GUIDANCE

Guidance on Information Requirements and Chemical Safety Assessment

Chapter R.11: PBT/vPvB assessment

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Guidance on Information Requirements and Chemical Safety Assessment
Chapter R.11: PBT/vPvB Assessment

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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:

http://echa.europa.eu/documents/10162/13559/mb_63_2013_consultation_procedure_for_guidance_revision_2_en.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].

[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 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*	November 2014

	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	Full revision of this document was necessary to take into account recent scientific and technical developments in the field. Main changes in the guidance document include the following: • XXX	XXX 201X

Convention for citing the REACH Regulation

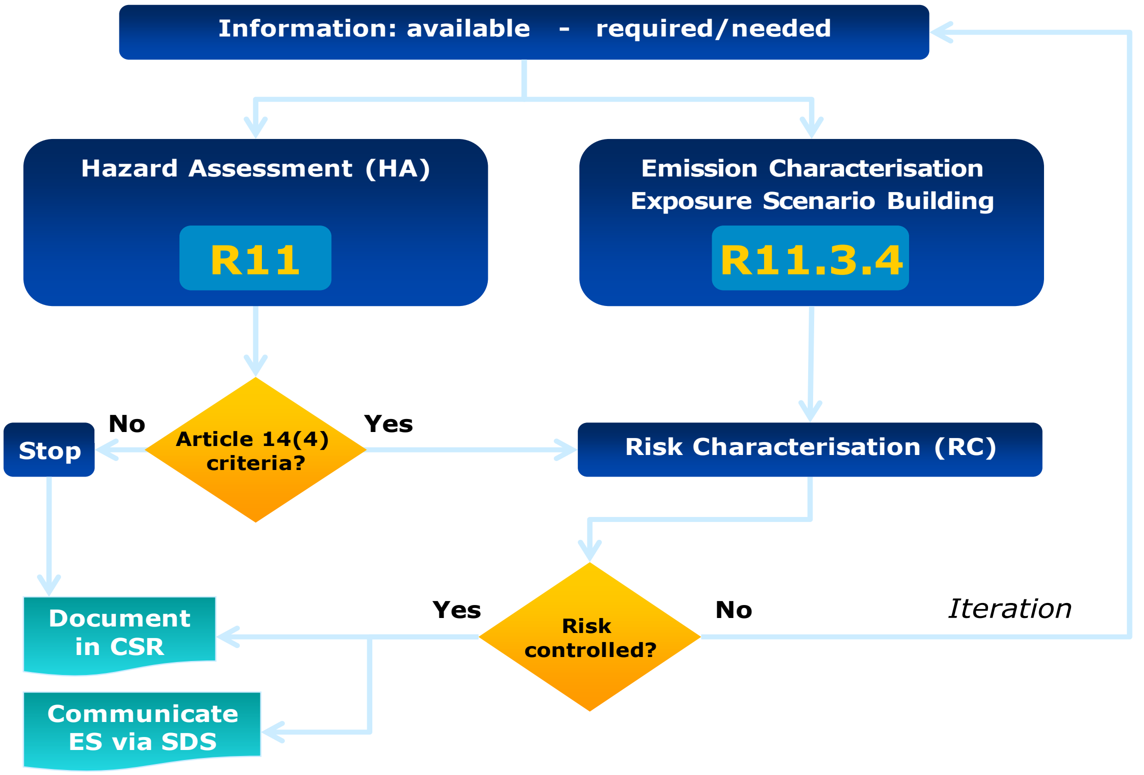
Where the REACH Regulation is cited literally, this is indicated by text in italics between quotes, or text in green boxes.

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 chemical safety assessment 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

- 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 chemical 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.

[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.

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 chemicals 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 chemicals, 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 consequences to the registrant of the conclusions are outlined in detail in Section R.11.3. Guidance on scientific methods 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

[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.

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.

The screening information can be understood as one subtype of assessment information, as Sections 3.2.1.(d), 3.2.2.(b) and 3.2.3(f) of Annex XIII to the REACH Regulation allow "other information" to be used as assessment information, provided that its suitability and reliability can be reasonably demonstrated. However, it should be noted that screening information cannot be directly (numerically) compared with the PBT/vPvB criteria, i.e. the screening information does not contain degradation half-life values or BCF values, which could be directly compared with the criteria. Screening information involves simple data, typically information from Annexes VII and VIII endpoints, that must be used to assess whether further information is needed.

A ***Weight-of-Evidence* determination by expert judgment** must be used in the PBT/vPvB assessment (see the green boxes above). 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 Articles 13(3) and 25(1)).

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.	-

* EC10 preferred over NOEC (see further explanation in Section R.11.4.1.3).

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*.

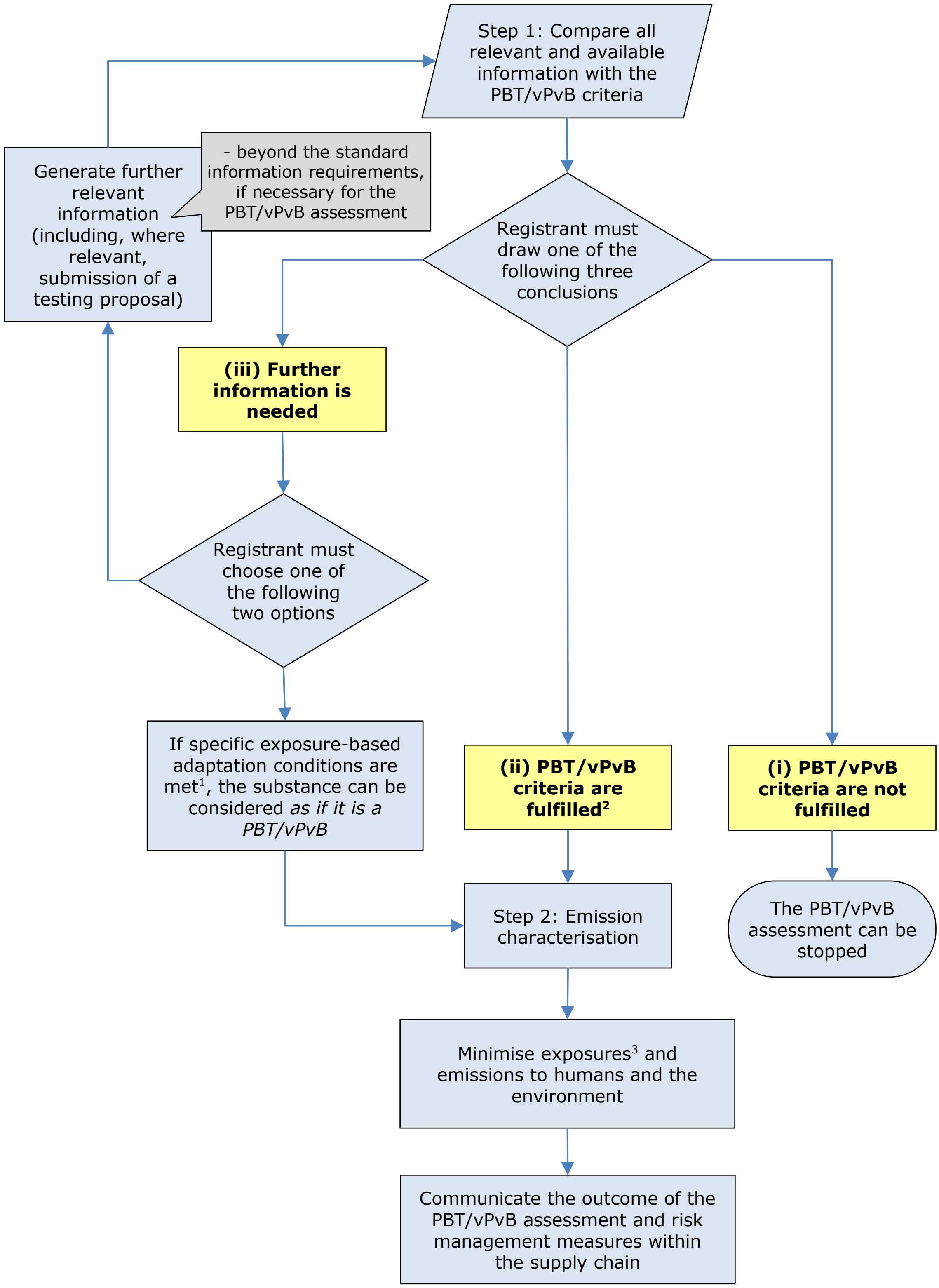
Figure R.11—1: Overview of the conclusions from Step 1 ("Comparison with the criteria") and their consequences.

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.

[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.

Relevant constituents, impurities, additives, degradation/transformation products must also be encompassed in this process.

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 (ii) "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 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.

[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.

R.11.3.2.2 Specific cases: substances fulfilling the PBT/vPvB criteria according to ECHA's Member State Committee in relation to the inclusion of substances in the Candidate List of Substances of Very High Concern

According to REACH Article 59, ECHA's Member State Committee (MSC) agrees on substances to be included to the Candidate List of Substances of Very High Concern (SVHC), i.a., if they fulfil the PBT and/or vPvB criteria. These agreements are published as ECHA decisions 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 MSC agreement. This PBT/vPvB assessment is 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 decision. If the registrant has new information available which was not referred to in the support document of the relevant ECHA 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. Although 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 the MSC, the registrant is further obliged to implement the conclusion of the MSC as the conclusion in force in his CSR. In case ECHA's Committee for Risk Assessment provides an opinion recommending restriction of a substance because it meets PBT/vPvB criteria, it is highly 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 downsteam users about the PBT/vPVB status.

If a registered substance contains a constituent, impurity or additive or transforms/degrades to a substance which is in the Candidate List because of meeting the PBT and/or vPvB criteria, the registrant must conclude his substance to meet 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)** **<u>or</u>** Treat the substance "as if it is a PBT or vPvB": after **conclusion (iii)**

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

In the following 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 degradation/transformation 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.

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 flowchart 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 Kow > 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 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 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.

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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

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 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 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 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 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 physicochemical properties of a substance.

For the assessment of the likelihood and severity of an event occurring due to the physicochemical properties of a PBT/vPvB substance, the same approach for risk characterisation applies as for any other substance (see Section R.7.1 in *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 waste water (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 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 5[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.

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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.

[10] The IUCLID 5 software is downloadable from the IUCLID website at http://iuclid.eu for free by all parties, if used for non-commercial purposes.

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 5 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 5 in practice is given in the IUCLID 5 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 5 is not to repeat information already provided in other IUCLID sections. A reference to other IUCLID sections can be made.

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 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.

[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).

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 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.

The use of quantitative *Weight-of-Evidence* approaches for the whole or a part of the available information is encouraged, although 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 *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. It should be noted that further development of the *Weigh-of-Evidence* approach is on-going and further Guidance may become available in the near future. **The registrant may choose, where necessary and justified, to apply a *Weight-of-Evidence* approach in his assessment, which provides more detailed guidance than the above source**. It is underlined that an essential prerequisite for applying a *Weight-of-Evidence* approach is that the reliability and suitability 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 suitability and relevance of information to the PBT/vPvB assessment is further described in the following sub-sections. 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 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.

Relevant constituents, impurities, additives and transformation/degradation products

The PBT/vPvB assessment should be performed on each relevant constituent, impurity and additive. It is not possible to draw 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

[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.

limit as a generic limit[13]. 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[14] as not relevant for the PBT/vPvB assessment.

It may not always be possible or even necessary to 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 above 0.1% (w/w). 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 application of this recommendation in a specific industry sector, please see the *Environmental assessment guidance on essential oils*[15].

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 with PBT/vPvB properties, and the total amount of these within the manufactured/imported substance should in no case exceed 1 tonne/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

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[13] The limit of 0.1% (w/w) is indicated elsewhere in the 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)(f), 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. By analogy, 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.

[14] 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).

[15] http://echa.europa.eu/support/substance-identification/sector-specific-support-for-substance-identification/essential-oils

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 *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 contains 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% (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**. The PBT/vPvB assessment should normally be carried out for each relevant transformation or degradation product.

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 degradation/transformation 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....*" The latter case 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[16]). 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.

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

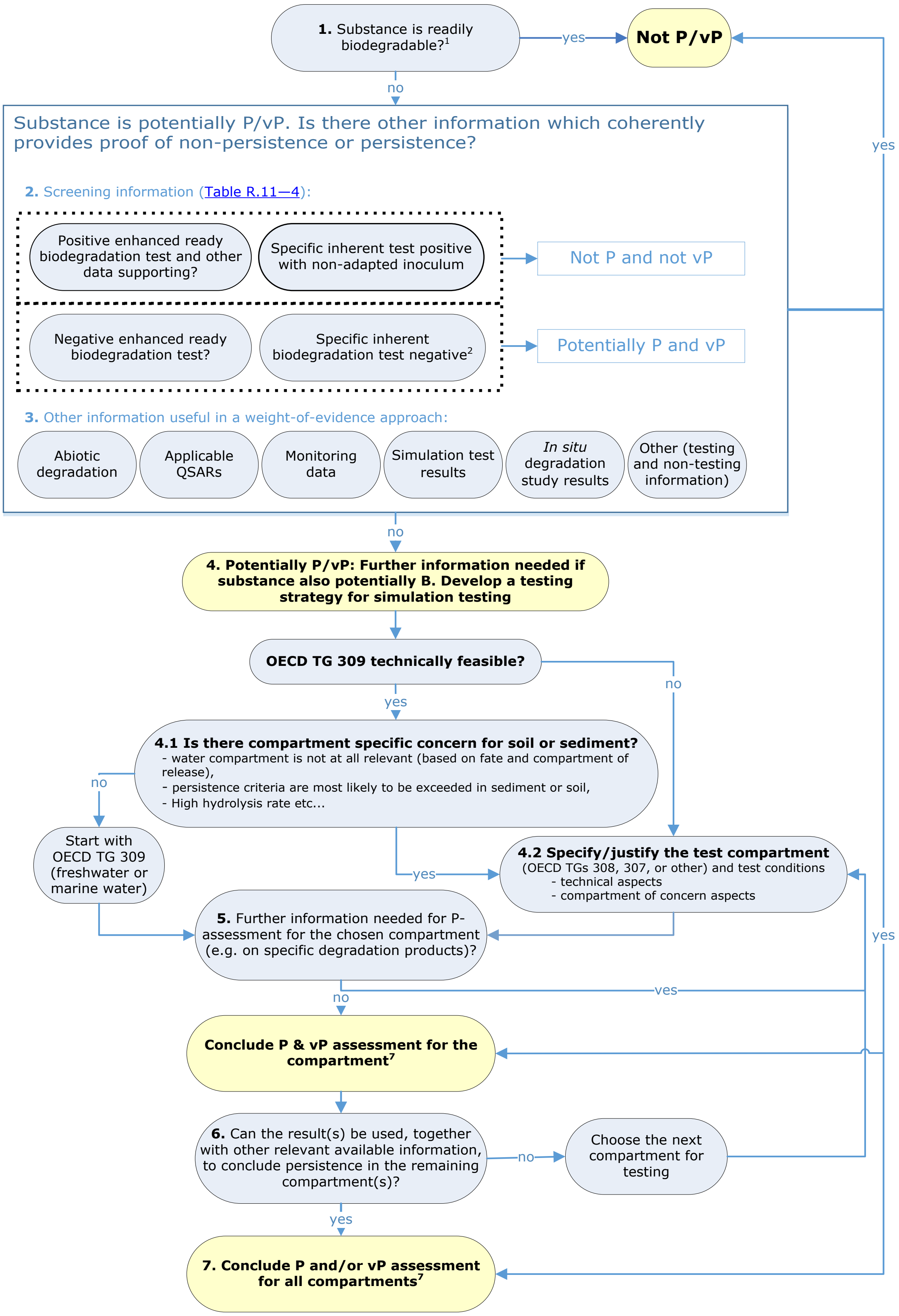
A strategy for degradation assessment and testing in the context of PBT/vPvB assessment is proposed in Figure R.11—3. A tiered approach to assessment and testing is necessary until a definitive conclusion on persistence can be drawn.

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". For deriving an unequivocal conclusion "P" or "vP", higher tier information generally needs to be available. Appropriate data need to be available to conclude the P/vP-assessment on all three compartments (or five, with marine compartments): water (marine water), sediment (marine sediment) and soil. Either an extrapolation of data from one or more compartment(s) to other compartment(s) needs to be justifiable 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 certain cases it may be possible to draw a conclusion "P" or "vP" based on screening information only as described later in this section and in the ITS in Figure R.11—3.

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.

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



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

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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 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).

2. Other sceening 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 in 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 in 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, CO2 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 CO2 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 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. 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 instrinsically persistent and in some cases further testing might be needed.

- **Enhanced screening tests –** In enhanced screening tests (with extended test duration or increased test vessel size), the test criteria set for a ready biodegradation test could be applied without the 10-day window exclusively for the purpose of assessing persistence or non-persistence (60% or 70%, depending on analyte) and in particular with extended test duration. Provided that the test was performed with non pre-adapted/exposed inocula, positive results can be used with other supporting data to conclude that the substance is not P/vP. On the contrary, a negative enhanced test does not meet these criteria and further information is needed. However, for enhanced screening tests with a test duration extended in order to investigate substances of low biovailability, observation of a steady increase in mineralisation over the exposure time without the presence of an apparent lag time should be interpreted as a sign of non-persistence. More information on such enhanced screening tests can be found in Sections R.7.9.4 and R.7.9.5 in C*hapter 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 developped following the ITS starting from step 4 below.

- **Use of (Q)SAR (both QSARs and SARs) estimates** – Refer to Section R.11.4.1.1.3 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 absorption 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. The degradation half-lives obtained in a hydrolysis test have to be compared to the persistence criteria of Annex XIII (i.e. a substance fulfils the P(vP) criterion if $T_{1/2} > 40$ (60) days). 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.11.4.1.1.4 below named "Field studies for persistence".

- **Monitoring data** – 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.11.4.1.1.4 below named "Field studies for persistence". 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 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 persistence 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 W*eight-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 developped. 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 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 compartment at all, this should also be taken into account in the testing strategy. This is not expected to be the case for most of the potential P/vP substances, as explained in the section below. If the OECD TG 309 is not technically feasible, selection of the most relevant first test compartment 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 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 evidence from substance emission data suggests otherwise). Once entering water, a substance may stay there for very long time before it reaches other compartments (air or sediment);

- Particularly for lower water solubility chemicals 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 degration. In general, a test using exclusively anaerobic conditions is not required as a first step. However, where anaerobic data are already available (cf. OECD TG 307 or 308), they might be useful as part of a *Weight-of-Evidence* assessment. For further information, see Section R.11.4.1.1.2 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 compartment, is not discussed. Further information on exposure-based exclusion of testing may be found in this Guidance under Section R.11.3).

Information on degradation and from environmental monitoring data, emissions estimated in the CSR, distribution modelling data (e.g. Mackay Level III) 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 assessement is that a conclusion needs to be derived for all three (five) compartments with the least possible testing efforts. The specific concern for persistence is normally present for the compartment for which the P/vP criteria are most likely to be exceeded or where the degradation half-life is the closest to the criteria (if the criteria are not exceeded). Consideration of compartment(s) of most relevant exposure may also play a role in the identification of the specific compartment for testing. Absence of exposure in a specific compartment may, in exceptional cases, be acceptable to exclude certain compartments from the P/vP assessment.

The following pieces of evidences 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 compartment;
- Environmental monitoring data suggesting persistence is likely in a particular compartment for a substance;
- Direct discharge to a compartment is expected to occur;
- The life-cycle is well characterised and clearly shows that a specific compartment is exposed.

If any compartment other than water is chosen as a first test environment, justification on the selected testing strategy 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.
- Generally, when water solubility of a substance is below 1 µg/L, testing on sediment and/or soil will be preferred. 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 metabolites).
- 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 concentration at which the test can be performed (i.e. due to different multiple water solubilities of the individual components).

Please see also further considerations on the simulation testing strategy in Section R.11.4.1.1 below.

5. Is there further information needed to conclude on persistence for the tested 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 compartment it should be verified that there is no concern in remaining compartments (see step 6).

6. Remaining concern in untested compartments

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

In general, a single simulation degradation study may be sufficient to extrapolate the results to the remaining four compartments, provided that the environmental media in environmentally realistic conditions are selected for the study and the extrapolation 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 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) compartments (see REACH Annex I, Section 3.0.2). In case extrapolation of the results from the tested compartment to the other compartments is not possible, further data generation is necessary to complete the assessment for the compartments for which a conclusion could not be drawn. Exclusion of (a) certain 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 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 are 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[17]), from European monitoring programmes (e.g. NORMAN Network[18]) 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 they 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.11.4.1.1.1 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 be taken. Conversely, if a simulation test indicates for example a half-life of over 200 days, this might be sufficient to decide that the substance meets the P and vP criteria. However, as described in Section R.7.9 in *Chapter R.7b* of the *Guidance on IR&CSA*, a negative 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 several references reporting that ready biodegradation tests 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.

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

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

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. 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 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 before drawing a conclusion.)

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

Persistence	Screening information	Conclusion
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 ThCo2 (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 ThCo2 (OECD TG 301 B) or ThOD (OECD TGs 301C, 301D, 301F,306 and 310)	Potentially P or vP
Enhanced screening tests****	biodegradable	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 in 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.
**** 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 »)
***** See section below for concluding ultimately on persistence in particular cases.

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.11.4.1.1.2 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 (surface water, sediment or 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 degradation products (metabolites, 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 ThCO2) or 70% for DOC removal assuming that the yield for growth of the microbial biomass is 30-40%. In the context of ready biodegradadability, test substance-specific analysis can also be used and primary degradation and formation of any metabolites 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". If sufficient degradation is shown in an enhanced biodegradation screening 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. More information on modifications of ready biodegradability tests with respect to such enhanced screening tests is contained in Sections R.7.9.4 and R.7.9.5 in C*hapter R.7b* of the *Guidance on IR&CSA*. Please note that these tests are referred to as "enhanced biodegradation screening tests".

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" in C*hapter* 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 in C*hapter 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 realistic 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 degradation (degradation products). The endpoints that need to be addressed are primary or ultimate degradation rate and degradation half-lives (DegT50) or dissipation half-lives (DT50) for the compartments included in the test system as well as the route of degradation, metabolites 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. 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-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 products would be needed since it cannot be excluded that a second transformation route forms a persistent metabolite in concentrations relevant for the P assessment. When the mineralisation half-life for the whole system is not below the P criterion, a full mass balance of the substance and any degradation products/metabolites 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 (Kästner *et al.*, 2014). Where primary degradation is observed, the identity of possible relevant metabolites 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 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*).

It should be noted that for direct comparison to the P/vP criteria only estimates of degradation half-life are appropriate. When the kinetics of transformation are first-order, single-first order (SFO) kinetic models can be used for predicting degradation half-lives. The predicted degradation half-lives should be used for comparison with the P/vP criteria. Use of bi-phasic kinetic models is recommended to be limited to cases where clear deviations from first-order kinetics occur. When the kinetics of transformation are bi-phasic, the best-fit model (FOMC, DFOP, HS) should be selected and used for predicting a DT_{50}. The DT_{50} predicted from the best-fit bi-phasic model should be used for comparison with the P/vP criteria. When applicable (DFOP or HS), the DT_{50} predicted from the slow phase should be preferred and used for comparison with the P/vP criteria. In case other DT_{50} are used, a justification should be provided with adequate and reliable documentation of the applied method.

Further information on degradation models 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). It is recommended to consult that guidance document for in-depth analysis of simulation degradation test results.

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 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), but for the purpose of reducing efforts of testing, the order of the test(s) should be designed so that the test reflecting the worst case of persistence potential (for which the expected degradation rate is the closest to the corresponding criterion and thereby the results would provide a "hit" with the highest certainty in case the criteria are exceeded in one of the compartments) should be conducted first. This would also ideally be the 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 releases patterns to the order of testing also need to be considered. In some cases, it may be correct and necessary to choose another test system than substance properties or the normal first preference (see discussion below) would suggest. This may be the case if it can be foreseen that reliable degradation data cannot be gained, e.g. in specific situations where a high level of NER is probable. In this case, data would mainly give information on mere dissipation, which is inadequate in P/vP assessment because degradation data need to be considered.

A flow diagram for considering the appropriate environmental compartment(s) for simulation degradation testing is illustrated in the ITS described in Figure R.11—3.

The further elements to be considered when choosing the compartment 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 identication and quantification, it may not be possible to conduct this study if the water solubility of the substance is well below 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 pathways part of the study may be employed at a higher test substance concentration to ease the analytical identification and characterisation of the metabolites. Further considerations on the OECD TG 309 study are provided below.

Soil/sediment simulation degradation testing is warranted if direct or indirect exposure to the substance is likely and a compartment-specific concern has been identified. In addition, as described in the ITS (Table R.11—3), the soil and sediment degradation simulation tests are needed when a conclusion on persistence cannot be drawn (for instance when the substance is concluded as not P) based on the simulation test in water or other available evidence. 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). Degradation rates/half-lives from simulation tests in soil can be used instead of generic values for the assessment of PEC_{soil}. While degradation rates/half-lives from simulation tests in sediment can be taken into account for the calculation of the $PEC_{regional}$, in practice this would have only a negligible influence on risk assessment.

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 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 before it reaches other compartments (air or sediment);
- Particularly for lower water solubility chemicals, which tend to be adsorptive, the OECD TG 309 avoids 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 direct or indirect emission;
- 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. Appropriate analytical methods should have suitable sensitivity to detect relevant changes in concentration (including metabolites).
- OECD TG 309 is performed at concentrations between 1 and 100 µg/L. Generally, when water solubility of a substance is below 1 µg/L, testing on sediment and/or soil will be preferred.

Once the appropriate simulation test(s) have been identified and conducted, data need to be interpreted to determine environmental degradation half-lives. 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 in C*hapter R.7b* of the *Guidance on IR&CSA*.

OECD TG 309

OECD TG 309 is performed at concentrations between 1 and 100 µg/L. 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 metabolites).

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 more water sources. 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 (no suspended solids) or as a suspended sediment test. The OECD test guideline indicates the method can be used to *simulate surface water free of coarse particles or turbid surface water (which might exist near the water-sediment interface)*. The amount of suspended solids in the pelagic test should approximately correspond the level of suspended solids in EU surface water (e.g. 10 – 20 mg_{dw}/L for fresh water). Default suspended solids concentration used for ERA is 15 mg_{dw}/L for freshwater and 5 mg_{dw}/L for marine water. If more than one water source is used in the assessment it is recommended that the amount of suspended solids reflects the EU surface waters with low and high concentration of the suspended solids. More than one water source could be considered instead of two concentrations of the test substance when the test concentration is well under the water solubility limit. In addition, a reference substance should be used to demonstrate the viability of the system.

The suspended solids option offers a suspended solids/sediment concentration range between 0.01 and 1g/L, but notes that the lower end is typical for most surface waters as specified above. If this option of added suspended solids is used, it is recommended to not use a magnetic stirrer bar as it may grind the solids/sediment and result in increased levels of NER and hence generally use of other means of aeration e.g. shaking of test vessels is instead recommended (Shrestha *et al.*, 2016).

According to Ingerslev and Nyholm (2000), conducting the tests with added suspended sediment significantly enhance the biodegradability of some of the test substances. In general this test design is 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.

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.

As different options exist in the TG in particular in respect to performing the test with or without suspended sediment, the possibility of shaking or stirring the test vessels, and the inclusion or exclusion of diffuse light. Given the options available, it is recommended that registrants provide justification of the choices made for the study in their IUCLID robust study summary and/or test plan proposals.

NOTE:

The current test strategy may need to be reconsidered if the measurement and assessment approach of NER is refined in the future. Scientific work is on-going to develop the understanding on NER but ECHA considers it appropriate to make the recommendation above based on current understanding and experience. Role of NER in P/vP assessment is discussed further in the section on non-extractable residues below in this document.

OECD TG 308

Generally where water solubility of a substance is below 1 μg/L, testing on sediment or soil will be preferred but generally the preferred choice is the OECD TG 309 in the first instance unless this is not technically feasible. Technically feasible means here that it has been impossible within 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. One of the reasons to the approach to prefer an OECD TG 309 as the primary testing choice is a desire to avoid NER formation, as described above.

The log K_{oc} may be used as an indicator of whether testing in a water-sediment system may be considered relevant and to include an aquatic sediment simulation test for substances with log $K_{oc} > 4$.

For highly adsorptive substances, the DegT50 for the total system can be approximated to the DegT50 for sediment. This is due to the expected rapid partitioning from the water compartment to sediment. DegT50 for the total system is, in terms of statistical reliability, a more reliable DegT50–estimate than DegT50-estimates from an OECD TG 308 study for separate compartments. This approach has the advantage of avoiding the need to determine compartment specific half-lives (Honti and Fenner, 2015)[19]. In this specific context, this approximation can be made, when the substance has log $K_{oc} \geq 3$ [20]. No other approximations are possible. In case the parent substance degrades to more soluble 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 test outcome can be affected by test vessel geometry and the associated water-sediment interface size. There is no specification of the vessel size or geometry in the test guideline and so it is recommended to record the dimensions of the test vessel, and include this in the IUCLID robust study summary.

ECETOC have investigated possible modifications to the OECD TG 308. One aspect includes agitating the test system by stirring the water above the sediment. The agitation generally results in a higher proportion of aerobic sediment, but also increased levels of NER. The research only assessed the effect on four chemicals and so the applicability of the findings to a broader range of substances is unknown. It also remains unclear how comparable results with a modified test system are with those determined from the standard system.

Sediment spiking instead of addition of the test substance *via* water is also possible. The overall half-life from such a test should be assumed to be the sediment half-life (unless there is significant desorption, which seems inlikely in the case of PBT substances). Advice on sediment spiking is available in Section R.7.8.10.1 "Laboratory data on toxicity to sediment organisms" in *Chapter R.7b* of the *Guidance on IR&CSA*. In addition to the advice provided in Chapter R.7b, the following option for sediment spiking may be considered: drying part of the sediment (e.g. 10%) and adding the test substance to the dry sediment as a vehicle for spiking. This decreases the volatilisation of the substance compared to sand spiking (Léon Paumen *et al.*, 2008).

Multiple simulation test results

Different compartments should be assessed separately. A substance can be concluded to be not-P only if it can be demonstrated that it is persistent in none of the 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 on the same 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 compartment, when four or less results are available, the most conservative result should be used for the 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, etc). Only test results corresponding to equivalent test conditions can be aggregated. In all cases, the approach should be well justified and documented and should be supported by the *Weight of Evidence* available. This should include a discussion of outlying results. In particular, the

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[19] 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/

[20] NB: the DegT50 water **cannot** be approximated to the DegT50 total system nor can it be approximated by the dissipation from the water phase.

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 be of importance in some persistence assessments e.g. if water covered soil environments are studied. On the contrary, in some cases, neither are considered to be especially relevant scenarios for the determination of persistence in the EU[21]. If anaerobic soil data are available, they may provide supporting information for 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 here therefore focuses on OECD TG 308.

Sediment test:

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[22]. Care should also be taken where the anaerobic data show rapid degradation of a substance. This is because there is no immediate discharge of a substance to anaerobic sediment or soil. Instead, the substance will need to cross an aerobic zone before reaching the anaerobic zone. This means it is important to understand the rate degradation across that aerobic zone to assess the persistence. However, if there is clear evidence of persistence under anaerobic conditions, the substance is expected to quickly shift to anaerobic zones (e.g. if the substance adsorb stongly to suspended matter and the sedimentation rate is fast) and degradation half-live

[21] For example, the PBT Expert Group decided not to use the anaerobic soil data in the assessment of persistence for phenothrin (10th meeting, September 2015).

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

under aerobic conditions is close to the P-criterion, then the substance may be assessed as persistent.

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. 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, metabolism route prediction[23] or prior knowledge[24] 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 metabolite may be of concern, e.g. dehalogenation (polybromodiphenylethers), or the degradation of sulphur-containing or nitro-containing molecules (SCHER, 2008). However, in general a test using exclusively anaerobic conditions is not required as a first step. For the OECD TG 308 sediment simulation test the "aerobic"' test will include anaerobic sediment. If a substance is expected to degrade only under anaerobic conditions, an OECD TG 308 may not be the most suitable test to study the persistency 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. If only an OECD TG 308 study is conducted, wrong conclusions on persistence may be drawn. In such cases, to exclude potential false negative results in relation to the P/vP assessment, strictly aerobic degradation should also be assessed.

Test temperature

Guidance on test temperature for the simulation test(s) is provided in Section R.7.9.4.1 in *Chapter R.7b* of the *Guidance on IR&CSA*.

Non-extractable residue

With regard to evaluation of soil or sediment simulation degradation test results, it is important to differentiate between actual degradation of a substance and formation of non-extractable residues (NERs) in the soil or sediment (or in an OECD TG 309 study with added suspended solids). The formation of NERs should not be confused with the degradation phenomenon.

The NER should ideally be differentiated in remobilisable and irreversibly bound fractions. While the irreversibly bound part (e.g. biogenically bound) can be assessed as a potential removal pathway, the remobilisable fraction (heavily sorbed, physical inclusion) pose a potential risk for the environment. There is, however, no simple relationship between

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

[24] 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.

extraction by the different individual extraction methods and the type of chemical binding to soil/sediment. This is discussed in Sections R.7.9.4 and R.7.9.5 in C*hapter R.7b* of the *Guidance on IR&CSA*.

Another issue to address is whether the parent substance, or its degradation products, *via* their interaction with sediment or soil organic matter become bound to or entrapped in the organic matrix. The environmental significance of NERs is related precisely to the extent to which they become "indistinguishable" from existing soil, sediment or organic matter. However, the term "indistinguishable" cannot currently be defined because the relationship between extraction by the different individual extraction methods and the type of chemical binding to soil/sediment is not simple to understand or to describe. For example, NER formation might be an indication of degradation only if the NER level decreases concurrently with gradual increase in mineralisation or metabolite formation. In contrast, a lack of degradation of the parent compound 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 compound has become non-extractable, and thus is not readily available to degradation. Information obtained by comparing results from NER formation in sterile and non-sterile soils/sediments can sometimes provide insight into the mechanisms of the process. If NER is only formed at high levels in non-sterile soils/sediments, this may indicate degradation of the parent substance. In this case the formed NER in the non-sterile soil/sediment is unlikely to consist of the parent substance.

There is currently no procedure to measure which part of the residue is not bound irreversibly (see Chapter R.7b of the *Guidance on IR&CSA* for more details). Neither is there a standard concept currently available to measure different fractions of the residue.

Therefore, the residues should be regarded, in the absence of systematic methodology, as non-degraded substance[25], unless, on a case-by-case basis, it can reasonably be justified or analytically demonstrated that a certain part of the residues can be considered to be irreversibly bound.

NOTE:

The NER-topic is under scientific development. ECHA nevertheless considers it appropriate to make the above recommendations based on current understanding. There may be a need to specify further these recommendations after the on-going scientific work has progressed.

Assessment of relevant degradation/transformation products ("relevant metabolites")

Where a substance is degraded by abiotic means or partly biodegraded, it may be necessary to consider whether there are any breakdown products or metabolites formed that could be potential PBTs/vPvBs. Where the original substance forms a breakdown product or metabolite that could be PBT/vPvB, there should be an assessment of the amount of this breakdown product or metabolite compared 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 products. Biotransformation/ degradation pathways may be complex and many different degradation products may be formed and some only in small amounts (or 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 products detected at

[25] Meaning non-degraded parent substance or as relevant metabolite(s) if such is or are formed.

≥10% of the applied concentration of the parent substance at any sampling time (principal metabolites) should at least be identified unless reasonably justified otherwise. The test guidelines furthermore stipulate that values even lower than 10% may be warranted depending on the specific case. However transformation 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 products should be considered on a case-by-case basis with justifications. See also the definition of relevant degradation/transformation products in Section R.11.4.1.

It should be noted that neither a readily biodegradable substance (based on ultimate degradation) nor its metabolites will normally need to be assessed because any metabolites can be assumed to be minimal and transient.

To assess whether the breakdown products or metabolites 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 breakdown products/metabolites 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 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 degradation/transformation 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 degradation/transformation 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 C*hapter 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. 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 metabolites to provide a mass balance are also needed. These both demonstrate that primary degradation has occurre, and allow subsequent PBT assessment of the degradants.

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. Further discussion on photodegradation is provided in C*hapter R.7b* of the *Guidance on IR&CSA*.

According to Castro-Jiménez and 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 chemicals 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 in C*hapter R.7b* of the *Guidance on IR&CSA*.

R.11.4.1.1.3 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 in C*hapter 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 C*hapter 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, the combined use of results from three estimation models in the EPI suite (US EPA, 2000) is suggested, as described above in the Explanatory Note 2 to the ITS for persistence assessment (Figure R.11—3).

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 chemicals 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, 2000) 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 (see the other boxes regarding the various types of other potentially available information).

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, and a few published models (Peijnenburg *et al.*, 1992, Stegeman *et al.,* 1993). These are reviewed in Section R.7.9.4 in *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 metabolites (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/.

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. Contrary to the result of Mackay fugacity level I modelling, Mackay level III modelling is also dependent on the release pattern (fraction of emission between air, water, soil) and thus also on the use of the substance.

Therefore, 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 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 http://www.trentu.ca/academic/aminss/envmodel/models/EQC.html), 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. This can easily be done by modelling the fate in a suitable STP model where the fractions at steady state are presented: volatilisation to air, adsorption to STP- sludge, STP-degradation and the emission fraction to surface water. Such models also typically employ the fugacity concept. The fraction adsorbed 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.

Finally, freely available multi-media models focussing on the potential for long range environmental (mainly air) transport also exist like the OECD Long Range Transport model (OECD, 2006). 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[26] of the results is needed. A robust study summary should be provided and give sufficient information on the modelling (i.e. default assumptions and input parameters of the model).

R.11.4.1.1.4 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

[26] 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. This is because these values may be predicted by the model, even if measured values have been input to the programme. 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 of how the chemical 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.

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.

Field studies do not represent higher-tier studies in the sense that their results would override other (lower-tier) results, but they 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. If a field study results in a DT50 exceeding the P/vP criteria, it may be possible to conclude that the substance is persistent because degradation on its own will need longer than the combined mechanisms.

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 in C*hapter 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 PPP but can also be used for any other chemical substance. The NAFTA guidance (NAFTA, 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.

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 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 and the EFSA guidance 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 radiolabeled 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. Guidance on how to derive DegT50 values from cosm 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.11.4.1.1.5 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 persistence 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 W*eight-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. To this end, 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 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 in *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)[27]

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 value. Detailed guidance regarding an ITS for BCF assessment is presented in Section R.7.10 in *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.

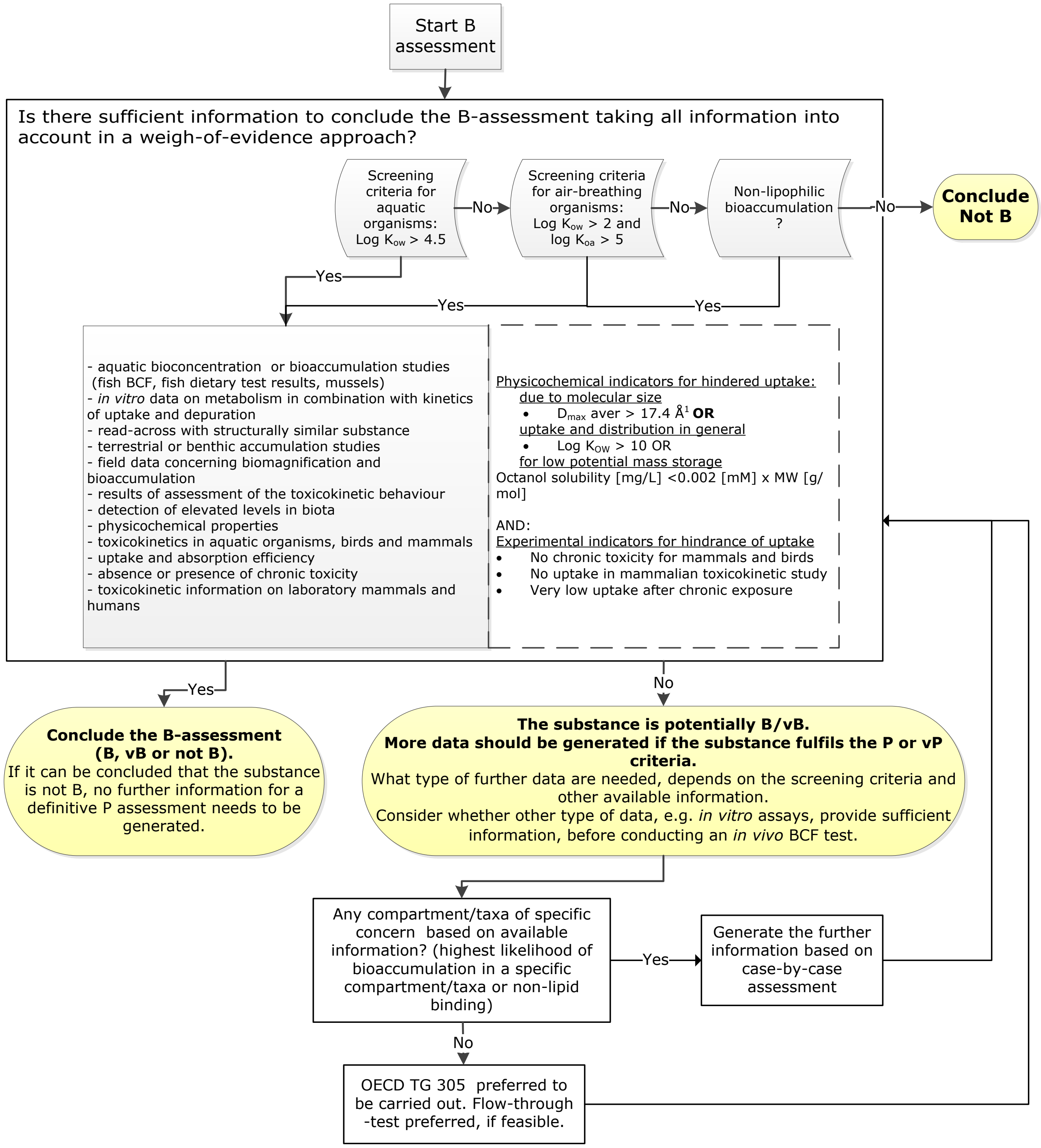
If the *Weight-of-Evidence* approach described under "Conclusions on the Endpoint" is not sufficient to draw a conclusion, the performance of an experimental bioaccumulation test or generation of other appropriate bioaccumulation information is required. However, before such a study is conducted for 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

[27] 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.

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.



1
2 **Figure R.11—4: Integrated assessment and testing strategy for B-assessment.**

R.11.4.1.2.2 Experimental aquatic bioconcentration factor (BCF) data

For the start, it should be noted that, in normal cases where experimental information on bioaccumulation is needed, a flow-through bioaccumulation test with fish according to OECD TG 305-I or OECD TG 305-II is preferred due to the best possibilities of reliably comparing the results from such test with the B/vB criteria.

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 chemical in the surrounding medium (C_w as mg/L). BCF is expressed in $L\cdot kg^{-1}$. 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 normalised to a fish with a 5% lipid content and corrected for growth during the study period as described in Annex 5 of the OECD TG 305.

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, 2012) or an equivalent test protocol in fish is preferred for producing experimental bioconcentration data. Valid results from this 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 incocnsistency impacts the overall conclusions on bioaccumulation potential.

Also use of other taxonomic groups than fish (e.g. mussel bioconcentration test, ASTM, 2003) is possible for measuring bioconcentration in the aquatic environment and the valid BCFs determined in other taxonomic groups can be used in assessing whether or not the B/vB criteria are met. Furthermore, in case a 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 in order to save animal lives. 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 not only in the animal tissues but also in water at the used test concentrations that should always be below the water solubility limit of the substance. In bioconcentration tests accumulation via the water phase must be the only route of exposure and any accumulation 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, and it may also be 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 will generally provide a statistically more robust value. If uptake follows first order kinetics and the BCF_{SS} was really based on steady state data, both methods 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. 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 Zebra fish (*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.

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 combined data for both the uptake phase and the depuration phase (see Annex 5 of the OECD TG 305), because this procedure represents the best fit for 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 sequential fit 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 fitting. K_2, i.e. depuration, 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 lognormal-transformed, a geometric mean for the water concentration should be used instead of an arithmetic mean.

Normally, the concentration of the test substance in fish tissues should be lipid normalised. A 5% lipid normalisation as recommended in OECD TG 305 should be performed unless it is evident that the substance does not primarily accumulate in lipid tissues; growth dilution should also be considered in the BCF estimation. The resulting BCF that is preferred for a comparison with the bioaccumulation criteria is the kinetic growth corrected and lipid normalised (to 5% lipids) BCF value (BCF_{KgL}). A justification is needed in case no normalisation is carried out.

It should be noted that the greatest weight under PBT assessment for REACH is placed on a valid BCF test due to the current understanding that BCF is in the most representative way of reflecting the bioaccumulation potential of a substance, where aquatic bioaccumulation is relevant. If BCF-values are incoherent with other data types, it is very important to address the reasons for such incoherence 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 biomagnification or trophic magnification occurs.

R.11.4.1.2.3 Experimental dietary biomagnification in fish (experimental dietary BMF)

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 and/or potential bioaccumulation may be predominantly expected from uptake via feed (e.g. for substances with extremely low water solubility and high K_{oc}, which will usually dissipate from water to organic matter). 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. However, an aqueous exposure test is preferred for substances that have a high log K_{ow} but still appreciable water solubility with respect to the sensitivity of available analytical techniques, and for which the maintenance of the aqueous concentration as well as the analysis of these concentrations do not pose any constraints. Therefore, an improved analytical technique or the use of a radiolabelled substance should be considered first to improve the detection limit in the aqueous test before deciding on whether a dietary test is indeed the only feasible option. Also, if the expected fish concentration (body burden) via water exposures within 60 days is expected to be below the detection limit, 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 and the whole body elimination rate constant (k_2) and half-life for substances for which this is impossible *via* the aqueous exposure route.

The following definitions are used in this guidance:

- The biomagnification factor (BMF) is the concentration of a substance in a predator relative to the concentration in the predator's prey (or food) at steady-state.
- The dietary biomagnification factor (dietary BMF) is the term used in OECD TG 305 to describe the result of dietary exposure test, in which exposure via the aqueous phase is carefully avoided and thus the dietary BMF from this test method differs from a BMF value from a field study in which both water and dietary exposure may be combined. 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. Another important difference that can occur between the lab BMF and the field BMF for chemicals with biomagnification potential is the variability of growth rates under laboratory and field conditions. However, it is possible to simulate field BMFs from lab BMFs to address these two differences using mass balance toxicokinetics (bioaccumulation) models.

Annex 8 of the OECD TG 305 summarises some approaches currently available to estimate tentative BCFs from data collected in the dietary exposure study. This calculation is based on a model predicted uptake rate constant (k_1) and the depuration rate constant (k2) determined from the dietary bioaccumulation study. For the PBT assessment, it is possible to translate the dietary experimental data to tentative BCFs for comparison against the BCF criteria outlined in Annex XIII. However, 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) and the experimental conditions (e.g., dissolved oxygen concentrations, water temperature, gill water pH for ionic chemicals). Thus assuming k_1 is accurately and appropriately predicted for the substance and the conditions of the experiment, the tentative BCF values could be determined. However, they should be considered as part of the body of evidence, and not used as the only values from which to draw conclusions in the PBT assessment.

For poorly soluble non-polar organic substances first order uptake and depuration kinetics is assumed, and more complex kinetic models should be used only for substances that do not follow first order kinetics. Several models are available to estimate a k_1 value needed to calculate an aqueous BCF from a dietary bioaccumulation study. Although there is some variation in the results of the k_1 models and the models are restricted to predominantly neutral organic substances, the 13 presented models span a range of a factor 2.7 for some examples of a hydrophobic potential PBT substances (Crookes and Brooke, 2011). As noted by Crookes and Brooke (2011) "The uncertainty in the estimated uptake rate constant was relatively large, however, even for the best performing methods." Therefore, the uncertainty of the k_1 models and their applicability domains (e.g. mostly restricted to neutral organic chemicals but including some weakly acidic or basic substances as well, log K_{ow} above 3.5 etc.) require consideration for the factors mentioned above. Accordingly, no one model can be recommended over the others and results must be used with caution, with reference to assumed applicability domains. If the method of deriving a BCF from a dietary BMF study is used, estimates of k_1 should be derived according to all the models available to give a range of BCFs.

Besides the calculation of a BCF from the depuration phase, the laboratory BMF derived from the test can be compared with laboratory BMF values for substances with known bioaccumulation potential in a benchmarking exercise. For example, such an approach has been described for dietary bioaccumulation studies with carp (Inoue, Hashizume *et al.*, 2012). Based on a regression between BCF and BMF for nine compounds tested in this set-up, it was shown that a BCF value of 5000 L/kg, normalized to a lipid content of 5%, corresponds to a lipid normalized BMF from the dietary test of 0.31 kg food/kg fish, and a BCF of 2000 L/kg corresponds to a BMF of 0.10 kg food/kg fish. Of the five substances that had a BCF value higher than 5000 L/kg, two of them had a BMF value in excess of 1. A different benchmarking could be obtained from aqueous and dietary bioaccumulation studies for perfluorinated compounds with rainbow trout (Martin et al., 2003a, b). A BCF value of 5000 L/kg corresponded to a BMF from the dietary test of 0.49 kg food/kg fish, and a BCF of 2000 L/kg corresponded to a BMF of 0.36kg food/kg fish. Of the three substances with a BCF > 2000, one had a BMF of 1.0, while the two others had substantially lower BMF values. These two different examples showed that there is no uniform relationship between BCF and BMF. Moreover, the 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. Further examination of differences between BCF data (and criteria) and BMF data (and criteria) with mass balance models and with larger datasets may in future provide further insights into relationships between the two bioaccumulation metrics and their respective bioaccumulation criteria. If benchmarking is used for comparing dietary BMF values with BMF values for substances with a known bioaccumulation potential, it must be ensured that these BMF values were obtained under similar conditions.

In conclusion, OECD TG 305 III: Dietary Exposure Bioaccumulation Fish Test provides a range of valuable information which should all be discussed in the bioaccumulation assessment. Paragraph 167 of the test guideline lists all the relevant measured and calculated data from the study which should be reported and considered for the bioaccumulation assessment, including the BMF values, substance assimilation efficiency and overall depuration rate constant. When interpreting the study results, the tentative calculated BCFs and a benchmarking exercise to compare the k_2 and BMF derived from the test with other substances with known bioaccumulation potential also provide useful evidence for the bioaccumulation assessment and are recommened to be reported. The k_2 (or half-life) value itself may be useful for the assessment of the bioaccumulation potential (see paragraph on "Overall depuration rate constants in fish" in Section R.11.4.1.2.9). Further guidance on the OECD TG 305 is available (OECD, 2016).

R.11.4.1.2.4 Experimental sediment bioaccumulation data (experimental Bioaccumulation Factors BAF and BSAF for sediment)

For the start, it should be noted that, in normal cases where experimental information on bioaccumulation is needed, a bioaccumulation test with fish (OECD TG 305) 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 fish bioaccumulation test is not expected to reflect sufficiently the bioaccumulation potential but testing of bioaccumulation potential in soil or sediment might provide the necessary information for deriving conclusions on the B/vB-assessment.. Whether in such specific situation a sediment bioaccumulation test or soil bioaccumulation test is the first option, should be considered case by case. Targeting the testing to compartment where bioaccumulation potential is expected to be the highest should be the main consideration. Additionally, relevance of exposure may also be considered for the choice between sediment and soil invertebrates bioaccumulation testing. The choices should always be well justified and take into account the need to minimise the number of animals used.

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

- The non-normalised biota-sediment accumulation 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 $kg_{sediment}·kg^{-1}_{worm}$.

- The steady state biota-sediment 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.

- Biota-sediment accumulation 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 determined by normalising the BAF_K (or BAFss) for the worm lipid content and the sediment total organic carbon content. 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 $kg_{sediment\ OC}·kg^{-1}_{worm\ lipid\ content}$.

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.

Bioaccumulation studies on sediment dwelling organisms can be used both for the screening and as part of the *Weigh-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 and this situation 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 biota-sediment accumulation 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) is the lipid-normalised steady state factor determined by normalising the BAF_K and should be additionally reported for highly lipophilic substances.

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, a normalised BSAF is derived from a non-normalised BSAF by converting the results to a sediment OC content. 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. 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. Especially chemicals having background sediment concentrations and potentially adaptable uptake mechanisms require careful consideration, as the sediment-dwelling organisms may have adapted to such chemicals which potentially affects the bioaccumulation process.

R.11.4.1.2.5 Experimental soil bioaccumulation data (experimental Bioaccumulation Factor BAF and BSAF for soil)

For the start, it should be noted that, in normal cases where experimental information on bioaccumulation is needed, a bioaccumulation test with fish (OECD TG 305) 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 fish bioaccumulation test is not expected to reflect sufficiently the bioaccumulation potential but testing of bioaccumulation potential in soil or sediment might provide the necessary information for deriving conclusions on the B/vB-assessment. Whether in such specific situation a soil bioaccumulation test or sediment bioaccumulation test is the first choice, should be considered case by case. Targeting the testing to compartment where bioaccumulation potential is expected to be the highest should be the main consideration. Additionally, relevance of exposure may also be considered for the choice between sediment and soil invertebrates bioaccumulation testing. The choices should always be well justified and take into account the need to minimise the number of animals used.

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

- The non-normalised biota-soil accumulation 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 weight of worm) divided by the concentration of the substance in the surrounding medium (C_s as $g \cdot kg^{-1}$ of dry weight of soil); the BSAF has the units of kg wet (or dry) soil$\cdot kg^{-1}$ wet (or dry) worm.

- The steady state biota-soil accumulation 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 weight of soil) being constant during this period of time.

- Biota-soil accumulation 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-sediment accumulation factor (BSAF) is determined by normalising the BAF_K (or BAFss) for the worm lipid content and the sediment total organic carbon content. 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 $kg_{OC} \cdot kg^{-1}_{lipid}$.

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.

Bioaccumulation studies with terrestrial organisms, especially those obtained from established experimental protocols, such as the OECD TG 317 *Bioaccumulation in Terrestrial Oligochaetes* can be used as part of the *Weight-of-Evidence* assessment of B and vB properties.

It should be considered that (soil or sediment) invertebrate species may have a lower metabolic capacity than fish species. Bioaccumulation in these invertebrates may therefore be higher than in fish under the same exposure conditions and this situation should be considered in a *Weight-of-Evidence* approach.

Earthworms and enchytraeids are the recommended taxonomic groups to be tested. In case of lipophilic substances the steady state biota-soil accumulation factor ($BSAF_{SS}$) and the kinetic biota-soil accumulation factor ($BSAF_K$) are preferably presented as the normalised biota-soil accumulation factor (BSAF), which is the lipid and soil organic carbon -normalised BSAF. 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 for ensuring that the concentrations in the soil and in the organisms are above the detection limit throughout the test.

The relevance of bioavailability of the substances potentially containing irreversibly binding fractions 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. 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 chemicals with background soil concentrations and potentially adaptable uptake mechanisms require particularly careful consideration, as the soil-dwelling organisms may have adapted to such chemicals 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 chemical spiked into soil, it has been demonstrated that at test end (provided that the relevant analytical procedures are available) the concentration of the test chemical in the adult worms can give an indication of uptake into the organism (Kinney *et al*., 2012). Care must be taken that the bioaccumulation assessment 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 chemical is not considered. As such, the results of this test should be interpreted with caution, but it can provide valuable screening information on chemical 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

Bioaccumulation factors (BAF calculated from monitoring data, field measurements or measurements in mesocosms) or specific accumulation in food chains/webs expressed as biomagnification factors (BMFs) or trophic magnification factors (TMFs) can provide supplementary information indicating that 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. 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). A relevant BMF or TMF value significantly 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 next to direct accumulation from water. However, as dietary and trophic biomagnification represent different processes than bioconcentration in aquatic organisms, BMF and/or TMF values <1 cannot be directly used to disregard a valid assessment based on reliable BCF data indicating that a substance meets the numerical B/vB criteria in Annex XIII to the REACH Regulation, but in this kind of cases all available data need to be considered together in a *Weight-of-Evidence* approach.

To be able to compare BMF values in a direct and objective manner, they should, as far as possible, be lipid normalized for the assessment of substances that partition into lipids in order to account for differences in lipid content between prey and predator. It should however be noted that non-lipophilic substances 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.

In principle, BMF values are not directly related to the BCF values, and in fact BMFs and BCFs represent complementary bioaccumulation pathways. 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 predatory/prey relationship for which it was measured. Biomagnification will vary between predatory/prey relationships, so a low BMF in one does not mean that it will be low in other predatory/prey relationship. Conversely, evidence of high biomagnification in one predatory/prey relationship is cause for significant concern and it is then in accordance with a cautious approach to assume that biomagnification may also occur in other (unmeasured) predatory/prey relationships. The same applies for bioaccumulation factors (BAF) calculated from field data (i.e. by relating concentrations in field sampled aquatic organisms to the concentration in their habitat). If such BAF values are above the criteria for B or vB it should be considered whether this information is sufficient to conclude that the substance meets the B or vB criteria. Care should be taken that the exposures from all relevant routes and compartments are considered when BAF values are evaluated.

The quality of field data needs to be assessed and interpreted correctly. Difficulties in interpretation arise especially for trophic magnification factors (TMFs), which describe the accumulation throughout the whole food chain. The TMF for a food chain 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. Currently, there is no standard procedure for studying TMFs. Hence, the conductance and sampling may vary considerably between different studies. As such, TMF represents the average biomagnification per trophic level within that food chain. The validity of the TMF is strongly dependent on the spatial and time scales over which the samples were retrieved. The most reliable TMFs are derived from data for non-migratory species originating from a confined area and sampled in the same period, or from food 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) and ECETOC (2014) for discussion on uncertainties.

The way data, on the basis of which the TMF values are calculated, are treated has a great impact on the outcome of the TMF value. Not only the magnitude of the TMF value can be impacted, but also whether biomagnification or biodilution occurs. In addition, the setup of the field study could have an influence on the resulting TMF values as well. These aspects cover both spatial and temporal variability in sampling, but also the selection of species belonging to the ecosystem. Spatial variability can lead to different organisms being exposed to different environmental concentrations. Temporal differences could have a strong impact on trophic magnification as well. Such temporal variability further complicates the interpretation of the observed TMF values. Further, it appears that TMF values could be strongly dependent on the inclusion or exclusion of certain species and on which part of the food chain is considered, for example pelagic species only or the benthopelagic food chain. Apart from that, even from similar food chains widely varying results can be obtained for the TMF (Houde *et al.*, 2008).

R.11.4.1.2.7 Addressing uncertainty of field data in the 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, covering all different study conditions and/or species) the data presented should be accompanied with a comprehensive discussion on the uncertainties. 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:

- Thorough elucidation of the food-web structure (feeding ecology; determination of the trophic level). The position in the food web is quantified using relative abundances of naturally occurring stable isotopes of N ($^{15}N/^{14}N$, referred to as $\delta^{15}N$). However the relative abundance of these isotopes and thus the determination of the trophic level and TMF is influenced by the physiology of the organism and its life trait history. Rapid growth with a higher protein demand for new tissue leads to lower enrichment factors than those with slower growth rates. Insufficient food supply and fasting and starvation leads to catabolism of body proteins and an increase of ^{15}N in organisms relative to those organisms with adequate food supply;

- Evidence to demonstrate that the steady-state has been achieved in the food web considered. Opportunistic feeders vary their diet over seasons or with life stage and point sources may influence observed TMFs. Additionally, apart from the diet there is always the possibility of a direct uptake of the substance under scrutiny and the relative importance of food versus e.g. water exposure can influence the magnitude of the TMF;

- Influence of sampling location(s) and timing(s), concentration gradients/migration behaviour;

- 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, 2001).

- Influence of species physiological characteristics (e.g., typical lipid content, whether air-inhaler or water inhaler);
- Influence of digestion rate/diet energy content, size and growth, ability to biotransform, sex, age;
- The number of organisms sampled at each point of the food web;
- Sample type. Sample collection is often restricted to tissue or serum samples in large predators due to ethical reasons and due to the challenging logistics with respect to sampling and laboratory constraints. Tissue-to-whole body extrapolations of measured concentrations, where this cannot be avoided, introduce additional uncertainties which need to be addressed;
- Analytical information such as detection and quantification limits;
- Quality assurance throughout the sampling, sample treatment, storage and analysis (including such as blanks and spiked samples);
- …

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).

R.11.4.1.2.8 Use of a fugacity approach for bioaccumulation assessment

The use of fugacity ratios (Burkhard *et al*., 2012; Mackay *et al*., 2013) has been proposed as a technique for bioaccumulation assessment. This method converts laboratory and field bioaccumulation metrics into a common fugacity ratio scale. 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.

The calculation of a fugacity ratio is an approximation based on certain assumptions. One of the assumptions made is that the partitioning to lipids is equal to the octanol-water partitioning and this may not always be the case. Therefore, use of a 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 porewater 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.

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. Polyc yclic 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 (e.g. 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 chemical biomagnifies.

NOTE:

ECHA has included the authorities' view into the text above and currently recommends the user of this Guidance to adhere to that recommendation.

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.

Overall depuration rate constants in fish

Upon prolonged exposure and after internal redistribution of a compound, the rate of elimination is independent of the uptake route: aqueous exposure, dietary exposure or both routes simultaneous as in the field. Besides that, uptake rates in fish are rather similar for neutral organic compounds and dependent on e.g. ventilation rates of gills for aqueous exposure and feeding rate for dietary exposure. So, the elimination rate is a discriminating factor in the bioaccumulation potential of such compounds. For this reason the half-life has been suggested as a useful metric for the bioaccumulation assessment (Goss, Brown *et al*., 2013).

The kinetic processes of especially bioconcentration from water, which are the uptake and elimination rate constants, are dependent on the size of a fish (e.g. Barber 2008, Brooke and Crookes, 2012). This implies that setting one value for the depuration rate constant for different organisms is not sufficient. 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 (see Section R.7.10.4.1 in *Chapter R.7c* of the *Guidance on IR&CSA*). 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}). A similar limit of 0.085 d^{-1} for the depuration rate corresponding with a BCF of 5000 was reported resulting from a comparison of lipid normalized BCF values with their corresponding depuration rate constants (Brooke and Crookes, 2012). These ranges could be used in interpreting and comparing data obtained from different studies (laboratory aqueous and dietary exposure, field exposure) in a *Weight-of-Evidence* approach for the assessment of bioaccumulation.

Chronic toxicity studies with mammals

If chronic toxicity studies with mammals are available, the complete absence of effects in the long-term is an indication that the compound is either chronically non-toxic and/or that it is not taken up to a significant extent. 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 B or vB.

Toxicokinetic studies with mammals

More direct information on the potential of a substance to bioaccumulate can be obtained from toxicokinetic studies with mammals, if available. Information on absorption, distribution, biotransformation and excretion of a substance in mammals may be used in a *Weight-of-Evidence* approach. Information on absorption and systemic bioavailability indicate if a substance is taken up after the exposure and, depending on other substance properties influencing toxicokinetics, whether there is a possibility for bioaccumulation. Distribution information may indicate possible location(s) of bioaccumulation. Some substances go through a biotransformation (i.e. metabolism). Also transformation products may accumulate and that possibility needs to be scrutinised in the PBT/vPvB assessment. The elimination process of a substance includes metabolism and excretion. Different elimination parameters may provide information on the bioaccumulation potential.

Elimination rates and half-lives are acknowledged as useful metrics indicative of the bioaccumulation potential (Arnot, Brown and Wania, 2014; Gobas et al., 2009; Goss, Brown and Endo, 2013; Gottardo, Hartmann and Sokull-Gluttgen, 2014; ECETOC, 2014; ECHA Member State Committee, 2015).

There is no universal elimination process–related threshold in B-assessment available which would cover all (aquatic/terrestrial - water breathing/air breathing) organisms because the elimination rate depends on several factors (e.g. species). Nor can any more specific cut off criteria be recommended to compare elimination data with the B/vB criteria. Nevertheless, prolonged elimination half-lives may indicate the potential of a substance to bioaccumulate.

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*.

NOTE:
The use of toxicokinetic data in B-assessment is under scientific development and this section may need to be revisited after the work has progressed. ECHA considers that only the qualitative recommendations provided above are appropriate based on current understanding.

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. The way in which the information on molecular size (average maximum diameter and maximum molecular length), molecular weight, Log K_{ow}, and octanol solubility should be used is briefly addressed in the following (background information on these parameters can be found in Appendix R.11—1). It should be noted from the outset that this information does not override valid information on aquatic bioaccumulation on the substance if the aquatic data indicate high bioaccumulation potential.

If average molecular size, log K_{ow}, and octanol solubility are above or below certain values (as described below), they can be considered as indicators for a limited bioaccumulation potential due to the lack of uptake. However, these parameters should never be used on its 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.

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, 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 Annex 1 to Appendix R.11—1 of this guidance document).

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. In addition to the normal criteria for application of read across, when applying read-across data in bioaccumulation assessment, two generally important aspects have to be considered, which are the hydrophobicity and the centre of metabolic action for both substances. An important parameter for PBT and vPvB assessment is the molecular size of the substance since it has an influence on the bioaccumulation behaviour (see Appendix R.11—1).

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. From a certain minimum length upwards it may be assumed that the substance disturbs the entire interior structure of the lipid bilayer of cell membranes and therefore does not accumulate to a significant amount, 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 as not being met. It is noted, that there is no agreed cut-off criterion for molecular length available at the moment and therefore the use of molecular length as one indicator of low bioaccumulation potential needs to be well justified.

Another parameter that directly reflects the molecular size of a substance is the average maximum diameter ($Dmax_{aver}$). Very bulky molecules will less easily pass through the cell membranes. This results in a reduced BCF of the substance. From one study of a diverse set of substances it appeared that for compounds with a $Dmax_{aver}$ larger than 1.7 nm[28] the BCF value will be less than 2000. 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 conformation of the substance as well as the method used.

[28] 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.

Log K_{ow}

For the PBT and vPvB assessment 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 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.

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 BCF value if this value is estimated from log K_{ow}. Perfluorinated compounds (PFCs) are examples of such partitioning behaviour, of which perfluorooctanoic acid (PFOA) is a well-known example.

For some groups of chemicals, such as organometals, ionisable substances and surface active substances, log K_{ow} is not a valid descriptor for assessing the bioaccumulation potential. Information on bioaccumulation of such substances should therefore take account of other descriptors or mechanisms than hydrophobicity.

At log K_{ow} values between 4 and 5, Log BCF increases linearly with log K_{ow}, if the chemical is absorbed at the same rate and if 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), a decreasing relationship between the two parameters is observed. Apart from experimental errors in the determination of BCF values for these very hydrophobic chemicals, 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 chemicals is normally also very uncertain due to experimental difficulties. 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, 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.

Log K_{oa}

For the PBT and vPvB assessment other than bioconcentration factors in aquatic organisms have to be considered as well. For bioaccumulation in aquatic organisms a screening threshold value has been established, which is log K_{ow} greater than 4.5. Equivalent to log K_{ow} for aquatic organisms, log K_{oa} (octanol-air partition coefficient) has been recognised as a parameter indicating that bioaccumulation can occur in air-breathing (terrestrial) organisms.

Available information on the combination of log K_{oa} and log K_{ow} as provided in the ITS, may indicate that the substance is potentially bioaccumulative in air-breathing organisms. In case such a substance is already confirmed as P or vP, it should be carefully considered whether aquatic bioaccumulation testing already is expected to reflect the “worst case” in terms of concluding on the B/vB -properties or whether it is instead more efficient to directly generate information on accumulation in air-breathing species.

In case a substance screens to be potentially bioaccumulative in air-breathing organisms and aquatic bioaccumulation testing indicates no bioaccumulation, further information and potentially further assessment on bioaccumulation in air-breathing organisms may be necessary. This could include monitoring data, mammalian toxicokinetics data (see Section “*Toxicokinetic studies with mammals” above)* and other information for air-breathing organisms as described above.

Reporting of log K_{oa} is not required under REACH but it can be calculated based on the information available in the registration dossier: K_{ow} and Henry's Law Constant (H). In case H is also unavailable, it can be estimated based on water solubility (WS), vapour pressure (VP), and molecular weight (MW). An efficiently absorbed, non-biotransformed neutral organic chemical with a log $K_{oa} \geq 5$ in combination with a log $K_{ow} \geq 2$ has the potential to biomagnify in terrestrial food chains and air-breathing marine wildlife as well as in humans, while the chemicals with log $K_{ow} < 2$ are being quickly eliminated by the urinary excretion, 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). Furthermore, all of the studies used to develop these partition coefficient combinations have emphasized that these partitioning property combinations relate to biomagnification potential only when predicated by the assumptions of high chemical absorption efficiency from the diet and no biotransformation after absorption and negligible active transport (in or out). In particular, considerations for absorption efficiency and biotransformation rates are thus also necessary for bioaccumulation assessment. Whole body half-lives (see e.g. Goss *et al*., 2013) and biotransformation rates (see e.g., Armitage and Gobas, 2007) have been proposed that would counteract biomagnification potential. However, these toxicokinetic values to mitigate biomagnification are a function of the defined conditions in which they were derived.

For example, for the soil-earthworm-shrew food-chain a model illustrates that chemicals with a log $K_{oa} > 5.25$ and with a log K_{ow} between 1.75 and 12 have a biomagnification potential unless they are metabolized at a sufficiently rapid rate, e.g., in excess of 0.3 d^{-1} or a half-life time of 2.5 d for shrews (Armitage and Gobas, 2007). Evaluative, representative biomagnification models for adult humans (e.g., Goss *et al*., 2013; Arnot *et al*., 2014) have indicated that biotransformation half-lives of about 70 days or faster may be sufficient to mitigate biomagnification potential. The differences between the half-lives required to mitigate biomagnification potential in the two systems (shrews and humans) relate primarily to differences in maximum gastrointestinal biomagnification and bioenergetics (Kelly *et al*., 2004; de Bruyn and Gobas, 2006) and body size (ca. 0.01 kg for shrews vs. ca. 70 kg for humans), i.e. allometry in physiological and metabolic processes (e.g. Hendriks *et al.*, 2001), emphasizing the requirement for context-specific data. However, it should be noted that the above mentioned cut-off values for elimination rates/half-lives are not currently recommended to be used in the B-assessment. Development of an approach to better understand toxicokinetic information is necessary and on-going (see also subsection "Toxicokinetic studies with mammals" above).

If sufficiently reliable and condition-specific data for chemical absorption efficiency and biotransformation rates are available from *in vivo*, *in vitro* or *in silico* methods, such data can be used to parameterize the models for terrestrial bioaccumulation assessment. As necessary, *in vitro* data and *in vitro* to *in vivo* extrapolation models can be used for evaluating chemicals that have K_{ow} values lower than the BCF screening threshold values (i.e., log $K_{ow} < 4.5$ and > 2), but with log K_{oa} values greater than about 5.5. *In vitro* methods for mammals are reasonably well-established as a result of decades of pharmaceutical testing and development (see below) and technical challenges relating to the solubility of such chemicals are expected to be minimal, i.e. chemicals with log K_{ow} <4.5 are generally amenable to *in vitro* testing. Additionally, *in silico* models for hepatic (Pirovano *et al*., 2016) and whole body clearance (Arnot *et al*., 2014; Berellini *et al*., 2012) may also provide valuable insights for bioaccumulation assessment of chemicals that fall into the aforementioned chemical partitioning range ($2 < \log K_{ow} < 4.5$ and $\log K_{oa} > 5.5$). The absorption efficiency is another critical parameter that can mitigate the biomagnification potential indicated by the proposed K_{ow} and K_{oa} values. In general, and when deemed necessary, combining the relevant information in the form of a mass balance bioaccumulation or toxicokinetic model is recommended.

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. 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.

***In vitro* data on biotransformation in fish**

In vitro methods such as fish liver S9 and primary hepatocyte assays provide information on metabolism and hence biotransformation in the organism. Because biotransformation is considered to be the dominant mechanism of elimination of hydrophobic substances, such in vitro tests have potential to support the assessment of bioaccumulation and may contribute to a reduction in (or refinement of) animal testing. For further details see Section R.7.10.3.1 *In vitro data on aquatic bioaccumulation* in *Chapter R.7c* of the Guidance on IR&CSA.

In evaluating the test results of an *in vitro* test care must be taken that the dissipation of the substance indeed relates to biotransformation. As the current procedures for *in vitro* metabolism tests are not suitable for constructing a mass balance, it cannot be excluded that the dissipation may be due to other processes. Especially for potential PBT substances that have generally a very low water solubility, the dissipation might be caused by processes such as adsorption and volatilisation.

To estimate a BCF the *in vitro* metabolism rate constant is extrapolated to an overall *in vivo* metabolism rate constant. This overall rate constant is used to calculate a kinetic BCF from the kinetic rate constants k_1 (gill uptake rate constant), k_2 (gill elimination rate constant), k_D (dietary uptake rate constant), k_E (faecal egestion rate constant), k_M (metabolic rate constant), k_G (growth rate constant), which are defined for the whole fish. The more hydrophobic a substance is, the slower the internal redistribution kinetics between lipid compartments and blood will become, which will likely reduce the overall metabolism rate. The *in vitro* to *in vivo* extrapolation to estimate the overall metabolism rate constant seems to be insufficiently validated at present for highly hydrophobic chemicals.

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 chemicals from water. These types of methods are at the moment only well described for hydrophobic substances. For more detailed information Section R.7.10.3.1 in *Chapter R.7c* of the Guidance on IR&CSA.

R.11.4.1.2.11 Conclusion on the endpoint

A substance meets the B or vB criterion if it is considered bioccumulative 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[28]***

    2. ***octanol-water partition coefficient as Log10 (Log K_{ow}) > 10 (calculated value, preferably by several estimation programs, for substances for which Log K_{ow} can be calculated and the model is reliable)***

    3. ***a measured octanol solubility (mg/L) < 0.002 mmol/L × MW (g/mol) (without observed toxicity or other indicators of bioaccumulation)***

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

    The supplementary information to confirm this limited uptake may comprise data from a chronic toxicity study with mammals (≥ 90 days, showing no toxicity), a toxicokinetic study with mammals or birds, a bioconcentration study with invertebrates, or reliable read-across from a structurally similar compound (all showing no uptake). These types of information should be examined in a *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. This approach is based on the report provided in Appendix R.11—1.

- If there is a reliable aqueous bioaccumulation study available, such as an aqueous exposure OECD TG 305 study, the result from this test 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 Kow > 4.5. Rapid metabolisation of a substance may lead to a lower BCF value. Methods such as fish liver S9 and fish hepatocyte assays may be useful as supporting information, but *in vitro* data alone should not be used to conclude on lack of bioaccumulation potential at the present point in time. However, 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 criterion. However, because there are no direct criteria to compare the outcome of the dietary exposure test with B criterion, such a conclusion can only be drawn if the substance clearly fulfils the B criterion or clearly does not (i.e. both the benchmarking approach in which BMF are compared to BMFs for known POPs and PBTs obtained under the same conditions and the method to derive a BCF calculated from the depuration rate from the dietary study in combination with an estimated uptake rate constant warrant a conclusion not B, B, or vB).
- 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-lives from monitoring studies in humans or wildlife. Often, this type of information yields variable results, which renders it insufficient to draw a conclusion on the bioaccumulation potential. Instead, this type of information will merely be used in a *Weight-of-Evidence* approach to support results from other studies.

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 to 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.

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—5). 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.

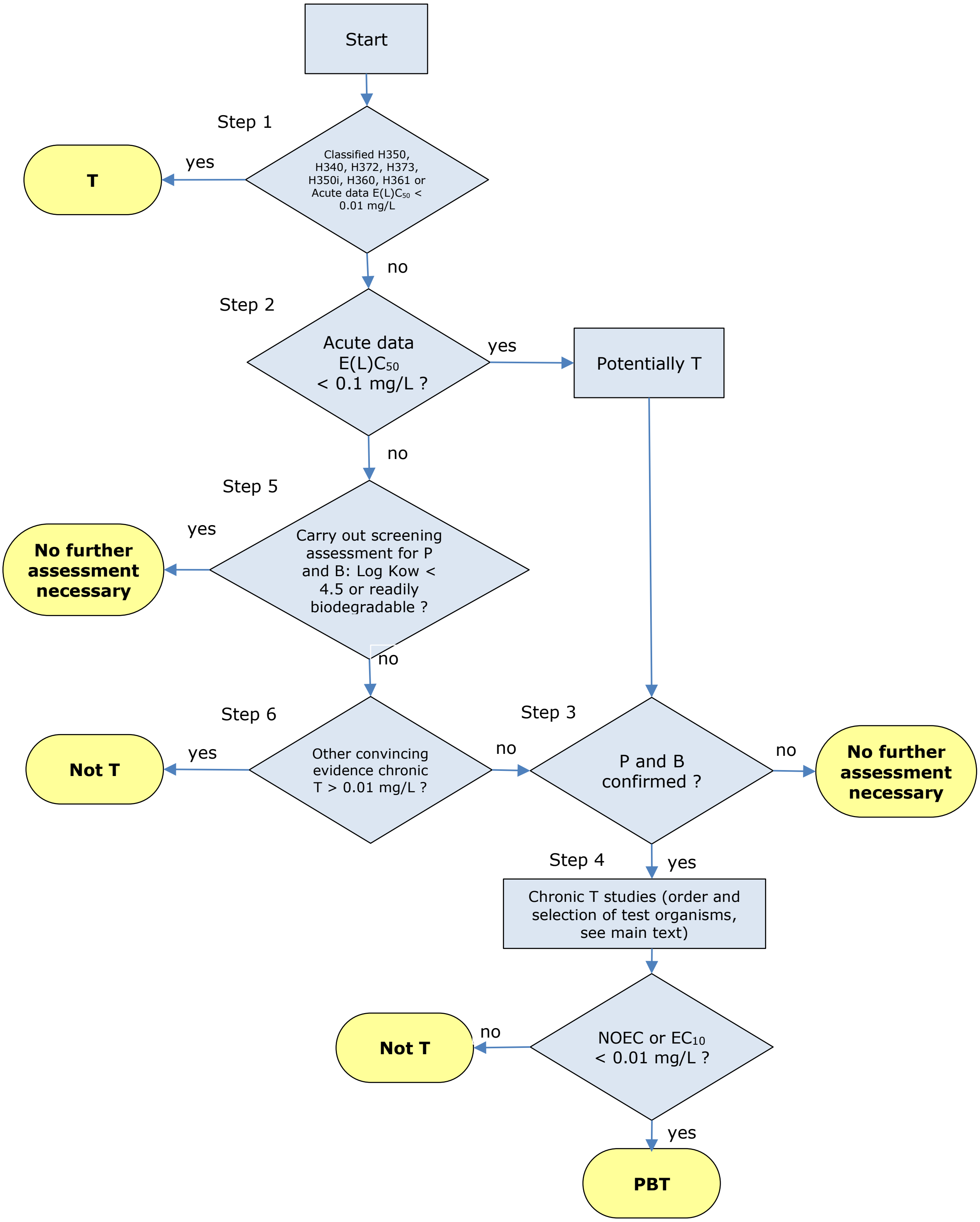


Figure R.11—5: T testing in support of PBT assessment for the aquatic environment.

According to Article 14 of REACH, PBT assessment starts at levels ≥ 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 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 Kow ≤ 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 chemicals 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:

- 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[29].

The evidence of CMR and chronic toxicity specified above does not only refer to substances that are already classified accordingly (i.e. DSD R-phrases: R45, R46, R48, R49, R60 – R63 or CLP hazard statements H350, H340, H372, H373, H350i, H360 and H361[30])[31] 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*)[32]. 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 (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 if data from invertebrates are equivalent to vertebrate data in the context of the PBT/vPvB-assessment. This is the case for aquatic toxicity testing but not for the B testing. 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

[29] 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.

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

[31] See Annex VII to CLP – (translation table from classification under DSD to classification under CLP)

[32] The criteria for classification of substances and mixtures in hazard classes and in their differentiations is provided in Annex I to the CLP Regulation, Mixtures must be classified and labelled according to the CLP Regulation from 1 June 2015 but may be classified according to Directive 1999/45/EC until then.

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 (EqP) theory. The EqP theory 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 EqP theory 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:

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

$NOEC(EC10)_{water}$ *(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 Koc of the substance as $Kp_{susp} = Foc_{susp}.Koc$ 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—6 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—6: 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[33]. 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} >5) 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—5).

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 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[34]. 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

[33] 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.

[34] 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.

been developed by OECD on toxicity testing of difficult substances (OECD, 2000b)[35]. 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. Only if this is not feasible due to the properties of the substance or due to disproportionate efforts, can the water accommodated fraction (WAF) be considered as last resort to generate exposure in a test (OECD, 2000b; 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 consistent basis for assessing the relative toxicity of poorly water soluble substances. Effects 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 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, 2000b; 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). By its nature the WAF-method is testing several constituents. Where toxicity is exhibited, this can be problematic when a test substance containing several constituents is used. In such a case, the toxicity cannot be allocated to specific constituents directly, but the 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 substance. The loading cannot be compared to the toxicity criterion. Only in the case of analytical verification of the water-soluble fraction this type of tests might be used in the T 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—6. In order to be suitable, the QSAR prediction should comply with the general principles described in Chapter R.6.1. 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

[35] As of December 2016, the OECD "guidance document on aquatic toxicity testing of difficult test chemicals" is under revision. The revised version will introduce additional recommendations for poorly water-soluble chemicals, and in particular with regard to the use of liquid/liquid saturator units and of passive dosing.

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, not for concluding “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. <u>Further information for the PBT/vPvB assessment is needed.</u>

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, 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.

Wheresupplementary 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 do not 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 <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.* A mono-constituent substance has a main constituent present at a concentration of 80% or more with PBT and/or vPvB properties;
- The substance is PBT/vPvB. It (as mono-constituent substance, well-defined multi-constituent substance or UVCB substance) contains one or more relevant[36] (group(s) of) constituent(s)[37] which fulfil the PBT and/or vPvB criteria[38];
- The substance is PBT/vPvB. One or more (group(s) of) constituent(s), impurity or additive degrades or is transformed into substance(s) which fulfil the PBT and/or vPvB criteria and these transformation or degradation products are formed in "relevant"[36] 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.

[36] "Relevant" is defined in section R.11.4.1.

[37] "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*.

[38] 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.

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
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[39].

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).

[39] 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.

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 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 degradation/transformation 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. For illustrative purposes certain antioxidants are used as examples (see List of Antioxidants, Appendix R.11—2).

General considerations

In Appendix R.11—1 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 see Appendix R.11—2,

Table R.11—10, Antioxidants No. 2, 4, 6-17 as examples) 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 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. Catabol (Mekenyan, 2006). For all relevant metabolites 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

The low bioaccumulation potential indicated by the Log K_{ow} > 10 should be supported by additional information (see Appendix R.11—1 "Indicators for limited bioaccumulation"). 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 criterion (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 criteria (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 metabolites are neither PBT nor vPvB. In this case the (parent) substance is not vPvB.

Log K_{ow} >10 and no ready biodegradability confirmed

If at least one additional indicator for limited bioaccumulation is fulfilled and potential metabolites 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.

Examples for the above assessment strategy are presented in Appendix R.11—2 "Assessment of substances requiring special consideration during testing".

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, Table R.11—12, in Appendix R.11—2).

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 Appendix R.11—1 "Indicators for limited bioaccumulation" 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 chemical 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 compound 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, 2000) often overestimate the actual K_{ow} of such substances e.g. organic pigments (Table R.11—7). 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—7). 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, Appendix R.11—2, Table R.11—15). 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—7 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, 2000) shows that the estimated K_{ow} exceeds C_o/C_w by between 1 and 8 orders of magnitude (more data see Appendix R.11—2).

Table R.11—7: Solubility of some pigments and comparison of their Co/Cw values with estimated K_{ow}s

(US EPA, 2000)

Colour Index Name	Mol weight (g/Mol)	Co (µg/L) at ambient temperature	Cw (µg/L) at ambient temperature	Log Co/Cw	Log K_{ow} (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 Appendix R.11—1 "Indicators for limited bioaccumulation", 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 (1) 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. An example for this type of assessment and conclusion is presented in Appendix R.11—2 under “2. Example for an assessment strategy for substances with low octanol and water solubility”.

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[40] 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 [add reference]. 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 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

40 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*.

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 tested in degradation and ecotoxicity tests could be narrowed further. Due to animal welfware 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).

Several examples of authority assessments of multi-constituent substances are provided in [add reference].

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 natural complex 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… [41] 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 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

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[41] 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

with new methods, e.g, as reviewd 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%. 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” [42]), 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.

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[42] Presentation by Magaud H *et al.* at SETAC Europe 25th Annual Meeting (3-7 May 2015 - Barcelona, Spain): Abstract 311 available at: https://c.ymcdn.com/sites/www.setac.org/resource/resmgr/Abstract_Books/SETAC-Barcelona-abstracts.pdf).

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 fulfill either the vPvB or PBT criteria, the entire (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) [add reference to the example submitted later].

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);
- 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[43]). 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 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 "Test items" 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).

   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).

   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.

ii. The so-called **block method**: this method is applied when a substance can be divided

[43] See for example this decision on substance evaluation: https://echa.europa.eu/documents/10162/d489cc70-7b49-46d8-b208-56e5b738a35e

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.

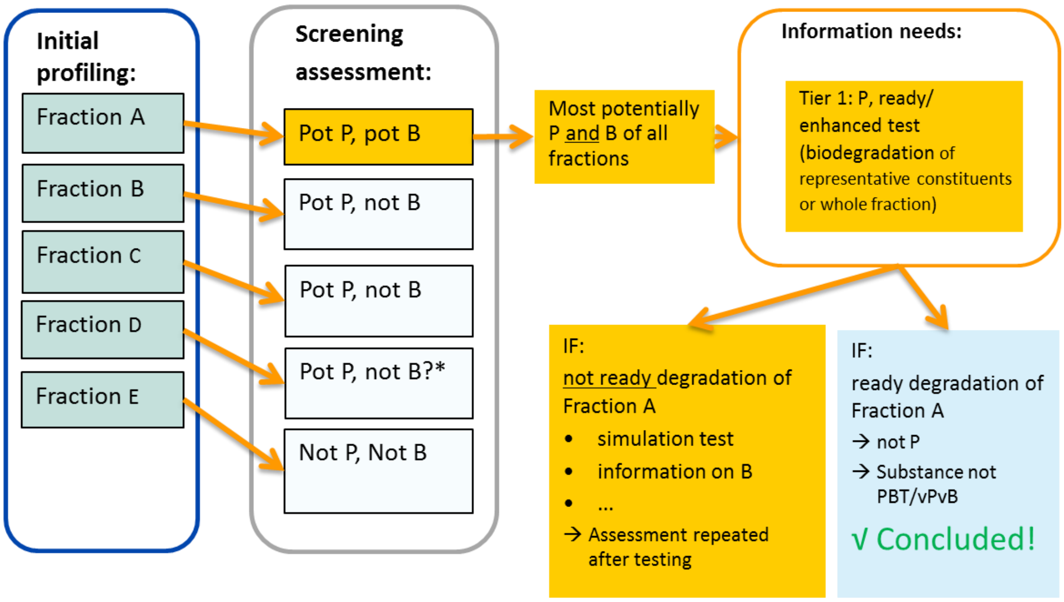
Advantages of the "fraction profiling approach" are, *i.a.*:

- 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—6 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—6: 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. For such similarity criteria, please refer to Chapter R.6 of the *Guidance on IR&CSA*.

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.

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 Kow 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 ecotox testing (see discussion in Section R.11.4.1.3).
- Isolation or synthesis of relevant constituen(s) may not be technically feasible.

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 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—3.

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.)

Finally, 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

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 genuinely 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 assessment to screen 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 Kow <4.5, it may be assumed that all constituents of the substance have logKow < 4.5. For interpretation of suchresults and estimates, please see Section R.11.4.1.2.

(iii) Toxicity

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) 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 concentrations of individual constituents.

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 synthetised 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 otherfraction 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 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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R.11.5 References

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Appendices

Appendix R.11—1: Indicators for limited bioconcentration for PBT assessment

Appendix R.11—2: Assessment of substances requiring special consideration during testing

Appendix R.11—3: PBT assessment of UVCB petroleum substances

Appendix R.11—4: Bioconcentration studies with benthic and terrestrial invertebrate species (BSAF)

Appendix R.11—1: Indicators for limited bioconcentration for PBT assessment.

Summary

This document was originally drafted as part of an ECETOC report on the use of alternatives in assessing the environmental safety of chemicals (ECETOC, 2005). Subsequently, the TC NES (Technical Committee for New and Existing Substances) subgroup addressing persistent, bioaccumulative and toxic (PBT) and very persistent/very bioaccumulative (vP/vB) chemicals (PBT working group) considered the recommendations and agreed to use them as part of the strategy of determining whether a chemical should be placed on a screening PBT/vPvB list and/or should be tested to determine whether it is B/vB. The document has been altered as a result of discussions in the PBT WG, and the following is the last version of the text being discussed by the TC-NES WG on PBTs[44].

The indicators below should not be considered as definitive, but should be considered with other information, e.g. data derived from toxicokinetic and/or chronic mammalian studies. Such data indicating extremely low or no uptake and/or no chronic systemic toxicity will increase confidence in the use of the guiding indicators below. The TC-NES WG on PBTs, therefore will consider the following provisional indicators case by case by employing expert judgement in assessing chemicals (note each term, their definition and derivation as well as the recommended values are further discussed later).

Used within a *Weight-of-Evidence* approach and with expert judgment a chemical may be considered as not **B** (i.e. unlikely to have a BCF > 2,000) using the following types of evidence:

1. ***An average maximum diameter (Dmax aver) of greater than 1.7 nm[45] plus a molecular weight of greater than 1100***
2. ***a maximum molecular length (MML) of greater than 4.3 nm[46]***
3. ***Octanol-water partition coefficient as Log10 (Log K_{ow}) > 10***
4. ***measured octanol solubility (mg/L) < 0.002 mmol/L × MW (g/mol) (without observed toxicity or other indicators of bioaccumulation)***

In addition to indicators 2, 3 and 4 above, and again within a *Weight-of-Evidence* approach and with expert judgment, an indicator for considering a chemical as possibly not being a **vB** (i.e. unlikely to have a BCF > 5,000) is if it has:

- a $D_{max\ aver}$ of greater than 1.7 nm[45] plus a molecular weight of greater than 700

In using the indicators above it should be noted that 1 and 2 are generally considered as potential barriers to uptake, 3 is considered a general indicator of uptake, distribution and availability (i.e. bioaccumulation in lipid containing parts of the organism) and the fourth parameter an indicator of potential mass storage in lipid tissues.

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[44] Please note that only editorial changes to the text of the TC-NES PBT WG were made during the first revision of this Guidance.

[45] 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.

[46] Please note that this indicator value 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).

Evidence of high biotransformation/metabolisation rate in fish may be used in support for the above mentioned indicators. Similar evidence in mammalian species may also be considered, though the possibility that mammalian species may transform chemicals at a higher rate than fish should be considered.

Evidence of significant uptake in fish or mammals after longer time exposure would imply that the indicators 1-3 above should not be used.

Discussion

Assessing the potential of chemicals to bioconcentrate - indications for reduced or hindered uptake

The magnitude of bioconcentration (i.e. the BCF) or bioaccumulation (i.e. the BAF) of a chemical in an (aquatic) organism is estimated by a ratio of the concentration of the chemical in the body of the animal to that of the environment or food. The BCF or BAF is the result of four processes, which occur when a chemical is taken up from an animal's surrounding environment or food. The BCF refers to the process where uptake is only via aqueous exposure, the BAF takes into account multiple uptake routes. The four processes are:

- Absorption - after the introduction of a chemical through food, water, air, sediment, or soil, its transport across a biological membrane into systemic circulation e.g. across fish gills, intestine, skin (Hodgeson and Levi, 1994).
- Distribution - after absorption, a chemical may bind to plasma proteins for circulation throughout the body, as well as to tissue components like fat or bone. The chemical may be distributed to a tissue and elicit a toxic response; other tissues may serve as permanent sinks, or as temporary depots allowing for slow release into circulation (Hodgeson and Levi, 1994).
- Metabolism - after reaching a tissue, enzymes may biotransform the chemical. During Phase I, a polar group is normally introduced into the molecule, which increases its water solubility and renders it a suitable substrate for Phase II reactions. In Phase II, the altered molecule combines with an endogenous substrate and is normally readily excreted. Metabolism is often a detoxification mechanism, but in some cases, metabolism may activate the parent compound and intermediates or final products may cause toxicity (Hodgeson and Levi, 1994).
- Excretion - a chemical with similar characteristics, primarily water solubility, to endogenous waste is eliminated by the same mechanisms. Chemicals with nutritional benefit may be broken down and ultimately exhaled as CO_2; volatile substances may also be exhaled directly through the lungs, Polar molecules that are freely soluble in plasma are removed through renal filtration and passed into urine. Fat soluble chemicals may be conjugated and excreted in bile (faeces) (Hodgeson and Levi, 1994).

In addition to excretion, growth of the organism may also be relevant in reducing the chemical concentration in the organism when the rates of other elimination processes are of the same order of magnitude as the dilution due to growth rate. Elimination through the transfer of chemical to the offspring through gestation or lactation may also be important.

This section describes several chemical properties that limit the absorption and distribution of a chemical, which would sufficiently hamper the uptake, distribution or the body burden of a chemical so that the BCF can be assumed to be of no or limited concern. Metabolism, excretion processes and growth also lead to a reduction of BCF/BAF but are not discussed in this paper.

Regulatory context

This text should be seen in the context of the European PBT and vPvB assessment of chemicals with a focus on the B or vB-assessment. Currently, if a substance has a calculated or measured BCF > 2,000 it fulfils the criterion for B. If it has a calculated or measured BCF > 5,000 it fulfils the criterion for vB. Based on a screening threshold value, a substance could be either B or vB when its (estimated) Log K_{ow} is > 4.5. In this case, if a substance meets the screening criterion for B or vB and it is also shown to be or likely to be (very) persistent, further consideration of its bioaccumulation potential is warranted. This may include critical review of its bioaccumulation potential according to (Q)SARs and bioaccumulation models taking into account its potential for uptake and metabolism (EC, 2003). The result of such an assessment may be so uncertain that further bioconcentration or bioaccumulation testing may have to be undertaken to determine whether the substance is B or vB.

Experimental testing to determine the BCF

The standard test to study the BCF in fish is the OECD TG 305 (bioconcentration test guideline). In this guideline, BCF is experimentally estimated using a flow through exposure regime with an initial uptake phase of up to 28 days followed by a depuration phase in clean water. The BCF can be estimated from the ratio C_f/C_w (C_f: concentration of test chemical in fish at steady state; C_w: concentration of test chemical in the exposure phase (water) or K_u/K_d (K_u: rate constant for uptake and K_d: rate constant for depuration; provided that first order – one compartment kinetics apply). In cases where substances meet the screening threshold value for B or vB, it is probable that these substances are very hydrophobic and have a very low aqueous solubility. Due to these properties it can be very difficult to test them in aqueous exposure systems such as an OECD TG 305 study. Alternatively, a recently developed dietary test (Anonymous, 2004) could be used to determine bioaccumulation potential through food or to derive data to estimate a BCF. However, many studies to determine the BCF of hydrophobic substances have been performed following aqueous exposure. The interpretation of such studies must be done with care. Many such studies were conducted following earlier versions of the OECD TG 305, and may include the following possible artefacts or shortcomings:

- Difficulties in measuring the 'true' aqueous concentration due to sorption of the substances to particulate and dissolved (organic) matter;
- Unstable concentration of the test substance in water and thus highly fluctuating exposure conditions
- Adsorption of the test chemical to glass walls or other materials;
- Volatilisation.
- Testing at concentrations clearly above the water solubility of the test chemical, normally via the inclusion of dispersants or vehicles which would lead to an underestimation of the BCF
- Determination of a BCF as the ratio between the concentration in fish and in water but under non steady state conditions

It is important to realise that in many of the studies that have investigated relationships between molecular dimensions and reduced uptake, i.e. based on 'lower' BCFs than expected, it was not always possible to exclude occurrence of some of the above mentioned shortcomings or artefacts and truly reduced uptake. Thus rules relating to molecular dimensions or mass proposed in the past and claiming reduced uptake should be critically reviewed.

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 uptake, i.e. from food, where other uptake mechanisms may play a role. 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 hydrophobic chemicals that significantly accumulate via the food, even for gill breathing organisms, but particularly for predatory fish higher in the food chain.

Mechanisms of absorption

The route a chemical follows from the point of initial exposure to the site of action or storage involves passage through a number of tissues and every step involves the translocation of the chemical across multiple membranous barriers (e.g. mucosa, capillary wall, cell membrane), each containing distinct lipid types and proteins. Four primary mechanisms operate to absorb a compound into the body from the environment (Hodgeson and Levi, 1994):

Passive transport - molecules diffuse across cell membranes into a cell, and they can pass between cells.

Active transport - like passive transport, works in both directions to absorb and exsorb a wide range of chemicals. This special protein, or carrier-mediated, transport is important for gastrointestinal absorption of essential nutrients. In rare instances, toxicants can be actively transported into the cell. Efflux proteins, such a P-glycoprotein, shunt molecules out of the cell. Because of the specificity of this mechanism, it cannot be generally modelled.

Filtration - small molecules can fit through channels, but molecules with molecular weights (MWs) greater than 100 g/Mol are excluded. Most compounds have limited access through these pores; filtration is considered more important for elimination than absorption.

Endocytosis - the cell membrane flows around the toxicant to engulf it and transfer it across the membrane. This mechanism is rare except in isolated instances for toxicants, such as for carrageenans with MW around 40,000 g/mol.

This appendix focuses on passive transport as the significant mechanism of absorption for most toxicants. This mechanism is the only one that can be modelled due to recent work to determine the physico-chemical parameters affecting simple diffusion across a membrane.

Molecular properties

Lipinski *et al.* (1997) first identified five physico-chemical characteristics that influence solubility and absorption across the intestinal lumen using more than 2,200 drug development tests. These characteristics have been rigorously reviewed (Wenlock *et al.*, 2003; Proudfoot, 2005), used to develop commercial models to estimate absorption in mammals, and are commonly used by the human and veterinary pharmaceutical industry. Although less research in absorption, distribution, metabolism and excretion (ADME) processes have been conducted in fish, data indicate significant similarity among all vertebrates, as described below.

'Lipinksi's Rule of 5' allows the prediction of poor solubility, and poor absorption or permeation from chemical structure. A chemical is not likely to cross a biological membrane in quantities sufficient to exert a pharmacological or toxic response when it has more than 5 Hydrogen (H)-bond donors, 10 H-bond acceptors, molecular weight > 500, and has a Log K_{ow} value > 5 (Lipinksi *et al.*, 1997). Wenlock *et al.* (2003) studied about 600 additional chemicals and found that 90% of the absorbed compounds had < 4 Hydrogen (H)-bond donors, < 7 H-bond acceptors, molecular weight < 473, and had a Log D value < 4.3. More recent work by Vieth *et al.* (2004) and Proudfoot (2005) supports the lower numbers. Molecular charge and the number of rotational bonds will also affect absorption by passive diffusion across a membrane or diffusion between cells.

Although these studies on almost 6,000 substances focussed on absorption, generally of per orally dosed drugs across the intestinal wall, the similarity in tissue structures of mammals and fish imply the equations and concepts can be reapplied to estimate absorption in fish. The 'leakiness' of a tissue, or its ability to allow a chemical to passively diffuse through it, can be measured using trans-epithelial electrical resistance (TEER) and can be used to compare tissue capabilities. A low TEER value indicates the tissue has greater absorption potential. Data indicate that fish and mammalian intestines are equally 'leaky' and that fish gills are more restrictive, similar to the mammalian blood brain barrier (Table R.11—8). The table also shows whether P-glycoprotein has been detected and could be a functional efflux protein active in the tissue.

Table R.11—8: Tissue absorption potentials

Tissue	P-glycoprotein efflux?	TEER ohm cm^2	References
Fish intestine	Yes	25-50	Trischitta *et al.* (1999)
Mammal intestine	Yes	20-100	Okada *et al.* (1977); Sinko *et al.* (1999)
Blood-brain barrier	Yes	400-2000	Borchardt *et al.* (1996)
Fish gill	Yes	3500	Wood and Pärt (1997)
Human skin	No	20,000	Potts and Guy (1997)

Octanol-water partition coefficient (Log K_{ow})

Following an assessment of the database used by Dimitrov *et al*. (2002), a cut-off for the Log K_{ow} of 10 has been suggested, which used within a *Weight-of-Evidence* scheme supports the observation that a substance may not be B/vB (see Appendix R.11—1 Annex 1).

It should be noted that there are very few reliable measured values of Log K_{ow} above 8 and that measurements in this region are very difficult (see Section R.7.1.8 in *Chapter R.7a* of the *Guidance on IR&CSA*). Consequently, measured values above 8 must be carefully assessed for their reliability. It is a consequence of this lack of data that most models predicting Log K_{ow} are not validated above a Log K_{ow} value of 8. Such predictions should therefore be considered in qualitative terms. As described in Appendix R.11—1 Annex 1, based on the current limited knowledge (both with respect to measured Log K_{ow} and BCFs), a calculated Log K_{ow} of 10 or above is taken as an indicator for showing reduced bioconcentration.

Molecular size

Molecular size may be considered as a more refined approach, taking into account molecular shape and flexibility explicitly rather than molecular weight alone. However, in the following section, certain definitions are needed;

- 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. In the document the average value for this 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.

These definitions are shown graphically in Annex 2 to this Appendix, together with examples of software that may be used for their calculations.

In the discussions although various values are referred to, the PBT WG recognise that firstly these values will probably alter as experience and the available data increase, and that secondly the actual value for a molecule's D_{max}, will depend on the conformer used and to a degree the software used. In interpreting the data these uncertainties need to be borne in mind.

Opperhuizen *et al.* (1985) found a limiting molecular size for gill membrane permeation of 0.95 nm, following aqueous exposure. In their study on polychlorinated naphthalenes (PCNs), bioconcentration increased with increasing hydrophobicity, i.e. the degree of chlorination, with uptake and elimination rate constants comparable to those of chlorinated benzenes and biphenyls. For the PCN-congeners studied, BCFs increased with increasing hydrophobicity up to higher Log K_{ow} values (>10^5). No further increase was observed at higher K_{ow} values. For the hepta- and the octachloronaphthalenes no detectable concentrations were found in fish. It was suggested that the absence of increasing bioconcentration was due to the inability of the hepta- and octachloronaphthalenes to permeate the gill lipid membrane, due to the molecular size of these compounds, brought about by the steric hindrance of the additional chlorine atoms. A cut-off of 0.95 nm was proposed as the cross-sectional diameter which limited the ability of a molecule to cross the biological (lipid) membrane.

Anliker and Moser (1987) studied the limits of bioconcentration of azo pigments in fish and their relation to the partition coefficient and the solubility in water and octanol. A tetrachloroisoindolinone type and a phenyl azo-2-hydroxy-naphthoicacid type, both had low solubility in octanol, < 1 and < 0.1 mg/L, respectively. Their cross-sectional diameters were 0.97 nm and 1.68 nm, respectively. Despite the high Log K_{ow} calculated for these chemicals, the experimentally determined Log BCFs were 0.48 and 0.70, respectively. The explanation for this apparent inconsistency of high Log K_{ow} and low BCF is the very limited absorption and fat (lipid) storage potential of these pigments, indicated by their low solubility in n-octanol (see next sub-chapter) and their large molecular size.

Anliker *et al.* (1988) assessed 23 disperse dyestuffs, two organic pigments and a fluorescent whitening agent, for which the experimental BCFs in fish were known. Sixteen halogenated aromatic hydrocarbons were included for comparison. Two characteristics were chosen to parameterise the size of the molecules: the molecular weight and the second largest van der Waals diameter of the molecules, measured on conformations optimised by force field calculations (Opperhuizen *et al.,* 1985). None of the disperse dyestuffs, even the highly lipophilic ones with Log K_{ow} > 3, accumulated significantly in fish. Their large molecular size was suggested to prevent their effective permeation through biological membranes and thus limit their uptake during the time of exposure. Anliker *et al.* (1988) proposed that a second largest cross section of over 1.05 nm with molecular weight of greater than 450 would suggest a lack of bioconcentration for organic colorants. While some doubts have been raised concerning the true value of the BCFs in these papers, as experiments were conducted at exposure concentrations in excess of the aqueous solubility, the data support the underlying hypothesis for reduced uptake for larger molecules.

Other studies addressing molecular dimensions have included Opperhuizen *et al.* (1987) who proposed that a substance greater than 4.3 nm would not pass membranes at all, either in the gills or in the gut based on a series of bioaccumulation and bioconcentration studies with linear and cyclic polydimethylsiloxanes (PDMS or "silicones") varying in chain length. To allow such large substances to pass is very unlikely since it would mean that the entire interior of the lipid membrane would be disturbed. Molecular weight did not explain reduced uptake, since one of the substances with a molecular weight of 1,050 was found in fish. The cross-sectional diameter of these substances could in itself also not explain the reduced uptake since those were smaller or equal to those of PCBs that did bioaccumulate strongly.

Opperhuizen *et al.* (1987) also referred to a study by Hardy *et al.* (1974) where uptake of long chain alkanes was disturbed for alkanes longer than $C_{27}H_{56}$ in codling. This chain length corresponds to a molecular dimension, i.e. molecular length, of 4.3 nm, equal to the length of the PDMS congener where reduced uptake was observed.

Loonen *et al.* (1994) studied the bioconcentration of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and found that the laterally substituted (2,3,7,8 substituted) were bioconcentrated while the non-laterally substituted were not. The main reason for this was attributed to metabolism (previously reported by Opperhuizen and Sijm, 1990, and Sijm *et al.*, 1993b), however, lower lipid solubility and lower membrane permeability were also considered to have played a role in the reduced BCFs observed. The non-accumulating structures would all have exceeded the effective cross-sectional diameter of 0.95 nm.

Although the lack of bioconcentration of some chemicals with a cross section of > 0.95 nm has been explained by limited membrane permeability, a number of other studies have demonstrated the uptake of pollutants with large cross sections (e.g. some relevant dioxin and PBDE congeners) by fish and other species. Therefore a simple parameter may not be sufficient to explain when reduced BCF/BAF occurs. Dimitrov *et al.* (2002, 2003, 2005**)** have tried to develop a more mechanistic approach to address this concept, using molecular weight, size, and flexibility in their BCF estimates.

In a review made by Dimitrov *et al.* (2002) it is suggested that for compounds with a Log K_{ow} > 5.0, a threshold value of 1.5 nm for the maximum diameter, $D_{max\ ave}$, could discriminate chemicals with Log BCF > 3.3 from those with Log BCF < 3.3. This critical value was stated to be comparable with the architecture of the cell membrane, i.e. half the thickness of the lipid bilayer of a cell membrane. This is consistent with a possible switch in uptake mechanism from passive diffusion through the bilayer to facilitated diffusion or active transport. In a later review paper, Dimitrov *et al.* (2003) used this parameter to assess experimental data on a wide range of chemicals. Their conclusion was that a chemical with $D_{max\ ave}$ larger than 1.5 nm would not have a BCF > 5,000, i.e. would not meet the EU PBT criteria for vB chemicals. More recently, Dimitrov *et al*., 2005, have revised this figure to 1.7 ± 0.02 nm following further assessment of the data set published. It is likely that the absolute value for this D_{max} may alter with further assessment and generation of database containing high quality BCF values.

Currently a value of 1.7 nm is recommended, however, with more experience and data this value may alter. Indeed it is recommended that the BCF data used in the various papers cited (Dimitrov *et al*., 2002, 2003 and 2005), and in particular the data for the larger molecules, for which the testing is undoubtly difficult, undergo critical quality and reliability review. Further assessment of these cut-offs should also be conducted following publication of the CEFIC LRI database containing high quality BCF data.

Conclusion: Again there would appear to be no clear cut-off. While recognising the uncertainties in the interpretation of experimental results, it is recommended that:

- Possibly not B : a $D_{max\ ave}$ of > 1.7 nm plus a molecular weight greater than 1100
- Possibly not vB : a $D_{max\ ave}$ of > 1.7 nm plus a molecular weight greater than 700
- Possibly not B and possibly not vB: A maximum molecular length of 4.3 nm may suggest significantly reduced or no uptake. This criterion appears, to be based on older studies and a limited number of chemical classes and should be treated with caution until further case studies are generated;

Solubility in octanol

The concept of having a value relating a chemical's solubility in octanol to reduced BCF/BAF is derived from two considerations: firstly, that octanol is a reasonable surrogate for fish lipids, and secondly, that, if a substance has a reduced solubility in octanol (and therefore by extrapolation in lipid) this may result in a reduced BCF/BAF. The former is reasonably well understood and indeed forms the basis of the majority of models for predicting BCF using Log K_{ow}. Further, octanol solubility (or better, the ratio of n-octanol/water solubilities) can characterise the transport of some small molecular sized, neutral compounds through biological membranes (Józan and Takács-Novák, 1997).

When a substance has a low solubility in octanol (S_{oct}) as well as a low solubility in water (S_w), the resulting ratio S_{oct}/S_w could range from very low to very high, with no clear idea on how this would affect the magnitude of the BCF/BAF. Still, it could be argued that a very low solubility in octanol could be used as an indication that only low body burdens can be built up in an aquatic organism (however, this may not apply to other mechanisms of uptake, and when the bioaccumulation may not be related to the lipophilicity of the chemical, e.g. when there is binding to proteins.

Chessells *et al.* (1992) looked at the influence of lipid solubility on the bioconcentration of hydrophobic compounds and demonstrated a decrease in lipid solubility with increasing K_{ow} values for superhydrophobic compounds (Log K_{ow} > 6). It was suggested that this led to reduced BCFs. Banerjee and Baughman (1991) demonstrated that by introducing a term for lowered octanol/lipid solubility into the Log K_{ow} BCF relationship, they could significantly improve the prediction of bioconcentration for highly hydrophobic chemicals.

Body burdens

The meaningful implication of bioaccumulation that needs to be addressed for PBT chemicals, e.g. as in the EU TGD (ECB, 2003), is to identify the maximum concentration(s) in organisms that would give rise to concern. The concept of critical body burdens (CBB) for acute effects is reasonably well established (McCarty and Mackay, 1993; McCarty, 1986) especially for chemicals that act via a narcosis mode of action. Recently there have been a number of reviews of this concept, Barron *et al.* (1997, 2002), Sijm and Hermens (2000) and Thompson and Stewart (2003). These reviews are summarised as follows:

- There are very few data available, especially for specifically acting chemicals and for chronic effects, upon which to make decisions relating to generic CBBs;
- The experimental data for CBBs show considerable variation both within specific modes of action and for those chemicals with a specific mode of toxic action. The variation appears to be around one order of magnitude for the least toxic type of chemicals (narcotic chemicals) but extends over several orders of magnitude for chemicals within the same types of specific toxic action. Much of the variability in CBBs can probably be explained by differences in species sensitivities, biotransformation, lipid content, whether the measurements relate to organ , whole body or lipid and whether the chemical was correctly assigned to a mode of action category;
- Some of the data in these reviews need to be checked for quality and need clear interpretation, particularly, those
    - Studies based on total radiolabel, and
    - Studies that quote no effect data which were derived from tests without establishing either a statistical NOEC (EC10) and/or a dose response curve.

Notwithstanding this, it may with some caution be possible to group ranges of CBB values for specific modes of toxic action. This is easier for narcosis type mode of actions, and becomes increasingly prone to error moving towards more specifically acting chemicals.

Table R.11—9 summarises three sources of information:

1. ***Sijm (2004) - an expert judgement view to arrive at an approximate single value based on three references, McCarty and Mackay (1993), Van Wezel and Opperhuizen (1995) and Sijm and Hermens (2000).***

2. ***Thompson and Stewart (2003) - based on a literature review, the data range beyond the narcosis mode of actions has been drawn from their report.***

3. ***Barron et al. (2002) - based on Figure 10 of Barron et al. (2002).***

When comparing the expert judgement of Sijm to the ranges indicated and to the figures in the respective publications, it is clear that the values chosen are in the approximate mid-point of the ranges/data. However, there is clearly a lot of variability and therefore uncertainty in deciding on the actual CBB value to use. Choosing the value of 0.001 mmol/kg ww (mid-point for respiratory inhibitors) allows for approximate protection for all the modes of action with the exception of the most toxic chemicals. The rationale for this choice would be that chemicals that act by the most specific mode of toxic action would probably be toxic (T) and hence sufficiently bioaccumulative to be of immediate concern.

Table R.11—9: Summary of various ranges of CBB - lethality (mmol/kg ww).

Mode of action and source	Narcosis	AChE inhibitors	Respiratory inhibitors
Sijm (2004)	2	0.01	0.001
Thompson and Stewart (2003)	2-8	0.000001 – 10	0.000001 – 10
Barron *et al.* (2002)	0.03 – 450	0.00004 – 29	0.00002 - 1.1 (CNS seizure agents)
McCarty and Mackay (1993)	1.7 – 8	0.05 - 2.7	0.00005 - 0.02 (CNS seizure agents)

Lipid normalising the chosen CBB of 0.001 mmol/kg ww, and assuming a lipid content of 5%, gives a lipid normalised CBB of 0.02 mmol/kg lipid or 0.02 × molecular weight mg/L lipid. However, given the uncertainty involved in deciding on the CBB that should be used, it is suggested that an application factor of 10, to account for species differences and organ versus body differences be applied to this solubility in lipid/octanol, giving an octanol solubility (mg/L lipid) of 0.002 × molecular weight. This would mean octanol solubilities of 1 and 2 mg/L n-octanol (or lipid), respectively, for substances with molecular weights of 500 and 1,000.

Conclusion: it is proposed that where a chemical has a solubility of less than (0.002 × molecular weight) mg/L in octanol it should be assumed that the compound has only a limited potential to establish high body burdens and to bioaccumulate. If it does bioaccumulate, it would be unlikely to give rise to levels in biota that would cause significant effects.

When there are fish or mammalian toxicity or toxicokinetic studies available, all showing no chronic toxicity or poor absorption efficiency, and a substance has, in addition, a low solubility in octanol, no further bioaccumulation testing would be needed, and the chemical can be assigned as no B, no vB. In theory, such a substance could elicit toxic effects after prolonged times in aquatic organisms. However, the chance such a thing would occur would be very low.

When there are no other studies available, and a substance has a low solubility in octanol, it is probable that other types of information (persistence, molecular size) would need be taken into account in deciding on bioaccumulation testing. It would also be helpful if testing, of the nature discussed above, were needed for other regulations, that might be useful in this evaluation, then the need for bioconcentration testing could be assessed when the new data became available.

Other indicators for further consideration

The two indicators, molecular size and lipid solubility, are the most frequently cited physical limitations for low bioconcentration. However, there are other indicators that could also be used for indicating whether the bioconcentration of a chemical is limited or reduced despite having a Log K_{ow} > 4.5. These include:

- Biotransformation - discussed in the TF report, ECETOC, 2005, (de Wolf *et al.*, 1992, 1993; Dyer *et al.*, 2003) and clearly needing development to improve how such information may be used;
- Other indicators for low uptake, these could for example include
  - lack of observed skin permeability (this alone not without substantiating that it is significant less than uptake in fish),
  - very low uptake in long term mammalian studies, and/or

- low chronic systemic toxicity in long term mammalian and/ or ecotoxicity (fish) studies.

Both these approaches would benefit from further research and investigation for their potential to indicate limited or reduced bioconcentration. While it is not recommended, based on the current level of information, to use such indicators alone to predict low bioconcentration, they can act as supporting information to other indicators in arriving at this conclusion.

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Appendix R.11—1 Annex 1

DEVELOPMENT OF A LOG K_{OW} CUT-OFF VALUE FOR THE B-CRITERION IN THE PBT-ASSESSMENT

The following assessment was based on the same data set used for development of the $D_{max\ ave}$ indicators (Dimitrov *et al.*, 2005, see main paper). Since publication the data set has been extended by Dimitrov. 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*.) and consequently the assessed data does not contain all the MITI data and may contain values that may not be considered as reliable by the TC-NES PBT WG. 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 (and those in the main paper) be re-evaluated when the CEFIC LRI Gold Standard database on BCF is available.

The fitted lines in Figure R.11—7, Figure R.11—8 and Figure R.11—9 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—7).

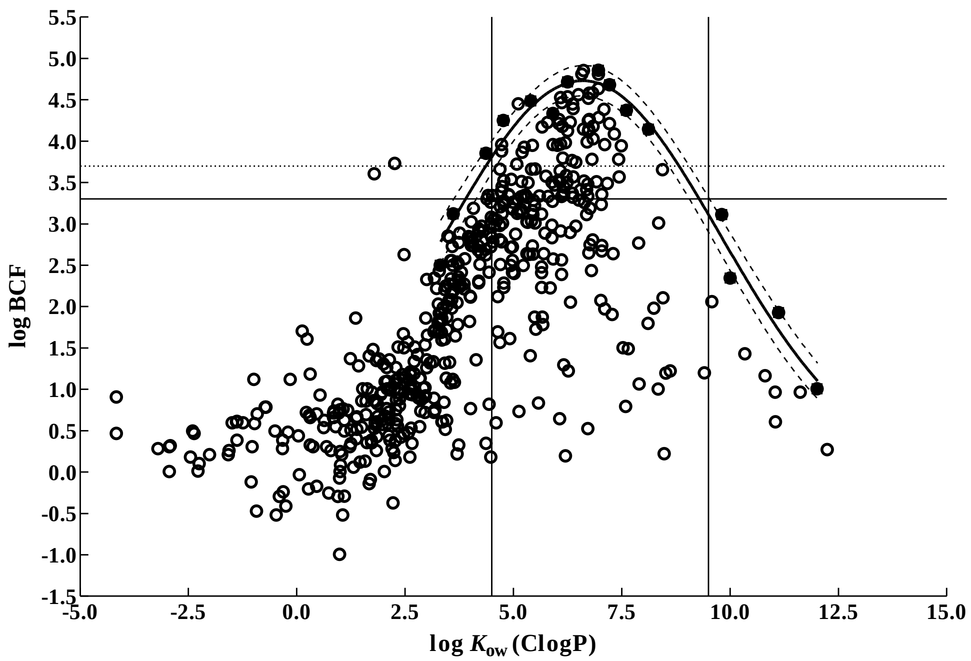


Figure R.11—7: Log BCF v calculated Log K_{ow}.

Figure R.11—8 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).

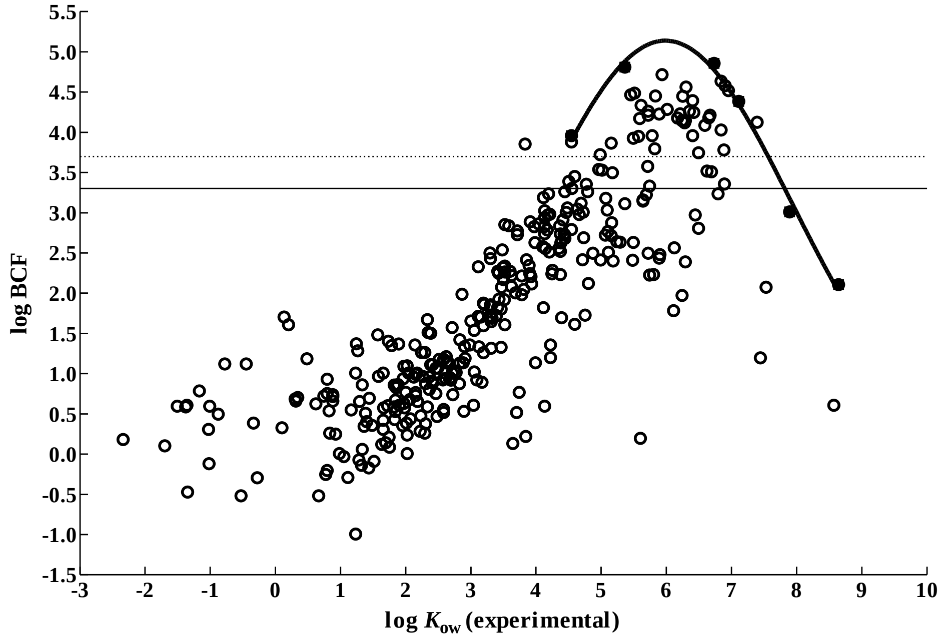


Figure R.11—8: LogBCF v measured log K_{ow}.

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—9). The data for very hydrophobic compounds 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—8. 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).

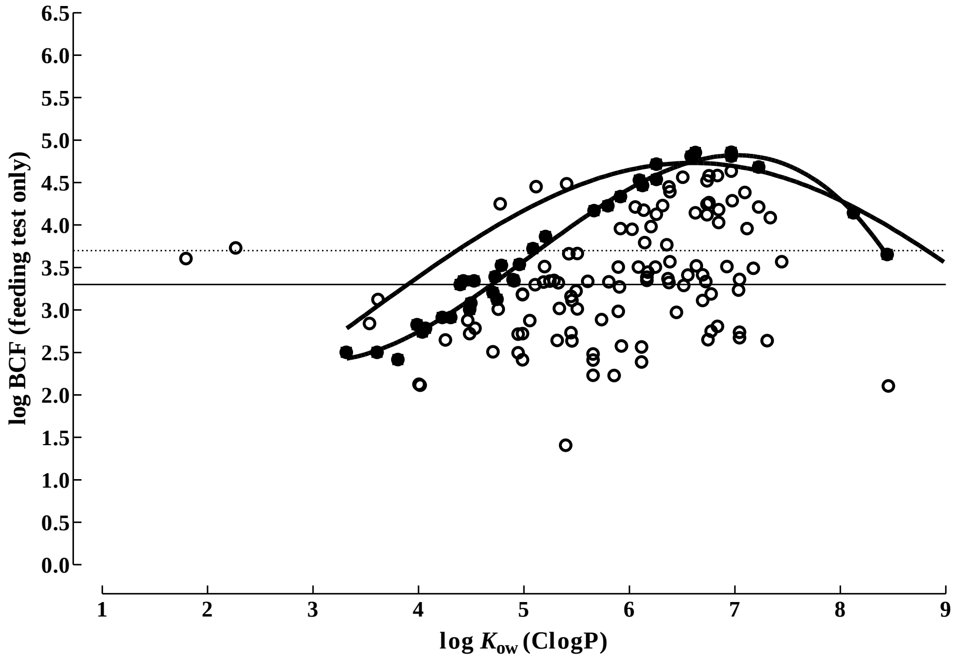


Figure R.11—9: LogBCF derived from feeding studies versus calculated Log K_{ow}.

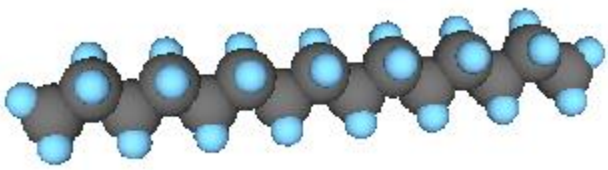
Summarized, the results of Figure R.11—7 to Figure R.11—9 suggest that the B-criterion is unlikely to be triggered for substances with a Log K_{ow} higher than 10. As with the other indicators described in the main paper, a Log K_{ow}-value higher than 10 should be used in a *Weight-of-Evidence* approach in combination with the other indicators.

Appendix R.11—1 Annex 2

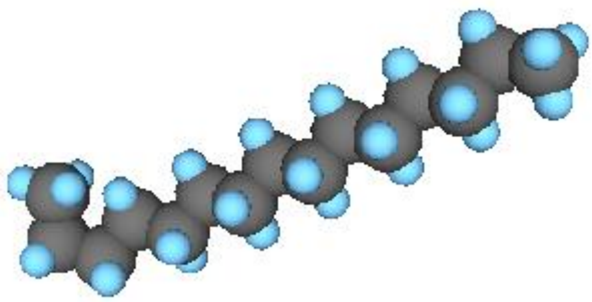
GRAPHIC DEFINITIONS FOR THE MOLECULAR DIMENSIONS USED IN THE MAIN PAPER

- 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.

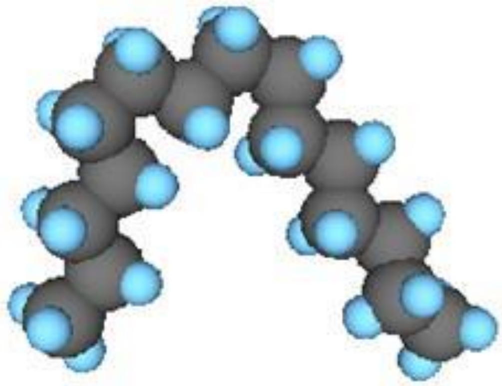
Conformer 1 (∆Ho = -84.5 kcal/mol), Dmax = 21.4; Deff = 4.99; Dmin = 4.92



Conformer 2 (∆Ho = -71.8 kcal/mol), Dmax = 19.8; Deff = 6.63; Dmin = 5.12



Conformer 3 (ΔHo = -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.

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.

OECD QSAR Toolbox

The development of this resource, which is currently in development, will include a database of chemical structures and associated information, CAS numbers etc. Currently, it is understood that included in the associated information will be a calculated D_{max}, derived by OASIS and based on a 2D structure. A value of this type should be used with extreme caution and as an indicator as to the possible utility of the approach. It is not recommended at this stage to use this value in the same way as a derived $D_{max\ ave}$ as described in the full paper.

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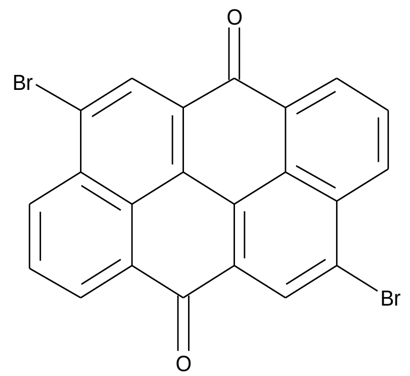
Appendix R.11—1 Annex 3

EXAMPLES - USE OF THE INDICATORS FOR LIMITED BIOACCUMULATION

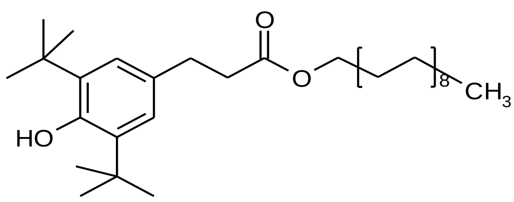
Example R.11-1

Indicator : n-Octanol solubility		
Name	Pigment Red 168	
CAS No.	4378-61-4	
Mol weight (g/Mol)	464	
Co (µg/L)	124	
CBB (µg/L)	928	
C_o < CBB	YES	
Log C_o/C_w	1.1	

Remark:

The n-octanol solubility C_o of Pigment Red 168 is well below the Critical Body Burden (CBB) which is an indicator of low bioaccumulation potential. In addition the Log C_o/C_w (octanol/water) is 1.1 which means low uptake through biological membrane.

Example R.11-2

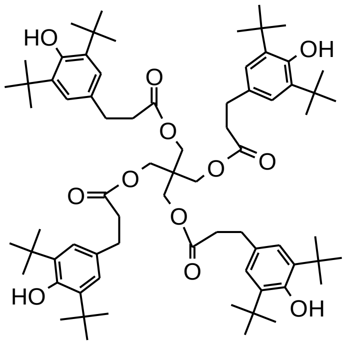
Indicator : K_{ow} > 10		
Name	ODBPA	
CAS No.	2082-79-3	
Mol weight (g/Mol)	531	
Log K_{ow}	13.4	

Remark:

ODBPA has a reduced potential for bioaccumulation.
In a Biodegradation test at low substance concentration and specific substance analysis ready biodegradability could be achieved. The transformation products formed are neither PBT nor vPvB.

Example R.11-3

Indicator : Average Size > 17A and MW > 1100 g/Mol PLUS Log K_{ow} > 10	
Name	PETP
CAS No.	6683-19-8
Mol weight (g/Mol)	1178
Average size (A)	17.9
log Kow	19.6

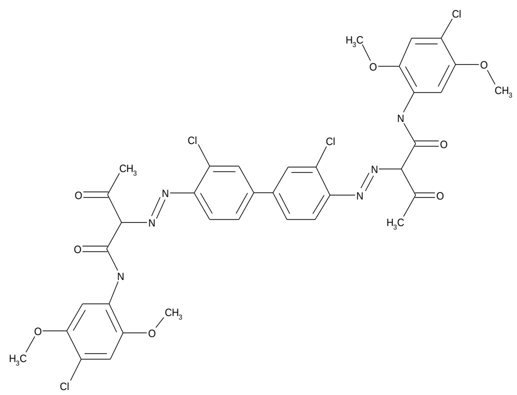


Remark:

The indicators average size > 17A and MW > 1100 g/Mol are fulfilled (substance is considered **not B**). In addition Log Kow is > 10 which means that the bioaccumulation potential is low. For more information see Appendix R.11—2, Example R.11-6.

Example R.11-4

Indicator : Average Size > 17A and MW > 700 g/Mol PLUS Octanol solubility	
Name	Pigment Red 83
CAS No.	5567-15-7
Mol weight (g/Mol)	818
Average size (A)	20
C_o (µg/L)	9
CBB (µg/L)	1636
C_o < CBB	YES



Remark:

The indicator average size > 17 A & MW > 700 g/Mol are fulfilled (substance is considered **not vB**). In addition the octanol solubility is very well below the Critical Body Burden (CBB) which means that the bioaccumulation potential is low.

Appendix R.11—2: Assessment of substances requiring special consideration during testing.

Table R.11—10: List of antioxidants (from Ullmann, 1995).

	Antioxidant type	CAS No.	MW (g/Mol)	calc. K_{ow} (KOWWin)
Hindered Phenols				
1	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl- (BHT)	128-37-0	220	5.1
2	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	2082-79-3	531	13.4
3	Phenol, 4,4',4"-[(2,4,6-Trimethyl-1,3,5-benzentriyl)tris(methylene)]	1709-70-2	775	17.2
4	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 2,2-bis[[3-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1-oxopropoxy]methyl]-1,3-propanediyl ester	6683-19-8	1178	19.6
Amines				
5	1,4-Benzenediamine, N-(1-methylethyl)-N'-phenyl-	101-72-4	226	3.3
Phosphites & Phosphonites				
6	2,4,8,10-Tetraoxa-3,9-diphosphaspiro 5.5 undecane, 3,9-bis 2,4-bis(1,1-dimethylethyl)phenoxy -	26741-53-7	605	10.9
7	12H-Dibenzo[d,g][1,3,2]dioxaphosphocin, 2,4,8,10-tetrakis(1,1-dimethylethyl)-6-fluoro-12-methyl- (9CI)	118337-09-0	487	12.8
8	12H-Dibenzo[d,g][1,3,2]dioxaphosphocin, 2,4,8,10-tetrakis(1,1-dimethylethyl)-6-[(2-ethylhexyl)oxy]-	126050-54-2	583	14.9
9	2,4,8,10-Tetraoxa-3,9-diphosphaspiro 5.5 undecane, 3,9-bis(octadecyloxy)-	3806-34-6	733	15.1
10	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	31570-04-4	647	18.1
11	Phenol, nonyl-, phosphite (3:1) (TNPP)	26523-78-4	689	20.1
12	Phosphonous acid, [1,1 -biphenyl]-4,4 -diylbis-, tetrakis[2,4-bis(1,1-dimethylethyl)phenyl] ester	38613-77-3	1035	27.2
Organosulfur compounds				
13	Propanoic acid, 3,3'-thiobis-, didodecyl ester	123-28-4	515	11.8
14	Propanoic acid, 3,3 -thiobis-, ditetradecyl ester	16545-54-3	571	13.8
15	Propanoic acid, 3,3'-thiobis-, dioctadecyl ester	693-36-7	683	17.7
16	Disulfide, dioctadecyl	2500-88-1	571	18.6
17	Propanoic acid, 3-(dodecylthio)-, 2,2-bis[[3-(dodecylthio)-1-oxopropoxy]methyl]-1,3-propanediyl ester	29598-76-3	1162	24.8
Oxamides				
18	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 2-[3-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1-oxopropyl]hydrazide	32687-78-8	553	7.8

1. Examples for Assessment of Substances with high Log K_{ow}

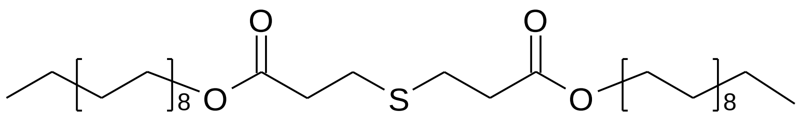
Example R.11-5

Propanioic acid, 3,3′-thiobis-, dioctadecyl ester, CAS No. 693-36-7

Table R.11—11: Properties of the antioxidant.

Parameter	Value
Molecular weight (g/Mol)	683
Water solubility (mg/L)	<< 1
Log K_{ow} (calculated)	17.7
Ready biodegradable (OECD TG 301B)	No
T Criteria fulfilled	No

Structure



STEP 1 **Calculated / measured Log K_{ow}**

Log K_{ow} calculated is 17.7

STEP 2 **Assessment type to be applied**

Log K_{ow} is > 10 and the T criteria is not fulfilled, this means a vPvB Assessment according Step 3

STEP 3 **vPvB Assessment**

STEP 3a **Persistence check**

The substance has two ester bonds. Cleaving the ester would lead to 2 Mol of 1-Octadecanol (1) and 1 Mol of 3,3′-Dithiobispropionic acid (2). Both substances (1) and (2) are readily biodegradable and are therefore no PBT or vPvB substances. The antioxidant itself is not readily biodegradable in a classical OECD TG 301B Sturm test at the usual high substance concentrations although the esters could be cleaved. The reason is the very low bioavailability of the substance. The biodegradation rate is therefore controlled by the dissolution rate. When the ready test (OECD TG 301D Closed Bottle Test) is carried out at low concentrations with stirring ready biodegradation can be achieved. In this case the assessment is finished with step 3a.

Conclusion **The antioxidant can be transformed in a ready test to metabolites which are itself readily biodegradable. Therefore the substance Propanoic acid, 3,3′-thiobis-, dioctadecyl ester, CAS No. 693-36-7 is not a vPvB Substance.**

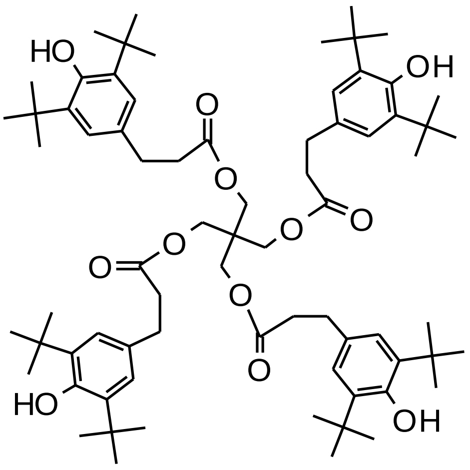
Example R.11-6

Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 2,2-bis[[3-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1-oxopropoxy]methyl]-1,3-propanediyl ester, CAS No. 6683-19-8

Table R.11—12: Properties of the antioxidant.

Parameter	Value
Mol weight (g/Mol)	1178
Water solubility (µg/L)	<< 1
Log K_{ow} (calculated)	19.6
Ready biodegradable (OECD TG 301B)	No
T criteria fulfilled	No

Structure



STEP 1 **Calculated / measured Log K_{ow}**

Log K_{ow} calculated is 19.6

STEP 2 **Assessment type to be applied**

Log K_{ow} is > 10 and T criteria is not fulfilled means vPvB Assessment according Step 3

STEP 3 **vPvB Assessment**

STEP 3a **Persistence check**

The substance has 4 ester bonds. Cleaving the ester would lead to 4 Mol of 3,5-bis(1,1-dimethylethyl)-4-hydroxy-benzenepropanoic acid (1) and Pentaerythrol (2). The acid (1) is not readily biodegradable but in an assessment it was demonstrated that (1) is not a PBT substance. Pentaerythrol (2) is readily biodegradable and is therefore not a PBT or vPvB substance. The antioxidant itself is not readily biodegradable in a classical OECD TG 301B Sturm test at high substance concentrations although the esters could be cleaved. The reason is the very low bioavailable of the substance. The biodegradation rate is therefore controlled by the dissolution rate. Due to the extremely low water solubility of the antioxidant a ready test at lower substance concentration will not result in ready biodegradation. In this case the assessment needs to proceed with step 3b.

STEP 3b **Bioaccumulation check**

Supporting information

Results from Animal studies

a) OECD TG 305 BCF Study

The Study is regarded as invalid as the substance was tested above water solubility but indicate low bioaccumulation

b) Animal ADE Studies

Adsorption, Distribution and Eliminations (ADE) Studies carried out with radiolabelled material show low adsorption of the substance. Adsorbed radioactivity is most likely starting material

MW and size criteria

D_{max} > 1.7 nm and MW > 700 g/Mol is fulfilled, substance has a D_{max} of 1.79 nm and a MW of 1178 g/Mol

Conclusion Although the antioxidant has ester bonds which could be cleaved ready biodegration cannot be achieved due to the very low (bio)availabilty of the substance. But there are several information available which support the low bioaccumulation potential based on the Log K_{ow} > 10. There are animal studies available (fish and rat) demonstrating low adsorption of the substance. In addition the MW and size criteria for low bioaccumulation potential are fulfilled as well (see Annex 1 'Indicators for limited Bioaccumulation').

Based on the available information with respect to the bioaccumulation potential and the likely metabolites it can be concluded in a *Weight-of-Evidence* approach that the antioxidant is not a vPvB substance.

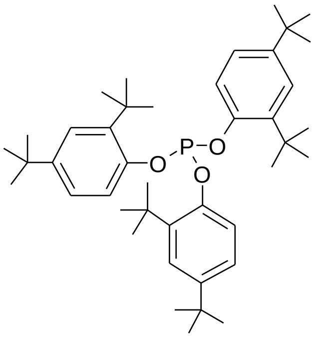
Example R.11-7

Tris(2,4-di-tert-butylphenyl)phosphite, CAS No. 31570-04-0

Table R.11—13: Properties of the antioxidant.

Parameter	Value
Mol weight (g/Mol)	632
Water solubility (mg/L)	<< 1
Log K_{ow} (calculated)	18.1
Ready biodegradable (OECD TG 301B)	No
T Criteria fulfilled	No

Structure



STEP 1 **Calculated / measured Log K_{ow}**

Log K_{ow} calculated is 18.1

STEP 2 **Assessment type to be applied**

Log K_{ow} is > 10 and the T criteria is not fulfilled, this means a vPvB Assessment according Step 3

STEP 3 **vPvB Assessment**

STEP 3a **Persistence check**

The substance has three ester bonds. Cleaving the ester would lead to 3 Mol of 2,4-Ditert.butylphenol (1) and 1 Mol of phosphite (2). (1) is not a PBT or vPvB Substance (EU, 2005) and (2) is an inorganic salt and no PBT or vPvB substance. The antioxidant itself is not readily biodegradable in a classical OECD TG 301B Sturm test. For metabolic reasons ready biodegration may not be achieved even at lower concentration. But hydrolysis at low concentration using radiolabelled material may result in abiotic transformation.

STEP 3b **Bioaccumulation check**

Log K_{ow} is > 10 but no further indication for limited bioaccumulation is fulfilled.

STEP 4 **Overall conclusion**

In this case the indicator Log K_{ow} > 10 is of limited value as the substances does not readily biodegrade even at low concentrations and no additional indicators for limited bioaccumulation are available.

In this case a hydrolysis study with radiolabelled material is warranted. If the half-life of the hydrolysis is > 40 days a bioaccumulation study needs to be carried out.

Table R.11—14: Octanol and water solubility of pigments, critical body burden for narcotic mode of action and Log $C_{octanol}/C_{water}$ (ETAD, 2006).

Pigment class	Colour index	MW (g/Mol)	Octanol solubility C_o (µg/L)	Critical Body Burden (CBB) (µg/L)	C_o<CBB	Water solubility C_w (µg/L)	Log C_o/C_w
Anthanthrone	P.R. 168	464	124	928	YES	10.8	1.1
Anthraquinone	P.R. 177	444	70	888	YES	230	-0.5
Benzimidazolone	P.R. 176	573	15	1146	YES	1.9	0.9
Benzimidazolone	P.R. 208	524	83	1048	YES	3.2	1,4
Benzimidazolone	P.Y. 151	381	210	762	YES	17.8	1.1
b-Naphthol	P.O. 5	338	1760	676	NO	7	2.4
b-Naphthol	P.R. 53:1 (salt)	445	1250	890	NO	1250	0.0
BONA *	P.R. 48:2 (salt)	461	170	922	YES	650	-0.6
BONA	P.R. 57:1 (salt)	426	850	852	YES	1800	-0.3
Diarylide Yellow*	P.Y. 12	630	48	1260	YES	0.8	1.8
Diarylide Yellow	P.Y. 12	630	50	1260	YES	0,4	2.1
Diarylide Yellow	P.Y. 13	686	22	1372	YES	0.8	1.4
Diarylide Yellow	P.Y. 14	658	3	1316	YES	analytical problems	
Diarylide Yellow	P.Y. 83	818	9	1636	YES	analytical problems	
Diketopyrrolopyrrole Pigment (DPP)	P.R. 254	357	30	714	YES	analytical problems	
Dioxazin	P.V. 23	589	330	1178	YES	25	1.1
Disazo Condensation	P.Y. 93	937	200	1874	YES	110	0.3

BONA = beta Oxynapthoic acid

* octanol is saturated with water, water is saturated with octanol

Table R.11—14 (continued) Octanol and water solubility of pigments, critical body burden for narcotic mode of action and Log $C_{octanol}/C_{water}$ (ETAD, 2006).

Pigment class	Colour index	MW (g/Mol)		Octanol solubility C_o (µg/L)		Critical Body Burden (CBB) (µg/L)	C_o<CBB		Water solubility C_w (µg/L)	Log C_o/C_w
Disazopyrazolone	P.O. 13	624		51		1248	YES		1.4	1.6
Isoindolinone	P.Y. 110	642		315		1284	YES		230	0.1
Monoazo Yellow	P.Y. 74	386		740		772	YES		7.6	2.0
Naphthol AS	P.R. 112	485		3310		970	NO		9.8	2.5
Naphthol AS	P.R. 170	454		225		908	YES		11.9	1.3
Perinone	P.O. 43	412		13		824	YES		7.2	0.3
Perylene	P.R. 149	599	<	12	>	1198	YES		analytical problems	
Perylene	P.Black31	599		96		1198	YES		analytical problems	
Perylene	P.R. 179	576	<	10	>	1152	YES	<	8	0.1
Perylene	P.R. 224	392	<	100	>	784	YES	<	5	1.3
Phthaloblue, metalfree	P.Blue16	515	<	10,1	>	1030	YES	<	10	0.0
Phthalocyanine	P.G. 7	1127	<	10	>	2254	YES	<	10	0.0
Phthalocyanine	P.B.15	576	<	7	>	1152	YES	<	7	0.0
Quinacridone	P.R. 122	340		600		680	YES		19.6	1.5
Quinacridone	P.V. 19	312		1360		624	NO		10.3	2.1
Quinophthalone	P.Y. 138	694		225		1388	YES		10	1.4

2. Example for an assessment strategy for substances with low octanol and water solubility

<u>Example</u> Pigment Yellow 12, CAS No. 6358-85-6

Table R.11—15: Data for Pigment Yellow 12.

Parameter	Value
Mol weight (g/Mol)	630
Water solubility (µg/L)	0.4
Octanol solubility (µg/L)	50
CBB (µg/L)	1260
C_o << CBB	YES
Log C_o/C_w	2.1
Log C_o/C_w << 4.5	YES
Aquatic ecotoxicity L(E)C50 (mg/L)	>> 0.1
14-C Pharmacokinetic male rat	No uptake Complete excretion through faeces

STEP 1 **Solubility measurement of Octanol and Water**

Octanol solubility is 50 µg/L and Water solubility 0.4 µg/L, Log C_o/C_W = 2.1

STEP 2 **B and T Assessment**

C_o < CBB and Log C_o/C_W < 4.5

Neither exceedance of CBB nor uptake via membrane is likely. Rat 14C Pharmacokinetic study confirms reduced uptake.

STEP 3 ***Weight-of-Evidence* approach**

In a *Weight-of-Evidence* approach based on C_o, Log C_o/C_W as well as on pharmacokinetic data it can be concluded that Pigment Yellow 12 is not a vPvB Substance and no further test is warranted.

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Appendix R.11—3: 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. 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). 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-lives and a third that could be used for assessing potential metabolites:

- 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. This model is freely available in EPISUITE as BIOHCWIN.

- Dimitrov *et al.* (2007) also describe a new 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 degradation metabolites (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 degradation metabolites. CATABOL 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 in *Chapter R.7c* of the *Guidance on IR&CSA* and Annex 1 to Appendix R.11—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 bioaccumulation data 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—4: 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 normalization (to 5% lipid content) should therefore always be performed, whenever possible for substance that are lipid binding.

The relationship between BSAF and BCF is epressed 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. 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.

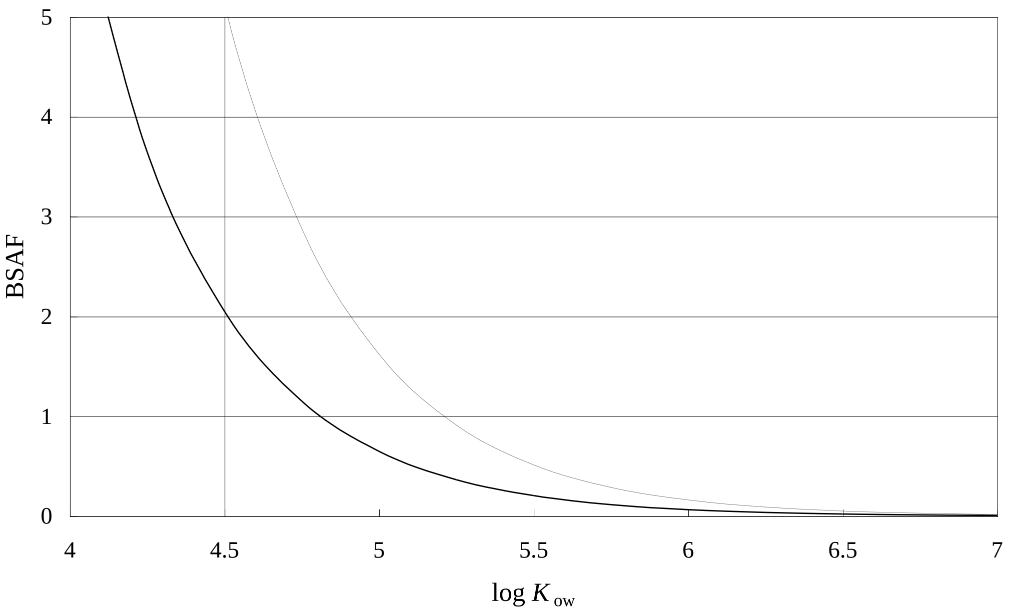

Figure R.11—10: Relationship between lipid and organic carbon normalised BSAF values and Log K_{ow} as indicator for the B and vB criterion.

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 dependend 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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