substances, please see the *Guidance for identification and naming of substances under REACH and CLP*.

sufficient and necessary to identify constituents of the registered substance according to section 2 of Annex VI to the REACH Regulation. However, the level of detail to be pursued is also dependent on the feasibility and proportionality of efforts and is therefore case dependent.

  - Testing selected constituent(s)/fractions of constituents (or in well justified cases the whole substance) for necessary properties. For substances containing various constituents the choice of appropriate **test items** is essential. Furthermore, the order in the normal tiered testing strategy (P first, then B, then T) may in some cases be changed, depending upon the ease and cost of generating such data and animal welfare considerations. Testing process may, e.g. start after a P and B–screening assessment with B–testing of the most relevant fractions with appropriate analytical characterisation of all constituents. Based on these results the specific fractions to be tested in degradation and ecotoxicity tests could be narrowed further. Due to animal welfare considerations such reverse order of testing should, however, only be carried out when it is likely that B-testing will anyway be needed and that the reverse order does in no case lead to more vertebrate testing than what would be the case when starting with degradation testing.

- Next tier of the assessment will include change/modification of the assessment approach, where needed, and repetition of the previous steps, if needed.

- Conclusion (see Section R.11.4.1.4).

R.11.4.2.2.1 Initial profiling of the substance composition

The complexity of the composition differs greatly between substances. Even for some UVCBs, the composition may be fully known. For other UVCBs as well as for large fractions of impurities of well-defined substances knowledge of the exact composition may be limited.

The *Guidance for identification and naming of substances under REACH and CLP* prescribes that unknown constituents are reported as far as possible by a generic description of their chemical nature for the identification of a substance. This description must be fit-for-purpose in light of determining the properties of the substance. For the PBT/vPvB -assessment, the description of these unknown constituents needs to be provided to the level of detail making screening PBT/vPvB -assessment possible and feasible. Type and expected variation of constituents (in terms of chemical groups or classes) will determine the level of detail. For example, for petroleum substances it would be hydrocarbon class, like mono-aromatics, n-alkanes, etc... For UVCB substances of botanical origin (e.g. essential oils) it could be terpenoid blocks, such as "monoterpene" and "sesquiterpene", subdivided by the appropriate functional descriptors "hydrocarbon", "alcohol", "ketone", etc and/or carbon skeletons "acyclic", "monocyclic", "bicyclic", etc… [69] The limitations of the analytical methods and proportionality of efforts to make other related information available may define the achievable level of detail and are case dependent. Therefore, the level of detail to be used to describe the

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[69] For further guidance provided by the fragrance industry, please, see: http://echa.europa.eu/support/substance-identification/sector-specific-support-for-substance-identification/essential-oils

constituents will vary from substance to substance and is case dependent. However, the level of available detail should allow defining chemical classes/functions present or modelling of the individual structures present.

Descriptors such as identity of the chemical functionalities present, molecular weight range, carbon number range, etc. may be useful as specifications. In some cases, these constituents may be best reported as a group (e.g. 'alkanes, C10-13, chloro' or "sesquiterpene hydrocarbons, $C_{15}H_{24}$"). Raw material(s) and manufacturing process details may help in generating the necessary information on substance composition. Profiling of the composition with new methods, e.g, as reviewed by Dimitrov *et al.* (2015) is recommended for the purpose of filling the data gaps at screening level.

An example of an initial profiling strategy of a fraction of unidentified constituents is given below:

1. Assess the available data that is used to characterise/describe the substance. Information derived by chemical identity characterisation is of highest value, but if such cannot be derived for technical feasibility reasons, other information sources can also be used. For example boiling point range is typically one of the main descriptors of petroleum substances and, if used combined with other more specific manufacturing information, it can be used to generate a list of structures that could reasonably be predicted to be present in the substance. For example with petroleum substances this would probably be hydrocarbon classes within specified chain lengths, degree of branching, and content of (iso)alkane, cyclic and aromatic constituents. For other classes of similar substances that are also UVCB (e.g. many surfactants, essential oils, halogenated mineral oil derived UVCBs) the composition could potentially be described as the distribution of non-polar and polar functional groups, as a function of molecular weight or chain length. Halogenated UVCBs could be described based on the nature of halogenation, chain length, degree of branching, saturation, cyclic and aromatic constituents and degree and nature of halogenation. Whatever approach is used to characterise the composition of the UVCB substance, a scientific and technical justification should be provided.

2. Determine the structures that are to be used as representative structures of each fraction for which full analytical identification is not available, detailing why these structures are regarded as representative and, if possible, give the approximate concentrations of the fraction for which they are considered representative.

3. In general it would not be necessary to generate representative structures if it were possible to demonstrate that the fraction for any representative structure were present at less than 0.1% (w/w). In practice this may be difficult to achieve.

R.11.4.2.2.2 Assessment approaches

Below the approaches which are recommended to be applied are described. These approaches are based on the idea that different "parts" (i.e. constituents or constituent fractions) of the substances are assessed separately (see the concept of "Assessment entity" [70]), unless the whole manufactured/imported substance is consisting of such similar constituents, that read across criteria can be applied amongst them for the purpose of the PBT/vPvB assessment. Whichever approach is considered suitable for a particular substance, the assessment document should contain a clear justification for the choice. Issues related to feasibility and/or proportionality of efforts may play a role in the choice of the assessment approach in addition to the technical elements listed under each approach. These should also be duly described in the assessment document, where appropriate.

The approaches described below do not necessarily cover all possible cases exhaustively, hence there may be situations where a different approach, not described below, could be justified.

"Known constituents" approach

This 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 the (v)P, (v)B and T properties of all constituents of the substance, and these specific constituents can be isolated or separately manufactured or otherwise acquired for the purpose of testing.

In this approach, the known constituents of the substance are first subjected to screening assessment individually. Hereby assessment approaches applied to pure (i.e. mono-constituent) substances can be applied (e.g. using experimental data, read across, QSARs). Specific constituents that are considered to be (the most) suspected ones with regard to the PBT/vPvB properties are targeted in the further steps. Testing, if necessary, is done by using individual constituents (or their surrogates) as test items. Each selected constituent is assessed for its P, B and T status, on its own, using available data on that constituent (or on read across–substances, if justified). The fact that a constituent can be more easily isolated or manufactured than another constituent may play a role in the choice of the constituent for assessment and testing but that should not be taken as the main criterion to test this specific constituent. The need to test a constituent should be driven by its relevance and representativeness for the overall PBT assessment of the substance (or fraction addressed).

In this approach known constituents present at ≥0.1 % w/w concentration in the substance should normally be considered as relevant (see section R.11.4.1 for further discussion on the concentration limit). The substance can be deemed as "not PBT/vPvB" if none of the relevant constituents individually is PBT or vPvB. This does not mean that all known constituents need to be tested but step-wise assessment and testing is crucial for focussing on the known constituents which represent the worst case in relation to the PBT/vPvB properties among all constituents of the substance.

In the opposite situation, if at least one of the relevant constituents meets the combination of P, B and T or vP and vB screening criteria, the assessment needs to progress to testing of those individual constituents following the normal P-, B- and then T-testing strategy. If one or more of the constituents are proven to fulfil either the vPvB or PBT criteria, the entire

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[70] Presentation by Magaud H *et al.* at SETAC Europe 25th Annual Meeting (3-7 May 2015 - Barcelona, Spain): Abstract 311 available at: https://www.setac.org/resource/setac-eu-barcelona-abstract-book.html).

(registered) substance must be concluded as "The substance fulfils the PBT and/or vPvB criteria" and the (group(s) of) constituent(s) causing this conclusion must be specified in the dossier.

This approach has been applied, e.g., in the SVHC identification of substances originating from coal tar distillation (e.g., coal tar pitch, high temperature; anthracene oil). It was also applied e.g. for phenol, styrenated (EC 262-975-0) under Substance evaluation.

Advantages of the known constituents-approach are, *i.a.*:

- Actual tests are performed on a pure (i.e. mono-constituent) discrete organic substance, and are easy to perform and interpret;
- In addition to being the preferred option, this approach may be the most efficient option in cases where substances contain constituents with diverse properties;
- It may in some cases require less effort to characterise the composition of the substance than the fraction profiling approach described below;
- The specific constituents may in some cases already be known for their properties and hence assessment effort can be reduced.

Disadvantages of the known constituents -approach are, *i.a.*:

- In many situations requires greater analytical ability to characterise the composition of the substance at the start of the PBT/vPvB assessment than the "whole substance -approach" described below;
- May require synthesis or other type of generation of specific constituent(s) for testing, if not otherwise available (e.g., from commercial providers of laboratory grade standards). Isolation or synthesis of relevant constituent(s) may not be technically feasible;.
- May require more than one test for each P, B, T –endpoint. This might raise testing costs and needs for vertebrate testing;
- Requires justification that any representative constituent chosen for testing is a reasonable worst case.

"Fraction profiling" (or "block profiling") approach

This approach is applied when, due to the complexity of the substance, it is not feasible to fully identify, assess or isolate single constituents but the substance can be divided into fractions/blocks, in which the constituents are structurally similar or in which the constituents are to such extent similar that their degradation, bioaccumulation and toxicity properties can be predicted to follow a regular predictable pattern(e.g., C14 chlorinated n-alkane with a chlorine content of 50-52 % by weight[71]). A prerequisite for application of this approach is that the PBT/vPvB-properties are assumed to be the same in the fraction (in this case the fraction should behave with regard to the PBT/vPvB-concern as if it were a single constituent or in a

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[71] See for example this decision on substance evaluation: https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1807e3841

predictable manner relative to the single constituents) or to follow a regular – predictable - pattern. The assessment report should justify why the constituents in the blocks can be considered to be sufficiently similar for the purpose of the PBT/vPvB assessment. For the purpose of testing, an actual physical fractionation or separate manufacturing of a fraction of the substance may be carried out to derive appropriate test substance(s) (for more details, see the subsection R.11.4.2.2.4 below).

A useful way to approach and document the assessment of the different fractions is via a matrix of the different blocks vs. P, B and T properties.

Two possible variations of this approach are described below:

i) The substance is conceptually divided into fractions containing similar constituents based on structural fragments and/ or other relevant molecular descriptors. The fraction itself is the main target of the testing and assessment, not individual (or surrogate) constituents therein, as is the case in the method described below in (ii).

ii) This approach can be applied in particular to complex UVCBs, however, application to other UVCBs or large impurity fractions of well-defined substances may also in some cases be appropriate. This approach has been used in the PBT assessment of, e.g. EC no 293-728-5 under the previous legislation and is applied in several ongoing PBT/vPvB assessments of the MSCAs (e.g., "tetrabutane", EC 292-461-1; medium chain chlorinated paraffins, EC 287-477-0).

iii) One example of this approach is where the substance is conceptually divided into fractions containing constituents having the same degradation behaviour (e.g. based on ready biodegradation tests). For these fractions the P assessment is clarified. The fractions identified as potentially P/vP may then be divided further into fractions containing similar constituents and assessed and tested in the same way as above.

iv) The so-called **block method**: this method is applied when a substance can be divided conceptually into fractions containing constituents which are very similar with regard to the properties to be assessed. Within a fraction read-across criteria can be applied among the constituents. For each of the fractions one or more representative constituent(s) is/are chosen for which testing and assessment is carried out. The constituent can be selected based on several considerations, e.g. that it can be easily retrieved for testing, there are already data on that constituent available or that it represents the worst case PBT-properties of the fraction (in case the constituents in the fraction are expected to exhibit a pattern of P, B, and/or T -properties within the boundaries of read across).

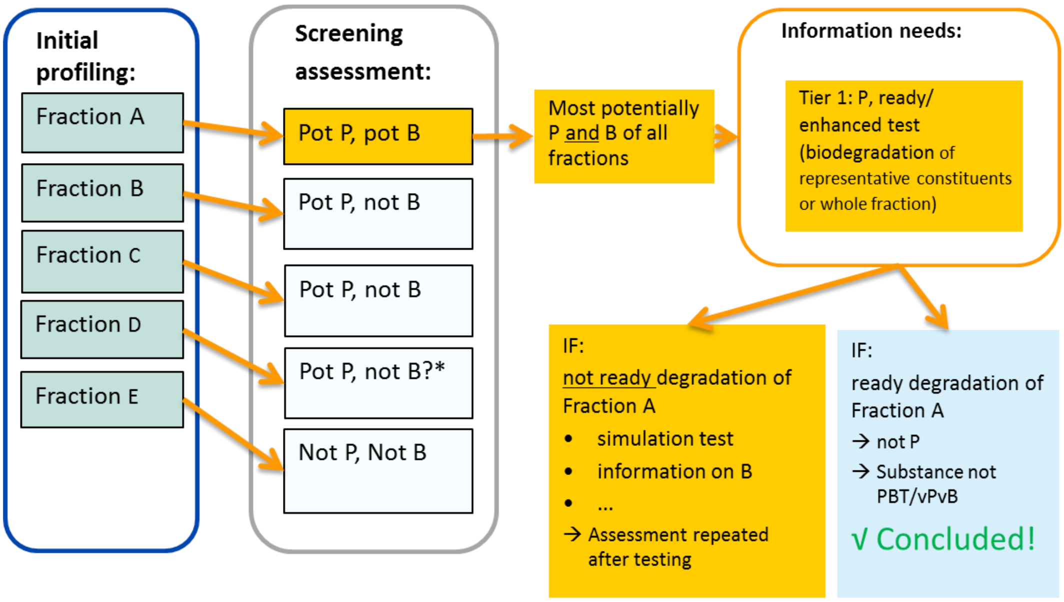
In all these variations of the "fraction profiling approach" fractions present at ≥0.1% w/w concentration in the UVCB are normally considered as relevant.

Advantages of the "fraction profiling approach" are, *i.a.*:

- More targeted and refined assessment compared to the "whole substance approach"
- Assessment of a complex substance fraction-wise allows efficient targeting of testing;
- May be the only practical option for some very complex UVCBs;

- Provides a refinement option if the "known constituents approach" is not feasible.
- Disadvantages of the "fraction profiling approach" are, *i.a.*:
- May require in some cases greater analytical effort to characterise the substance composition at start of PBT assessment than the "whole substance approach";
- May requires synthesis or other type of generation of specific substance/test item for testing, if not otherwise available (e.g. raw material may in some situations be used as representative of a fraction which consists of unreacted raw material);
- May require more than one test for each P, B, T endpoint. This might increase needs for vertebrate testing.

Requires demonstration that any test item chosen for testing is a reasonable worst case. Figure R.11—7 below shows an anonymised example of the first assessment tier of a UVCB substance for which fraction profiling has been applied.



* If screening assessment leaves uncertainties, these need to be addressed. Either utilise the new data on Fraction A to conclude (pattern finding, fragment read across, etc.) or test fraction D, if needed

Figure R.11—7: Example of the first assessment tier of a UVCB substance for which fraction profiling has been applied

Whole substance approach

The substance is considered to be one chemical substance for the purpose of the assessment and testing. This is possible, if all the constituents therein can be justified to be very similar with regard to the PBT-properties relevant for the assessment based on information on, e.g. manufacturing method, raw materials and/or chemical composition/analyses.

Due to the disadvantages and limitations, the application of the "whole substance" approach may only be possible in certain limited cases for the complete PBT/vPvB assessment of a substance. If one of the above mentioned approaches is feasible, these should be used instead of the 'whole substance' -approach as they are generally more transparent and regarded as providing information of higher certainty. For certain tests and for certain endpoint-specific assessments it may be possible to address the substance as a whole despite some slight differences in the properties of the constituents. For example, if it is known or can be reasonably assumed (e.g. based on the known chemical composition and/or relevant description of raw materials and production process but in addition also relative to the known or likely chemical identity of constituents) that (all) the constituents are structurally similar and therefore can be expected to have a reasonably similar PBT-properties, using the whole substance as test item may be considered – especially if such an analysis can be supported by non-testing or experimental data.

In cases where "not PBT/vPvB" is concluded based on results from tests with the whole substance, there should be a clear case made in the assessment for why all constituents are structurally sufficiently similar and hence also similar with regard to the PBT properties to justify such a conclusion. Same principles in establishing similarity of constituents apply for monoconstituent, multiconstituent and UVCB substances. For such similarity criteria, please refer to Chapter R.6 of the *Guidance on IR&CSA, Read-Across Assessment Framework (RAAF) and* Advice on using read-across for UVCB substances.

The "whole substance approach" is often applied by the registrants. It has been observed that the use of this approach should be better justified in the CSRs. Such justification and rationale supporting similar P, B and T properties between constituents may include for example analytical data, QSAR data, physical-chemical information, bioavailability considerations, mode of action etc..

Advantages of the "whole substance approach" are, *i.a.*:

- The registered substance itself is used for testing and thus there is no need for generation of new material;
- It may be the only option if it is technically not feasible within reasonable efforts to establish the exact identity of the constituents in the registered substance to the level needed;
- In some cases the analytical requirements for whole substance identification may be simpler than for identification of individual constituents.

Some disadvantages and considerations of situations where the "whole substance approach" should not be applied are described below:

- Conclusion provides a single profile for the whole substance. This may be too inaccurate in some cases. Test results may not be representative of all constituents: Possible risk of miss-screening, for instance using a single log K_{ow} value to represent a range of constituents or assuming ready biodegradability for a UVCB, where constituents are not sufficiently similar in reality.
- Some tests using the whole substance as test item may not produce reliable results (e.g. if physico-chemical properties of the constituents vary significantly, the exposure

concentrations cannot in some cases be maintained in such way that the test would be considered valid according to the test guideline);

- Available whole substance test data may not be relevant and/or may be unreliable and/or be difficult to interpret (either due to differences of physico-chemical properties between constituents or because the composition may be partly unknown/uncertain /vary, and hence data may not be shown to be representative enough for the registered substance);
- May trigger the need for the water accommodated fraction (WAF) approach for ecotoxicity testing (see discussion in Section R.11.4.1.3).

Combination of more/several approaches described above

It may be most efficient with regard to resources and time needed to combine several approaches in the assessment of one substance. E.g., for a complex UVCB it may be necessary to carry out an assessment of certain known constituents always present in the substance, but also to carry out a profiling fraction-wise for the remaining parts of the composition of the substance, if the remaining parts are anticipated to be so different from the known constituents that they may make a difference for the assessment conclusion.

CONCAWE (2001) has used an approach which combines information from tests where the whole substance has been tested and information from tests utilising the block approach. This approach is presented in Appendix R.11—2.

Different approaches may also be applied at different stages of the assessment, e.g. if information and knowledge on the substance increases during the assessment.

A particular example is that for bioaccumulation, simultaneous testing at low concentration of several constituents each below its water solubility and sampling and analysis of their concentration in water and in the organism (fish), if technically feasible, may be a cost efficient testing option. The approach may also be applied in the dietary bioaccumulation study. It may be employed on separate fractions or blocks – or in some cases even on the whole substance. A prerequisite for obtaining reliable results is that the co-occurrence of each constituent does not interfere with the bioaccumulation behaviour of other constituents also being tested (e.g. through enzyme induction, etc.)

Choice of the assessment approach

Finally, taking all the above into account including the substance composition and technical feasibility and suitability of the presented approaches for a particular substance the most appropriate approach for the assessment should be chosen. The choice of the assessment approach may be dependent on the data already available. In any case, results from relevant studies carried out by using the whole (registered) substance as test item should always be included into the dataset, where these are already available, regardless of the assessment approach chosen. Such results may in some cases support profiling of the substance, even in such cases where the “whole substance approach” will not be chosen as the main assessment approach for the case. Additionally, readily available test results on individual constituents need to be taken into account in the assessment even if the “whole substance approach” is applied. In such cases the results on individual constituents need to support the choice of the "whole substance approach". If they do not support the use of the "whole substance approach", another approach would need to be considered.

R.11.4.2.2.3 Specific aspects

The PBT/vPvB properties of a substance are evaluated in a stepwise approach, starting with P-assessment. Depending on the assessment approach chosen, the PBT/vPvB assessment generally focusses on individual constituents or groups of constituents/fractions (see Section R.11.4.2.2.2).

When assessing P, B and T it is important to understand that there is a difference in testing and interpretation of the data, that relates to the concentration of the test substance and that this has consequences for the assessment of substances containing various constituents. For degradation (hence persistence) and bioaccumulation, the concentration of the substance in the test vessel is not included within the measure of the endpoint (Mackay *et al.*, 2001). This is not the case for toxicity which is expressed in terms of concentration. The impact this has when assessing P, B and T is discussed under each of the endpoints below.

When evaluating P, B and T -related studies it is important to pay attention to the available physico-chemical data and its representativeness. For example, a water solubility or K_{ow} test carried out with the whole substance where whole substance–related analytics has been followed does not give information on the specific water solubility or K_{ow} of individual constituents, in case these have different properties (due to structural differences). Therefore, the basic physico-chemical data may also need to be generated for the constituents or constituent fractions depending on the assessment approach chosen, before other results can be evaluated or further testing decided.

QSARs-profiling, where applicable, is often crucial for the screening assessment of the potential properties of expected constituents and hence for the search for the worst case fractions/constituents which can be targeted for further assessment and testing. QSAR results of P, B, T and relevant physico-chemical properties of the expected constituents or representatives of fractions often have important role in justifying selected assessment approach and test items. It should be remembered that individual QSAR-model predictions are not normally able to accommodate the multi-constituent nature of a substance, but they represent the results for a particular chemical structure (i.e, for one selected constituent at a time). Otherwise, for the use of QSARs in the assessment of constituents the same principles apply as for the use of QSARs in the assessment of pure (i.e. mono-constituent) chemical substances.

The following specific considerations on data interpretation take as prerequisite that there is differentiation between the test item and the registered substance (of course, in the whole substance-approach these are the one and same).

Where new data are generated for a fraction profiling or known constituent-approach, it should be kept in mind that the most persistent constituent may not be the most bioaccumulative or toxic – and *vice versa*.

i) Persistence

One cannot easily assess the persistence of complex substances that contain many constituents using biodegradation testing methods that measure parameters (e.g. CO_2

evolution), since these tests measure the properties of the whole substance but do not provide information on the individual constituents.

If the selected test item consists of sufficiently similar structures and is shown to meet the stringent ultimate ready biodegradation test criterion (>60% in 28 days), it can be concluded that the underlying constituents comprising the complex substances are not expected to be persistent (OECD, 2001).

If the test item composition does not consist of similar structures or is not well characterised, it may still contain a certain amount of constituents that are persistent although the amount of easily degradable constituents is high enough to lead to an overall degradation percentage sufficient to meet the criteria for ready biodegradation.

ii) Potential for Bioaccumulation

Similar difficulties apply to bioaccumulation assessment.

Estimates for the individual constituents based on K_{ow}, QSARs or other methods may be used. Also multi-component measuring techniques such as SPME or HPLC could be useful to give an initial estimate of bioaccumulation potential. For example, if all the peaks in the HPLC chromatogram have a log K_{ow} <4.5, it may be assumed that all constituents of the substance have log K_{ow} < 4.5. For interpretation of such results and estimates, please see Section R.11.4.1.2.

iii) Toxicity

Extrapolating toxicity data from the whole substance to its constituents is often challenging and may lead to under- or overestimation of the toxicity of an individual constituent. Toxicity is defined via a concentration response and is dependent on the bioavailability. If the tested substance contains many constituents having differences in the response and bioavailability, this makes the interpretation very difficult. For example, the physical form may prevent the dissolution of the individual constituents of such a substance to any significant extent where the whole substance is applied directly, as required in normal ecotoxicity test guidelines, to the test medium. The apparent exposure concentration(s)/dose(s) in the test system may lead to incorrect interpretation on toxicity of individual constituents. Therefore, care should be taken to interpret the observed (lack of) effect(s) in relation to actual exposure concentration(s)/dose(s) of individual constituents.

In the event that a constituent/fraction is concluded as P and subsequently as B, then a toxicity assessment must be performed on that same constituent/fraction for which P and B are also evaluated.

Before starting toxicity testing on relevant constituents/fractions of the substance, all available information, including results of testing with whole substance, should be gathered and assessed if either T or not T conclusion can be reached in *Weight-of-evidence* approach for the relevant constituents/fractions. *Weight-of-evidence* to support the assessment may include assessment of all available *in vivo* and *in vitro* (e.g. mutagenicity) information on the substance and individual constituent(s), similarity of the toxic effect among constituents, physical-chemical properties, similar substances including read-across, other substances containing the same constituent(s), structural alerts for (eco)toxicity, mode of action and QSAR.

When (eco)toxicity information is available for a whole substance containing multiple constituents (for instance via WAF for aquatic toxicity, see Section R.11.4.1.3.5), the results may not be adequate for the PBT-assessment of individual constituents/fraction. During whole substance testing, for example the solubility and bioavailability of the constituent/fraction of interest may have been influenced by other constituents in the substance and the bioavailable composition may vary over time during the exposure period, resulting in additional interpretational uncertainty. In cases where a whole substance approach for (eco)toxicity is justified, whole substance data can be directly compared to the T-criteria, and can be used to derive conclusions on the T properties for PBT-assessment purposes.

(Eco)toxicity data for a whole susbtance can be used when it can be determined with sufficient certainty which constituent(s)/fraction(s) (and to which extent) contribute to the observed toxicity. Assessment must be accompanied with adequate justification to link both the presence or absence of the toxicity of the whole substance to the constituent/fraction. Information on whole substance for concluding T of constituents has been used for example for EC 401-850-9[72].

If no definitive conclusion for the constituent/fraction can be drawn, from the available information on the whole substance or other available information, further constituent specific experimental information may be needed. The generation of new relevant information should be started whenever possible by testing with non-vertebrate organisms. Generation of new information on vertebrate toxicity for the constituent must be avoided, if possible.

If there is a need for new experimental information, it is recommended to reflect if the aquatic toxicity is relevant to be tested first taking into account indications for low aquatic toxicity or concern for relevant human health endpoints. For aquatic toxicity testing the procedures described in Section R.11.4.1.3 can be followed. As described in the ITS for aquatic toxicity, vertebrate animal testing should be generally minimised by first testing non-vertebrate species, if relevant.

To avoid unnecessary animal testing, no further constituent specific information on mammalian toxicity is needed for the PBT assessment if the constituent is concluded vPvB or if the constituent is concluded T based on information on ecotoxicity or if based on case-by-case *Weight-of-Evidence* assessment as described above the conclusion on T can be reached. If based on the *Weight-of-Evidence* assessment a conclusion on T is still not possible to reach for a constituent fulfilling P and B criteria, the need of generating new information on vertebrate toxicity for that constituent must be carefully considered.

In case the whole substance is classified as carcinogenic (1A or 1B), mutagenic (1A or 1B), toxic to reproduction (1A, 1B or 2) or STOT RE (1 or 2) but the contribution of the PB-constituent on the classification cannot be confirmed in the *Weight-of-Evidence* assessment, the Registrant could also consider treating the substance as if it is a PBT/vPvB substance in accordance with the conditions set out in Annex XIII, section 2.1.

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[72] SVHC Support document – EC 410-850-9

R.11.4.2.2.4 Test items

If new testing is considered necessary, the set of tests, test sequence and test item(s) should be determined so that the results serve in the most efficient way the assessment with the chosen approach.

The test items are allowed to deviate from the registered UVCB substance, if that is justified by the selected assessment approach. It should be noted, that the test item(s) may itself/themselves be UVCB(s), well-defined multi-constituent substance(s) or mono-constituent substance(s), depending on the case and purpose.

The choice of the test item(s) is always dependent on the type of the substance but also on the case-specific understanding of which testing strategy is most efficient to conclude on the PBT/vPvB properties. Furthermore, feasibility and proportionality of efforts may also play a role in selecting the test item. It may in some cases be necessary to run a test on a particular property, e.g., simulation degradation test, for several test items, where one or more test items per fraction are used in parallel or in sequential tests.

In the "known constituent–approach" the test item consists of a single chemical structure. It can be extracted from the substance itself or be a separately synthesised as surrogate for a constituent (a similar chemical substance to the constituent). In block method the test item(s) per block targeted for testing and assessment may consist of one or more substances which are present as constituent(s) in the block or surrogate substances. Test item of a block may also be the whole block or similar multi-constituent substance. In the other fraction profiling approaches, the test item is either the whole fraction itself or a fraction of the fraction hence always consisting of multiple constituents. In that case also, the test item can be extracted from the substance or be separately synthesised. Similarly, also in fraction profiling, the test item may be a representative multi-constituent substance/mixture, if no extraction or synthesis of the target fraction of the registered substance is feasible.

Justification of test item selection should also be documented in the CSR or authority's assessment report.

The choice of the assessment approach and the test item may in some cases also affect the selection of the test method. For instance an aqueous BCF study can only in practice be performed with a substance where exposure concentration of constituents can be verified by measurements. Any uncertainty due differences in constituent properties of a test item (e.g., such as increased leaching of test substance from food pellets due to variation in physchem properties) need to be considered when interpreting the results. For this purpose a GC-characterisation of the test substance in the test system and/or in different test system matrixes before, during and after the test has been conducted might be useful.

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Appendices

Appendix R.11-1.1:	Development of a log Kow cut-off value for the B-criterion in the PBT-assessment
Appendix R.11-1.2:	Graphic definitions for the molecular dimensions
Appendix R.11-2:	PBT assessment of UVCB petroleum substances
Appendix R.11-3:	Bioconcentration studies with benthic and terrestrial invertebrate species (BSAF)
Appendix R.11-4:	Non-extractable residues (NER)
Appendix R.11-5:	Comparison of HPLC and KOWWIN v1.68 QSAR generated log Kow values
Appendix R.11-6:	Relevance and scientific justification of correction for growth dilution when deriving BCF
Appendix R.11-7:	Volatilisation correction approaches for the kinetic analysis of simulation studies

Appendix R.11—1.1: Development of a log K_{ow} cut-off value for the B-criterion in the PBT-assessment (developed based on fish BCF)

The following assessment was based on the same data set used for development of the $D_{max\ ave}$ indicators (Dimitrov *et al.*, 2005). Since publication the data set has been extended by Dimitrov, S. This was the dataset used for this exercise. With respect to the database used for the development of the cut-off value it is important to realize that the database comprises two data sets obtained from ExxonMobil and MITI. A quality assessment was made of the MITI data (as described in Dimitrov *et al.* 2005) and consequently the assessed data does not contain all the MITI data and may contain values that may not be considered as reliable. The experimental data from ExxonMobil are generated from fish-feeding studies, but only cover substances with Log K_{ow} values of < 7. For these reasons, it is recommended that this indicator be re-evaluated.

The fitted lines in Figure R.11—8, Figure R.11—9 and Figure R.11—10 are based on subsets of the BCF-dataset and are use to illustrate a limited bioconcentration potential for substances with high K_{ow}-values. However, they are not to be used as a QSAR to estimate BCF from Log K_{ow} (see Section R.7.10 in *Chapter R.7c* of the *Guidance on IR&CSA*).

For substances with a Log K_{ow} higher than 9.3 (based on CLogP) it was estimated that the maximum BCF value is equal to 2000. The 95% confidence interval for this exercise is 9.5 (Figure R.11—8).

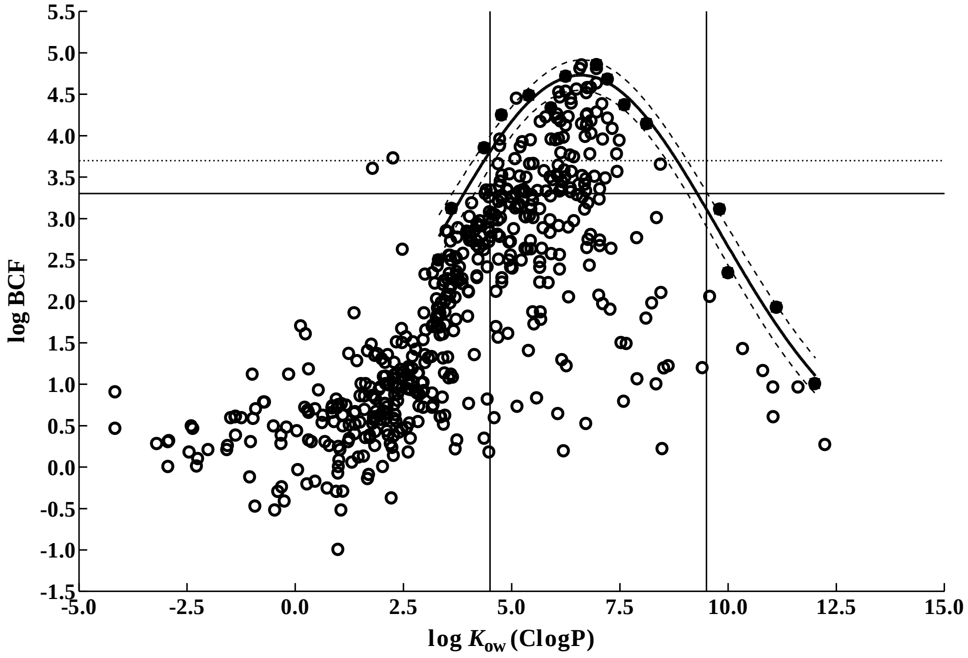

Figure R.11—8: Log BCF v calculated Log K_{ow}.

Figure R.11—9 plots the available BCF data against measured Log K_{ow} values. No experimental were available above Log K_{ow} of 8.5 apart from estimates by HPLC. This supports the belief that this is the limit of current state-of-the-art techniques for the determination of Log K_{ow} (i.e. slow-stirring and column elution).

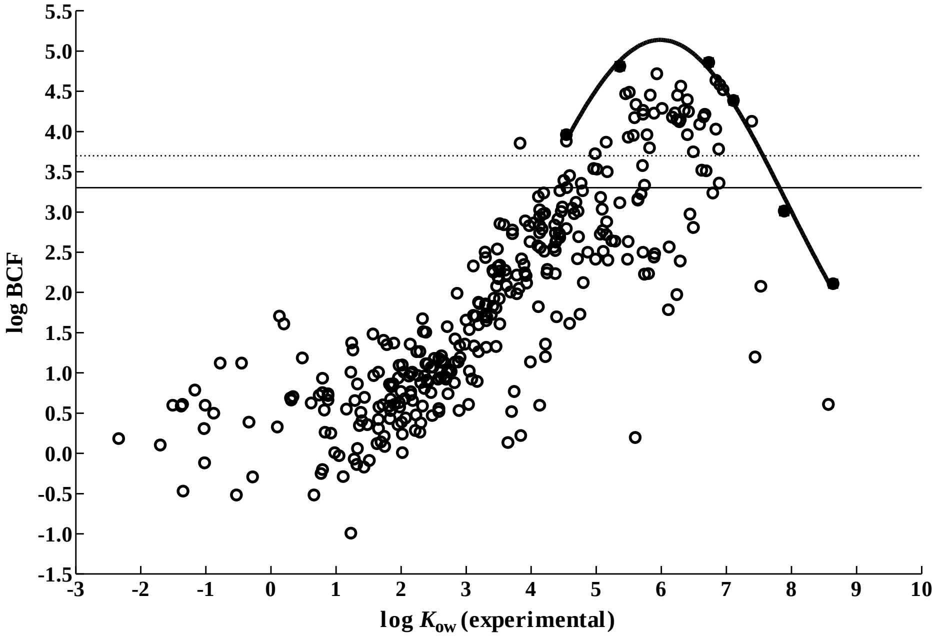

Figure R.11—9: LogBCF v measured log K_{ow}.

The relevance and experimental difficulties of conducting aqueous exposure on substances with very high Log K_{ow} must be questioned. Therefore it was decided to repeat the calculation with the BCFs from feeding experiments only (Figure R.11—10). The data for very hydrophobic substances are limited and there were 15 values for substances with calculated Log K_{ow} values above 7. None of these 15 reached the same level of BCF as the highest BCFs between Log K_{ow} values of 6.5 and 7.0 when compared to the parabolic relationship in Figure R.11—9. Of these 15, three substances had calculated Log K_{ow} values above 8, one is a vB substance and one is a B substance (very close to vB).

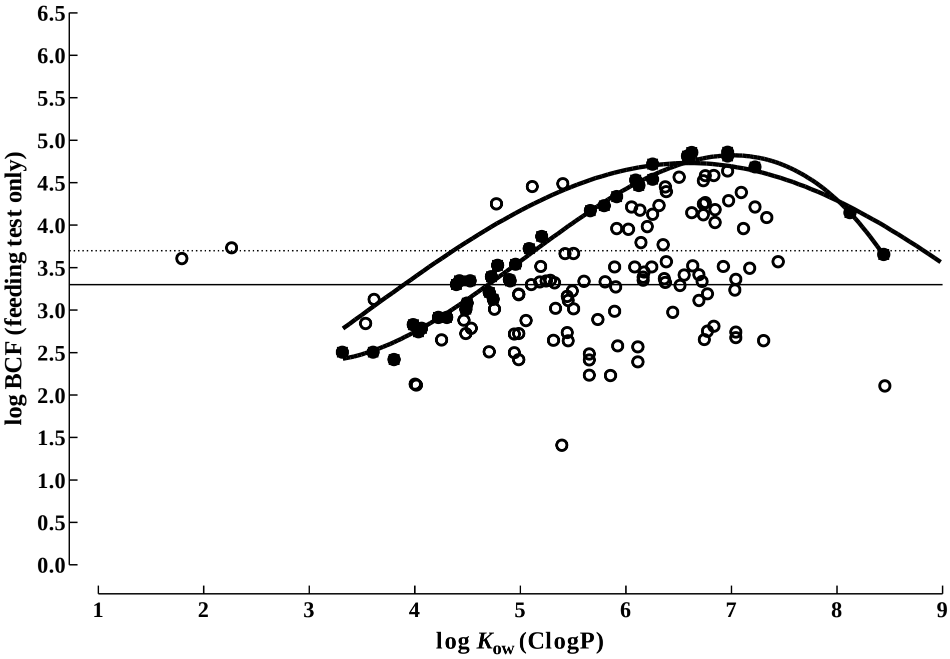

Figure R.11—10: LogBCF derived from feeding studies versus calculated Log K_{ow}.

Summarized, the results of Figure R.11—8 to Figure R.11—10 suggest that the B-criterion is unlikely to be triggered for substances with a Log K_{ow} higher than 10. As with the other indicators for hindered uptake, a Log K_{ow}-value higher than 10 should be used in a *Weight-of-Evidence* approach in combination with the other indicators.

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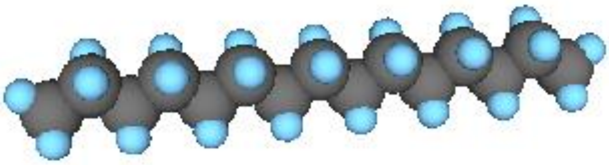
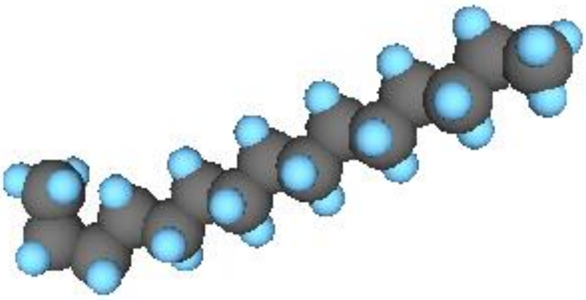
Appendix R.11—1.2 Graphic definitions for the molecular dimensions used in the main paper

- Maximum molecular length (MML) – the diameter of the smallest sphere into which the molecule would reside, as written, i.e. not accounting for conformers
- Maximum diameter, D_{max} – the diameter of the smallest sphere into which the molecule may be placed. Often this will be the same as the MML, especially for rigid molecules. However, when flexible molecules are assessed, energetically reasonable conformers could be present for which this is very different. The average value of D_{max} for "energetically stable" conformers is used, i.e. $D_{max\ ave}$.
- (Maximum) Cross-sectional diameter – the diameter of the smallest cylinder into which the molecule may be placed. Again different conformers will have different cross-sectional diameters.

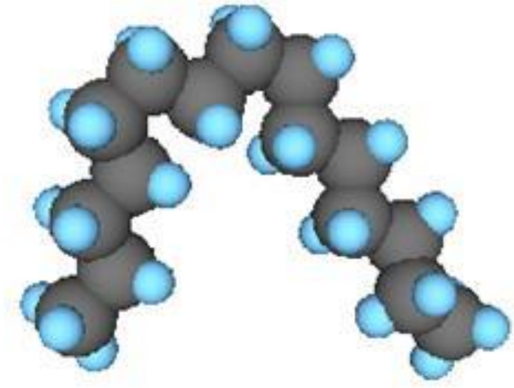
Conformer 1 (∆Ho = -84.5 kcal/mol), Dmax = 21.4; Deff = 4.99; Dmin = 4.92



Conformer 2 (∆Ho = -71.8 kcal/mol), Dmax = 19.8; Deff = 6.63; Dmin = 5.12



Conformer 3 (∆Ho = -68.5 kcal/mol), Dmax = 14.0; Deff = 11.5; Dmin = 5.52



Example Softwares:

- **OASIS**

To calculate $D_{max\ ave}$ conformational analysis of the molecule needs to be conducted. This is done by estimating D_{max} of each conformers and then the average Dmax values across the conformers. An OASIS software module is used to generate the energetically stable conformers representing conformational space of the molecules. The method is based on genetic algorithm (GA) generating a final number of structurally diverse conformers to best represent conformational space of the molecules (Mekenyan *et al.*, 1999 and 2005). For this purpose the algorithm minimizes 3D similarity among the generated conformers. The application of GA makes the problem computationally feasible even for large, flexible molecules, at the cost of non-deterministic character of the algorithm. In contrast to traditional GA, the fitness of a conformer is not quantified individually, but only in conjunction with the population it belongs to. The approach handles the following stereochemical and conformational degrees of freedom:

- rotation around acyclic single and double bonds,
- inversion of stereocenters,
- flip of free corners in saturated rings,
- reflection of pyramids on the junction of two or three saturated rings.

The latter two were introduced to encompass structural diversity of polycyclic structures. When strained conformers are obtained by any of the algorithms the possible violations of imposed geometric constraints are corrected with a strain-relief procedure (pseudo molecular mechanics; PMM) based on a truncated force field energy-like function, where the electrostatic terms are omitted (Ivanov *et al.*, 1994). Geometry optimization is further completed by quantum-chemical methods. MOPAC 93 (Stewart, 1990 and 1993) is employed by making use of the AM1 Hamiltonian. Next, the conformers are screened to eliminate those, whose heat of formation, DHfo, is greater from the DHfo associated with the conformer with absolute energy minimum by user defined threshold - to be within the range of 20 kcal/Mol (or 15 kcal/mol) threshold from the low(est) energy conformers (Wiese and Brooks, 1994). Subsequently, conformational degeneracy, due to molecular symmetry and geometry convergence is detected within a user defined torsion angle resolution.

A freely available version of this Dmax calculation is available in the OECD QSAR Toolbox under 3D parameters (Dimitrov *et al*, 2002; Mekenyan *et al*, 2005).

- **Calculation of the 3D Dimension of a Molecule**

A molecular modelling program, e.g. Molecular Modelling Pro, uses a 2D molecular structure as a starting point for the calculation. In the 1st step the program calculates the least strained 3D conformer using e.g. MOLY Minimizer as built in the Molecular Modelling Pro. Normally this minimizing of strain requires multiple steps. If the strain energy is minimized the program calculates the 2nd step the 3D molecular dimensions (x length, y width, z depth) e.g. in Angstrom. Based on these x,y,z dimensions Molecular Modelling Pro is able to calculate a global maximum and minimum which can be used a Dmax. Another example of a software which may be used to estimate D_{max} is Marvin Sketch.

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Appendix R.11—2: PBT assessment of UVCB petroleum substances.

UVCB petroleum substances are assessed using the same principles as other UVCBs, as introduced in Section R.11.4.2.2. However, at the time of developing PBT assessment principles for UVCBs the available knowledge on the composition and behaviour of petroleum substances was broader than the knowledge available on other types of UVCBs, thereby warranting the development of a specific methodology to assess petroleum substances. The following subsections introduce how such knowledge can be used. The specific assessment path presented is called the hydrocarbon block method, developed by CONCAWE (2001). An analogous assessment path may be used for other UVCB categories, if appropriate.

Step 1: Characterisation of the petroleum substance

Due to their derivation from natural crude oils and the refining processes used in their production, petroleum substances are complex mixtures of hydrocarbons, often of variable composition. Many petroleum substances are produced in very high tonnages to a range of technical specifications, with the precise chemical composition of particular substances, rarely if ever characterized. Since these substances are typically separated on the basis of distillation, the technical specifications usually include a boiling range. These boiling ranges correlate with carbon number ranges, while the nature of the original crude oil and subsequence refinery processing influence the types of hydrocarbon structures present. The CAS name definitions established for the various petroleum substance streams generally reflect this, including final refinery process; boiling range; carbon number range and predominant hydrocarbon types present.

For most petroleum substances, the complexity of the chemical composition is such that it is beyond the capability of routine analytical methodology to obtain complete characterisation. Typical substances may consist of predominantly mixtures of straight and branched chain alkanes, single and multiple naphthenic ring structures (often with alkyl side chains), single and multiple aromatic ring structures (often with alkyl side chains). As the molecular weights of the constituent hydrocarbons increase, the number and complexity of possible structures (isomeric forms) increases exponentially.

For the purposes of a PBT assessment of petroleum substances, when required, it is suggested that an analytical approach using GCxGC is used when feasible. This method offers a high resolution that may also be helpful in being more precise as to the exact type of structures present, (Forbes *et al.*, 2006), in contrast to more generic methods based on Total Petroleum Hydrocarbon (e.g. TNRCC Method 1005, 2001). Still other methods could be used to characterize the composition of petroleum substances as the GCxGC method has the caveat that it can only be used for carbon numbers up to around C_{30}.

The outcome of this step should be a matrix of hydrocarbon blocks, containing the % contribution of the block to the petroleum substance. With GCxGC this characterisation will be extended to include broad descriptions of structures including alkanes, isoalkanes, naphthenics, aromatics, etc.

Step 2: Assessment

The next step is to collate the available information on persistence, bioaccumulation and toxicity of the petroleum substance(s) being assessed. Where this is done as part of a category, there will be need for a good justification, which could also include analytical characterisation of a category. The assessment of the data will follow similar lines as for any data examination, including the extent to which the petroleum substances were characterised or described, the type of protocol followed and the quality of the information obtained for the respective endpoints.

Persistence (P)

The first part of the P assessment would be to examine the available data, and in particular attempt to identify whether the data on the petroleum substance(s) under investigation can be considered representative for the whole composition. The principles as provided for applying the "whole substance" approach as specified in Section R.11.4.2.2 and elements as discussed in Section R.11.4.1.1 (Persistence) need to be considered. Where there is convincing evidence of ready biodegradation of the whole substance under these principles, it can be reasonably assumed that the individual components are unlikely to be persistent.

If there is insufficient evidence for ready biodegradation or the substance composition is not sufficiently homogenous (i.e. the known or assumed constituents are structurally too dissimilar) to interpret data on the whole substance, then the assessment should proceed to the next stage. This involves generating typical structures either from the chemical analysis conducted or from other sources of information relevant to the petroleum substances being assessed. For example, Redman *et al.* (2012, 2014) describe how a set of over 1500 structures are available for assessing hydrocarbon blocks of petroleum substances. The structures cover a wide range of hydrocarbon types including isoparaffinic, normal paraffinic, mono-naphthenic (1-ring cycloalkanes), di-naphthenic (2-ring cycloalkanes) and poly-naphthenic, mono-aromatic, di-aromatic and aromatic (3 to 6-ring cycloalkanes) classes and mixed aromatic/napthenic hydrocarbons. By correlating the predicted boiling point of these structures to the available analytical information, a series of blocks can be generated in which these structures are representative of the types potentially present in the petroleum substance.

The assessment can then proceed by evaluating available degradation half-life information on any known individual constituents, e.g. benzene, hexane, pristane etc. This information will in every case be insufficient for the assessment of petroleum UVCB substances due to the wide range of potential structures and the relatively limited information currently available on most of the individual structures that have normally not been tested, as they are rarely isolated or manufactured. Consequently, the information will need to be supplemented with data from predictive models.

For hydrocarbons, there are two QSAR models that could be considered for assessing environmental degradation half-lifes and a third that could be used for assessing potential transformation/degradation products:

- Howard *et al.* (2005) describe a model that predicts the degradation half-life of a hydrocarbon in the environment. The model is well described, including information on the test/training sets. In using the model it would be advisable to assess the training and tests sets to ensure suitable coverage of the structures being assessed. Some data used in BIOHCWIN are based partially on half-lifes obtained for single substances studied as multi-constituent substances, e.g. cycloalkanes, thus the predicted half-lifes can be affected by co-metabolism and therefore may overestimate the rate of degradation in such cases (see Section R 7.9.3.1 in *Chapter R.7c* of the *Guidance on IR&CSA*). BIOHCWIN half-lifes which are below the P/vP criteria should not be used to support a conclusion "not P/vP". Care must also be taken with branched substances, as it appears that their environmental half-lives may be underestimated by BIOHCWIN (Rorije *et al.*, 2012). This model is freely available in EPI Suite as BIOHCWIN (US EPA, 2012).

- Dimitrov *et al.* (2007) describe a model that combines CATABOL (Jaworska *et al.*, 2002) with assumptions of first order catabolic transformations. The training and test sets include information of petroleum substances as well as observed catabolic pathways compiled from various sources including public web sites such as EAWAG BBD (http://eawag-bbd.ethz.ch).
- Finally, for demonstrating that there are no concerns, caused by potential transformation/degradation products (the previous assessments are all addressing primary biodegradation), it is recommended that available information is collected and predictions made of relevant PBT properties of potential transformation/degradation products. CATABOL (successor CATALOGIC) is an example of integration of such an approach in a commercial modelling system (Jawoska *et al.*, 2002).

If these assessments indicate that there are structures or blocks that are of concern, the assessment can either proceed to the generation of new information as described in the main report, or conclude that the assessed blocks can be considered persistent and proceed to the bioaccumulation assessment.

Bioaccumulation (B)

The B assessment essentially follows the same process as that described for the P assessment except that it is highly unlikely that there will be good quality experimental data on petroleum UVCB substances. Instead the B assessment is more likely to address the individual structures for their potential to bioaccumulate. This, as with the P assessment, will start with addressing where there is available experimental evidence to be able to draw a conclusion on the B properties of blocks or individual constituents.

Where there are insufficient experimental data to be able to make a judgement there are several QSAR models available for continuing the process. These are discussed in Section R.7.10.3.2 of *Chapter R.7c* of the *Guidance on IR&CSA* and Appendix R.11—1.1 of this Guidance document. An assessment of the predictions from these QSAR models, with available experimental information should lead to the identification of those blocks where there are concerns for their potential (or realised, if specific structures are assessed) ability to bioconcentrate. The use of experimental fish or aquatic invertebrate bioaccumulation data from tests following standard test methods/guidelines is preferred over that from other sources, including invertebrates, because fish bioaccumulation data are generally more reliable as standard test methods/guidelines are used to determine them. Fish bioaccumulation data include the effect of biotransformation in fish which can be substantial for some hydrocarbons. Such data also provide indications of whether the potential for food-chain magnification at higher trophic levels exists. This type of data, with further information on trophic level biomagnification or dilution, can be used in a *Weight-of-Evidence* approach to demonstrate whether the longer term uncertainties associated with bioaccumulation of constituents may exist.

Toxicity (T)

Assessment of the toxicity of all individual constituents within a petroleum substance would in many cases be extremely difficult or practically impossible. While the whole substance assessment using the Water Accommodated Fraction (WAF) methodology has been accepted for classification purposes (OECD, 2001), the use of this information for the T assessment is problematic.

For petroleum substances, a model, PETROTOX, has been developed (Redman *et al.*, 2012), based on previous work assuming a non-polar narcosis mode of action (i.e. baseline toxicity, McGrath *et al.*, 2004, 2005). The equations underlying the hazard portion of this model, which was developed to predict the acute and chronic ecotoxicity of petroleum substances and hydrocarbon blocks, may be used to address the predicted baseline toxicity of individual structures when no experimental data are available.

It should be noted that for the ultimate conclusion on the T property, long-term toxicity test results are generally necessary as, at present, no appropriate prediction tools for long-term ecotoxicity are available. The prediction tools may, however, be used as supporting tools for designing tests and for the interpretation of experimental results. Before initiating experimental fish toxicity tests it should be considered whether data exist allowing a robust conclusion to be drawn on whether the substance fulfils the $T_{mammalian}$ criteria (see Section R.11.4.1.3).

How to proceed further

Where there are constituents or blocks that show a concern for both P and B properties, there is a need to generate further higher tier information on these properties. Exceptions to this conclusion might be in case there are sufficient ecotoxicological data on specific constituents or representative structures in the blocks that demonstrate no concern for the T criterion and where the P and B properties are concluded not to indicate vPvB-properties.

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Appendix R.11—3: Bioconcentration studies with benthic and terrestrial invertebrate species (BSAF).

In case data are available from bioconcentration studies on benthic and terrestrial invertebrate species they may be used as indicator for a high bioaccumulation potential. Results of these studies are expressed as biota-to soil/sediment accumulation factor (BSAF). In order to compare BSAF with BCF values care must be taken if a species with a very low lipid content was used because BCF values are normaly reported on a wet weight basis. Lipid normalisation (to 5% lipid content) should therefore always be performed, whenever possible for substance that are lipid binding.

The relationship between BSAF and BCF is expressed in the following equation, in which BCF could be replaced by the criterion for B or vB.

$$BSAF = \frac{BCF(lipid)}{K_{oc}} = \frac{2000 / 0.05}{K_{oc}} \text{ for indication of } B \text{ or } \frac{5000 / 0.05}{K_{oc}} \text{ for indication of } vB$$

A terrestrial or benthic (lipid and organic carbon normalized) BSAF value for a substance with a Log K_{ow} of 4.5 that exceeds the value of 2 is an indication of a BCF of 2000 and higher, based on pore water concentration (Figure R.11—11). Similar for a substance with a Log K_{ow} of 4.5 a BSAF value higher than 5 is an indication that the BCF exceeds the value of 5000, based on pore water concentration.

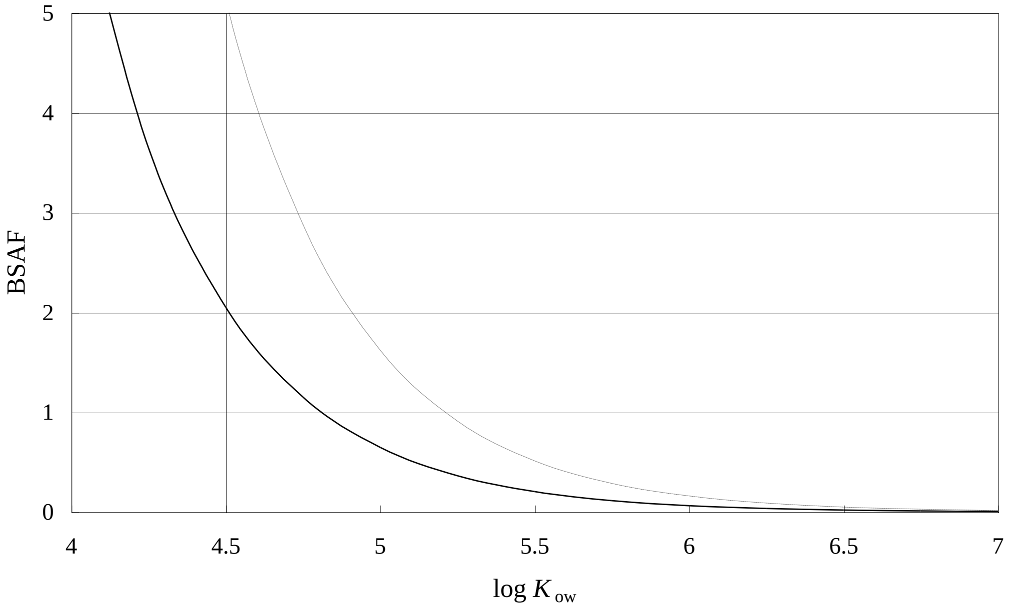

Figure R.11—11: Relationship between lipid and organic carbon normalised BSAF values and Log K_{ow} as indicator for the B and vB criterion.

The solid line is calculated with a BCF value (5% lipids) from pore water of 2000, the dotted line is calculated with a BCF value of 5000. The Log K_{oc} has been calculated according to the equation Log K_{oc} = Log K_{ow} - 0.21 by Karickhoff *et al.* (1979).

Due to increasing sorption with Log K_{ow}, the BSAF values for calculated BCF values of 2000 and 5000 rapidly decrease. Therefore, for a substance exceeding Log K_{ow} of 5.5, a BSAF value in the order of 0.5 and above indicates high bioaccumulation potential.

However, lower BSAF values are difficult to interpret in the context of the B and vB assessment due to several confounding factors. Sorption and bioconcentration increase with hydrophobicity, and as it is not necessarily in the same manner, sorption is an important parameter dependent on soil and substance properties. Bioconcentration might be reduced compared to what is expected from Log K_{ow} value but even low BSAF values of 0.1 and lower do not necessarily mean that the BCF value based on pore water concentration do not exceed 5000, because of the strongly increased sorption for highly hydrophobic substances. Moreover, sorption might be higher than what is expected from Log K_{ow} because sorption to carbonaceous materials may play an important role. Besides that, for these low BSAF values it is often difficult to distinguish between real uptake and adsorption to the organisms or interference of gut content in the determination of the BSAF values.

In conclusion, lipid and organic carbon normalized BSAF values of 0.5 and higher are an indication of high bioaccumulation. In some cases these values might be considered to be enough evidence in itself to assess the substance as B and vB, especially if reliable experimental data on pore water concentrations are available and the system is in equilibrium. However, lower BSAF values should not be used to the contrary, because low uptake from sediment or soil does not imply a low aquatic BCF value.

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Appendix R.11-4: Approach on non-extractable residues (NER) quantification and characterisation in persistence assessment

To complete the mass balance as a part of a simulation test with radiolabelled substances (^{14}C), quantification of the amount of total non-extractable residues (Total NER) is necessary. The assessment may be refined by characterising and quantifying the different NER types. This Appendix provides a stepwise approach for characterising and quantifying NER (based on Kästner *et al.* 2018). A tiered extraction scheme for quantification of Total NER (Step 1) and the characterisation of the different NER types (Step 2 and 3) is described below (Figure R. 11—12).

If any other method is used, the relevance and adequacy of the method needs to be demonstrated. Determination and characterisation of the Total NER with the methods described in this Appendix, rely on the use of radio-labelled substance. In the absence of any information on the nature/types of NER, the residues should be regarded as non-degraded parent substance.

Types of NER in environmental matrices (Löffler *et al.*, 2022, ECHA, 2019, Kästner *et al.*, 2018):

- **NER Type I (strongly sorbed and physically entrapped)**: strongly sorbed or physically entrapped into the matrix, contain the parent substance, transformation/degradation products or both. NER Type I have the potential to be remobilised, and should be considered for assessment of persistence. If chemical analyses are conducted, it may be possible to distinguish whether NER Type I consist of unmodified parent substance or of transformation/degradation products.

- **NER Type II (covalently bound)**: residues that are covalently bound to the matrix (e.g. to humic matter) in surface water, soils or sediments and that are considered to have low remobilisation rates. Unless there are indications from the available literature or monitoring data regarding their potential remobilisation, covalently bound residues may be regarded as irreversibly bound.

- **NER Type III (bioNER)**: incorporated into biomass (biogenic NER, also called bioNER), NER Type III result from the anabolic formation of biomolecules (amino acids, phospholipids, and other biomass compounds) from the degradation products of the parent substance. Dead biomass, and therefore biogenic NER, are eventually fixed in organic matter derived from decaying microbial biomass. NER Type III are considered to be of no concern.

The sum of NER Type I and II can be also referred as XenoNER. XenoNER can be roughly estimated as Total NER - NER Type III (bioNER).

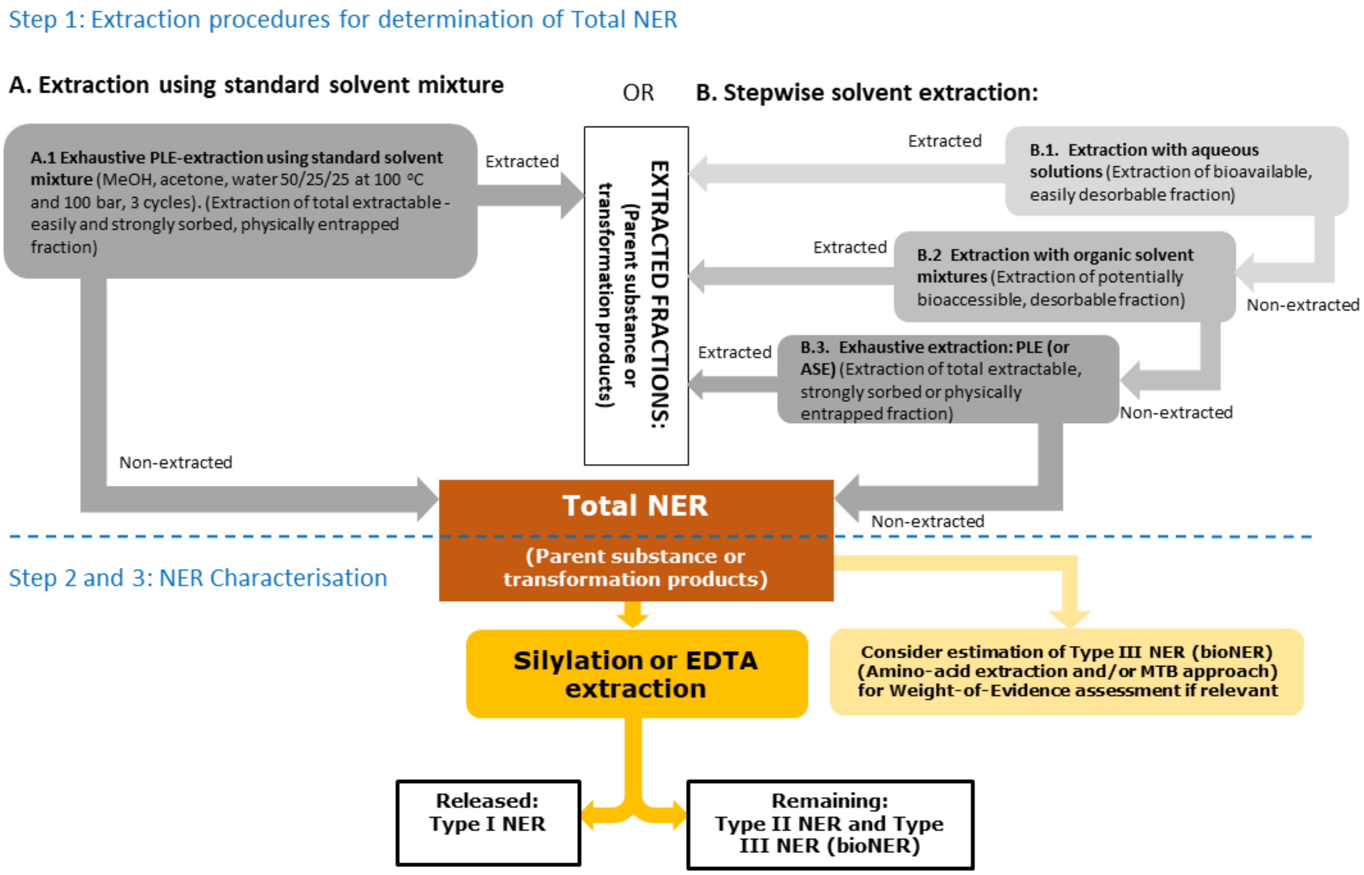

Figure R. 11—12: Approach for the quantification of the total extractable and non-extractable residues (NER) and the characterisation of the NER types in environmental matrices (water/soil/sediment) (adapted from Kästner *et al.* 2018).

ASE: Accelerated Solvent Extraction; PLE: Pressurised Liquid Extraction at elevated pressure and temperature (equivalent to ASE); MTB approach: Microbial Turnover to Biomass approach; EDTA: Ethylenediaminetetraacetic acid.

The Figure R. 11—12 provides an overview approach for the quantification of the total extractable and non-extractable residues (NER) and the characterisation of the NER types in environmental matrices. Detailed description of each step is provided in the sections below and in Figure R. 11—13, Figure R. 11—14 and Figure R. 11—15.

Quantification of NER: extraction methodologies (Step 1)

This step allows the distinction into the extractable fraction and the non-extractable residues, the latter representing the substance or its transformation/degradation products remaining in the matrix (soil, sediment, suspended matter). The extraction efficiency needs to be optimized for the test substance and/or its potential transformation/degradation products. An efficient extraction procedure for the test substance may not be equally efficient for its transformation/degradation products.

Total NER are defined as the residues remaining in the matrix after defined exhaustive extractions steps described below (one standardised extraction procedure with a ternary solvent mixture or three consecutive extraction steps). The amount of NER is operationally defined by the extraction method employed. Different extraction regimes (i.e. different solvents and different extraction conditions) are possible for the same substance.

The following extraction procedures refer to Step 1 (A or B) of Figure R. 11—12.

The selection of the most appropriate extraction strategy to define the amount of extractable residues in simulation test is case specific and it is affected by the substance itself and the test matrix. The stepwise extraction procedure below (Step 1: A.1, B.1, B.2 and B.3) describes a way to release all the extractable fractions. Each of these fractions can be used to analyse the amounts and to identify the structures of the extractable residues. The residues remaining in the matrix after those three steps are defined as Total NER.

The choice of extraction procedure depends on the applicability and efficiency of the extraction procedures. The extraction method should not destroy the parent substance and/or the transformation/degradation products as quantification and characterisation could be subsequently performed. Control experiments should be conducted to assess whether or not the parent substance and/or the transformation/degradation product(s) are stable under the extraction conditions (e.g. effects of high temperature). Selection of the most appropriate approach should be evaluated in a pre-study and justification provided.

A.1 The pressurized liquid extraction (PLE) (also called Accelerated Solvent Extraction (ASE)) with a standard solvent mixture (MeOH, acetone, water 50/25/25, v, v, v) at 100°C and 100 bar with 3 extraction cycles is one of the two recommended extraction procedures for determination of Total NER. Alternatively, the sample can also undergo a stepwise solvent extraction first (**B.1** and **B.2**) to be followed by PLE, both with a substance specific solvent (**B.3**).

The aqueous extraction (**B.1**) is intended to be a surrogate of the pore water in order to determine the 'easily extractable' fraction that is bioavailable for organisms living in the matrix, e.g. soil or sediment. For the aqueous extraction at every sampling day, the sample can be extracted by shaking for 24 hours with $CaCl_2$ solution (0.01M) (Houba *et al.*, 2000; Peijnenburg *et al.*, 2007; Beulke and van Beinum (2012)). Settling of the solids during shaking should be avoided. The soil (or sediment):solution ratio should be the same at every sampling time point. The solid and liquid phases should be separated by centrifugation and the concentration of parent substance and potential transformation product(s) in the liquid phase analysed. The liquid should be recovered from the sample as much as possible if consecutive aqueous and solvent extractions are performed on the same sample. Other solutions of salts like calcium or sodium nitrate, ammonium acetate or nitrate etc. have also been used for this purpose.

B.2 includes extraction with organic solvent mixtures which are intended to be a surrogate for the potentially bioaccessible[73] or desorbable fraction from the matrix). The remaining residues from step B.1 could be sequentially extracted at ambient temperature with carefully selected aqueous:organic solvent mixtures (e.g. 50:50 or 20:80 water:acetonitrile; v/v), which at times may be modified with minute amounts (0.1-2.5 % v/v) of formic acid, acetic acid and/or ammonia in order to enhance the solubility of the substance and/or its transformation/degradation product(s).

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[73] Bioaccessibility of residues in organisms encompasses what is directly bioavailable and what may potentially become bioavailable, for instance after release from a surrounding matrix or the matrix degradation.

Samples should be extracted for prolonged time periods (4 h – 24 h) using physical agitation, (e.g., an overhead shaker or a horizontal shaker) ensuring complete suspension of the matrice (e.g. soil, sediment) that is being investigated. Shorter extractions times could be appropriate and should be evaluated on a case by case basis. Ultra-sonication may enhance the extraction efficiency, but the temperature of the sample should then be monitored. During studies with radiolabelled substances, sequential extractions with the same solvent should be performed until <5 % of the radioactivity released from the first extraction is obtained. This usually occurs between three and five extractions with one solvent system (Nießner and Schäffer, 2017).

The selection of the proper organic solvents is a critical step. The physico-chemical properties of the analyte, i.e., its volatility, water solubility, the solubility in the organic solvent to be used, the pKa, and the stability, as well as the test matrix properties (such as the moisture and organic matter content of soils and sediments), must be considered (ECETOC, 2013). Properties of some extraction solvents and their relations to properties of analytes are provided in Kästner, *et al.*, 2018.

Pure organic solvents should be avoided in the first extraction steps because molecules distributed in the interlayers of clay particles in soil may be entrapped by shrinking of the clay when water is removed. Therefore, in the first extraction steps water miscible organic solvents should be used mixed with low volumes of water, followed by exhaustive extraction with the use of pure solvents (or solvent mixtures).

Since transformation/degradation product(s) usually differ from the parent substance in terms of polarity (most often more polar, sometimes less polar) and of chemical reactivity, extraction procedures have to be developed during the course of a degradation study. An effective extraction solvent for the parent substance may not be effective for transformation product(s). Polar solvents or solvent mixtures should be tested for extraction depending on the nature of the residues.

B.3 includes exhaustive extractions which are intended to be a surrogate for the slowly desorbable fraction. Compared to the extraction methods described in the previous steps (Steps B.1 and B.2), exhaustive extractions are conducted under harsher conditions (e.g. elevated temperature and/or elevated pressure). The exhaustive extraction will release part of the molecules strongly sorbed to the matrix and thus provide information on the total extractable fraction. Remaining residues after exhaustive extractions will define the total amount of NER.

The recommended method for the last exhaustive extraction step is Pressurised Liquid Extraction (PLE) also called Accelerated Solvent Extraction (ASE). If the efficiency of the PLE method is not satisfactory other harsh extraction procedures (i.e Soxhlet, Microwave Assisted Extraction or Supercritical fluid extraction) with solvent variations can be applied.

The results from Step 1 can be used for DegT50 derivation as shown in the Figure R. 11—13. The parent in total extractable fraction is considered first for deriving the DegT50 and is compared with the vP criterion. If the vP criterion is fulfilled no further characterisation of NER is needed. If the vP criterion is not fulfilled a DegT50 based on the sum of the total extractable fraction and the Total NER is derived and compared with the P/vP criterion. If the criterion is fulfilled the substance could be considered as P or vP or if the Total NER is ≥ 10 % quantification of the NER types should be considered to refine the assessment (Step 2).

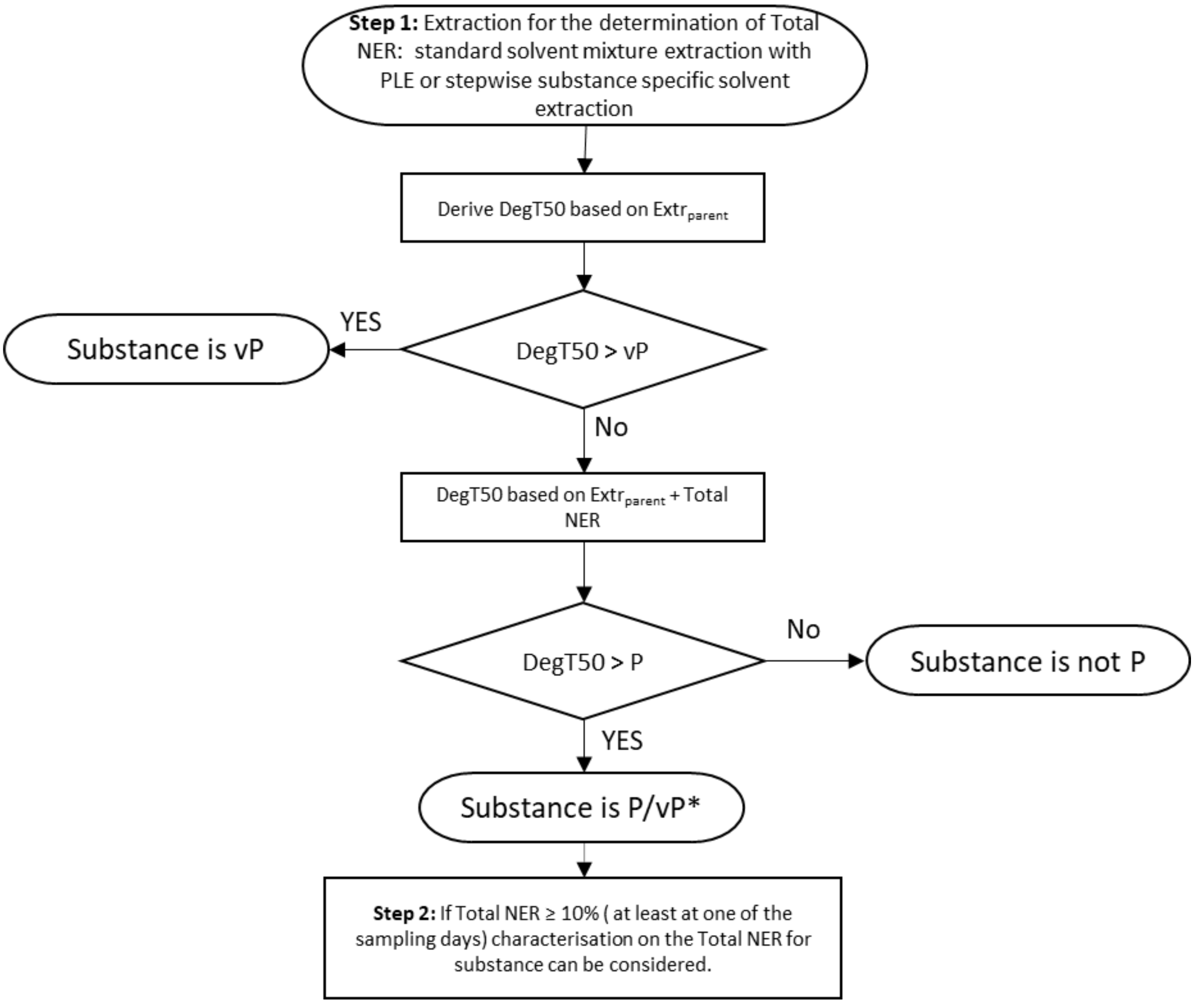

Figure R. 11—13: Assessment scheme for Step 1: Quantification of the total extractable and non-extractable residues (NER) in environmental matrices (water/soil/sediment).

* Conclusion on persistence in the absence of information on the amount of Type I in Total NER.

Characterisation of NER (Steps 2 and 3)

Step 2 describes the characterisation of the NER into potentially remobilisable fraction (NER type I) and NER remaining in the matrix (NER type II and bioNER/NER Type III) (based on Kästner *et al.* 2018, Löffler *et al.* 2020). Estimation on bioNER (Type III) may also be considered if relevant. Subsequently Step 3 allows the quantification of the parent substance in Type I NER for all sampling days/points.

The identification and quantification of the parent substance and transformation/degradation products should be carried out both in the extractable fraction and in the NER type I fraction (obtained by silylation/EDTA extraction) in all sampling days/points. If it is not possible to differentiate parent from transformation/degradation products, the total radioactivity measured in this fraction shall be considered as parent.

The differentiation of NER type I and II does not need to be performed when this information is not expected to change the conclusion for the persistence assessment, for example when based on all available information it is already possible to conclude.

Step 2: Determination of NER type I by silylation or EDTA extraction.

As stated above Step 2 allows the differentiation of the Total NER into potentially remobilisable fraction (NER type I) and NER remaining in the matrix (NER type II and bioNER/Type III). Characterisation of NER in all sampling days/points is considered relevant when the amount of Total NER is ≥10 % of the total applied radioactivity (AR) at least at one of the sampling days. When the total NER is <10 %, the possibility for further characterisation could still be explored as the technical feasibility of the characterisation might also depend on the test methodology (spiking radioactivity) and availability of analytical methods. Determination and characterisation of the Total NER described in this Appendix rely on the use of radiolabelled substances.

The potentially remobilisable fraction (NER type I) can be quantified by silylation or EDTA extraction of the extraction residues (i.e. sample containing Total NER). The extraction efficiency and the technical feasibility in relation to the test substance and transformation/degradation products should be considered when selecting the method, i.e. the extraction procedure shall not destroy or react with the test substance. This should also be checked in a preliminary study. NER remaining in the matrix (soil, sediment, suspended matter) after silylation/EDTA extraction have a low remobilisation potential, it does not need to be considered in the persistence assessment and can be seen as a removal pathway (Kästner *et al.*, 2018).

Step 2 (Figure R. 11—14) describes quantification and characterisation of the NERType I by Silylation or EDTA extraction of the matrix.

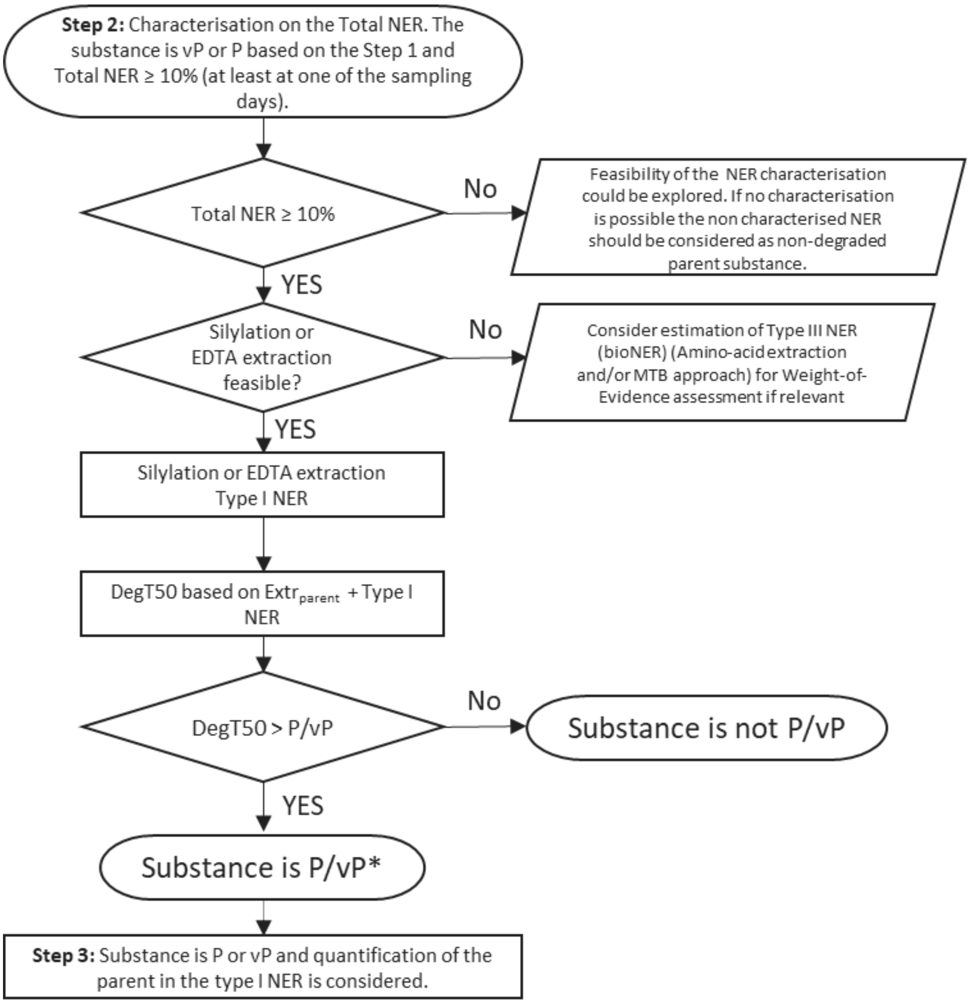

Figure R. 11—14: Assessment scheme for Step 2: Quantification and characterisation of Type I NER by Silylation or EDTA extraction of the matrix.

* Conclusion on persistence in the absence of information on the amount or parent and degradation product in NER I.

Silylation is the introduction of a substituted silyl group (R3Si-) to a molecule carrying functional groups with exchangeable protons. Silylation will replace the exchangeable hydrogen atoms of functional groups in soil organic matter - e.g. carboxylic, hydroxy or amino groups - with trimethylsilyl groups (Haider *et al.*, 2000). The majority of the NER entrapped in the humic matrix (type I NER) are released after silylation, while NER formed by covalent binding (type II NER) remain bound to the matrix. In addition, both fractions derived from this procedure may also contain bioNER. The released and the remaining fractions can be quantified when radioactively labelled chemicals have been used. However, it is recommended that subsequent analyses for released parent substances or transformation product(s) are performed in addition in order to quantify specifically type I NER, e.g. by mass spectrometric approaches. For additional structural information, size exclusion chromatography or spectroscopic methods like nuclear magnetic resonance (NMR) can be applied. Alternatively, stable isotope labelling (^{13}C, ^{15}N) can be applied with subsequent GC-MS or LC-MS analyses. Berns *et al.* (2005), Wang *et al.* (2017), Löffler *et al.* (2020) and Hennecke *et al.*, 2023 provide a detailed description of the silylation procedure.

In general, similarly to the extraction method mentioned above the characterisation methods should not chemically alter the parent substance or its transformation/degradation products.

Limitations of the silylation method (including following analytical methods):

- The silylation method has not been standardised.
- Silylation may also destroy the target molecules (parent substance and/or transformation product(s)) if it contains the functional groups modified by the silylation procedure. Therefore, appropriate control extraction treatments should always be run while performing the silylation.
- Both the released fraction and the remaining fraction derived from this procedure may also contain bioNER. Therefore in order to quantify specifically the amount of type I NER, it is necessary either to analyse for released parent substances or transformation product(s) e.g. by mass spectrometric approaches, or to quantify the amount of bioNER (see Step 2.2 below)
- Analyses of parent substance and transformation product(s) in the released fraction might be challenging in particular if there are many matrix components. By default, NER type I should be considered to be parent substance if the analysis cannot be performed.
- Structural identification of type I residues using stable isotope labelling for subsequent stable isotope NMR (13C, 15N) is otherwise possible but has the disadvantage that high concentrations of the test substance have to be used due to the low sensitivity of the NMR method. Therefore, for such experiments, control experiments analysing the degradation kinetics at low concentrations should be performed.

EDTA extraction destabilizes the soil matrix by complexing with the cations in the soil e.g. Ca^{2+}. The physically trapped substance is then subsequently released which is considered to be comprised of mainly type I NER. It is noted that EDTA extraction and silylation are releasing different substances/fractions as the release mechanism is different. Löffler et. al. (2020) provides a detailed description of the EDTA extraction procedure.

The results from Step 2 can then be used in DegT50 derivation. The total extractable parent fraction together with the Type I NER are considered for deriving the DegT50. If the criterion is fulfilled the substance could be considered as P or vP. In this case further refinement of the assessment could be performed by quantifying the parent substance concentration in the Type I NER (Step 3).

In case neither silylation nor EDTA extraction (Step 2) are feasible, normally the Total NER should be regarded as non-degraded parent substance and taken into account as such for DegT50 derivation. However, further investigation of the extent of the bioNER formation could be performed using the Microbial Turnover to Biomass (MTB) – model and/or the amino-acid extraction method. MTB could clarify whether additional experimental testing to estimate the amount of bioNER would be relevant. For example, if the amount of bioNER is predicted to be negligible, then extraction of amino acids, would likely not have additional value to the assessment. If the amount of bioNER is predicted to be significant this might indicate that a large fraction of the Total NER might be associated with the bioNER and thus extraction of amino acids could provide some additional information. The impact of the measured bioNER fraction to the DegT50 could then need to be investigated. In this case the DegT50 can be estimated on the basis of the total extractable fraction and the XenoNER (Total NER- NER Type III (bioNER)). The quantification of the bioNER using the extraction of amino acids method is highly uncertain and thus the results and the DegT50 estimated from such investigation can only be considered undet Weight-of-Evidence approach.

MTB - Microbial Turnover to Biomass

Microbial Turnover to Biomass (MTB) approach is an in silico screening tool and may be used for assessing whether type III (bioNER) is likely to be formed. It can clarify whether additional testing for the evaluation of bioNER is relevant.

The MTB approach is based on the carbon conversion to microbial biomass and estimates the microbial yield and bioNER formation potential of microbes growing on a specific carbon source (chemical). The estimation of bioNER formation with the MTB method is a two-steps process described in Trapp *et al.*, 2018 and Trapp *et al.*, 2022.

Limitations of the MTB approach:

- Predicted range of the amount of bioNER may deviate from the experimental values in e.g. following situations:
    - degradation is incomplete (transformation product(s) accumulate),
    - degradation is partially or fully anaerobic,
    - significant amount of C from the substance is stored within carbonates,
    - used inoculum does not contain microbes (enzymes) capable of efficient degradation,
    - substrate is toxic and inhibits the degradation, or
    - the model does not usually take into account the position of labelling. Depending on the labelling position, the measurement of mineralisation ($^{14}CO_2$ evolution) and of NER can differ significantly for the same substance. In particular, position of the ^{13}C- or ^{14}C label in carboxylic moieties or other highly oxidised positions of the parent substance should be avoided.

If both the MTB method and other information (e.g. evolved CO_2) indicate that bioNER may be formed and that the quantification of the bioNER may be relevant for the assessment, extraction of labelled amino acids as described below could be performed.

Extraction of amino acids

The amount of bioNER can be experimentally estimated by full hydrolysis (acid hydrolysis with 6 M HCl at 105 °C in closed glass vessels for 24 hours) of the matrix using isotope labelled substances with subsequent amino acid analysis. The amount of bioNER can be back-calculated from the amount of amino-acids extracted and identified (Kästner *et al.* 2018).

Both labelling with radioactive (^{14}C) (Possberg *et al.*, 2016) and stable isotopes (^{13}C, ^{15}N) (Kästner *et al.*, 2014; Nowak *et al.*, 2011; Wang *et al.*, 2016; Wang *et al.*, 2017.) have been applied to quantify the amounts of type III NER using the amino acids extraction method.

Limitations of the extraction of amino acids (including following analytical methods):

- The method to identify bioNER type III has not been standardised but rather represents a method derived from basic research.

- Structural identification of the amino-acids in the type III NER using stable isotope labelling for subsequent stable isotope NMR (^{13}C, ^{15}N) is possible but has the disadvantage that high concentrations of the test substance have to be used due to the low sensitivity of the NMR method.

Step 3: Quantification of parent on Type I NER.

Step 3 (Figure R. 11—15) allows the quantification of the parent substance in Type I NER for all sampling days/points. The results from Step 3 can then be used for DegT50 derivation. The total extractable parent fraction together with the parent Type I NER are considered for deriving the DegT50. If the criterion is fulfilled the substance could be considered as P or vP.

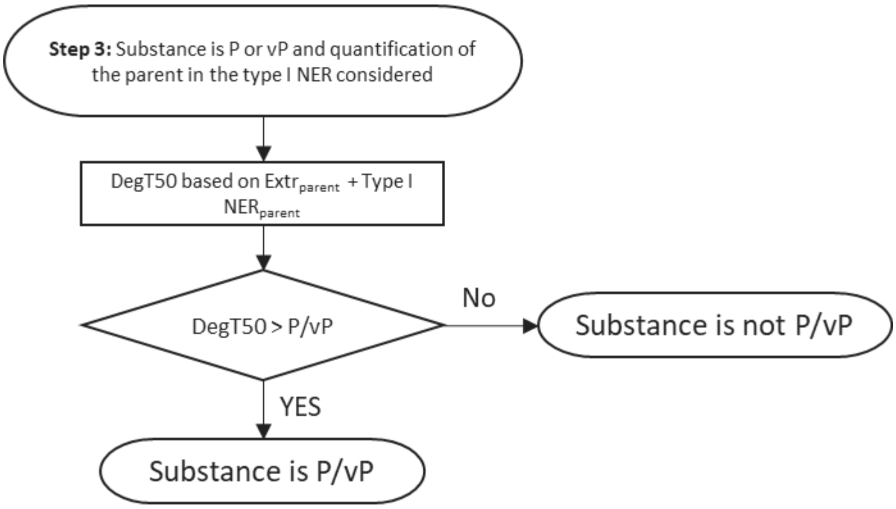

Figure R. 11—15: Assessment scheme for Step 3: Quantification of parent substance in Type I NER.

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Appendix R.11- 5: Comparison of HPLC and KOWWIN v1.68 QSAR generated log K_{ow} values

The log K_{ow} generated by the HPLC-method according to OECD TG 117 (OECD, 2022a) is an estimation method that is equivalent to theoretical models using descriptive information (like chemical structure, i.e. QSARs) to estimate the log K_{ow}. A first attempt to compare the performance of the HPLC method against QSAR estimation of log K_{ow} using 450 HPLC generated log K_{ow} entries in IUCLID (January 2021) for which experimental, curated log K_{ow} data was available, shows that the HPLC method is less accurate in predicting these 450 substances (R2 of 0.87 for HPLC method vs 0.95 for KOWWIN v1.68 QSAR, Figure R. 11—16 and Figure R. 11—17**)** as well as slightly biased (given the y-axis offset of 0.18 log units for HPLC vs 0.03 log units for the KOWWIN v1.698 QSAR). This is expected to give slight over-prediction of log K_{ow} values for substances with a low log K_{ow}, and under-prediction of substances with log K_{ow} above 4.0. The under-prediction will however be very small close to log K_{ow} of 4, and increase with increasing log K_{ow}. HPLC and KOWWIN v1.68 QSAR are however very close to each other in predictivity. This is confirming observations on the precision of both HPLC and QSAR generated log K_{ow} estimates in literature; Finizio *et al.* (1997) showed for 87 pesticides that HPLC gives good results, whereas the ClogP QSAR (BioByte Corp.) gives excellent results. It was concluded that QSAR (in this case ClogP) seemed to be the only reliable method for substances with log K_{ow} > 5. Hodges *et al.* (2019) compared HPLC and QSAR generated log K_{ow} values for non-ionic, anionic, cationic and amphoteric surfactants. HPLC generated consistently higher log K_{ow} estimates than the slow-stirring method for non-ionic surfactants (OECD, 2022b). QSAR gave very different results than HPLC for ionized surfactants. The ECETOC technical report nr.9 (ECETOC, 1983) concluded that HPLC has been adequately validated. A method of calculating partition coefficients from molecular fragmental constants was also noted (already in 1983) as being (as) useful (Rekker, 1977; Hansch and Leo, 1979). 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.

When comparing KOWWIN v1.68 QSAR vs HPLC generated log K_{ow} estimates for the larger dataset of 2077 substances in IUCLID for which no experimental log K_{ow} is available (see Figure R.11—8) it becomes clear that there can be huge differences between the two estimation methods. Bicherel *et al.* (2016) found a relatively poor relationship between the log K_{ow} based on HPLC data and the water solubility compared to shake-flask and slow stir data, confirming limitations of the HPLC derived logK_{ow} data. One of the reasons for the differences may be intramolecular hydrogen bonds. The formation of intramolecular hydrogen bonds (IMHBs) competes with intermolecular hydrogen bonding and thus increases the chemical potential of substances in solvents where intermolecular hydrogen bonds can be formed (water). IMHBs may impact the log K_{ow} by several orders of magnitude. Since EPI Suite does not consider the potential formation of IMHBs, the estimates for such substances are less reliable (see e.g. Wang *et al.*, 2011, Buser *et al.,* 2013).

When trying to establish specific groups of substances for which the HPLC and QSAR method are disagreeing most it turns out that specifically ionogenic substances and salts are predicted very differently – probably due to partly neglecting ionization in the QSAR estimates, but also due to the choice of column in the HPLC method not being 'fit for purpose' of measuring ionogenic substances.

It is hard to identify clearly specific functional groups that are consistently over- or underpredicted by HPLC or a specific QSAR method. Therefore, taking into account multiple sources of (predicted) log K_{ow} information, and weight-of-evidence becomes essential when estimates are within a log unit below or above the screening value of 4.5.

Therefore, it is advised to always generate QSAR estimations of log K_{ow} together with HPLC generated estimates, especially if the HPLC generated estimate of log K_{ow} is in the range of one log unit below or above the screening value of log K_{ow} = 4.5. Given the large scatter observed when comparing HPLC generated values to KOwWIN v1.68 QSAR generated values (see Figure R. 11—18), one should always try to include QSAR (including other QSARs than KOWWIN v1.68) values in the *Weight-of-Evidence* approach when interpreting an HPLC generated log K_{ow} estimate. Especially ionising substances, salts, metal complexes and complex mixtures would benefit from additional estimated data (next to HPLC generated data) from a variety of log K_{ow} QSAR models and a *Weight-of-Evidence* evaluation.

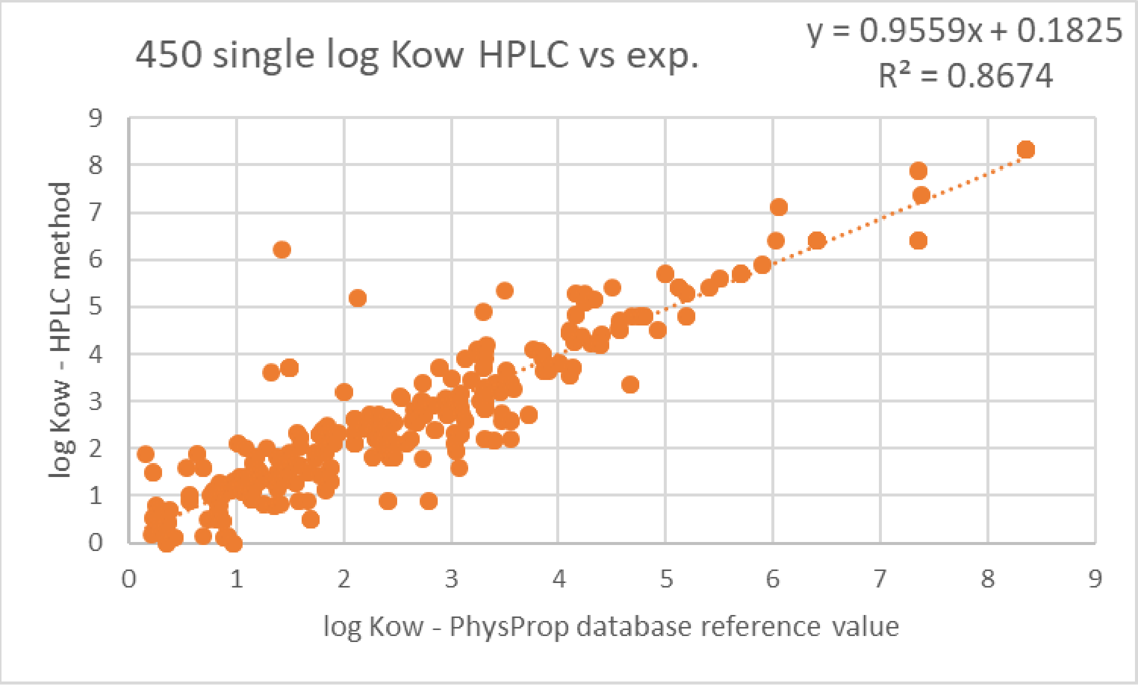

Figure R. 11—16: Predictivity of the OECD TG 117 HPLC method for generating log K_{ow} estimates using 450 substances with HPLC generated log K_{ow} values in IUCLID (January 2021) for which experimental data on log K_{ow} is also available. The linear regression equation is given on top with its R^2 value as a measure of the variance explained, and plotted as the dotted orange line.

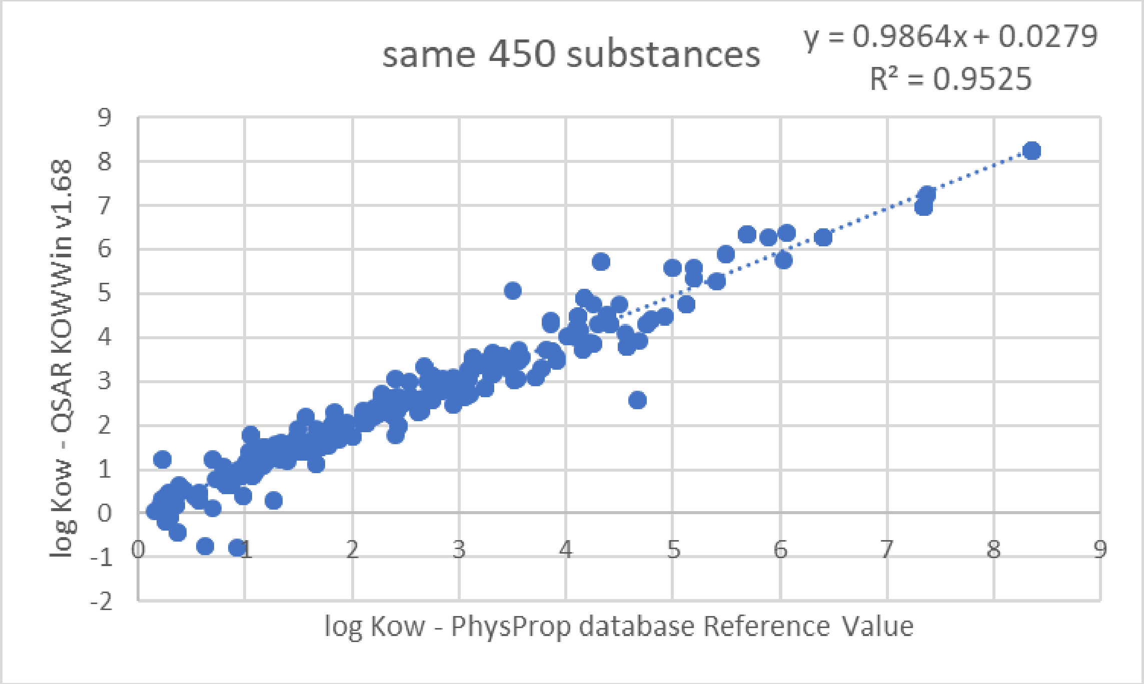

Figure R. 11—17: Predictivity of the KowWIN v1.68 QSAR estimating log K_{ow} for the 450 substances for which HPLC-derived log Kow values were given in IUCLID, and for which also experimentally determined log K_{ow} values were available. The linear regression equation is given on top with its R^2 value as a measure of the variance explained and plotted as the dotted blue line.

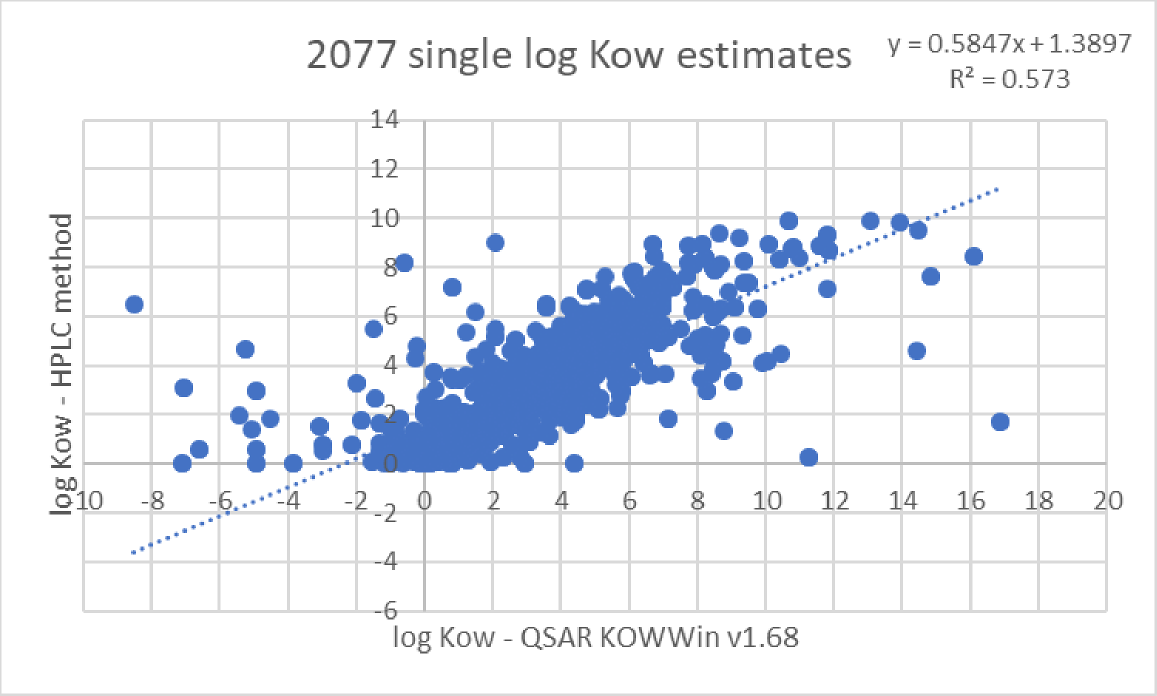

Figure R. 11—18: HPLC generated log K_{ow} estimates in IUCLID for which no experimental data was available, compared to the KowWIN v1.68 estimate of log K_{ow}. The linear regression equation is given on top with its R2 value as a measure of the variance explained and plotted as the dotted blue line.

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Appendix R.11- 6: Relevance and scientific justification of correction for growth dilution when deriving BCF

Overview of used terminology

The terminology of the OECD 305 and REACH guidance documents is followed below:

- k_2 is the overall elimination rate constant, i.e. the elimination rate constant fitted directly from the experimental data
- k_V is the elimination rate constant due to passive diffusion by gill ventilation. This value is denoted as k_2 in e.g. Gobas & Lee (2019)
- k_E is the elimination rate constant due to fecal egestion
- k_{met} is the elimination rate constant due to metabolic transformation
- k_T is the overall elimination rate constants of these three routes of actual elimination from the body of the fish (k_V+k_E+k_{met}); k_2 considers also growth
- k_G is the exponential growth rate of the fish
- k_1 is the overall uptake rate constant from the aqueous phase into the fish
- I is the ingestion rate
- α is the assimilation efficiency
- C_{water} is the concentration in the exposure water
- C_{food} is the concentration in the administered food
- C_{fish} is the concentration in fish
- V_{fish} is the volume of fish
- m_{fish} is the mass of fish
- m is the mass of the substance

Theoretical background

In steady-state, the concentration of a substance in fish (C_{fish}) remains constant over time (t). This is expressed by the following formula:

$$\frac{dC_{fish}}{dt} = 0 \qquad (1)$$

If fish is considered as a one-compartment model and fish is not growing, this means that the sum of all depuration mass flows is equal to the sum of all uptake mass flows (either from the aqueous phase or via dietary uptake):

$$k_1 \cdot C_{water} = k_T \cdot C_{fish} \qquad (2)$$

$$\alpha \cdot I \cdot C_{food} = k_T \cdot C_{fish} \qquad (3)$$

However, for growing fish, the volume of the compartment increases over time and to maintain a constant concentration (i.e. steady-state), there must be a net mass inflow in the fish.

$$\frac{dC_{fish}}{dt} = \frac{d\frac{m}{V_{fish}}}{dt} = 0, with\ \frac{dV_{fish}}{dt} > 0\ and\ thus\ \frac{dm}{dt} > 0 \qquad (4)$$

Hence, to maintain steady-state, and to conserve the mass balance, the mass inflow resulting from the uptake from the exposure media (water and/or food) must equal the sum of the mass outflow due to total elimination from the fish body and the mass flow compensating for the growing fish. To include fish growth, the equation for steady-state is extended to

$$k_1 \cdot C_{water} = k_T \cdot C_{fish} + k_G \cdot C_{fish} \qquad (5)$$

It is noted that the rate constant k_G does not represent a substance mass transport.

This equation is based on concentrations of the substance in the fish, e.g. in µg/kg_{fish}/d. If both sides of the equation are multiplied by the mass of fish (m_{fish} [kg]), this mass balance (in units of mass of the substance per day, e.g. µg/d) can be written as:

$$k_1 \cdot C_{water} \cdot m_{fish} = k_T \cdot C_{fish} \cdot m_{fish} + k_G \cdot C_f \cdot m_{fish} \qquad (6)$$

With

k_1 = (respiratory) uptake rate constant from water [L/kg_{fish}/d]

k_T = overall depuration rate constant [/d] from real depuration processes

C_{water} = concentration of the substance in water [mg/L]

C_{fish} = concentration of the substance in fish [mg/kg_{fish}]

m_{fish} = mass of the fish [kg_{fish}]

k_G = exponential growth rate constant [/d]

In the one compartment model that describes bioaccumulation in fish, the parameters k_1 and k_T refer to mass transfer processes in and out of the fish and k_G describes the increasing compartment volume in terms of mass of fish. Thus, to obtain a constant concentration in growing fish, $k_1 \cdot C_{water}$ has to be greater than $k_T \cdot C_{fish}$. In steady state, this means that the apparent bioconcentration factor (C_{fish}/C_{water}) can be described as:

$$\frac{C_{fish}}{C_{water}} = \frac{k_1}{k_T + k_G} = \frac{k_1}{k_2} \qquad (7)$$

This is the equation that forms the basis for the growth corrected bioconcentration factor, which is included in the OECD technical guideline 305. This equation correctly describes the steady state situation for growing fish expressed on the basis of (substance) mass flows.

Elimination processes and their relation to fish growth

The purpose of the growth correction is to obtain an estimate of the steady state BCF under non-growing conditions. It should thus be investigated, whether the rate constants k_1 and k_T, or more precisely k_1/k_T, for the growing fish are representative for non-growing fish too. To examine this, the parameter k_T, which represents the sum of all real elimination processes of substance mass out of the fish, should be further investigated. k_T consists of three separate elimination processes, being elimination by passive diffusion by ventilation through the gills (k_V), fecal egestion (k_E) and metabolism of the substance (k_{met}).

$$k_T = k_V + k_E + k_{met} \qquad (8)$$

In the case of bioconcentration, uptake is solely through ventilation via the gills. The ventilation rate is determining the uptake rate constant. It is plausible that this ventilation rate will be higher in growing fish due to an increased metabolism (Gobas & Lee, 2019). However, the ventilation rate is not only determining the uptake rate constant in a BCF test, but also the elimination rate constant via the gills. In other words, the ratio of the respiratory uptake and elimination rates is independent of an increased ventilation rate due to growing of the fish.

From the model for fish bioaccumulation, which forms the basis for BCFBAF from Epi Suite of the U.S. EPA (Arnot and Gobas 2003), it appears that for small fish (1 g) with a lipid content of 5%, the elimination via the gills is a more important route than fecal egestion for substances with a log K_{ow} up to 6.7, emphasizing the importance of elimination through the gills.

Also the feeding rate is linked to the growth rate, but in the dietary bioaccumulation experiment this is reversed: the feeding rate is a controlled parameter, consequently the growth rate is dependent on the feeding rate. For bioconcentration experiments with aqueous exposure this is different, as the ventilation rate is not a controlled parameter, but rather a result of the metabolism of the fish. In addition to the feeding rate, the fecal egestion rate could be assumed to be higher for growing fish too. Indeed, in the BCFBAF model (Arnot and Gobas 2003), the fecal egestion rate is directly coupled to the dietary uptake rate.

From the limited data presented by Gobas and Lee (2019) on growth rate in relation to feeding rate and oxygen consumption, originating from different tests with different organisms (rainbow trout and sockeye salmon), it appears that both processes are more or less proportionally related to growth rate (Figure R. 11—19). In other words, if fish feed at a higher rate, their ventilation rate will increase proportionally. Also, as described above, the fecal excretion rate will increase in a more or less similar proportional manner. Depending on the nature of the substance, one of these two elimination processes is more important (i.e. elimination via the gills or fecal egestion), but for dependence of the overall elimination rate on growth rate, it seems not to matter too much which of the two processes is dominant.

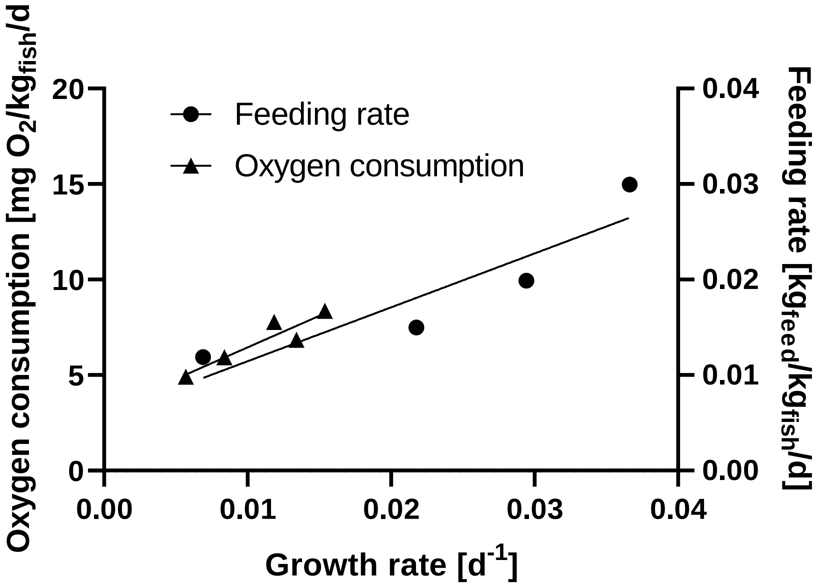

Figure R. 11—19: Relationships of oxygen consumption and feeding rate with growth rate, based on data presented in Gobas and Lee (2019).

The effect of growth on the third parameter in k_T, the metabolism rate constant k_{met}, is largely unknown. However, if general metabolism is higher in fast growing fish, this might have consequences for the metabolic transformation of xenobiotic substances as well. It seems reasonable to assume that growth has also an influence on the metabolic transformation rate as well. However, in general, the substances for which growth rate plays an important role on the BCF are usually not the ones that are metabolized in significant amounts.

In general, it can be concluded that with fast growing fish, not only the uptake parameters are affected but the elimination from the fish as well (i.e. k_T). For gill ventilation, it is reasonable to state that the ratio of k_1 and k_V is the same for growing and non-growing fish. If egestion is taken into account as well, it might be assumed that the ratio between k_1 and the sum of k_V and k_E is still rather similar for growing and non-growing fish, because both ventilation rate and feeding rate seem to be proportionally dependent on growth rate (Figure R. 11—19). For metabolic transformation, the relationship with growth rate is least well-known, but it seems reasonable to consider an equal dependence on growth rate as well. In summary the ratio, between k_1 and k_T seems rather independent of the growth of fish. Therefore, the ratio between k_1 and k_T can be considered as the estimate of the kinetic BCF for non-growing fish.

Summary and implications for bioaccumulation assessment

In conclusion, the kinetic rate constants for growing fish will generally be higher than for non-growing fish. The increase in uptake rate constant and elimination rate constants for mass transfer in and out of fish is proportional. Growth is not a mass transfer out of the fish, but an increase of the volume of compartment (fish). To keep the fish concentration equal (steady state), a part of the mass of the substance taken up by the fish is needed to compensate for the increasing volume of the fish. This will result in a shorter period to reach steady-state and a steady-state situation with overall lower concentrations for growing fish than for non-growing fish (and thus a lower apparent steady-state BCF for growing fish). Growth correction by subtracting k_G from the observed k_2, indicated by k_{2G}, yields the sum of the overall elimination rates that denote real elimination from the fish ($k_T=k_V+k_E+k_{met}$). This value is almost proportionally dependent on growth as k_1 and therefore the overall growth corrected BCF, which is equal to $k_1/(k_V+k_E+k_{met})$, seems to be a reasonable estimate of the kinetic BCF for non-growing fish. This leads to the conclusion that growth correction should be applied to the kinetic BCF in case of growing fish, to have a reasonable estimate for non-growing fish.

It should be noted that not only growth and an increased metabolism has its influence on the ventilation rate. Also temperature is a very important parameter for the ventilation rate. However, nearly all models that are developed to estimate the respiratory uptake rate constant k_1 do only account for the weight of fish and possibly log K_{ow}. If the models that exist to estimate a k_1 value are not suitable for fast-growing fish, they are likely to underestimate the k_1 for fast-growing fish. The BCF estimated from a dietary OECD 305 test might therefore be underestimated if an estimated k_1 value is combined with the high k_T value determined experimentally for fast growing fish.

Verification of the procedure to estimate k_T as k_{2G} is provided by Brooke and Crookes (2012). In this report an alternative method was tested in which the mass of the substance in each individual fish was fitted against time rather than concentrations. It appeared that there was an almost one-to-one relationship between k_T that was estimated from the mass of the substance in individual fish and k_{2G} that was calculated by subtracting the overall growth rate from k_2 that was derived from the concentrations of the individual fish. Only at very low values of k_2, when k_T approaches zero, the subtraction of the growth rate yields slightly underestimated values compared to the alternative method. This method of alternatively determining k_T to estimate the BCF is also included in the OECD TG 305 (OECD, 2012) and the Guidance Document to the OECD TG 305 (OECD, 2017).

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Appendix R.11-7: Volatilisation correction approaches for the kinetic analysis of simulation studies

Three different volatilisation correction approaches for the kinetic analysis of simulation studies are presented below. In all of these procedures it is assumed that the volatile losses of parent are adequately identified and quantified in the volatile trapping systems. If part of the volatilised test substance was not trapped and was lost from the test system, the results of the kinetic analysis have more uncertainty. Also losses through other processes, such as adsorption, increase uncertainties in the kinetic analyses. If repartitioning from the headspace to water (or water-sediment) or soil phase occur, e.g. if the test substance is not trapped during the test and is lost from the test system only when the test bottle is acidified and purged for CO_2 analysis, the volatilisation correction approaches presented below should not be used as they consider the volatilised fraction as a sink.

A. Separate fitting of data on total dissipation and volatile traps (Verbruggen, E., personal communication, 16 March 2022) – instead of Appendix 11 of FOCUS guidance (2014)

B. Simultaneous fitting of data on parent substance in water-sediment and volatile traps to SFO kinetics in OECD 308 using ModelMaker (Jene, 2007b in Annex I of the CLP report on EC 254-938-2 (2018))[74]

C. Simultaneous fitting of data on parent substance in soil and volatile traps to SFO kinetics in OECD 307 using CAKE (Shrestha *et al.* 2019)

A. Separate fitting of data on total dissipation and volatile traps

The Appendix 11 of the FOCUS Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (2014) includes correction procedures to account for volatilisation in the kinetic analysis. However, this approach where k_{vol} is derived from the fit of the amount accumulated in the volatile traps is incorrect since k_{vol} should give a result close to the k_{tot} fitted from the dissipation of the substance from the test system. Deriving k_{vol} from the amount accumulated in the volatile traps may lead to cases where kinetics calculations end up with even slightly negative degradation rates, because the overall dissipation rate fitted in one way is subtracted from the overall dissipation rate fitted in another way. Therefore, a proposal to substitute the FOCUS Guidance Appendix 11 regarding estimation of k_{deg} is presented in this section. The approach applies for cases where both the total dissipation and volatilisation follow first order kinetics.

In a simulation test, the decrease in concentration of the parent substance in the test system reflects the overall dissipation of the test substance in the test system. From the concentrations of parent substance in the test system no distinction can be made between the different processes that have caused this dissipation if the processes occur concurrently. The pattern of decrease follows thus the overall kinetics of the simultaneous processes.

If the two simultaneous processes (degradation and volatilisation) are occurring with the same kinetic pattern (e.g. first-order degradation of the parent substance will be accompanied by a first-order volatilisation of the parent substance), the amount dissipated due to one of the two

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[74] Annex I to the CLH report (EC 254-938-2) https://echa.europa.eu/documents/10162/17218/clh_rep_annex_pendimethalin_en.pdf/76b2443b-b1a3-802f-1b0d-ab04f42ff9e8

processes is a constant fraction of the total dissipation. This holds also true for the amount accumulated in the volatile traps. The amount of volatiles trapped at any time is assumed to be a constant fraction of the amount dissipated from the test system. This means that the accumulated amount follows the overall dissipation. However, data should be checked to verify that this assumption is met.

Hence, for first-order kinetics, if $k_{tot} = k_{vol} + k_{deg}$, the pattern of volatilisation accumulated in the volatile traps thus follows k_{tot} and not k_{vol}. That is because the amount that is degraded is also not available anymore for volatilisation. Therefore, it is not correct to derive k_{vol} from the fit of the data of the volatile traps.

In the case of first-order kinetics, the formula for the total overall dissipation losses can be expressed, as:

$k_{tot} \quad k_{deg} + k_{vol}$

with

k_{tot} is first-order overall dissipation rate

k_{vol} is first-order overall volatilisation rate

k_{deg} is first-order overall degradation rate

If the starting mass in the test system is m_{tot}, then in principle with a complete mass balance (e.g. for radioactivity), the amount of parent substance degraded if all the substance has dissipated is:

$m_{tot} = m_{deg}(\infty) + m_{vol}(\infty)$

$m_{deg}(\infty) = m_{tot} - m_{vol}(\infty)$

where,

$m_{deg}(\infty)$, mass degraded in the test system when all the substance has dissipated,

m_{tot}, starting mass in the test system and,

$m_{vol}(\infty)$, mass volatilised and accumulated in the traps when all the substance has dissipated.

The mass of the parent substance volatilised and accumulated in the gas trap can be fitted as:

$m_{vol}(t) = m_{vol}(\infty)*(1-\exp(-k_{tot}*t)$

Thus, the amount $m_{vol}(\infty)$ can be derived from the fit of the volatilised mass in the gas traps. Because m_{tot} and $m_{vol}(\infty)$ are known, $m_{deg}(\infty)$ can be calculated.

Because $k_{deg} / k_{tot} = m_{deg}(\infty) / m_{tot}$, then k_{deg} can be calculated as:

$k_{deg} = k_{tot} * (m_{tot} - m_{vol}(\infty)) / m_{tot}$

In summary, to be able to estimate k_{deg}, what is needed is the overall dissipation rate constant (k_{tot}), the starting mass of radioactivity (m_{tot}) and the fitted amount of volatilised radioactivity if all substance has dissipated ($m_{vol}(\infty)$).

For additional clarification, intermediate equations and explanations are provided in the Annex I of the *ECHA note on Volatile substances*[75] available on ECHA Website.

B. Simultaneous fitting of data on parent substance in water-sediment and volatile traps to SFO kinetics in OECD 308 using ModelMaker

In the OECD TG 308 study (STUDY CA 7.2.2.3/4) included in the CLH report of pendimethalin, ModelMaker (v3.0.4) was used to simultaneously fit the total residue data of the whole system and the cumulative volatilisation data in order to derive DegT50 that described the volatilisation corrected total degradation of the substance.

A compartment model was set up to describe the total dissipation indicated by the total dissipation rate kTOT as sum of the degradation and the volatilisation indicated by the degradation rate kDEG and the volatilisation rate kVOL. A schematic diagram of the model is shown below (Figure R. 11—20). The model was implemented in ModelMaker (v4.0) and the Chi^2 error level was calculated using the FOCUS kinetics tool FOCUS_DEGKIN_v2.

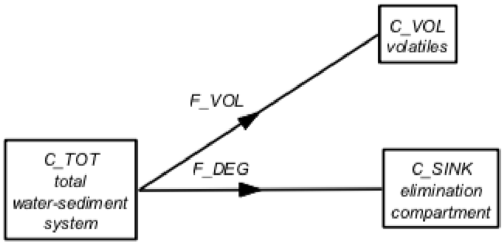

Figure R. 11—20: Compartment model for the parent in total water-sediment system (C_TOT) including volatilisation (C_VOL) and sink (elimination compartment) implemented in ModelMaker (Figure 4.1.4.1-6 in the Annex I of the CLP report on EC 254-938-2).

The underlying differential equation system is given by:

$$\frac{\partial}{\partial t} C_{TOT} = -k_{DEG}\, C_{TOT} - k_{VOL}\, C_{TOT} = -k_{TOT}\, C_{TOT}$$

$$\frac{\partial}{\partial t} C_{VOL} = +k_{VOL}\, C_{TOT}$$

$$\frac{\partial}{\partial t} C_{SINK} = +k_{DEG}\, C_{TOT}$$

Where,

C_{TOT} = *total measured concentration in water-sediment system*

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[75] ECHA note: Options to assess persistence of volatile substances in regulatory PBT assessment (2022) https://echa.europa.eu/documents/10162/17228/note_volatiles_in_simulation_tests_en.pdf/d218ddcb-e5da-7c0a-e5d0-3eae3e1c26dc?t=1669388686441

C_{VOL} = *cumulative volatilisation*

C_{SINK} = *cumulative degradation products (and other elimination processes, e.g. NER)*

k_{DEG} = *degradation rate of the system*

k_{VOL} = *volatilisation rate of the system*

k_{TOT} = *total dissipation rate of the system*

C. Simultaneous fitting of data on parent substance in soil and volatile traps to SFO kinetics in OECD 307 using CAKE

Shrestha *et al.* (2019) presented an extended kinetic modelling to enable considering volatilisation in the modelling of degradation kinetics in OECD TG 307 tests. In the model, the volatilisation losses are considered as an additional product that neither decline nor repartition into the soil. The volatilisation is thus treated as a separate sink for the parent substance, and it is considered to occur in parallel to the biodegradation. Therefore, in this extended model the degradation and the volatilisation of the substance were considered as two processes and separated so that individual rate constants could be calculated for the volatilisation process as well as the degradation process (Figure R. 11—21).

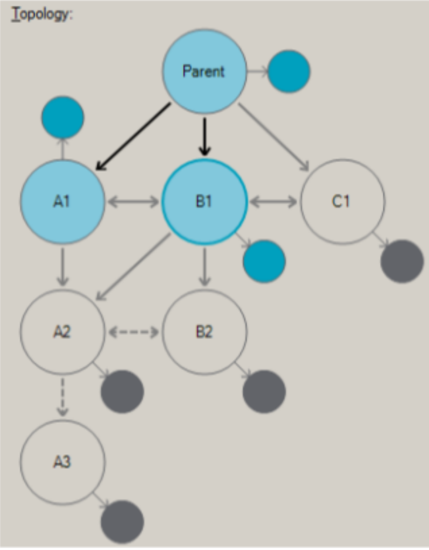

Figure R. 11—21: Structure of the model used in the CAKE tool. Parent: concentration of parent substance measured in soil; A1: sum of extractable transformation/degradation products; B1: volatilised parent substance.

In general, the model assumes first order kinetics with k as an overall dissipation rate and c the concentration of test substance according to following equation:

$$\frac{dc}{dt} = -kc$$

However, for the extended model it is assumed that k consists of two rate constants kV (volatilisation rate) and kT (transformation rate):

$$k = kT + kV$$

To describe the ratio of the two parallel processes the model internally uses "fractions" F^V (volatilisation fraction) and F^T (transformation fraction) which can be calculated based on the individual rates for volatilisation and transformation together with the overall decline rate as follows:

$$F^T = \frac{k^T}{k}$$

$$F^V = \frac{k^V}{k}$$

When the model is run in CAKE, the values for the two fractions (F^T and F^V) are estimated by the tool. They describe how the optimization tool evaluate the importance of the respective processes, transformation and volatilisation, in the experiment. Based on the fraction for volatilisation and the overall DT50 estimated by CAKE, half-lives for volatilisation ($DT50^{,vol}$: half-life due to the volatilisation of the substance) and for transformation (DegT50: half-life due to all (primary) transformation processes) can be calculated using the following equations:

$$DT^{50,vol} = \frac{DT^{50}}{F^V}$$

$$DegT^{50} = \frac{DT^{50}}{1 - F^V}$$

It is noted that this DegT50 does not only include the formation of degradation products but also other processes (e.g., formation of NER or loss of the substance through adsorption to test vessel, leakage etc).

References

FOCUS (2014) Appendix 11: Correction procedures to account for dissipation by volatilisation. In Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. https://esdac.jrc.ec.europa.eu/public_path/projects_data/focus/dk/docs/FOCUSkineticsvc1.1Dec2014.pdf

Shrestha P, Meisterjahn B, Klein M, Mayer P, Birch H, Hughes CB, Hennecke D (2019) Biodegradation of volatile chemicals in soil: separating volatilisation and degradation in an improved test setup (OECD 307). Environ. Sci. Technol. 53 (1), 20e28. https://doi.org/10.1021/acs.est.8b05079

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