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GUIDANCE

# Guidance on Information Requirements and Chemical Safety Assessment

# Chapter R.11: PBT/vPvB assessment

Version 2.0

November 2014

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This document aims to assist users in complying with their obligations under Regulation (EC) No 1907/2006 (REACH Regulation). However, users are reminded that the text of the REACH Regulation is the only authentic legal reference and that the information in this document does not constitute legal advice. Usage of the information remains under the sole responsibility of the user. The European Chemicals Agency does not accept any liability with regard to the contents of this document.

**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:<br>(i) replacing references to DSD/DPD by references to CLP;<br>(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:<br>• Chapter R.11 title has been changed to "PBT/vPvB assessment";<br>• 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);<br>• Description of the registrant's obligations in Section R.11.3 has been expanded upon;<br>• 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;<br>• 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*";<br>• 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"*;<br>• 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.<br>• 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;<br>• 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 criterion has been removed;<br>• As the screening criteria 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.<br>• The document has been re-formatted to ECHA new corporate identity. | |

### Convention for citing the REACH Regulation

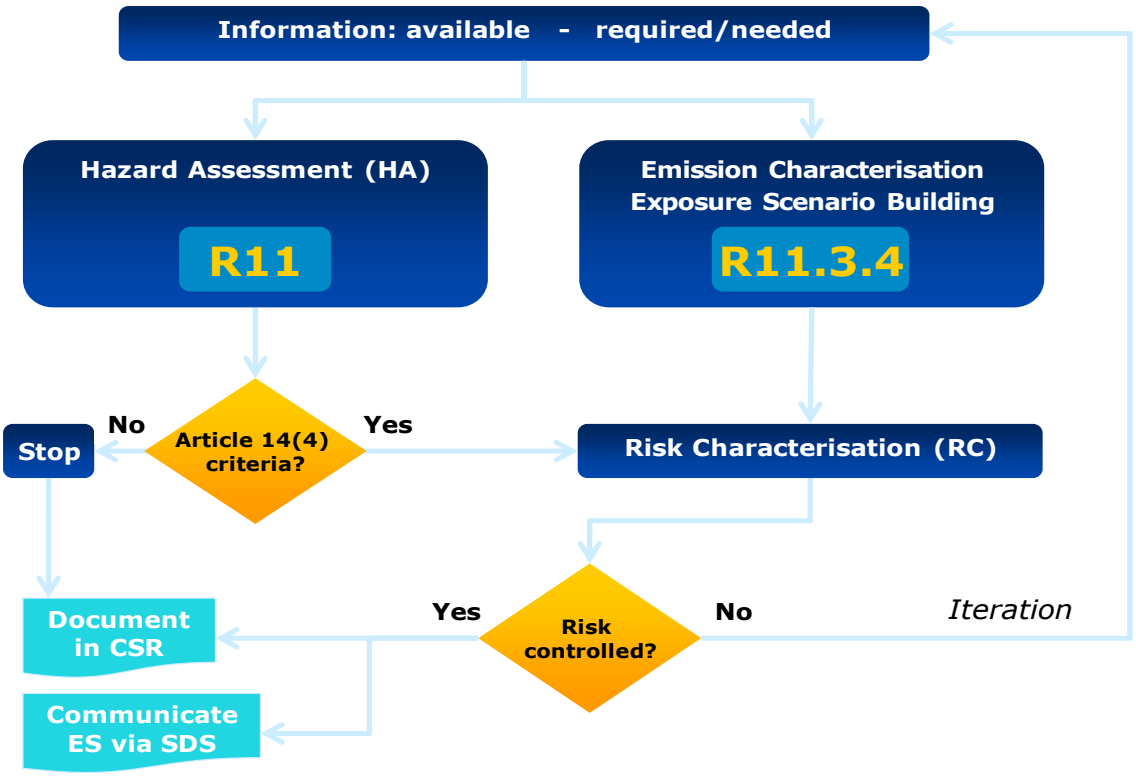
Where the REACH Regulation is cited literally, this is indicated by text in italics between quotes, or text in green boxes.

### 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

This guidance document contains a description of scientific principles for the persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) assessment in accordance with Section 4 of Annex I to REACH, 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 lead to toxic effects, even if they are not necessarily proven yet. These properties are defined by the criteria laid down in Section 1 of Annex XIII to REACH (*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*, http://echa.europa.eu/guidance-documents/guidance-on-reach). 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.

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.

[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.

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 REACH, the objective of the PBT/vPvB assessment is to determine if the substance fulfils the criteria given in Annex XIII to REACH ("*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 REACH 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 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. 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 data that numerically match with the Annex XIII criteria. Generating such data may for instance not be possible because the properties of the substance do not permit the respective tests to be conducted. In these cases a conclusion may need to be drawn on the basis of screening information and all further evidence available. In many cases further information may need to be generated before it can be judged whether the substance fulfils the Annex XIII criteria, and the guidance provides detailed testing strategies that the registrant should use for each endpoint in Section R.11.4.

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

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

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

Certain substances fulfilling the PBT/vPvB criteria may also be eligible to be included in the Stockholm Convention or the UNECE Protocol on Persistent Organic Pollutants (POPs). Even if the criteria for identifying POPs are not exactly the same as the PBT/vPvB criteria, they do partly overlap. The POPs criteria also include potential for long-range transport as an additional criterion compared to the PBT/vPvB criteria. Any Party to the Convention or to the Protocol may propose further substances to be included in Annex A, B, or C of the Convention. Such proposals could use information provided as part of registration dossiers under REACH. Indeed, EU/EEA MSCAs that are party to the Convention or members of the inter-sessional working groups to the Convention can use such information whilst respecting the confidentiality claims contained in the registration dossiers.

## R.11.2 Overview of Annex XIII to REACH

The purpose of this section is to introduce the content and terminology of Annex XIII to REACH. 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 REACH

The introductory section of Annex XIII to REACH 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 REACH 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 REACH 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 REACH 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 REACH 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 REACH are provided in Section R.11.4. In addition, *Practical Guide 2 "How to report Weight-of-Evidence"* 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 REACH must be considered further to Annex I to REACH:

**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.

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* (see http://echa.europa.eu/guidance-documents/guidance-on-reach). 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 REACH

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 REACH.

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

| Property | PBT criteria | vPvB criteria |
|---|---|---|
| **Persistence** | A substance fulfils the persistence criterion (P) in any of the following situations:<br>(a) the degradation half-life in marine water is higher than 60 days;<br>(b) the degradation half-life in fresh or estuarine water is higher than 40 days;<br>(c) the degradation half-life in marine sediment is higher than 180 days;<br>(d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days;<br>(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:<br>(a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days;<br>(b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days;<br>(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:<br>(a) the long-term no-observed effect concentration (NOEC) or EC10 for marine or freshwater organisms is less than 0.01 mg/L;<br>(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;<br>(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). Only long-term/chronic aquatic data can be used for the comparison with the aquatic toxicity criterion.

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

| | |
|---|---|
| **Indication of P and vP properties** | (a) Results from tests on ready biodegradation in accordance with Section 9.2.1.1 of Annex VII;<br>(b) Results from other screening tests (e.g. enhanced ready test, tests on inherent biodegradability);<br>(c) Results obtained from biodegradation (Q)SAR models in accordance with Section 1.3 of Annex XI;<br>(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;<br>(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;<br>(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 only be used for determining that the substance may fulfil the T criterion. 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 REACH.**

| | |
|---|---|
| **Assessment of P or vP properties** | (a) Results from simulation testing on degradation in surface water;<br>(b) Results from simulation testing on degradation in soil;<br>(c) Results from simulation testing on degradation in sediment;<br>(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;<br>(b) Other information on the bioaccumulation potential provided that its suitability and reliability can be reasonably demonstrated, such as:<br>- Results from a bioaccumulation study in terrestrial species;<br>- Data from scientific analysis of human body fluids or tissues, such as blood, milk, or fat;<br>- Detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment;<br>- Results from a chronic toxicity study on animals;<br>- Assessment of the toxicokinetic behaviour of the substance;<br>(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;<br>(b) Results from long-term toxicity testing on fish as set out in Section 9.1.6 of Annex IX;<br>(c) Results from growth inhibition study on aquatic plants as set out in Section 9.1.2 of Annex VII;<br>(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;<br>(e) Results from long-term or reproductive toxicity testing with birds as set out in Section 9.6.1 of Annex X;<br>(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.

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

Section 4.0.1 of Annex I to REACH 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 REACH, 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 REACH 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 REACH. 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 and carry out Step 1 again, OR
- The registrant must treat the substance *as if it is a PBT or vPvB*.

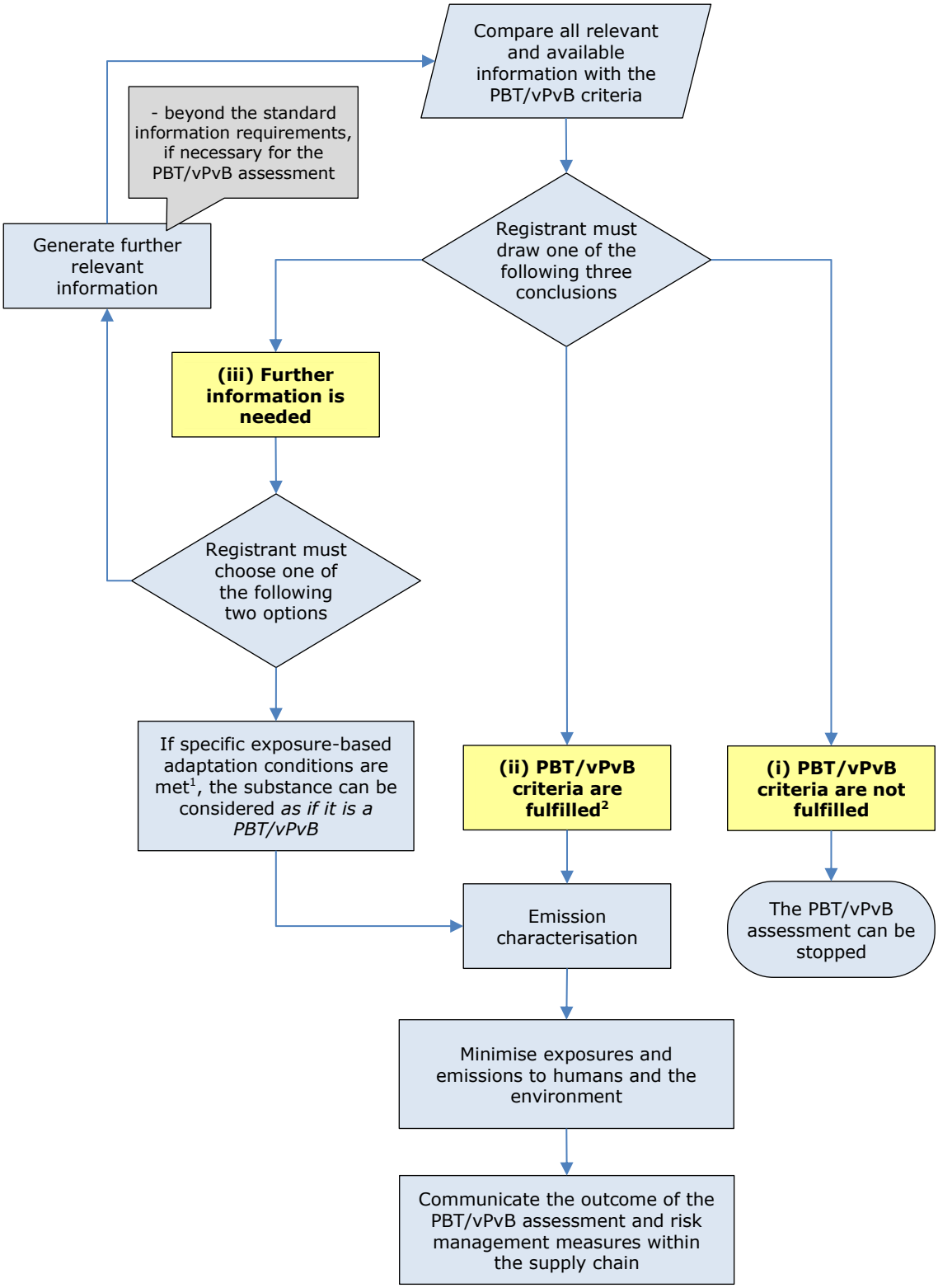
**Figure R.11—1: Overview of the conclusions from Step 1 ("Comparison with the criteria") and their consequences.**

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 REACH 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 REACH.

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

**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)* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment). It should be noted that any data adaptations according to Column 2 of Annexes VII to X to REACH or Annex XI to REACH should be justified according to the relevant ECHA documents (e.g. *Practical Guides 4, 5 and 6,* and *Guidance on IR&CSA, Chapter 5 and Chapter 6*, see http://echa.europa.eu/practical-guides and http://echa.europa.eu/guidance-documents/guidance-on-reach). 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 REACH. 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 criteria and conditions for applying them are described, which the registrant should consider while drawing a conclusion for screening. The screening criteria 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 REACH 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 REACH, 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* (see http://echa.europa.eu/guidance-documents/guidance-on-reach).

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 generally 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 all transformation/degradation products. It should be noted that also Section 9.2.3 of Annex IX to REACH 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.

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 and continue under Step 1: after **conclusion (iii)** **or** Treat the substance "as if it is a PBT or vPvB": after **conclusion (iii)**

In the following the consequences are described more in detail.

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

**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.

**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 Article 59 REACH 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.

**Generate relevant additional information**

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 to REACH and Column 2 of Annexes VII to X to REACH. 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 REACH. Especially for such Column 2 waivers of Annexes VII to X to REACH 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 Section 9.3.2 of Annex IX to REACH testing requirement (bioaccumulation in aquatic species). 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 REACH. 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 REACH allows the use of such other information. The other type of information can be experimental information not falling under Annex IX or X to REACH, 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 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 REACH, 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 REACH 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.

**Treat the substance "as if it is a PBT or vPvB"**

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

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

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

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

Additionally, for a substance to be considered "as if it is a PBT/vPvB" (i.e., the substance is regarded as a PBT/vPvB without finalising the PBT/vPvB assessment), appropriate parts of the CSR and the technical dossier must clearly demonstrate that the registrant fulfils the conditions for exposure based adaptation. This is the prerequisite as defined by Section 2.1 of Annex XIII to REACH 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 REACH. For a description of the required conditions please refer to the *Guidance on intermediates* (see http://echa.europa.eu/guidance-

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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

documents/guidance-on-reach) and *Guidance on IR&CSA, Chapter R.5: Adaptation of information requirements* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

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 REACH). 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* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) 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 REACH 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 of the *Guidance on IR&CSA, Chapter R.7a*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

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.

**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 for intermediates,* http://echa.europa.eu/guidance-documents/guidance-on-reach).

***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, for example, incinerating the waste water or extracting the "*PBT or vPvB substance*" from it. 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 96/82/EC on the control of major-accident hazards involving dangerous substances and the Directive 94/9/EC 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.

**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* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

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.

[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. 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 REACH: '*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.

**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.

### 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 REACH 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/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 is also emphasized that *Weight-of-Evidence* determination is not a

mechanism to justify disregarding valid, standard test data that show that a chemical fulfils the criteria for PBT or vPvB.

For particular cases, further described in Section R.11.4.1.4, the *Weight-of-Evidence* determination should consider all three properties in conjunction. In particular, if for one or more of the properties only screening information is available and screening criteria as provided in the following sub-sections are applied to draw a conclusion, all three properties persistence, bioaccumulation and toxicity must be considered in conjunction.

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].

*Practical Guide 2 "How to report Weight-of-Evidence"* provides a general scheme for building a *Weight-of-Evidence* approach (see http://echa.europa.eu/web/guest/practical-guides). 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* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment). 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.

**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 are 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 rooted in a principle recognised in European Union legislation[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.

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

[13] For example, for another category of substances of very high concern according to Article 57 of REACH, 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).

However, it may in specific cases 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, e.g., of 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, additives and transformation/degradation products with PBT/vPvB properties, and the total amount of these within the manufactured/imported substance should in no case exceed 1 tonne/year. Additionally, a reduced threshold might be necessary to derive information relevant for PBT/vPvB assessment, e.g., for very toxic substances, and the information on the toxicity derived for the classification and labelling purposes could be used for defining such a lower concentration limit for PBT/vPvB assessment.

It may not always be possible to sufficiently characterize and identify UVCBs (substances of Unknown or Variable composition, Complex reaction products or Biological materials) based on the information given in Section 2 of Annex VI to REACH for substance identification because (i) the number of constituents 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 substance identification is possible or not, the registrant should carry out a PBT/vPvB assessment for all constituents 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.

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 criteria 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 the registrant considers it more appropriate to generate new degradation information in accordance with Section 2.1 of Annex XIII to REACH other than degradation simulation test data, 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. 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)

When assessing data concerning the persistence of a potential PBT/vPvB substance and, if necessary, determining the next steps, there are a number of stages to go through. The first part of the assessment should address the extent to which the available data enable(s) 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 or a simulation test indicates a half-life ($T_{1/2}$, for the definition of half-life see Table R 7.9-1 of Section R.7.9.1.1 of the *Guidance on IR&CSA, Chapter R.7b*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) of less than 1 day for the aqueous biodegradation, the decision that a substance is not P could be taken. Similarly if the opposite is the case, i.e. an OECD TG 301 study indicates <10% biodegradation or a simulation test indicates a half-life of over 200 days, this is normally sufficient to decide that the substance meets the P criteria and possibly the vP criteria.

However, often the data are not so clear-cut, and frequently they are contradictory, especially for biodegradation. Therefore a careful consideration is needed before a decision is reached in order to avoid a false negative conclusion. The strategy outlined in this chapter should be read as guidance 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 chemical being assessed. As a minimum, and where possible and technically feasible, information on the vapour pressure, water solubility, octanol/water partition coefficient, basic dissociation behaviour (if relevant), surface active properties (if relevant) and Henry's law constant must be available, and the impact of these data on the test design and data interpretation should be considered.

With regard to persistence, it is insufficient to consider removal alone where this may simply represent the transfer of a substance from one environmental compartment to another (e.g. from the water phase to the sediment). Degradation may be biotic and/or abiotic (e.g. hydrolysis) and result in complete mineralisation, or simply in the transformation of the parent

substance (primary degradation). Where only primary degradation is observed, it is necessary to identify the degradation products and to assess whether they possess PBT/vPvB properties.

The following three sections give guidance on how to address data from biodegradation studies, abiotic 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. The final section explicates the Integrated Testing Strategy (ITS) for persistence assessment. 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 guidance on degradation which should be consulted (see Section R.7.9 of the *Guidance on IR&CSA*, *Chapter R.7b,* http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

In case only screening information is available, screening criteria listed in

Table R.11—4 can be used to judge whether an ultimate conclusion on the persistence of the substance can be made or whether further information is needed. It is noted that the 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. Additionally, although it might be theoretically possible to calculate degradation half-life values from screening information, such values cannot be directly compared with the P/vP criteria of Annex XIII to REACH, but the screening information should be discussed as such and compared with the screening criteria. The use of screening information and screening criteria are discussed further in the following sub-sections.

**Table R.11—4: Screening criteria for P, vP, B, vB and T.**

| 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** | **or** | |
| Biowin 6 (MITI 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 |
| Ready biodegradability test | ≥70% biodegradation measured as DOC removal (OECD TGs 301A and 301E) or ≥60% biodegradation measured as ThCo2 (OECD TG 301B) or ThOD (OECD TGs 301C, 301D and 301F)*** | Not P and not vP |
| | <70% biodegradation measured as DOC removal (OECD TGs 301A and 301E) or <60% biodegradation measured as ThCo2 (OECD TG 301 B) or ThOD (OECD TGs 301C, 301D and 301F) | Potentially P or vP |
| Modified ready biodegradability tests or 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

** For substances fulfilling this but BIOWIN 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 of the *Guidance on IR&CSA, Chapter R.7b*. Expert judgement and or use of *Weight of Evidence* (WoE) also employing other information may be required to reach a conclusion ( i .e. concerning « biodegradable/ not biodegradable ») also because some of the current guidance in the Chapter on degradability is not so prescriptive.
***** See section below for concluding ultimately on persistence in particular cases.

**Assessment of biodegradation data**

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

1. Tests on ready biodegradation (e.g. OECD TG 301 series, enhanced ready test)
2. Tests on inherent biodegradation
3. Tests on simulation biodegradation 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 and degradation products (metabolites, bound 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 of the *Guidance on IR&CSA*, *Chapter R.7b*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

***Tests on ready biodegradation***

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 chemical will not be degraded under environmental conditions. Tests on ready biodegradation are described in OECD TG 301 A-F. Degradation is followed by determination of sum parameters such as dissolved organic carbon (DOC), $CO_2$ production or oxygen uptake. Substance-specific analysis can also be used to assess primary degradation and to determine the concentration of any metabolites formed. Given the time, costs and in some cases practical difficulties associated with a simulation test, an enhanced ready biodegradation test design offers a cost effective intermediate screening test. If sufficient degradation is shown in such a test, i.e. the pass level is reached, the substance can be considered as "not P". For more information on modifications that can be made to a ready test Sections R.7.9.4 and R.7.9.5 of the *Guidance on IR&CSA*, *Chapter R.7b* should be consulted. Please note that these tests are referred to as "enhanced 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 performed using more favourable conditions than ready biodegradability tests, and are hence optimised to show whether a potential of degradability exists.

Lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD TG 302 series would provide sufficient information to confirm persistence without the need for further simulation testing. 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 convincing 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 solubility of a test substance may reduce the availability of the substance in the test medium. These issues are discussed in more detail in Sections R.7.9.4 and R.7.9.5 of the *Guidance on IR&CSA*, *Chapter R.7b*.

***Tests on simulation of biodegradation***

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 of degradation (degradation products). The endpoints usually addressed are primary or ultimate degradation rate and degradation half-lives or DT50s for the compartments included in the test system as well as the route of degradation, metabolites and bound residues. In addition, a mass balance is included and therefore possible losses from the test

system during the test period can also be quantified. It should be noted that for comparison to the P criteria only estimates of degradation half-life are appropriate. When the kinetics of transformation are biphasic, non-first order DT50s calculated from these studies must not be compared to these triggers. Where kinetics are biphasic, dividing an appropriately estimated DT90 by 3.32 gives a half-life estimate that can be compared to the P criteria. This "rule" is proposed in the Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (European Commission DG-SANCO, 2006). It is recommended to consult this guidance document for in-depth analysis of simulation degradation test results.

Before testing, the compartment of concern needs to be identified in order to decide which simulation test is the most appropriate method for addressing degradation especially for difficult substances. This is discussed later on in this guidance.

Tests should report the degradation rate in each medium determined through mineralisation, e.g. volatile $^{14}C$, and/or direct substance analysis. Where possible, a full mass balance of the substance and any degradation products/metabolites should be determined, and include a determination of the level of bound residues. 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 of the *Guidance on IR&CSA*, C*hapter R.7b*).

Please note that since its 32$^{nd}$ meeting the Member State Committee has started to require new simulation degradation studies to be carried out around neutral pH values and at 12°C, which is understood as the mean temperature of European surface waters. Accordingly, temperature correction of degradation half-lives from already available study results to 12°C is recommended. In the absence of equations/models reflecting temperature dependence of biodegradation, the Arrhenius equation as provided under the section on "Temperature dependence of hydrolysis" of this Guidance (or a similar appropriate equation designed to normalise physico-chemical degradation rates) can be used as a possible means of normalisation.

Another issue to address is whether parent molecules, or their degradation products, via their interaction with sediment or soil organic matter become bound to or entrapped in the organic matrix. The environmental significance of bound residues is related precisely to the extent to which they become indistinguishable from existing organic matter. This is discussed in Sections R.7.9.4 and R.7.9.5 of the *Guidance on IR&CSA*, C*hapter R.7b*).

**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 a substance with a degradation half-life in a simulation test not fulfilling the P-criterion. If abiotic degradation tests are available, there may be 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 of the *Guidance on IR&CSA*, C*hapter R.7b*).

There are several abiotic degradation/transformation processes in the environment to consider including hydrolysis, direct and indirect photodegradation, oxidation/reduction, surface-controlled catalytic reactions, molecular internal conversions etc. The most important of these is usually hydrolysis, which is relatively insensitive of 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 fast hydrolysis rates which are well known to be persistent in soil and/or sediment. Therefore, fast hydrolysis rates cannot alone lead to concluding that a substance is not persistent. Test results showing

fast hydrolysis rates always need to be evaluated carefully in context with other information on the substance, such as partitioning and ionising properties.

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

**Assessment based on estimation models (QSAR, SAR)**

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

***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 of the *Guidance on IR&CSA*, *Chapter R.6,* http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

(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 of three estimation models in the EPI suite (US-EPA 2000) is suggested as described later in this section in Explanatory Note 5 to the ITS for persistence assessment.

***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.

An approach based on consensus modelling has been used in the Canadian exercise, screening the DSL[15] (Arnot *et al.*, 2005). In this approach the authors recommend the following approach:

1. ***Gather all available empirical data for the substance of interest in all relevant media.***
2. ***Run the four BIOWIN models (1, 3, 4, and 5) and the CATABOL model, average the BIOWIN half-lives and check that the results are generally consistent with the CATABOL results.***
3. ***The empirical and model data are then combined using expert judgment to suggest a range of half-lives which may be applicable to that substance.***
4. ***Apply factors to relate water, soil, and sediment half-lives and possibly sewage treatment plant (STP) half-lives. This can be done directly or using the slide rule pictorial approach (discussed in the report).***

Clearly this approach needs to be further investigated for its usefulness in relation to P assessment and should be used with care and sufficient justification.

For specific classes of chemicals it may also be possible to run specific QSARs. For example HCBIOWIN, based on hydrocarbons (Howard *et al.*, 2005), alcohols (Yonezawa and Urushigawa, 1979a), *n*-alkyl phthalates (Yonezawa and Urushigawa, 1979b), chlorophenols

[15] DSL: Domestic Substance List which is a comprehensive inventory of known substances in Canadian commerce (past and current) and currently includes approximately 24000 substances.

and chloroanisoles (Banerjee *et al*., 1984), *para*-substituted phenols (Paris *et al*., 1983), and *meta*-substituted anilines (Paris *et al.,* 1987).

The use of QSAR model predictions are of particular relevance and interest 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 practical and cost implications.

***Abiotic degradation models***

There are very few software models available for predicting aquatic photodegradation, and a few published models (Peijnenburg *et al.*, 1992, Stegeman *et al.,* 1993). These are reviewed in Section R.7.9.4 of the *Guidance on IR&CSA*, *Chapter R.7b* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

**Choice of compartment for simulation degradation testing**

In Annex IX of REACH statements are made in relation to the choice of environmental compartment for simulation degradation testing when required for the CSA (which includes the risk assessment and the PBT/vPvB assessment).

For a PBT and vPvB assessment, the identification of the relevant environmental compartment(s) and, hence, the subsequent selection of suitable simulation test(s), should be based on the identified uses and releases patterns as well as the intrinsic properties of the substance (e.g. water solubility, vapour pressure, Log $K_{ow}$, Kp) significantly influencing the environmental fate of the substance.

A flow diagram for selecting the appropriate environmental compartment(s) and the subsequent selection of simulation test(s) is illustrated in the ITS described below. The Kp (sediment) may be used as an indicator of whether testing in a water-sediment system may be warranted, e.g. it may be considered to include an aquatic sediment simulation test in addition to a pelagic simulation test for substances with Kp (sediment) > 2000. Results from multi-media modelling (e.g. Mackay level 3 models) could also be explored in order to evaluate the environmental compartment(s) of primary concern. It is noted that the results of such models should be used with care as they strongly depend on the relative size of the environmental compartments and the emission parameters employed in the modelling. Contrary to the result of Mackay level 1 modelling, Mackay level 3 modelling is also dependent of the release pattern (fraction of emission between air, water, soil) and thus also on the use of the substance. Nevertheless a case-by-case evaluation of the results of such models may be useful and may even indicate whether or not pristine environmental compartments (e.g. open sea) may be exposed to a significant extent (i.e. indicate a potential for long range environmental transport via the atmosphere).

A number of multimedia models are available as well as a number of studies on comparison of these different models. One of the most relevant studies in the current context is the study performed by an OECD expert group which describes a comprehensive comparison of 9 multimedia models (Fenner *et al.*, 2005). Furthermore a software tool has been developed in this context which includes a level III multimedia model that is representative of the 9 models in the comparison study and presents model results in the format recommended by the OECD expert group (OECD, 2006). This tool might be useful to assess the distribution of the substance over different environmental compartments.

When identifying which compartment is of relevance for simulation testing, potential atmospheric deposition should also be taken into account. For chemicals with a high Henry´s Law Constant or $K_{OA}$ value there may be considerable transport to the atmospheric phase. Nevertheless concern for the non-air compartments may in general arise:

a. ***If the substance has a degradation half-life in air > 2 days it may have a potential for long range atmospheric transport (see the Stockholm convention on POPs) and may be deposited to remote areas. For such substances information on degradation in the expected receiving compartment(s) is recommended. One obvious possibility is to select a simulation degradation test based on open-ocean conditions i.e. a test with low organic loading, low bacterial density and high salinity ("ocean die-away test") according to OECD TG 309.***

b. ***If the substance has a degradation half-life in air < 2 days it is not expected to stay in the atmosphere for long as it will degrade rapidly. Thus there will be a limited potential for long range atmospheric transport. Depending on the behaviour of the chemicals (e.g. adsorption) it should be considered if the volatility of the substance is sufficiently high to consider that the substance will not be present in the other environmental compartments (e.g. water).***

When significant atmospheric transport can be ruled out as a distribution process on the basis of multimedia modelling or due to a short degradation half-life in air, then the relevant compartment to be investigated is that exposed via the water phase, i.e. receiving waters such as rivers, lakes, estuaries, the coastal zone, and/or their respective sediments. The surface water environmental compartment receiving the bulk of the input volume of a chemical should be focused upon. This requires an adequate knowledge of production, supply, use, discharge and losses of the substance. In those situations where there is a direct discharge to the marine environment, estuarine or coastal water compartments should be selected as the basis for the simulation test design.

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 which provides reliable results, and at very low concentrations technical issues may make it very difficult to establish a reliable degradation curve in the study.

Furthermore the relevance of such a study, even if it could be conducted, may not be high, as the environmental distribution and occurrence of the substance in the pelagic compartment would be very low. Thus depending on the physico-chemical properties and availability of good quality analytical methods, it may not be warranted 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 "less than 1 to 100 µg/L". REACH does not contain any other specifications on when a surface water degradation simulation test should not be performed if the CSA indicates the need. The reason why may well be that generally surface water will be exposed significantly if the water solubility of the substance is not very low and if emissions and losses to the environment occur.

Soil/sediment simulation degradation testing is warranted if direct or indirect exposure to the substance is likely. Soil and sediment degradation simulation tests should only be considered if these compartments are directly exposed (cf. the emission characteristics of the chemical) or if they are indirectly exposed due to the environmental fate characteristics of the substance. The latter case includes, when the substance is released to surface water but due to high sorption partitions to the sediment or to STP sludge, which is spread on soil.

Once the appropriate simulation test(s) have been identified and conducted, the data need to be interpreted to determine environmental degradation half-lives. Guidance on how to interpret data from simulation test is available in Section R.7.9.4 of the *Guidance on IR&CSA*, C*hapter R.7b* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

In the ITS for persistence assessment described below it is indicated which types of simulation degradation tests should be considered based on exposure pattern. 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 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<br>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<br>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 |

**Conclusion on the endpoint: ITS for persistence assessment**

A strategy for degradation testing in the context of PBT/vPvB assessment is proposed in Figure R.11—3. Such a strategy requires a tiered approach to testing including the use of simulation testing methods unless a substance, if relevant based on *Weight-of-Evidence* judgements, has been shown to be or not to be persistent.

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. However, in certain cases it may be possible to draw a conclusion "P" or "vP" based on screening information only.

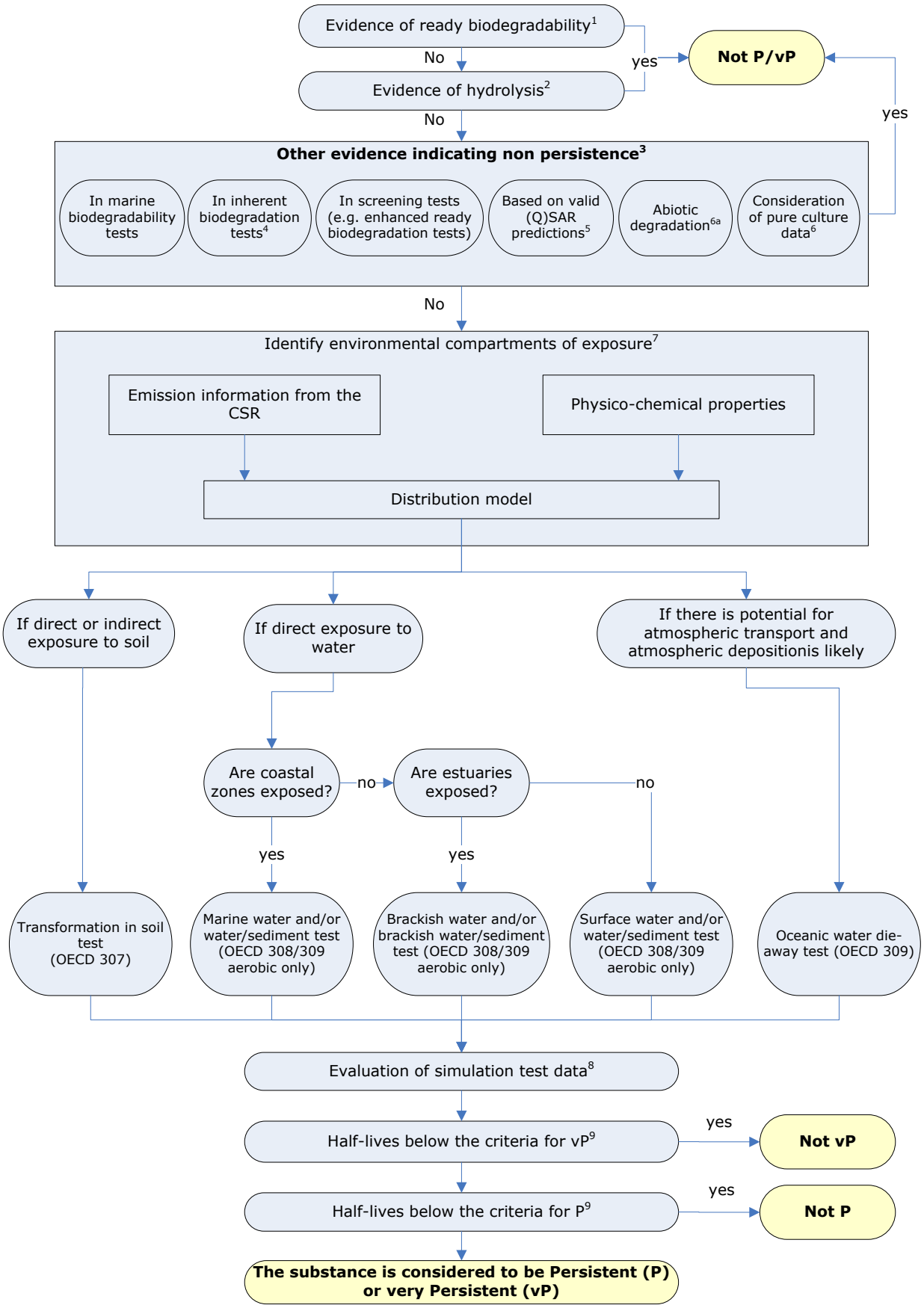



**Figure R.11—3: Integrated Testing Strategy for persistence assessment – maximising data use and targeting testing.**

**Conclusion on Persistence - Explanatory Notes to the Flowchart**

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 does not fulfil the criteria for Persistence (P) (see Sections R.7.9.4 and R.7.9.5 of the Guidance on IR&CSA, Chapter R.7b, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

2. ***Evidence of hydrolysis –*** If significant and substantial abiotic degradation has been confirmed and the hydrolysis transformation products have been assessed and concluded not to be PBT/vPvBs and it is certain that the fate properties of the substance do not attenuate the hydrolysis rate in sediment or soil, no further testing of degradation is required for the PBT/vPvB assessment. Additional studies, e.g. examining the influence of dissolved organic carbon on hydrolysis rates, may be necessary to provide this certainty. The degradation half-lives obtained in a hydrolysis test have to be compared to persistence criteria of Annex XIII (i.e. a substance fulfils the P(vP) criterion if $T_{1/2}$ > 40 (60) days). Careful consideration will need to be given to the 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).

3. ***Other evidence indicating non-persistence -*** 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. For example, a result of more than 60% ultimate biodegradability (ThOD, $CO_2$ evolution) or 70% ultimate biodegradability (DOC removal) obtained during 28 days 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 of the Guidance on IR&CSA, Chapter R.7b). This is also applicable to standardised marine biodegradability tests (OECD TG 306, Marine $CO_2$ Evolution test, Marine BODIS test, and the Marine $CO_2$ Headspace test).

   Before concluding under consideration of Explanatory Notes 3 – 6(a) that a substance is "not P" or "not vP", it should be carefully examined if there exists conflicting evidence from monitoring data (see Note 9 for more information).

4. ***Assessment of inherent biodegradation test data -*** Results of specified tests of inherent biodegradability, i.e. only Zahn-Wellens test (OECD TG 302B) or MITI II test (OECD TG 302C) 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% mineralization (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). If test results are available showing that a substance is not inherently biodegradable under the mentioned conditions this is a clear indication that the substance will not biodegrade in the marine environment and, hence, must be regarded as persistent.

5. ***Use of (Q)SAR (both QSARs and SARs) estimates –*** Such estimates may be used for preliminary identification of substances with a potential for persistence (see as well 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)

When the QSAR predictions using these models are reliable and the estimation results clearly indicate that the substance is not persistent, further information will normally not be required for the PBT and vPvB assessment, and it may be considered as not fulfilling the criteria for P. This implies that borderline cases should be carefully examined, e.g. when the estimate of the ultimate degradation time gives a result in the range 2.25 to 2.75 (see Sections R.7.9.4 and R.7.9.5 of the *Guidance on IR&CSA, Chapter R.7b*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment). 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).

***6. Use of pure culture data –*** The data derived from studies with pure culture cannot on its own be used within persistence assessment, however these types of data should be considered as part of the *Weight-of-Evidence* approach.

*6.a* ***Use of other abiotic data*** - Data derived from these studies (e.g. photodegradation, oxidation, reduction) cannot on their own be used within persistence assessment, but may be used as part of a *Weight-of-Evidence* approach.

***7. Identification of the environmental compartment of exposure*** for simulation testing (see sub-section Choice of compartment for simulation degradation testing above).

***8. Evaluation of simulation test data -*** In order to evaluate the outcome of the simulation test the following information is required:

a. Test conditions

b. First order, pseudo-first order rate constant, degradation half-life or DT50

c. Length of the lag phase

d. Fraction of mineralised label, and, if specific analyses are used, the final level of primary degradation

e. Mass balance during and at the end of the study

f. Identification and concentration of major transformation products, where appropriate

g. An indication of the level of bound residues

h. A proposed pathway of transformation, where appropriate

i. Rate of elimination (e.g. for risk assessment purposes)

***9. Evaluation versus the P and vP criteria***

Before concluding finally 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 or internationally acknowledged organisations such as e.g. 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.11.4.1.2 Bioaccumulation assessment (B and vB)

This section deals with assessment of bioaccumulation 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. This comprises results from bioaccumulation experiments, monitoring data from the field and information from toxicity studies on accumulation as well as other testing and non-testing indications of bioaccumulation. Where bioaccumulation/bioconcentration factors are presented, efforts should be made to present these in relation to whole body concentrations and the values should preferably be normalized as well, e.g. to lipid content. In some cases, e.g. because of the absence of data relevant for normalization, it may, however, be necessary to investigate and use these factors related to tissue/organ specific concentrations. In those cases, a rationale for this preference must be provided.

Guidance on the evaluation and validation of both testing data and non-testing information can be found in Section R.7.10 of the *Guidance on IR&CSA, Chapter R.7c* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

**Experimental aquatic bioconcentration factor (BCF) data**

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 $L \bullet kg^{-1}$. Please note that corrections for growth and/or a standard lipid content are not accounted for.
- 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.
- 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 the 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 cannot directly be compared to a BMF value from a field study (in which both water and dietary exposure may be combined).

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 a *Weight-of-Evidence* determination using expert judgement to derive the conclusion.

Also use of other taxonomic groups than fish (e.g. mussel bioconcentration test ASTM 2003) is possible for measuring bioconcentration in the aquatic environment. 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.. Conditions for selecting the minimised 305-II instead of the 305-I are described in the OECD TG and it should be noted that the 305-II –test can also be used within those conditions for the bioaccumulation assessment.

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. 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. A $BCF_k$ value 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. If uptake follows first order kinetics and the $BCF_{SS}$ was really based on steady state data, both methods should lead to the same result. If the $BCF_k$ is significantly different from the $BCF_{SS}$, growth dilution and loss process should be specifically checked, and the $BCF_{Kg}$ should be used. $BCF_k$ is preferred for PBT substances due to i) the slow kinetics possibly leading to non-equilibrium, and especially ii) the correction for growth dilution, which is not included in the $BCF_{SS}$. More emphasis on $BCF_k$ is also given in the OECD TG 305.

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, see below, should also be considered in the BCF estimation. A justification is needed in case no normalisation is carried out.

The increase in fish mass during the test will result in a decrease of the test substance concentration in growing fish (= growth dilution) and thus the BCF may be underestimated if no correction is made. Growth dilution may affect both $BCF_{SS}$ and $BCF_K$. No agreed method is available to correct $BCF_{SS}$ for growth. Therefore the $BCF_K$ should also be calculated and corrected for growth dilution, $BCF_{kg}$, if data allow an estimation. The OECD TG 305 contains a procedure for growth correction.

It should be noted that a valid fish BCF > 2000 or 5000 indicates significant accumulation in the test organism. This means that the substance is defined as "B" or "vB" regardless of whether biomagnification or trophic magnification occurs. This is because the fish itself is assumed to experience adverse perturbation through the uptake and storage of the chemical. This could cause unpredictable effects on the organism and population of the organism. In turn this has unknown impacts on the food chain, for example due to reduced food for predators.

**Experimental dietary biomagnification in fish (experimental dietary BMF)**

A dietary exposure test, preferably the OECD TG 305-III: Dietary Exposure Bioaccumulation Fish Test, should be considered for substances for which it is not possible to establish aqueous exposure 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. 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.

Annex 8 of the OECD TG 305 summarises some approaches to estimate tentative BCFs from data collected in the dietary exposure study. For the PBT assessment, it is recommended to calculate and present such tentative BCFs to enhance the transparency of the dataset. The tentative values should be considered as part of the body of evidence, and not used as the only values from which to draw conclusions in the assessment. For poorly soluble non-polar organic substances first order uptake and depuration kinetics is assumed, and more complex kinetic models should be used for substances that do not follow first order kinetics.

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

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

- The bioaccumulation factor (BAF) at any time during the uptake phase of this bioaccumulation test is the concentration of test substance in/on the test organism (Ca in g•kg-1 wet or dry weight) divided by the concentration of the substance in the surrounding medium (Cs as g•kg-1 of wet or dry weight of sediment). In order to refer to the units of Ca and Cs, the BAF has the units of kg sediment•kg-1 worm.
- The steady state bioaccumulation factor (BAFss) is the BAF at steady state and does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium (Cs as g•kg-1 of wet or dry weight of sediment) being constant during this period of time.
- Bioaccumulation factors calculated directly from the ratio of the sediment uptake rate constant divided by the elimination constant kinetic rate constants (ks and ke, respectively - see Annex 1 of the OECD TG 315) are termed kinetic bioaccumulation factor (BAFK).
- The biota-sediment accumulation factor (BSAF) is the lipid-normalised steady state concentration of test substance in/on the test organism divided by the organic carbon-normalised concentration of the sediment at steady state. Ca is then expressed as g•kg-1 lipid content of the organism, and Cs as g•kg-1 organic content of the sediment. BSAF is expressed in kg 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 for the screening and assessment of bioaccumulation properties. These studies are particularly relevant when a valid fish bioconcentration test result (including the fish-feeding method) is not available, or when exposure from sediment is expected to be more relevant than from the water column. It should be considered that (soil or sediment) invertebrate species in general 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.

The OECD TG 315 Bioaccumulation in Sediment-dwelling Benthic Oligochaetes is the preferred method for generating additional information. The recommended oligochaeta species are *Tubifex tubifex* (Tubificidae) and *Lumbriculus variegatus* (Lumbriculidae). The species *Branchiura sowerbyi* (Tubificidae) is also indicated but it should be noted that it has not been validated in ring tests at the time of writing. The bioaccumulation factor (expressed in kg wet (or dry) sediment•$kg^{-1}$ wet (or dry) worm) is the main relevant outcome and can be reported as a steady state bioaccumulation factor $BAF_{ss}$ or as the kinetic bioaccumulation factor ($BAF_K$). In both cases the sediment uptake rate constant $k_s$ (expressed in g 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 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. For lipophilic substances, BAFs often vary with the organic carbon content of the sediment. Typically a substance will have greater availability to the organism when the sediment OC is low, compared to a higher OC. It should be considered to test at least two natural sediments with different organic matter content, 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 BSAF is derived from a BAF by using a normalised OC content of 2%. This value is chosen based on the standard artificial sediment used in OECD sediment toxicity tests. 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.

Relevance of bioavailability of the substance for the test organism should also be considered and if relevant and possible the BAF should be corrected for the bioavailable fraction.

It should be noted that at the present time it is not possible to give any threshold values for BAF and BSAF in sediment as currently there are not enough scientific data available. 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 BAF and BSAF values an appropriate weight in the B and vB assessment.

In addition to the BAF and/or BSAF factors, other indications such as bioaccumulation process not reaching the steady state at the end of the exposure period or a low depuration rate are relevant when considering, with a *Weight-of-Evidence* approach, whether B or vB criteria are fulfilled. Substances having background sediment concentrations and adaptable uptake mechanisms require careful consideration.

**Experimental soil bioaccumulation data (experimental Bioaccumulation Factor BAF soil)**

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

- The bioaccumulation factor (BAF) at any time during the uptake phase of this bioaccumulation test is the concentration of test substance in/on the test organism (Ca in g•kg-1 dry weight of worm) divided by the concentration of the substance in the

surrounding medium (Cs as g•kg-1 of dry weight of soil); the BAF has the units of kg wet (or dry) soil•kg-1 wet (or dry) worm.

- The steady state bioaccumulation factor (BAFss) is the BAF at steady state and does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium (Cs as g•kg-1 of dry weight of soil) being constant during this period of time.
- Bioaccumulation factors calculated directly from the ratio of the soil uptake rate constant and the elimination rate constant (ks and ke,) are termed kinetic bioaccumulation factor (BAFK).
- The biota-soil accumulation factor (BSAF) is the lipid-normalised concentration of the test substance in/on the test organism divided by the organic carbon-normalised concentration of the test substance in the soil at steady state. Ca is then expressed as g•kg-1 lipid content of the organism, and Cs as g•kg-1 organic content of the soil; the BSAF has the units of kg OC•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 for the assessment of B and vB properties.

These studies are particularly relevant when a valid fish bioconcentration test result (including the fish-feeding method) is not available, or when exposure from sediment or soil is expected to be more relevant than that from the water column. It should be considered that (soil or sediment) invertebrate species in general 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. The steady state bioaccumulation factor $(BAF)_{ss}$ and the kinetic bioaccumulation factor ($BAF_K$) are preferably presented as dry/wet weight estimations and should be reported as well as the uptake and elimination rates. For highly lipophilic substances the biota-soil accumulation factor (BSAF), which is the lipid-normalised BAFss, should also be reported. The dependence of these values on the concentrations of the substance in soil, and when relevant, the soil characteristics should be specifically reported.

BAF and BSAF often vary 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 from a BAF by using a normalised OC content of 5%. This value is chosen based on the standard artificial soil used in OECD terrestrial invertebrate toxicity tests. This allows tests on the same substance, and tests on different substances to be comparable provided that the lipid content of the organisms employed in the different tests is similar. 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 is 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 the BAF should be corrected for the bioavailable fraction.

It should be noted that at the present time it is not possible to give any threshold values for BAF and BSAF in soil as currently there are not enough scientific data available. 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 BAF and BSAF values an appropriate weight in the B and vB assessment.

In addition to the BAF and/or BSAF factors, indications such as, a bioaccumulation process not reaching the steady state at the end of the exposure period, or a low depuration rate are relevant when considering, with a *Weight-of-Evidence* approach, whether the B or vB criteria are fulfilled. It should be noted that organo-metals and other substances with background soil concentrations and adaptable uptake mechanisms require particularly careful consideration.

**Field data and biomagnification**

In accordance with Annex I all available information/evidence on bioaccumulation, such as for example field data, must be considered in a *Weight-of-Evidence* approach. Indicators like bioaccumulation factors (BAF calculated from monitoring data, field measurements or measurements in mesocosms of 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 (although the quantity and quality of field data may be limited and their interpretation difficult): Furthermore, the information may be used to support the assessment of persistency, 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 higher than 1 (see also *Section R.7.10 of the Guidance on IR&CSA, Chapter R.7c*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) 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 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 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.

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, therefore an indication of a biomagnification potential can on its own be considered as a basis to conclude that a substance meets the B or vB criteria but 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 food chain for which it was measured. Biomagnification will vary between food chains, so a low BMF in one food chain does not mean that it will be low in other food chains. Conversely, evidence of high biomagnification in one food chain 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) food chains. 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.

**Other testing data**

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

***Chronic toxicity studies with mammals***

If chronic toxicity studies with mammals are available, the complete absence of 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 the absorption efficiency from such studies is relevant for PBT/vPvB assessment.. This parameter indicates whether or not the test substance is taken up from the digestive tract. If the substance is not taken up by mammals, or if only trace amounts of the substance are incorporated, then it is also likely that the substance will not easily pass across fish gill membranes and therefore may not have a high bioconcentration factor (BCF) in fish. Thus, such information may be used in a *Weight-of-Evidence* approach together with non-testing information on molecular size to conclude that the substance is not taken up in sufficient amounts to meet the B or vB criteria.

Other useful information that may be extracted from mammalian studies is the excretion rate of the parent compound and the metabolism rate. However, especially with regard to the latter, this information cannot be extrapolated directly to bioaccumulation of the substance in aquatic organisms such as fish, because mammals generally have a higher metabolic capacity than fish (Sijm and Opperhuizen, 1989; Sijm *et al.*, 1997). For further information see Section R.7.10.3.4.

**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.

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.

***Read-across with other substances***

If a valid BCF value for a structurally closely-related substance is available, read-across can be applied. When applying read-across two generally important aspects have to be considered, which are the lipophilicity 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).

Care must be taken when lowering the value. For the PBT or vPvB assessment this will not pose a problem if the known BCF value is already below 2000 or 5000. Hence, for the PBT or vPvB assessment values obtained by read-across should not be based on BCF values well above the criteria of 2000 and 5000 that then were corrected downwards to values below 2000 or 5000 (see Section R.7.10.3.2 *of the Guidance on IR&CSA, Chapter R.7c*,

http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

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 of the *Guidance on IR&CSA, Chapter R.7c*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

***Molecular size and weight***

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[16] the BCF value will be less than 5000.

Molecular weight is a parameter that is not directly related to the molecular size of a compound. However, it is a parameter that can be easily obtained from the molecular structure of a substance. A molecular weight higher than 1100 g/mol is an indicator that the aquatic BCF of the respective substance is lower than 2000. If the substance has a molecular weight higher than 700 g/Mol this is an indicator that the BCF is below 5000. Together with other information this information can be used in a *Weight-of-Evidence* approach to conclude that the substance is not B/vB (see "conclusions on the endpoint").

***Log $K_{ow}$***

For the PBT and vPvB assessment a screening criterion has been established, which is Log $K_{ow}$ greater than 4.5. The assumption behind this is that the uptake of an organic substance is driven by its hydrophobicity. For organic substances with a Log $K_{ow}$ value below 4.5 it is assumed that the affinity for the lipids of an organism is insufficient to exceed the B criterion, i.e. a BCF value of 2000 (based on wet weight of the organism, which refers to fish in most cases).

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 $K_{ow}$.

For some groups of chemicals, such as metals and surface active compounds, Log $K_{ow}$ is not a valid descriptor for assessing the bioaccumulation potential. Information on bioaccumulation of

[16] 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.

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 $K_{ow}$. This linear relationship is the basis for the B screening criterion 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 modelled $K_{ow}$ values > 10 is not known. Ideally the results of several model predictions 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 $K_{ow}$ 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.

***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. 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 aquatic bioaccumulation***

In vitro methods such as fish liver S9 and primary hepatocyte assays provide information on metabolism and hence biotransformation in the organism. Because metabolism 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. Currently their applicability is limited due to the lack of standardized protocols and limited validation. For further details see Section R.7.10.3.1 *in vitro data on aquatic bioaccumulation* of the *Guidance on IR&CSA, Chapter R.7c* (http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

***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 of the *Guidance on IR&CSA, Chapter R.7c*.

**Conclusion on the endpoint**

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. The relevant information includes laboratory bioconcentration tests (aquatic, terrestrial and benthic) and information on biomagnification and bioaccumulation from field studies. If

available, such information might be sufficient to conclude whether the substance is vB, B, or not B.

- If such information is not available for a substance produced or imported at levels below 100 t/y and the substance has a Log $K_{ow}$ lower than 4.5 and no specific mechanism of uptake apart from lipophilic partitioning is known, 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.
- However, for a substance produced or imported at a level of 100 t/y or more, information on bioconcentration in aquatic species has to be made available by the registrant and to be considered in the assessment, unless this information can be waived according to Column 2 of Annex IX or according to Annex XI (e.g. low bioaccumulation potential, no exposure, testing technically not possible).

In any other case, 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 data on bioconcentration (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.
- direct data on bioconcentration 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 (for all substances subject to PBT/vPvB assessment).

In this further evaluation, non-testing data should be used in combination with supplementary evidence to examine whether the substance potentially meets the B and vB criteria. Because non-testing information generally is considered to be insufficient to abstain from confirmatory testing, the availability of other reliable information indicating a low bioaccumulation potential is essential. This supplementary information may comprise data from a chronic toxicity study with mammals (≥ 90 days, showing no toxicity), a toxicokinetic study (showing no uptake), a bioconcentration study with invertebrates, or reliable read-across from a structurally similar compound. 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. This approach is based on the report provided in Appendix R.11—1.

If the above mentioned supplementary information is available, based on *Weight-of-Evidence* and expert judgement a substance may be considered as not B (i.e. unlikely to have a BCF > 2,000 ) on the basis of the following types of indicators:

1. an average maximum diameter ($Dmax_{aver}$) of greater than 1.7 nm[16] and a molecular weight of greater than 1100 g/mol
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)

An indicator for considering a substance as possibly not being vB (i.e. unlikely to have a BCF > 5,000) is, apart from indicators 2., and 3. above:

4. a Dmax aver of greater than 1.7 nm[16] plus a molecular weight of greater than 700 g/mol

Indicators 1. and 4. recommended here as non-testing information influence 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. Evidence of significant

uptake of a substance in vertebrates after prolonged exposure is a contraindication to using the above indicators.

Also, rapid metabolisation of a substance may lead to a lower BCF value. Methods such as fish liver S9 and fish hepatocyte assays might have the potential to support refinement of BCF estimations but there is still a need for further evaluation of these methods before they can be recommended for regulatory purposes.

**Integrated Testing Strategy (ITS)[17]**

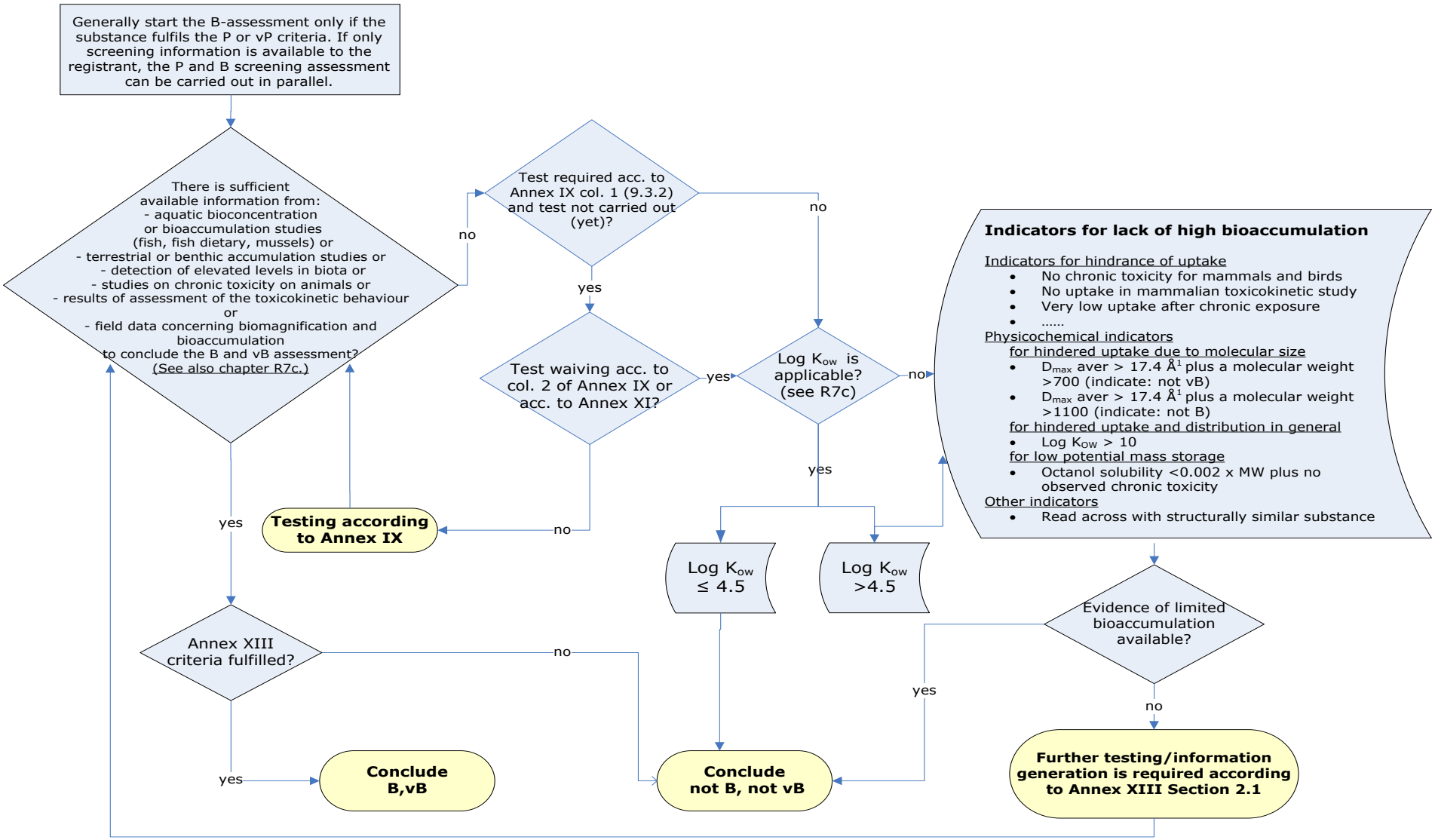
If a substance is imported or produced in an amount of more than 100 t/y, a bioaccumulation test is mandatory (REACH Annex IX 9.3.2). The option of waiving the bioaccumulation test according to Column 2 of REACH Annex IX can only be taken if the information from the experimental test is not required for the conclusion on the PBT/vPvB-properties. Similarly, the standard aquatic bioaccumulation test requirement cannot be adapted according to REACH Annex XI, if the PBT/vPvB assessment shows that abioaccumulation 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 of the *Guidance on IR&CSA*, *Chapter R.7c* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment). 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 test is conducted for assessing the B and vB criteria, the P criterion should be investigated in order to prevent unnecessary testing of animals. Further bioaccumulation testing is only necessary, if the P criterion has been confirmed to be fulfilled for the substance.

If a BCF test still must be performed, the OECD TG 305-I test should be preferred. However, for the purpose of the PBT/vPvB assessment, the minimised test OECD TG 305-II test with less fish may be considered, depending on a range of factors including the required level of precision of the determination of the BCF value for the particular substance. The OECD TG 305 indicates some criteria for selecting the minimised test. 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 305-1 test would be appropriate.

[17] 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.




Note that a weight-of-evidence approach must be applied at all steps of the ITS. The relevant constituents, impurities, additives and degradation/transformation products must be taken into account.

[1]Please note that the indicator value of 17.4 Å for the average maximum diameter was derived using the descriptor $D_{max}$ from OASIS. Indeed, it appears from the Environment Agency (2009) that the use of different software tools could lead to variable results for the same substance.

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

### R.11.4.1.3 Toxicity assessment (T)

**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[18].

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[19])[20] 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*)[21]. 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 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

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[18] 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.

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

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

21 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.

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 substances with very high Log $K_{ow}$ (depending on the class of chemical but as a general rule Log $K_{ow}$ > 6) 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 of the *Guidance on IR&CSA, Chapter R.7b*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

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 ($K_{susp\text{-}water}$) (see Section R.7.8 of the *Guidance on IR&CSA, Chapter R.7b*).

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:

$$NOEC(EC10)_{water} = \frac{RHO_{susp}}{K_{susp-water} \cdot 1000} \cdot NOEC(EC10)_{sed\,wwt} \quad \textbf{Equation 11-1}$$

*$NOEC(EC10)_{water}$ ($mg.L^{-1}$)*
*$RHO_{susp}$ (bulk density of wet suspended matter expressed in $kg.m^{-3}$)*
*$K_{susp\text{-}water}$ ($m^3.m^{-3}$)*
*$NOEC(EC10)_{sed\ wwt}$ ($mg.kg_{wwt}^{-1}$)*

It should be noted that since usually $NOEC_{sed}$ derived from experimental studies are given in dry weight (as mg/kg dwt), therefore, where this is the case, a conversion to wet weight (as mg/kg wwt) must be done before applying the equation 11-1. The conversion factor from dry weight to wet weight is: $NOEC(EC10)_{sed\ wwt} = NOEC(EC10)_{sed\ dwt}/4.6$[22] (see Section R.16.6.4 *Characterization of environmental compartments* of the *Guidance on IR&CSA, Chapter R.16*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

[22] The conversion factor of 4.6 is derived from the following equation: $NOEC(EC10)_{\mathbf{sed\ wwt}} = (NOEC(EC10)_{\mathbf{sed\ dwt}} * Fsolid_{susp} * RHOsolid) / RHO_{susp}$
with:
- Volume fraction solids in susp. matter: $Fsolid_{susp} = 0.1\ m_{solid}^3.m_{susp}^{-3}$
- Density of the solid phase: $RHOsolid = 2500\ kg_{solid}.m_{solid}^{-3}$
- Bulk density of (wet) suspended matter: $RHO_{susp} = 1150\ kg.m^{-3}$

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.

$$C_{sed,\lim} = \frac{K_{susp-water}}{RHO_{susp}} \cdot C_{watersol} \cdot 1000$$ **Equation 11-2**

$C_{watersol}$ *(mg.L$^{-1}$)*
$RHO_{susp}$ *(bulk density of wet suspended matter expressed in kg.m$^{-3}$)*
$K_{susp-water}$ *(m$^3$.m$^{-3}$)*
$C_{sed,lim}$ *(mg.kg$^{-1}$)*

If no chronic effects are found from this limit test, the result can be considered as experimental evidence that the substance does not meet the pelagic T criterion, 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 of the *Guidance on IR&CSA*, *Chapter R.7b* (see http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment)). 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).

**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.

**Screening information and screening criteria**

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 criteria 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*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

The toxicity criterion (T) for PBT assessment cannot be decided upon the basis of acute studies alone. If the screening criterion 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[23]. 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, as these substances may not have had sufficient time in the acute test to be significantly taken up by the test organisms and to reach equilibrium partitioning. 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[24]. 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

[23] 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.

[24] 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.

provides a NOEC close to but above 0.01 mg/L, a long-term fish study is likely to be needed to confirm "not T" unless, taking into consideration the above-mentioned approach, convincing evidence exists that the fish NOEC will be higher than 0.01 mg/L. Supporting evidence in such considerations could be an acute fish value that is a factor of 10 or more greater than that of the other two trophic levels under the provision that the acute daphnid test showed toxicity at least one order of magnitude lower than the limit of solubility.

Certain chemical characteristics (such as high adsorption or extremely low solubility) are likely to make any toxicity testing extremely laborious if not technically impossible. Guidance has been developed by OECD on toxicity testing of difficult substances (OECD, 2000). 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*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

**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 criteria for T (EC50 or LC50 < 0.1 mg/L). It may, on a case by-case-basis, be decided whether confirmatory chronic testing on fish is necessary if valid QSAR prediction indicates that the acute E(L)C50 is < 0.01 mg/L. Alternatively either first an acute fish toxicity limit test could be performed to check whether the acute toxicity is below 0.1 mg/L or the QSAR-prediction could be accepted as providing sufficient evidence of the T criterion being fulfilled.

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

**Integrated testing strategy for T-testing in support of PBT assessment for the aquatic environment**

In this section guidance on the recommended testing strategy is provided as an annotated flow chart (Figure R.11—5).

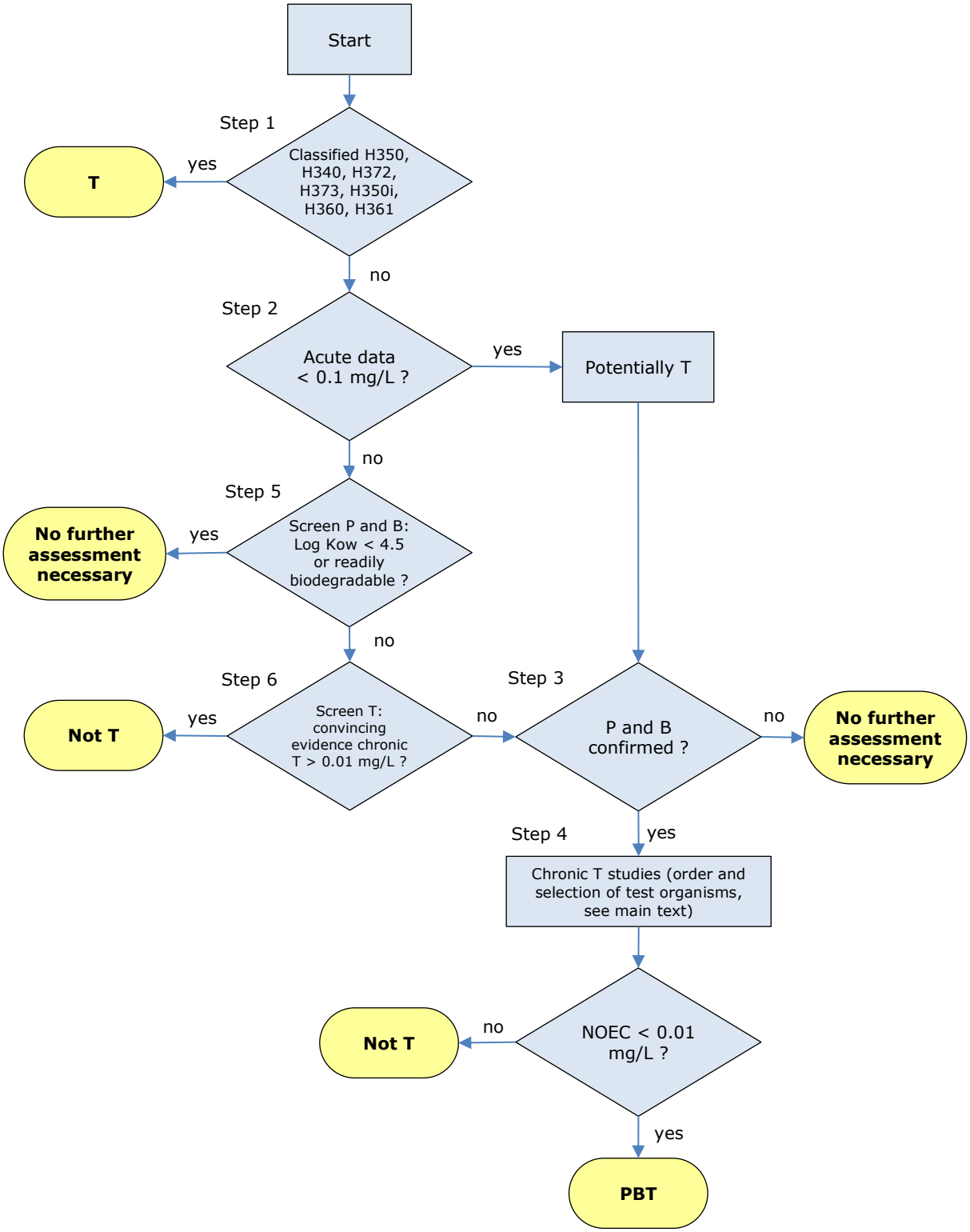


**Figure R.11—5: T testing in support of PBT assessment for the aquatic environment.**

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;

- 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, or STOT RE 2, 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, THEN move to step 2.

**Step 2:** Assessment of acute aquatic toxicity data;

- IF any $EC_{50}$ < 0.1 mg/L, THEN the substance is a Potential T candidate. Move to step 3.
- IF all $EC_{50}$ ≥ 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. 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 < 0.01 mg/L, THEN PBT confirmed
- IF NOEC ≥ 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:** Further screening of long term T-evidence (e.g. by means of read across and *Weight-of-Evidence* or group approach)

- IF information lacking, 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.11.4.1.4 Conclusions on PBT or vPvB properties

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

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

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

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

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

**(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 does 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 criteria as provided in Section R.11.4 were not met for any particular endpoint based on screening information. Furthermore, when the screening criteria for persistence or bioaccumulation as defined in the following sub-sections are not fulfilled, further PBT/vPvB assessment can stop when there is a well justified lack of counter evidence which would raise concern for the substance to have PBT or vPvB properties. In this case, the registrant can also draw the conclusion (i).

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

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

In the case of aquatic toxicity, there will be occasions when acute aquatic toxicity data are not available or the available acute aquatic toxicity data will be insufficient to judge whether chronic effects might occur at or below the 0.01 mg/L level. Such cases may occur when the water solubility is very low and/or the octanol/water partition coefficient is very high. In such cases acute, i.e. short term, aquatic tests may not give a true measure of toxicity because steady state conditions could not be reached within the duration of the test.

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

Where toxicity is a critical parameter for PBT assessment, i.e. the substance is persistent and bioaccumulative but there are insufficient 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).

**(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.*

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 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 evidence may be sufficient to conclude 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). However, there may be 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.

In some circumstances, the available data may not allow a direct numerical comparison to Annex XIII for each of the criteria, but there may be other relevant data available, which provide evidence that the substance fulfils the PBT or vPvB criteria. It is necessary for the registrant to consider in a *Weight-of-Evidence* approach and by use of expert judgement, all the information that is available on the property or properties for which a direct numerical comparison is not possible to determine whether further information must be generated or whether a conclusion can be drawn.

It may be possible to decide on a scientific basis that a test for determining a particular property is not necessary. This applies if already available information provides sufficient evidence that the particular criteria would be met if the appropriate test were conducted. For example, a substance may not fulfil the bioaccumulation criteria based on available screening information, but it is persistent and toxic according to the criteria and there is evidence from field measurements for significant bioaccumulation in organisms at or near the top of the food chain. In addition, evidence of high bioconcentration from structurally similar compounds may allow a conclusion to be drawn.

Subject to the conditions stated in Section R.11.4.1.1,where a substance shows < 20% degradation in a standard test for inherent biodegradation, this can be considered as confirmation that the substance will not degrade with a degradation half-life lower than the Annex XIII criteria, and hence no further confirmation of persistence is needed.

There are other circumstances where a conclusion can be drawn that the substance fulfils the Annex XIII criteria. For example:

- Substances that are not themselves persistent but have degradation products or metabolites that have PBT or vPvB properties as defined by Annex XIII (see further in relation to both PBT/vPvB assessment efforts (Sections R.11.3.2 and R.11.3.3) and to emission and risk characterisation and management measures (Section R.11.3.4);
- Read-across of data from a structurally similar substance with known PBT, vPvB properties.

In some cases the particular data-set for a substance, when compared to Annex XIII, may show that the specific criteria are not met, but other evidence, such as monitoring data may exist and provide evidence to the contrary. These data should be examined carefully in a *Weight-of-Evidence* approach and an expert judgement made whether the criteria should be considered as being met and the substance consequently be identified as PBT or vPvB.

For determining whether the available evidence leads to the conclusion that the substance is a PBT/vPvB althought the data do not allow a direct comparison with all the criteria in Annex XIII, it is clear that no specific criteria can be identified, but rather a set of contributing factors that could be considered on a case-by-case basis. These contributing factors may, of course, become *de facto* criteria over time but will also have had more rigorous scrutiny during this period. All assessment has, by definition, some uncertainty. The level of uncertainty that can be accepted is a political/policy decision but generally it is recognised that underestimates of adverse effects are possible, even if unlikely. One aspect that influences the acceptability of uncertainty is, of course, the potential consequences of being wrong in defining the level of effect. For example, if the adverse consequences can be easily reversed by regulatory action, e.g. by imposing some form of exposure control, some uncertainty in the risk characterisation is likely to be acceptable.

What distinguishes the PBT and vPvB substances from other substances is that i) the level of uncertainty in identifying long-term risk cannot be estimated with sufficient accuracy and ii) consequences of an underestimation of adverse effects are not easily reversible by regulatory action, i.e. the effect is occurring or is likely to occur at a certain point in time and, even if there is immediate regulatory action to prevent further emission, the adverse effects will continue.

Under these circumstances, the uncertainty in the prediction of risk is less acceptable. The acceptability is further complicated by the fact that the combination of properties ensures that such substances over longer timeframes will distribute widely in both environmental media and biota, and thus the impact, should it occur, will be both prolonged and widespread.

Given that the criteria in Annex XIII are specific, whereas the properties that give rise to the above concern cannot be so rigidly defined by science, expert judgement must be applied with a *Weight-of-Evidence* determination to identify substances of concern. One key concern for PBT/vPvB substances is their potential for widespread distribution and, where there is evidence that this can occur or has occurred, this should be taken into account. One example of where

this can be considered important is where there is a potential for long-range transport through the air, with accompanying evidence that wide distribution could occur. This, in addition to specific real or 'borderline' PBT/vPvB properties, can be considered as evidence giving rise to PBT or vPvB concern and hence to consider the substance as fulfilling the PBT or vPvB criteria.

A key property in determining whether widespread distribution and environmental accumulation could occur is that of persistence. Normally, only persistent substances would undergo widespread spatial transport and present the potential for long-term contamination of large areas that are characteristic of PBT/vPvB type substances. In general, the more persistent a substance is shown to be, the more it will be necessary to consider carefully all available evidence in assessing the potential for bioaccumulation and toxicity in order to decide whether a substance should be considered as a PBT or vPvB.

If a substance is not persistent according to the criteria of Annex XIII, it would normally not need to be considered further as being a potential PBT or vPvB. However, before taking that decision, any additional evidence that may be available particularly from monitoring data covering locations remote from known emission sources should be carefully examined. Evidence from monitoring showing occurrence in remote areas is not, on its own, evidence of persistence, although it may be evidence of widespread distribution. Where a time trend from such remote monitoring is available and this shows that the 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. It should be noted that in many such cases the substance may be considered in the absence of information on experimental degradation half-lives as persistent. If the substance also meets the BT or vB criteria, it must be considered as PBT or vPvB.

If a substance clearly meets the persistence criteria of Annex XIII, then a number of other factors relating to bioaccumulation and toxicity should be carefully considered.

Where the substance has been shown to have a very long environmental persistence, i.e. the half-life in relevant environmental media is very much greater than that defined in Annex XIII, then evidence of bioconcentration close to but below that in Annex XIII should be considered as potential evidence for identifying the substance as a PBT/vPvB. If there is additional evidence from monitoring in biota, and in particular top predators from remote regions, this would lend further weight to a conclusion that this substance is a PBT or vPvB. In these cases, if it is concluded that the substance is not considered as PBT or vPvB, this should be clearly justified in the PBT/vPvB assessment.

Evidence of bioconcentration from water alone may not be sufficient to fully describe the potential for uptake, particularly where the substance has a high adsorption capacity. Other routes of exposure may predominate in the environment and be reflected through monitoring and widespread detection in biota. Detection of a substance in the tissue of an organism provides a clear indication that it has been taken up by that organism, but does not by itself indicate that significant bioconcentration or bioaccumulation has occurred. For that, the sources, contemporary exposure levels and uptake routes (for example through water as well as food) must be known or reasonably estimated. Nevertheless, widespread occurrence in biota unrelated to local sources, particularly top predators and biota in remote areas, should be examined carefully to determine whether this should be considered as evidence suggesting the substance is a PBT/vPvB. A normal quantitative risk assessment can consider accumulation in biota via the secondary poisoning scenario (see Section R.7.10 of the *Guidance on IR&CSA, Chapter R.7c*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) and this may address the concern. Where this is considered the case, clear justification for this approach must be documented in the CSA. Where there is convincing evidence that a substance can biomagnify in the food chain, the substance should be considered as fulfilling the bioaccumulation criterion irrespective of the measured BCF. Further discussion of the use of BMF indicators is included in Section R.11.4.1.2. Field measurements of concentrations in organisms at various trophic

levels in defined food chains or food webs can be used to evaluate biomagnification, but the interpretation of such data may be difficult.

***Terminology***

For the purposes of this Guidance, the following terminology is used for substances which have been concluded to fulfil the PBT or vPvB criteria:

- *PBT or vPvB substance*: A substance having a constituent with PBT or vPvB properties, which is present at a concentration of 80 % or more;
- *Substance containing maximum X % (or X% - Y%) PBTs or vPvBs*: A substance having one or more constituents or impurities with PBT or vPvB properties in individual amounts equal or above 0.1 % (but less than 80%). The percentage can be a maximum percentage (X) or a range (X-Y), whatever is applicable.
- *Substance forming PBTs or vPvBs*: If any constituent, impurity or additive of a substance degrades or is transformed into substances which fulfil the PBT or vPvB criteria and if these transformation or degradation products are formed in "relevant" amounts. The term "relevant" has been defined for the registrant's substance in Section R.11.4.1. For the purpose of the REACH Article 59 process for identification of Substances of Very High Concern, the assessment of what are "relevant" transformation/degradation products may be done case-by-case. The percentage of degradation or transformation products may be indicated as for impurities or constituents with PBT- or vPvB- properties, if applicable (more guidance on degradation/transformation products is given in Section R.11.4.2.2).

The consequences of conclusion (ii) for the registrant are described in Section R.11.3.

**(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.*

Where an analysis of 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[25].

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 or B properties, the registrant should draw conclusion (ii) and generate the necessary additional information until the available information allows to draw one of the two ultimate conclusions (see Section R.11.4.1 for description of "relevant").

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

[25] 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.

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 main constituent, impurity or additive 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.

This may for example occur with UVCBs where it might be possible to conduct a confirmatory test but where the outcome may be difficult to interpret in terms of the conclusions on the PBT properties of all (unknown) constituents.

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.

The consequences of this conclusion for the registrant are described in Section R.11.3.3.

### 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.

**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 of the *Guidance on IR&CSA, Chapter R.7b*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) 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:

1. Results from an animal study (mammalian or fish) confirming no or low bioaccumulation
2. $D_{max\ aver}$ of the molecule is > 1.7 nm[26] and a Mol weight > 700 g/Mol

**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'.

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

**Substances with low solubility in octanol and water**

The assessment strategy should be applicable to substances with low solubility in octanol and water and in general having a narcotic mode of action (see Section R.6.2.1 of the *Guidance on IR&CSA, Chapter R.6* for guidance on identification of MoA, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) 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).

***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 with **narcotic mode** of action 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*<br>50 | 0.8<br>0.4 | **1.8***<br>**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 might also be applicable for substances with low solubility in octanol and water:

1. Results from an animal study (mammalian or fish) confirming no or low uptake into the organism
2. $D_{max\ aver}$ of the molecule is > 1.7 nm[26] and a Mol weight > 700 g/Mol

***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). 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 $C_o/C_w$ ≤ 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 & 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 & 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 multi-constituent substances

**Characterising multi-constituent substances (MCS) and UVCBs**

The process of assessing multi-constituent substances (MCS) and UVCB substances comprises several stages, including identification of the constituents, impurities and additives, where relevant, as provided in the *Guidance for identification and naming of substances under REACH and CLP* (see http://echa.europa.eu/guidance-documents/guidance-on-reach). It also involves gathering available data, relating these to the P, B & T properties of constituents and impurities, and, where necessary, generating new information.

The most critical stage in the assessment is characterising the MCS/UVCB to a sufficient level to allow a PBT/vPvB assessment to be conducted. Clear information on the composition of the substance is required within analytical and practical possibilities.

***Multi-constituent substances***

For MCSs this characterisation should be relatively straightforward and will entail a listing of the relevant constituents and the approximate percentages at which each constituent is present. Following such a listing the assessment should then proceed to address each of the constituents thus described, for a PBT/vPvB assessment. One potential advantage of addressing MCS constituents in this way is that there may be potential for read-across or grouping and/or use of QSAR model predictions on relevant known or suspected constituents (see also *Chapter R.6* of the *Guidance on IR&CSA,* http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment). This possibility could be explored in the same way as any other read-across or grouping approach.

***UVCBs***

For UVCBs, the characterisation will not be so easy, as by definition the composition of a UVCB may be largely unknown and variable. For a UVCB substance, all known constituents, present at concentrations ≥ 10 % should be specified by at least the English-language IUPAC name but preferably a CAS number; the typical concentrations and concentrations ranges of the known constituents should be given as well. Constituents that are relevant for the classification of the substance and/or for PBT/vPvB assessment must always be identified by the same identifiers. This means that substances with PBT or vPvB properties need to be considered for the PBT/vPvB assessment down to a threshold level of ≥ 0.1 % (w/w). Where it is scientifically practical, unidentifiable constituents should be assessed using the following strategy:

1. Assess the available data that is used to characterise/describe the UVCB. For example boiling point range is one of the main descriptors of petroleum substances and, if used with other more specific manufacturing information, can be used to generate a list of structures that could reasonably be predicted to be present in the UVCB. 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 substances. For other classes of similar chemicals that are also UVCB (e.g. surfactants) 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 specified based on chain length, degree of branching and halogenation. Whatever approach is used to characterise the composition of the UVCB substance, a scientific and technical justification should be provided.

2. Identify the structures that are to be used as representative structures of the unknown fraction, detailing why they are representative and, if possible, give the approximate concentrations of the fraction for which they are 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.

**Gathering and assessing available information**

The next stage of an assessment of an MCS or a UVCB is to gather all the relevant information relating to the constituents defined (in an MCS) or as described above, for UVCBs. In addition, information regarding the use of the substance and emission patterns should be gathered as it is possible that ultimately this information will be necessary to address the level of concern that might be expressed, (see Sections R.11.3.2.1 and R.11.3.4) for example about high tonnage complex substances. Toxicology information for the substance, both mammalian and aquatic, should be gathered as well as the data that relates to persistence and potential to bioaccumulate. Similarly, when toxicology or persistence data are present, or information related to bioaccumulation potential that cover the individual constituents or representative structures are available, these should also be collected. Depending upon the type of UVCB, or the consistency of properties of constituents in an MCS, it may be possible to set up blocks, e.g. as in the hydrocarbon block method, that allow for the assessment to proceed, based on information from representative constituents/structures and read across to the blocks. Thus the composition of a UVCB can be defined in terms of representative structures for groups of closely related molecules, while for an MCS this would be blocks based on the identified constituents. Examples of UVCBs are petroleum substances, in which different hydrocarbon classes form homologous series with gradual, predictable progressions of properties with increasing carbon number or number of branches. Part of the process is then to define the key structural classes (or blocks), into which constituents can be sub-divided. In this way it is possible to "map" UVCB substances into a common set of blocks which can be evaluated with respect to the following properties.

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 compound and that this has consequences for the assessment of UVCBs. For degradation (hence persistence) and bioaccumulation, the concentration of the chemical 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.

***(i) Persistence***

A consequence of the statement above is that one cannot easily assess the persistence of complex substances that contain many constituents using biodegradation testing methods that measure summary 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.

In the case of UVCB substances, the following general strategy is suggested for P assessment. If the UVCB substance consists of homologous 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). However, care should be taken if the range of chain length is very broad. The UVCB substance may still contain a certain amount of constituents that are persistent if the amount of easily degradable constituents is high enough and thus may lead to an overall degradation percentage sufficient to meet the criteria for ready biodegradation. For UVCBs that do not consist of homologous structures, ready biodegradation test data should be judged on a case-by-case basis depending on relative composition and degradability of individual constituents. In cases where the UVCB substance is not readily biodegradable or ready biodegradability data are lacking, a second tier of P assessment is proposed.

In the second Tier, based on the blocks previously defined, the evaluation with respect to P properties can proceed by reference to experimental data or valid (Q)SAR predictions for the chosen representative structures/constituents in each block.

***(ii) Potential for Bioaccumulation***

Similar difficulties apply to bioaccumulation assessment. Moreover, most bioaccumulation test methods are not applicable (or at least difficult to apply) to MCS or UVCB substances. Thus the 'mapping' or 'blocking' approach described above for the evaluation for persistence of individual constituents can also be used for assessing bioaccumulation potential by use of test data or valid (Q)SAR predictions on the chosen representative structures/constituents in each block.

In a first tier, estimates for the individual components 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. If initial estimates of the blocks do not indicate a potential for bioaccumulation, further assessment is not necessary.

For those blocks for which further assessment is required the second tier proceeds with testing of representative structures that help in making a decision for those blocks.

***(iii) Toxicity***

Toxicity is defined via a concentration response (Mackay *et al*., 2001) and is dependent on the bioavailability of the individual constituents in an MCS or an UVCB test substance. This may make interpretation for some substances 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 to the test medium. The consequence of this would be that toxicity may not be seen in the test system (e.g. coal tar pitch), whereas in the real world the toxic constituents would be released into the environment in a manner that meant they were no longer confined by the physico-chemical structure of the substance as a whole and hence could cause toxic effects.

For petroleum derived UVCBs, the lethal loading test procedure (WAF) provides the technical basis for assessing the short term aquatic toxicity of petroleum substances (OECD, 2000; Girling *et al*., 1992, see also Appendix R.7.8-1). Test results are expressed as a lethal or effective loading that causes a given adverse effect after a specified exposure period. The principal advantage of this test procedure is that the observed aquatic toxicity reflects the multi-component dissolution behaviour of the constituent hydrocarbons comprising the petroleum substance at a given substance to water loading. In the case of petroleum substances, expressing aquatic toxicity in terms of lethal loading enables petroleum substances comprised primarily of constituents that are not acutely toxic to aquatic organisms at their water solubility limits to be distinguished from petroleum substances that contain more soluble hydrocarbons and which may elicit acute aquatic toxicity. As a consequence, this test procedure provides a consistent basis for assessing the relative toxicity of poorly water soluble UVCBs and has been adopted for use in environmental hazard classification (OECD, 2000; UNECE, 2003). UVCB 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). This is problematic when addressing T within a PBT assessment. Consequently, the blocks that have been assessed for P and B, should be evaluated using valid QSAR models and available experimental data.

**Generation of new information**

Degradability and chronic toxicity testing of MCSs and UVCBs thought to contain PBT constituents, is generally not advocated, as the results can often be difficult to assess. For this reason QSAR estimation and read-across are often chosen approaches for generating new information, other than the testing of strategically selected individual constituents, if needed. With respect to the order of testing, for the PBT assessment of a mono-constituent substance,

this would generally proceed stepwise with the assessment of potential persistence addressed first, followed by bioaccumulation (if the P criteria is met) and then toxicity testing (if both P and B are met). For MCSs and UVCBs this assessment strategy may need to be further evaluated and treated on a case- by-case basis, depending upon the ease and cost of generating such data and animal welfare considerations. Thus for UVCBs and MCS, this process would probably start with a B assessment including initial assessments of potential for uptake and metabolism (see Section R.11.4.1.2 on B assessment).

**Final assessment**

For those substances containing many constituents a case-by-case approach is necessary and only some general guidance can be given. In relation to the question, "how much information is required", a *Weight-of-Evidence* approach should be applied which will include expert judgement addressing many other issues including feasibility etc.

The further steps in terms of information gathering, and implementation of RMM should be related to the magnitude of impact to human health and environment (e.g. percentage of PBT/vPvB impurities, release potential including consideration of the tonnage and the use categories).

An example approach, based on the Hydrocarbon Block approach and the scheme outlined above, is given in Appendix R.11—3.

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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 latest version of the text being discussed by the TC-NES WG on PBTs[27].

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[28] plus a molecular weight of greater than 1100***
2. ***a maximum molecular length (MML) of greater than 4.3 nm[29]***
3. ***Octanol-water partition coefficient as Log10 (Log $K_{ow}$) > 10***
4. ***a 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[28] 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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[27] Please note that only editorial changes to the text of the TC-NES PBT WG were made during the first revision of this Guidance.

[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.

[29] 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.

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 criterion, 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 criterion 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 the OECD TG 305 test. 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 (MW) 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 of the *Guidance on IR&CSA, Chapter R.7a*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment). 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 weight

A number of values have been suggested for the molecular weight (mwt) cut-off for absorption across fish tissues. The EU TGD (EC, 2003) indicates that molecules with a mwt greater than 700 g/Mol are less likely to be absorbed and bioconcentrate. The US EPA, exempts chemicals with a molecular weight of above 1,100 g/Mol in the PBT assessment conducted under the Toxic Substances Control Act (US EPA, 1999). Anliker *et al.* (1988) suggested that a pigment could be excluded from needing a fish bioaccumulation test if it has both a molecular weight of greater than 450 and a cross section of over 1.05 nm (as the second smallest van der Waals diameter or $C_{eff}$). Rekker and Mannhold (1992) suggested that a calculated Log $K_{ow}$ of > 8 can be used on its own, or in combination with a molecular weight of > 700-1,000 to conclude (with confidence) that the compound is unlikely to bioaccumulate. While there has been limited experimental evidence for a molecular weight cut-off, Burreau *et al.* (2004) did demonstrate reduced bioconcentration and no biomagnification for high molecular weight polybrominated diphenyl ethers, with six or more bromines, molecular weight 644-959.

Conclusion: Evidence from both mammalian and fish studies indicate that molecular weights have been suggested or used to estimate a chemical's limited bioaccumulation potential. Considering that molecular size and shape vary versus molecular weight, molecular weight alone is insufficient. However, it does suggest that once the molecular weight is in the region of 700-1,100, depending on other factors, a reduced BCF may be expected.

While recognising the uncertainties in the interpretation of experimental results, it is recommended that to demonstrate a reduced BCF a substance should have either:

- Possibly not vB : a molecular weight in excess of 700 g/mol, or
- a molecular weight of greater than 700 g/Mol with other indicators (see later discussion).

**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 (EC, 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, and will be published in 2007. 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—6, Figure R.11—7 and Figure R.11—8 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 of the *Guidance on IR&CSA, Chapter R.7c*, http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

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—6).

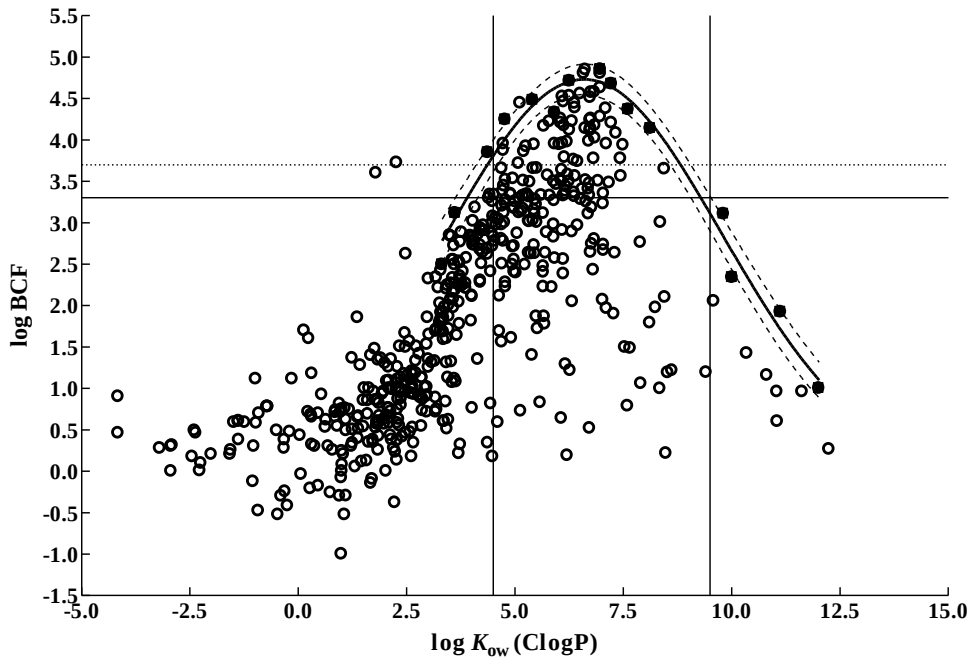



**Figure R.11—6: Log BCF v calculated Log $K_{ow}$.**

Figure R.11—7 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).

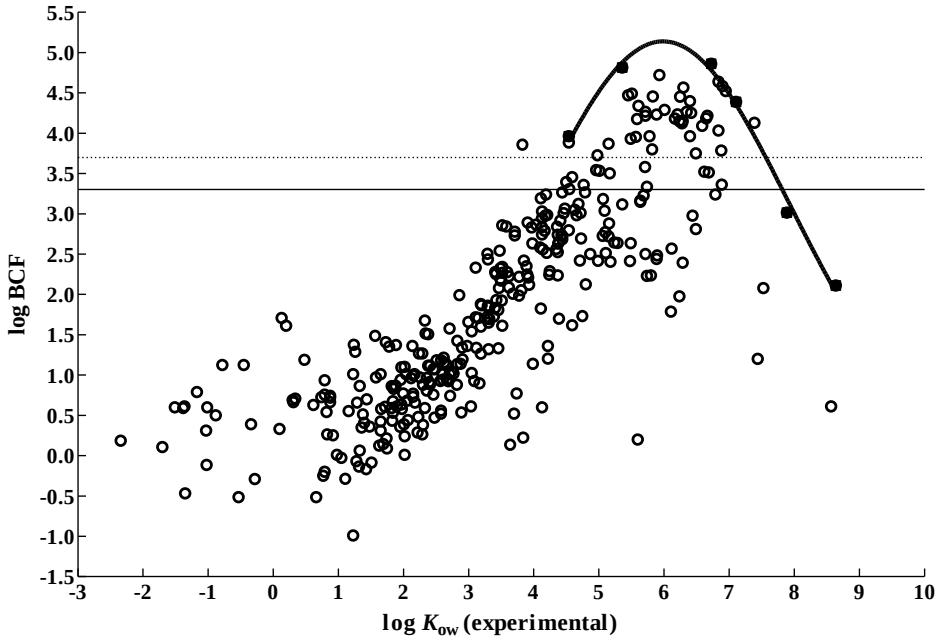



**Figure R.11—7: LogBCF v measured log $K_{ow}$.**

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—8). 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 2. 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).

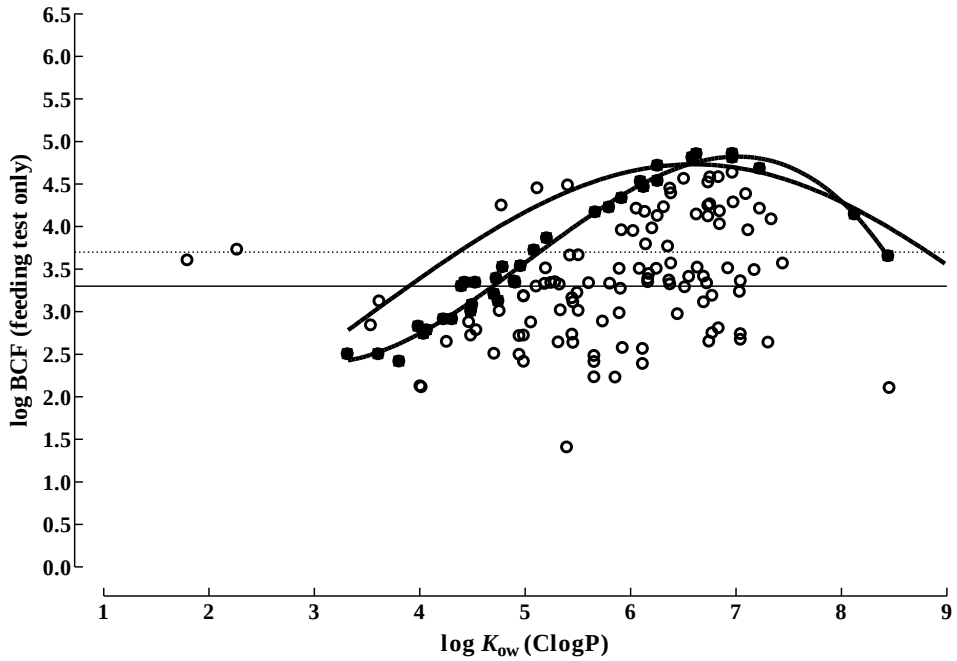



**Figure R.11—8: LogBCF derived from feeding studies versus calculated Log $K_{ow}$.**

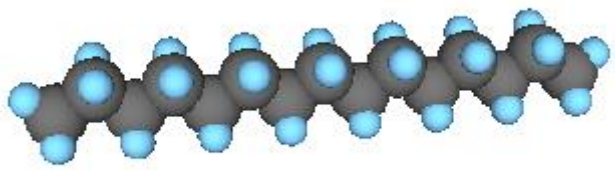
Summarized, the results of Figure R.11—6 to Figure R.11—8 suggest that the B-criterion is unlikely to be triggered for substances with a Log $K_{ow}$ higher than 10. As with the other indicators described in the main paper, a Log $K_{ow}$-value higher than 10 should be used in a *Weight-of-Evidence* approach in combination with the other indicators.

**Appendix R.11—1 Annex 2**

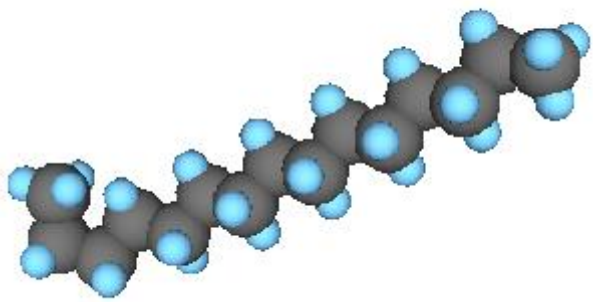
**GRAPHIC DEFINITIONS FOR THE MOLECULAR DIMENSIONS USED IN THE MAIN PAPER**

- 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.

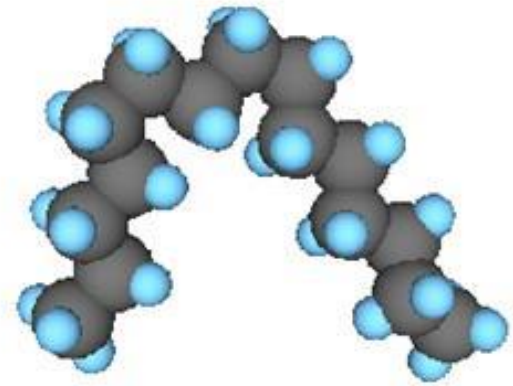
Conformer 1 (ΔHo = -84.5 kcal/mol), Dmax = 21.4; Deff = 4.99; Dmin = 4.92



Conformer 2 (ΔHo = -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.

**References**

Ivanov JM, Karabunarliev SH, Mekenyan OG (1994) 3DGEN: A system For an Exhaustive 3D Molecular Design. J Chem Inf Comput Sci 34:234-43.

Mekenyan OG, Pavlov T, Grancharov V, Todorov M, Schmieder P and Veith G (2005) 2D-3D Migration of Large Chemical Inventories with Conformational Multiplication. Application of the Genetic Algorithm. J Chem Inf Model 45:283 -92.

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Stewart JJP (1993) MOPAC 93. Fujitsu Limited, 9-3, Nakase 1-Chome, Mihama-ku, Chiba-city, Chiba 261, Japan, and Stewart Computational Chemistry. 15210 Paddington Circle, Colorado Springs, Colorado 80921, USA.

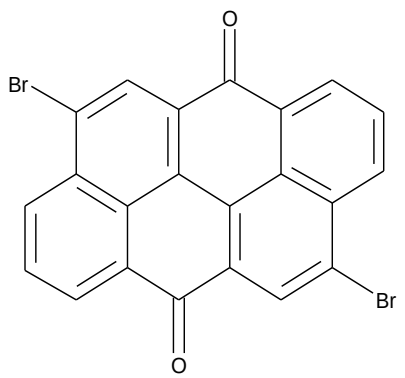
Stewart JJP (1990) MOPAC: A semi empirical molecular orbital program. J Comput-Aided Mol Des 4:1-105.

Wiese T and Brooks SC (1994) Molecular modelling of steroidal estrogens: novel conformations and their role in biological activity. J Steroid Biochem Molec Biol 50:61-72.

**Appendix R.11—1 Annex 3**

**EXAMPLES - USE OF THE INDICATORS FOR LIMITED BIOACCUMULATION**

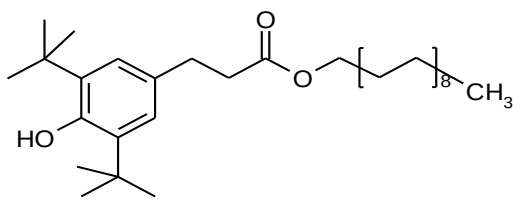
**Example R.11-1**

| **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.

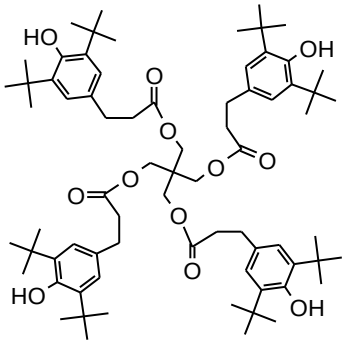
**Example R.11-2**

| **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.

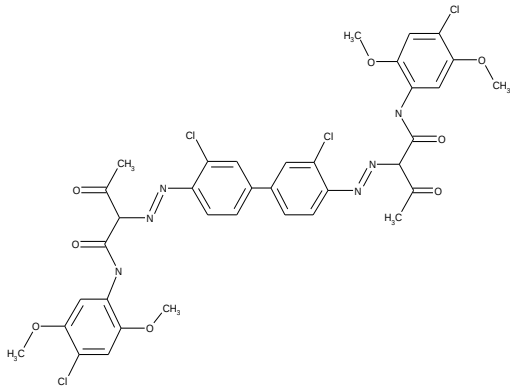
**Example R.11-3**

| 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 $K_{ow}$

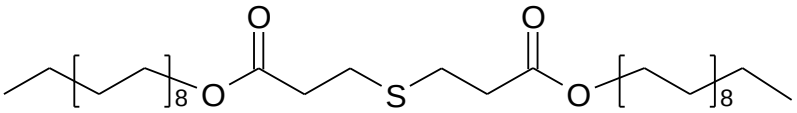
**Example R.11-5**

**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.**

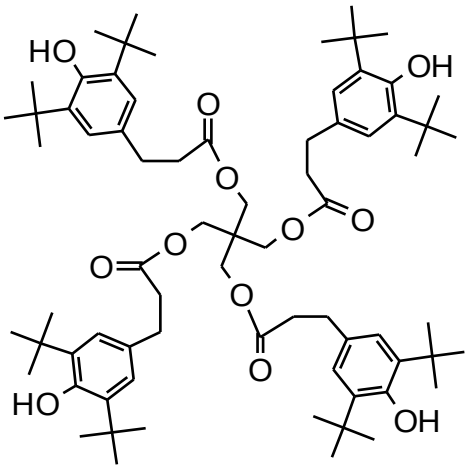
**Example R.11-6**

**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.**

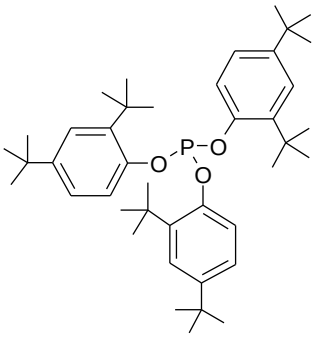
**Example R.11-7**

**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

Example 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<br>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.

### References

ETAD (2006) Measurements of Octanol and Water solubility of Pigments, carried out by ETAD Member companies, Data ownership is with ETAD.

Ullmann (1995) Encyclopaedia of Industrial Chemistry, Section Antioxidants.

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

**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 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 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 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, when required, it is suggested that an analytical approach based on Total Petroleum Hydrocarbon (e.g. TNRCC Method 1005) methods should be used. Other alternative methods (e.g. 2D-GC) are also becoming available that offer higher resolution that may also be helpful in being more precise in the exact type of structures present, (Forbes *et al*., 2006).

The outcome of this step should be a matrix of hydrocarbon blocks, with a minimum of boiling point range and %contribution to the petroleum substance. With 2D-GC this characterisation can be extended to include broad descriptions of structures including alkanes, isoalkanes, naphthenics, etc.

**Step 2: Assessment of available data**

The next step is to collate the available information on the petroleum substances 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 than 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.

**Step 3: Assessment of persistence (P)**

The first part of the P assessment would be to examine the available data, and in particular attempt to identify whether the petroleum substances under investigation could be considered to be ready biodegradable. As discussed in Section R.11.4.1.1 ((i) Persistence), for homologous substances, where there is convincing evidence of ready biodegradation of the whole substance, e.g. in an OECD TG 301 type test, it can be reasonably assumed that the individual components are unlikely to be persistent.

If there is insufficient evidence for ready biodegradation, then the assessment should proceed to the next stage. This involves generating typical structures either from the analysis conducted or from other sources of information relevant to the petroleum substances being

assessed. Thus for example, Comber *et al*.(2006) describe how a set of over 1400 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. 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 type potentially present in the petroleum substance.

The assessment can then proceed with assessment of available information on any known individual chemicals, e.g. benzene, hexane, pristine etc. This information will in every case be insufficient for the assessment of petroleum substances due to the wide range of potential structures and the relatively limited information currently available on individual structures that are normally not part of an assessment process, 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 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.

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 UM-BBD (Ellis *et al.*, 2006).

Finally, to demonstrate that there are no concerns, caused by potential metabolites (the previous assessments are all addressing primary biodegradation), it is recommended that a prediction of potential metabolites be made and these also assessed (although the extent of this assessment needs to be carefully considered and depend on the type of structures being assessed). An example of such a model is CATABOL (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 to the bioaccumulation assessment.

**Step 4: Assessment of 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 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 structures.

Where there are insufficient experimental data to be able to make a judgement there are several QSAR models available for continuing the process.

Stewart *et al*. (2005) describe the work done to BCFWIN v2.16, to re-calibrate the model for hydrocarbon type structures by ensuring that the data used was of the highest quality and that recently generated information was also incorporated.

The second model that can be used, Dimitrov *et al*. (2005) is based on a wide range of good quality information and specifically addresses biotransformation, while making an assumption about the maximum uptake possible at specific Log $K_{ow}$s.

An assessment of the predictions from these 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.

Where there are blocks that are showing a concern for both P and B properties, it will normally lead to the need to generate further higher tier information on these properties. The exceptions to this conclusion might be where there are sufficient ecotoxicological data on specific structures in the blocks that demonstrate no concern for the T criteria and where the P and B properties are sufficiently defined that an evaluation for vPvB is unnecessary.

**Step 5: Assessment of toxicity (T)**

As previously discussed, the assessment of the toxicity of individual substances within a petroleum substance is extremely difficult. While the whole substance assessment 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*., 2006), based on previous work assuming a non-polar narcosis mode of action (McGrath *et al*., 2004, 2005). This model, which was developed to predict the ecotoxicity of petroleum substances and hydrocarbon blocks, could be used to address individual structures where no experimental data is 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 be, however, used as supporting tools for designing tests and for the interpretation of experimental results.

**References**

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TNRCC (2001) TNRCC Method 1005, TOTAL PETROLEUM HYDROCARBONS, Texas Natural Resource Conservation Commission, Revision 03, June 1, 2001.

**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.

$$BSAF = \frac{BCF(lipid)}{K_{oc}} = \frac{2000/0.05}{K_{oc}} \text{ for indication of B or } \frac{5000/0.05}{K_{oc}} \text{ for indication of vB}$$

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.

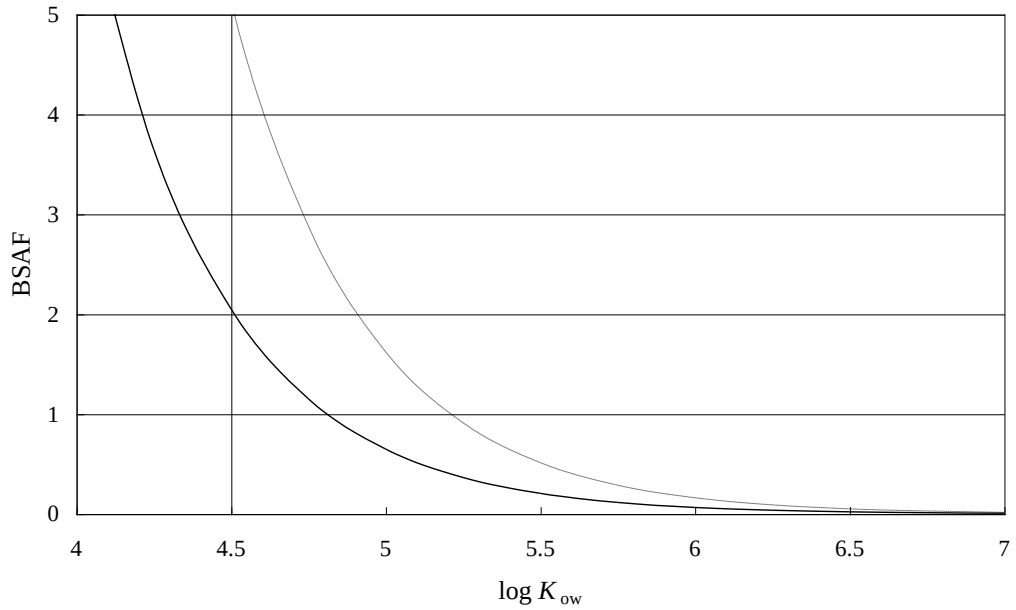


**Figure R.11—9: Relationship between lipid and organic carbon normalised BSAF values and Log $K_{ow}$ as indicator for the B and vB criterion.**

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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