Guidance on information requirements and chemical safety assessment

Chapter R.11: PBT Assessment

May 2008

LEGAL NOTICE

This document contains guidance on REACH explaining the REACH obligations and how to fulfil them. 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. The European Chemicals Agency does not accept any liability with regard to the contents of this document.

PREFACE

This document describes the information requirements under REACH 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 REACH.

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. These guidance documents can be obtained via the website of the European Chemicals Agency (http://echa.europa.eu/reach_en.asp). 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 Corrigendum to 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); amended by Council Regulation (EC) No 1354/2007 of 15 November 2007 adapting Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) by reason of the accession of Bulgaria and Romania (OJ L 304, 22.11.2007, p. 1).

Convention for citing the REACH regulation

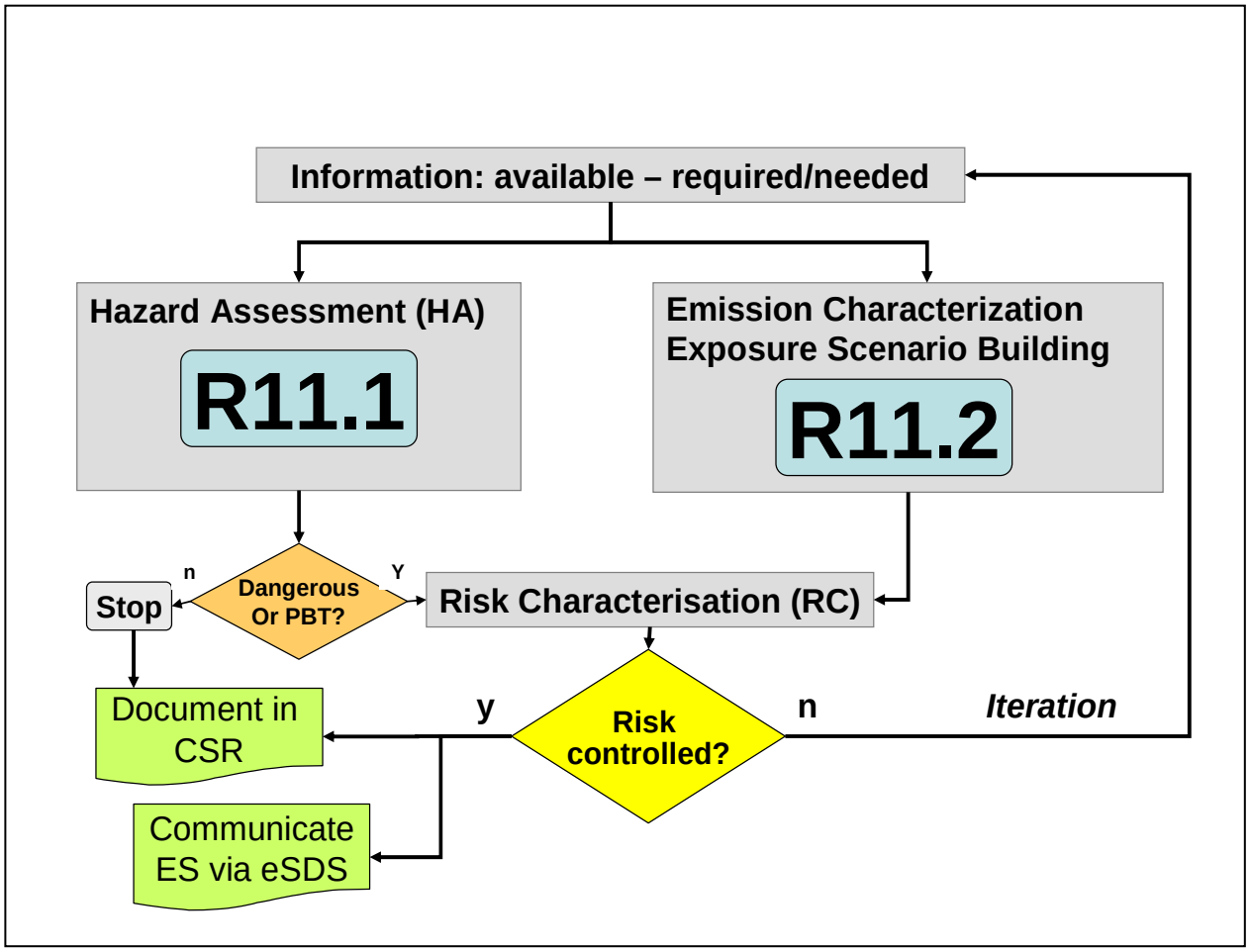
Where the REACH regulation is cited literally, this is indicated by text in italics between quotes.

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 ASESSMENT

A PBT/vPvB assessment is required for all substances for which a chemical safety assessment (CSA) must be conducted and reported in the chemical safety report (CSR). These are 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 REACH. However, some further exemptions apply as described in Article 14(2), e.g. for substances present in a preparation if the concentration is less than 0.1 % weight by weight (w/w), for on-site or transported isolated intermediates, and for Product and Process Oriented Research and Development (see *Guidance on Registration*, section 1.8.1 for further information).

PBT substances are substances that are persistent, bioaccumulative and toxic, while vPvB substances are characterised by a particular high persistency in combination with a high tendency to bio-accumulate, but not necessarily proven toxicity. These properties are defined by the criteria laid down in Annex XIII of the Regulation.

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 practically 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 case of vPvB chemicals, there is concern that even if no toxicity is demonstrated in laboratory testing, long-term effects might be possible since high but unpredictable levels may be reached in man or the environment over extended time periods.

The properties of the PBT/vPvB substances lead to an increased uncertainty in the estimation of risk to human health and the environment when applying quantitative risk assessment methodologies. For PBT and vPvB substances a "safe" concentration in the environment cannot be established using the methods currently available with sufficient reliability for an acceptable risk to be determined in a quantitative way[2]. Therefore, a separate PBT/vPvB assessment is required under REACH (Art. 14(3d)) 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 Annex I(4) of the Regulation, the objective of the PBT/vPvB assessment is to determine if the substance fulfils the criteria given in Annex XIII, 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. In addition, it is necessary to identify the likely routes by which humans and the environment are exposed to the substance. According to

[2] 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.

Annex I (6.5) 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 (RMM) which minimise emissions and subsequent exposures of humans and the environment throughout the lifecycle of the substance that results from manufacture or identified uses.

In practice, the PBT and vPvB assessment comprises 3 steps (1) comparison with the criteria, (2) emission characterisation and (3) risk characterisation, which are outlined in detail in sections R.11.1 and R.11.2 of this Chapter.

In the first step, the registrant has to compare the available information on intrinsic properties with the criteria for persistency, bioaccumulation and long-term toxicity given in Annex XIII. The registrant needs to consider all the information that is available in his technical dossier (all available and relevant information and as a minimum the information required by the relevant Annexes VII to X). In cases where the information in the technical dossier does not allow a *direct* comparison with the criteria in Annex XIII, Annex I (4.1) requires the registrant to:

- consider, on a case-by-case basis, other available evidence like monitoring data giving rise to an equivalent level of concern and
- consider all information relevant for screening of the P, B and T properties of his substance.

The sections on the assessment strategy and the assessment of the P, B and T properties of a substance provide guidance on how a registrant can make best use of the different types of information available. These 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 requiring the generation of data that literally 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 data and all further evidence available. In many cases further information as detailed in Annexes IX and X of the Regulation 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 sub-sections of R.11.1.3.

Substances are considered as PBT or vPvB substances when they fulfil the criteria for all three (or two) inherent properties P, B and T or 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.

There are a number of possible outcomes from the comparison with the criteria with different consequences on the further steps of the PBT/vPvB assessment.

i) The data show that the properties of the substance meet the specific criteria detailed in Annex XIII, or do not allow a direct comparison with all the criteria in Annex XIII, but nevertheless indicate that the substance is likely to have these properties.

   In this case an emission and risk characterisation in accordance with the stipulations of Annex I (4.2 & 6.5) is required (i.e. characterisation of all emissions throughout the lifecycle of the substance and implementation, respectively recommendation of RMM and operational

conditions (OC) that minimise exposure of humans and the environment). Guidance on these two consecutive steps is given in Section R.11.2 below.

ii) The data show that the properties of the substance do not meet the specific criteria detailed in Annex XIII or do not allow a direct comparison with all the criteria in Annex XIII but nevertheless indicate that the substance is not likely to have these properties and, consequently, that the substance is not considered a PBT/vPvB.

In this case the PBT/vPvB assessment stops at this point. A normal exposure assessment and risk characterisation in accordance with Sections 5 & 6 of Annex I may however be required if the substance is dangerous in accordance with the classification criteria of Council Directive 67/548/EEC (see Parts D and E of this guidance document).

iii) The data on the properties of the substance do not allow a direct comparison with all the criteria in Annex XIII and further information is needed.

In this case a registrant has two options:

1. The registrant generates the required information (depending on the information needed, the submission of a testing proposal may be required) and concludes on the PBT/vPvB properties of the substance concerned once the lacking data are available (i.e. conclusion (i) or (ii)); or
2. The registrant refrains from generating further information and treats his substances as if it were a PBT/vPvB (see conclusion (iv)).

iv) Further information would be needed to conclude on the PBT/vPvB properties of the substance. However, the registrant (for several reasons) has decided not to conduct confirmatory testing.

If a clear decision on the properties of a substance cannot be made, either because it is not possible to characterise a substance, or since it is technically not possible to conduct testing, this lack of a clear decision does not obviate the requirement on a registrant to propose appropriate and proportionate RMMs and OCs. Further guidance on this case is provided in Section R.11.1.5.

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). The criteria for identifying POPs are overlapping with the PBT/vPvB criteria, but include the potential for long-range transport. Any Party to the Convention or to the Protocol may propose further substances to be included. In future, such proposals could use information provided as part of registration dossiers under REACH.

R.11.1 Assessment strategy

The PBT and vPvB assessment of a substance shall be based on all the relevant information available, which is normally the information that shall be submitted as part of the technical dossier, including the physicochemical, hazard and exposure information generated in the context of the CSA (i.e. information on physicochemical properties, toxicological or environmental hazard endpoints and human or environmental exposure concentrations). If the technical dossier, for one or more endpoints, contains only the information as required in Annexes VII and VIII, the registrant shall, based on screening data or other information available, consider whether further information needs to be generated to fulfil the objective of the PBT and vPvB assessment, i.e. to assess whether the substance fulfils the criteria.

For substances satisfying the PBT and vPvB criteria, an exposure and risk characterisation with the objective to minimise emissions and subsequent exposures of humans and the environment from manufacture or identified uses shall be performed (see Section R.11.2). In this context it is important to note that a substance may consist of more than one constituent or that it may form transformation or degradation products. If the substance contains one or more constituents with PBT/vPvB properties in individual amounts ≥ 0.1 % (w/w) or if transformation/degradation products with the respective properties in individual amounts ≥ 0.1 % are being generated (see Section R.11.1.1 for details), the substance must be treated like a PBT/vPvB with regard to emission estimation and exposure control, i.e. in accordance with the principles laid down in Section R.11.1.2. However, it may be considered, for the sake of relevance of risk posed by such an amount and the proportionality of assessment effort, to elevate the threshold value above 0.1%. In the considerations whether application of an elevated percentage trigger could be appropriate, the use pattern of the multi constituent substance and the potential emissions of the constituents or degradation/transformation products having PBT or vPvB properties must be accounted for. Thus, careful consideration must be given as to whether the lower 0.1 % threshold should apply where uses leading to significant emissions are anticipated. An elevated threshold value should not exceed 10% (w/w) for the total amount of all constituents and transformation/degradation products with PBT/vPvB properties, and the total amount of such constituents and transformation/degradation products should not exceed 1 tonne/year.

The PBT or vPvB assessment should cover a consideration of each parameter (P or vP, B or vB, and T) in order to arrive at an informed decision on the PBT or vPvB properties of a substance (or its individual constituents / transformation products). In principle, substances are only considered as PBT or vPvB when they are deemed to fulfil the criteria for all three (or two) inherent properties P, B and T or vP and vB, respectively. 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. Further guidance on this issue is given in Section R.11.1.5.

The assessment strategies set out in Sections R.11.1.3 and R.11.1.4 should normally be followed and further information be searched for or generated, if necessary. In deciding which information is required (on P, B or T) care must be taken to avoid animal testing where 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 the B criterion, eventually followed by toxicity testing to clarify the T criterion.

However, for substances for which persistency testing is difficult or practically impossible, like e.g. for certain multi-constituent or very poorly water soluble substances, it may sometimes be more reasonable to start the PBT/vPvB assessment by evaluating the B criterion. The B-assessment may

in these cases start with non testing information such as log K_{ow} data, molecular size, diameter and weight. In other cases, where it concerns multi-constituent substances or very poorly soluble substances, further specific bioaccumulation tests could form the start of the PBT or vPvB assessment. Guidance on the assessment of substances whose specific substance properties such as being a mixture of several constituents, formation of transformation products, low water solubility, high adsorption or volatility require derogation from the standard assessment and testing strategy is given in Section R.11.1.4 whereas the standard assessment approach is outlined in Section R.11.1.3.

R.11.1.1 Terminology for and management of substances containing PBT or vPvB constituents

R.11.1.1.1 Substance Identity

The identity of any substance for which a registration dossier is prepared should be clearly described in accordance with the respective guidance for identification and naming of substances as developed in the *Guidance on Substance Identification*.

As a general rule, for well defined substances (mono- and multi-constituent substances), it should be aimed to know and cover the composition up to 100%, and for each constituent a complete chemical specification, including structural information, should be given. Constituents relevant for the PBT/vPvB assessment should be specified if present in a concentration of ≥ 0.1% (*Guidance on Substance Identification*). Individual concentrations < 0.1 % need normally not to be considered (for details see Section R.11.1.1.3). If for one or several impurities ≥ 0.1% the identification and quantification is not possible, a justification shall be included in the CSR.

It may not be possible to sufficiently identify UVCBs (substances of Unknown or Variable composition, Complex reaction products or Biological materials) by the identification parameters of REACH Annex VI(2) 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. However, the chemical composition and the identity of the constituents should still be given as far as is known. For a UVCB substance, all known constituents present at concentrations ≥ 10 % should be specified by at least the English IUPAC name and preferably a CAS number. The typical concentrations and concentrations ranges of the known constituents should be given as well.

R.11.1.1.2 Terminology to be used for substances containing PBTs and vPvBs

The following terminology should be used for substances containing PBTs or vPvBs:

- *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 of a substance degrades, or is transformed into substances which have PBT or vPvB properties and if these transformation or degradation products are formed in individual amounts above 0.1% (of the weight of the initial substance). 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.11.1.3.

It is emphasised that management measures have to be considered as soon as a substance contains or degrades to PBT or vPvB substances above the threshold of 0.1%, irrespective of which of the three groups described above the substance belongs to.

R.11.1.1.3 Implications for Risk Management

The principal requirements for identification and naming of mono- or multi-constituent substances and UVCBs are laid down in the *Guidance on Substance Identification*. Further guidance on how to conduct a PBT/vPvB-assessment for multi-constituent substances and UVCBs is given in Section R.11.1.4.2. Assessment of metabolites and transformation products is described in Section R.7.9. Together these documents provide the general framework to which extent constituents and degradation/transformation products should be identified.

If a constituent or degradation/transformation product has been shown to have PBT/vPvB properties, in principle a ≥ 0.1 % (w/w) threshold applies for assessing and managing the substance containing or generating this constituent or product like a PBT/vPvB. In this case, an emission and risk characterisation for each constituent or degradation/transformation product would normally be required, including documentation of the results in the CSR. However, assessment efforts should be proportionate to the magnitude of the potential impact to human health and the environment (e.g. depending on the percentage of PBT/vPvB constituents and exposure potential based on tonnage and use; see more details in Section 11.1 on the assessment strategy).

For substances with PBT/vPvB constituents in individual amounts < 0.1 % of the substance, normally no further action is necessary. This is in line with the threshold used for considering PBT and vPvB substances in preparations (Article 14(2)(f)). However, there may be particular cases of PBT/vPvB constituents for which specification of percentages below 0.1 % is required. But this requirement is then driven by the toxicological profile of the constituent (e.g. high potency CMR) and the provisions for classification and labelling and not by the fact that the respective constituent is concomitantly a PBT/vPvB.

R.11.1.2 PBT and vPvB criteria

R.11.1.2.1 Definitive criteria

The criteria to be used to decide if a substance (or one of its constituents or transformation products in individual amounts ≥ 0.1 % (w/w)) must be regarded as a PBT or vPvB substance are set out in Annex XIII to the Regulation. Table R. 11-1 provides an overview of these criteria. Two sets of criteria exist, one for PBT substances and a second category for vPvB substances. This second category is developed under the recognition that for substances that are very persistent and bioaccumulate significantly in the food chain, high but unpredictable levels may be reached in wildlife or man over extended time periods. For such substances it is not necessary to demonstrate toxicity in laboratory testing as long-term effects can be anticipated anyway.

The PBT and vPvB criteria of Annex XIII to the Regulation do not apply to inorganic substances but shall apply to organo-metals.

Table R. 11-1: PBT and vPvB criteria according to Annex XIII

Property	PBT-criteria	vPvB-criteria
Persistence The assessment of the persistency in the environment shall be based on available half-life data collected under the adequate conditions, which shall be described by the registrant.	- $T_{1/2}$ > 60 days in marine water, or - $T_{1/2}$ > 40 days in fresh- or estuarine water, or - $T_{1/2}$ > 180 days in marine sediment, or - $T_{1/2}$ > 120 days in fresh- or estuarine sediment, or - $T_{1/2}$ > 120 days in soil.	- $T_{1/2}$ > 60 days in marine, fresh- or estuarine water, or - $T_{1/2}$ > 180 days in marine, fresh- or estuarine sediment, or - $T_{1/2}$ > 180 days in soil.
Bioaccumulation The assessment of bioaccumulation shall be based on measured data on bioconcentration in aquatic species. Data from freshwater as well as marine water species can be used.	BCF > 2000 L/kg	BCF > 5000 L/kg
Toxicity	- NOEC (long-term) < 0.01 mg/L for marine or freshwater organisms, or - substance is classified as carcinogenic (category 1 or 2), mutagenic (category 1 or 2), or toxic for reproduction (category 1, 2 or 3), or - there is other evidence of chronic toxicity, as identified by the classifications: T, R48, or Xn, R48 according to Directive 67/548/EEC.	-

R.11.1.2.2 Screening criteria and information for identification of PBTs and vPvBs

For many substances the available data may not allow a definitive conclusion on the PBT or vPvB properties. In this case so-called screening criteria may be used as surrogate information to decide whether a substance may potentially fulfil the PBT or vPvB criteria. A summary of these screening criteria is provided in Table R. 11-2 More details on their use are provided in Sections R.11.1.3, R.11.1.4 and R.11.1.5.

In order to decide whether the substance must be considered as a potential PBT/vPvB substance, the whole available information needs to be taken into account. In particular, the screening criteria for each of the three properties persistency, bioaccumulation and toxicity need to be considered in conjunction. It has to be kept in mind that the fact that a substance does not meet the T criterion is not enough to stop the evaluation of the remaining endpoints in the PBT/vPvB screening step.

Table R. 11-2 : Screening criteria for P, vP, B, vB and T

Type of data	Criterion	Screening assignment	See section
Persistence			R.11.1.3.1
Ready biodegradability test	readily biodegradable	Not P and not vP	
Enhanced ready biodegradability test	readily biodegradable	Not P and not vP	
Specified tests on inherent biodegradability			
Zahn-Wellens (OECD 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	
MITI II test (OECD 302C)	≥70% mineralisation (O2 uptake) within 14 days; log phase no longer than 3d; no pre-adapted inoculum	Not P	
Biowin 2 (non-linear model prediction) and Biowin 3 (ultimate biodegradation time) **or** Biowin 6 (MITI non-linear model prediction) and Biowin 3 (ultimate biodegradation time)	Does not biodegrade fast (probability < 0.5)[3] and ultimate biodegradation timeframe prediction: ≥ months (value < 2.2) **or** Does not biodegrade fast (probability < 0.5)[1] and ultimate biodegradation timeframe prediction: ≥ months (value < 2.2)	P P	
Bioaccumulation			R.11.1.3.2
Convincing evidence that a substance can biomagnify in the food chain (e.g. field data [4])	e.g. BMF > 1	B or vB, definitive assignment possible	
Octanol-water partitioning coefficient (experimentally determined or estimated by valid QSAR)	Log Kow ≤ 4.5	Not B and not vB	
Toxicity			R.11.1.3.3
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	T	
Avian toxicity (subchronic or chronic toxicity or toxic for reproduction)	NOEC < 30 mg/kg food	T	

[3] The probability is low that it biodegrades fast

[4] See Guidance on information requirements

R.11.1.3 Assessment of PBT/vPvB properties – standard approach

R.11.1.3.1 Persistence assessment (P and vP)

When assessing data concerning the persistence of a potential PBT/vPvB 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 and a simulation test indicates a half-life ($T_{1/2}$) 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 and 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 as clear cut, and frequently they are contradictory, especially for biodegradation. Therefore a careful consideration is needed before a decision is reached. 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 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 a primary degradation is observed, it may therefore be 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 persistency assessment. As mentioned above, the sequence in which 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).

<u>Assessment of biodegradation data</u>

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

1. Tests on ready biodegradation (e.g. OECD 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, 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 (Sections R.7.9.3 and R.7.9.4).

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 301 A-F. Degradation is followed by determination of sum parameters such as DOC, CO2 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 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 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 details in Sections R.7.9.4 and R.7.9.5.

Tests on simulation of biodegradation

The simulation tests as described in OECD 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 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.

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 media 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 should 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).

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.

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 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).

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).

The tests used and their interpretation are all discussed in Sections R.7.9.4 and R.7.9.5.

Assessment based on estimation models (QSAR, SAR)

The use of QSAR and SAR predictions for identifying substances for persistency (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.

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).

(Q)SAR estimates may be used to preliminary identify substances with a potential for persistency. To 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 persistency 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. This section briefly highlights key issues:

- Many models are based on the same data set, derived from MITI, called the MITI-I dataset.
- Of the models available, those in the EPI suite, BIODEG, CATABOL and some based on MultiCASE/TOPKAT, have been extensively evaluated in the context of regulatory schemes.
- Currently, most of the models are only recommended for use for "negative" screening (i.e. mainly for concluding on the non biodegradability of the substance).
- Identification of metabolites and understanding the role of them is important.
- Consensus modelling may be the way forward.

An approach based on consensus modelling has recently been used in the Canadian exercise, screening the DSL[5] (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 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 and chloroanisoles (Banerjee *et a*l, 1984), *para*-substituted phenols (Paris *et a*l, 1983), and *meta*-substituted anilines (Paris *et al,* 1987).

The use of QSAR model predictions are in particular of 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.

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 Kow, Kp) significantly influencing the environmental fate of the substance.

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

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, 2006b). 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 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 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 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 half-lives. Guidance on how to interpret data from simulation test is available in Section R.7.9.4.

In the ITS for persistency assessment described below it is indicated which types of simulation degradation tests should be considered based on exposure pattern. The information in Table R. 11-3 below presents the criteria for the assessment of persistence (P/vP) and identifies relevant test systems for determining environmental half-lives.

Table R. 11-3: 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 half-life in marine water is higher than 60 days, or The half-life in fresh- or estuarine water is higher than 40 days, or	The 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 half-life in marine sediment is higher than 180 days, or The half-life in fresh- or estuarine water sediment is higher than 120 days, or	The 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 half-life in soil is higher than 120 days	The half-life in soil is higher than 180 days	OECD TG 307: Aerobic and anaerobic transformation in soil

Conclusion on the endpoint: ITS for persistency assessment

A strategy for degradation testing in the context of PBT/vPvB assessment is proposed in Figure R. 11-1. 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 shown to be or not to be persistent. A conclusion on persistence may be based on non-test data ((Q)SAR model predictions, read across, chemical categorisation), available non-standard test or standard test data and, if needed, on performance of simple and cheap tests, such as e.g. the OECD TG 301 series (with or without enhancements as described in Sections R.7.9.4 and R.7.9.5.

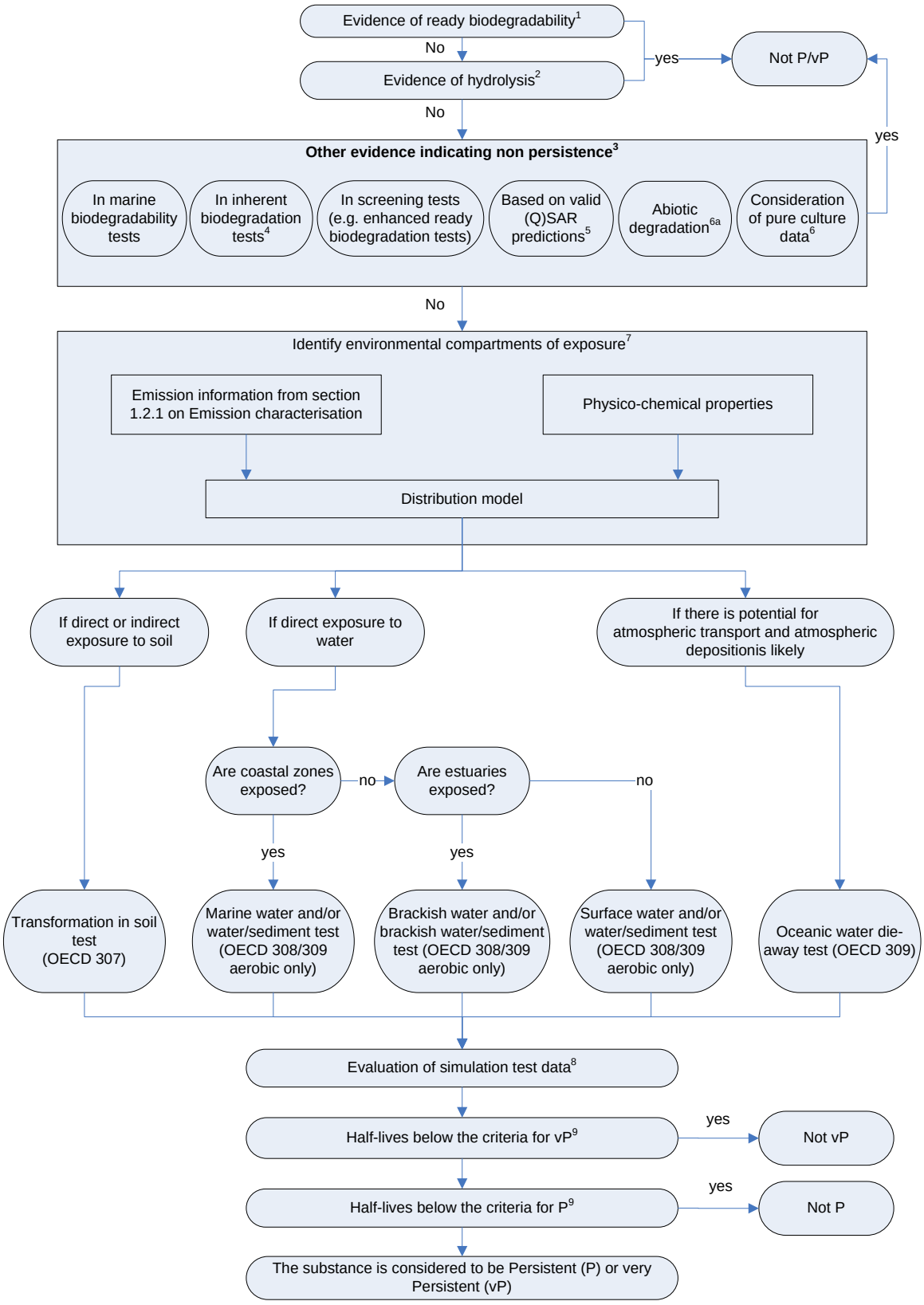


Figure R. 11-1: ITS for persistency assessment – maximising data use and targeting testing

Conclusion on Persistency - 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 exception of the 10-day window, there is no reason to perform further biodegradation tests for the PBT/vPvB assessment. The conclusion is that the substance is not fulfilling the criteria for Persistence (P) (see Sections R.7.9.4 and R.7.9).

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, no further testing of degradation is required for the PBT/vPvB assessment. The half-lives obtained in an 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. An attempt should be made to identify at least degradation products of >10% of the concentration of the parent substance (see. Sections R.7.9.4 and R.7.9.5).

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). 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 is *not* fulfilling 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, shall 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 persistency (see as well Section R.11.1.3.1 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 < 2.2), **or**

- MITI non-linear model prediction (BIOWIN 6): does not biodegrade fast (probability < 0.5) and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value < 2.2)

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 2.2 to 2.7 (see Section R.7.9.4 and R.7.9.5). 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 persistency status of the substance (cf. 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 persistency 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 this studies (e.g. photodegradation, oxidation, reduction) cannot on their own be used within persistency assessment, but in a weight of evidence approach.

Identification of the environmental compartment of exposure for simulation testing (see this Section R.11.1.3.1 , above)
valuation 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)

Evaluation ***versus the P and vP criteria*** (Section R.11.1.2)

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. This could include, for example, findings of significant concentrations of the substance under consideration in remote and pristine environments such as the arctic sea or Alpine lakes. 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.11.1.3.2 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. Guidance on the evaluation and validation of both testing data and non-testing information can be found in Section R.7.10.

Experimental aquatic BCF data

Bioconcentration studies with aquatic organisms, especially those obtained from established experimental protocols, such as the OECD 305 fish bioconcentration test (OECD, 1996) and the ASTM E1022-94 mussel bioconcentration test (ASTM, 2003) can be directly used for comparison with the B and vB criteria. If reliable data are available from other bioconcentration tests the results of such studies can also be used. BCF data shall be lipid normalised to a fat content of 5 % and whole body weight shall be considered to take into account growth dilution (for details see Section R.7.10). In the case of potential PBT/vPvB substances, which are generally rather hydrophobic, the analysis and maintenance of the aqueous concentration will be of special concern. To circumvent difficulties with maintaining and analysing the aqueous concentration of the test substance, a dietary accumulation test with fish may be used (Anon. 2004a, 2004b). Results of all mentioned tests can be directly compared with the B and vB criteria (results of the fish dietary test must however be recalculated to aquatic BCF values - details see Section R.7.10).

Field data and biomagnification

In accordance with Annex I all available information/evidence on bioaccumulation, like for example field data, shall be considered in a weight of evidence approach. Indicators like bioaccumulation factors (BCF) 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. (see also the *Guidance for the preparation of an Annex XV dossier on the identification of substances of very high concern*. 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) can as well be considered as an indication of 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.

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 in a way that they can be directly calculated from each other, unless certain assumptions and recalculations are made as in the case of the fish dietary accumulation test (Anon. 2004a, 2004b and Section R.7.10). However, because food chain transfer and secondary poisoning are basic concerns in relation to PBT and vPvB substances, an indication of a biomagnification potential can on its own right be considered 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. 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

BIOCONCENTRATION STUDIES WITH BENTHIC AND TERRESTRIAL INVERTEBRATE SPECIES

For some substances data may be available from bioconcentration studies with other species than fish. The bioaccumulation from soil or sediment to terrestrial/benthic species is expressed as the biota-to soil/sediment accumulation factor (BSAF). BSAF values can be useful as simple screening tools to indicate the bioaccumulation potential, although great variation in BSAF values may be observed across different methods. It also 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 (see Appendix R. 11-1).

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 for the potential of a substance to bioaccumulate within aquatic organisms can be obtained from toxicokinetic studies with mammals, if available. Relevant from such a study for PBT/vPvB assessment is information on the absorption efficiency. 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 were 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 kind of 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 can not 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 Kow, and octanol solubility should be used is briefly addressed in the following (background information on these parameters can be found in Appendix R. 11-2).

Other methods such as in vitro methods or biomimetic extraction procedures may as well 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 that has 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 L/kg. 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 L/kg that then were corrected downwards to values below 2000 or 5000 L/kg (see Section R.7.10.3.2).

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).

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. If this length exceeds 4.3 nm, it is 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 L/kg. Folding of long linear structures may alter the length of the molecule of the substance, which renders it easier 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, if a substance has a molecular length larger than 4.3 nm and other information indicating a low bioaccumulation potential is available, the criterion for B and hence also for vB can be considered as not being met.

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 the cell membranes. This results in a reduced BCF of the substance. From a diverse set of chemicals it appeared that for compounds with a $Dmax_{aver}$ larger than 1.7 nm the BCF value was less than 5000 L/kg.

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 L/kg. If the substance has a molecular weight higher than 700 g/Mol this is an indicator that the BCF is below 5000 L/kg. 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 KOW

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 L/kg (based on wet weight of the organism, which refers to fish in most cases).

Care must be taken in case that 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 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 Kow > 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 L/kg 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.

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. This indicative trigger value may however not apply to chemicals with specific toxicity (specific mode of action). 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 on "in vitro data on aquatic bioaccumulation").

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.

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 (for substances at levels below 100 t/y).
- 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. 11-2.

If the above mentioned supplementary information is available, based on WoE and expert judgement a chemical 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 and a molecular weight of greater than 1100 g/mol
2. a maximum molecular length (MML) of greater than 4.3 nm
3. octanol-water partition coefficient as log10 (log K_{ow}) > 10 (calculated value, preferably by several estimation programs, for substances for which log Kow can be calculated and the model is reliable)
4. 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 chemical as possibly not being vB (i.e. unlikely to have a BCF > 5,000) is, apart from indicators 2., 3. and 4. above:

5. a Dmax aver of greater than 1.7 nm plus a molecular weight of greater than 700 g/mol

Indicators 1., 2. & 5. recommended here as non-testing information influence uptake and distribution of substances. The log K_{ow} (3.) is a general indicator for uptake, distribution and excretion whereas the octanol solubility (4.) 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 fish or mammals 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)[6]

If a substance is imported or produced in an amount of more than 100 t/y and it is not possible to waive testing according to column 2 of Annex IX or according to Annex XI, a bioaccumulation test is mandatory. 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 Chapter R.7.10. Figure R. 11-2 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 considered if the available testing and non-testing data are sufficient to conclude on the B-properties for those substances < 100 t/y or if bioaccumulation testing is 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 must be considered.

[6] 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.

However, before such a test is conducted for assessing the B and vB criteria, the P criterion should be investigated first in order to prevent unnecessary testing of animals.

If a BCF test still must be performed, the OECD 305 test should be preferred. Note that any modification of a standard test protocol should only be done with the agreement of the appropriate regulatory authority. However, for the purpose of the PBT/vPvB assessment, a limited 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. For instance, if it is estimated that the BCF-value may be close to the threshold values of either 2000 L/kg for 'B' or 5000 L/kg for 'vB', the BCF determination by a limited test might not be warranted because the result may be associated with too much uncertainty. In such a case a full OECD 305 test would be appropriate. However, if a limited test is considered sufficient, usage of less fish could for example be achieved by testing at only one concentration (often the characteristics of the PBT/vPvB compound render a determination at two concentrations differing by a factor of 10 complicated) or by reducing the sampling frequency.

If the substance has a very low solubility, which makes the determination of the BCF according to OECD 305 complicated or impossible, an alternative test is the fish dietary bioaccumulation study. In the background document to the protocol of dietary test (Anon. 2004b) the method is advised for substances with water solubilities below 0.01 to 0.1 mg/L.

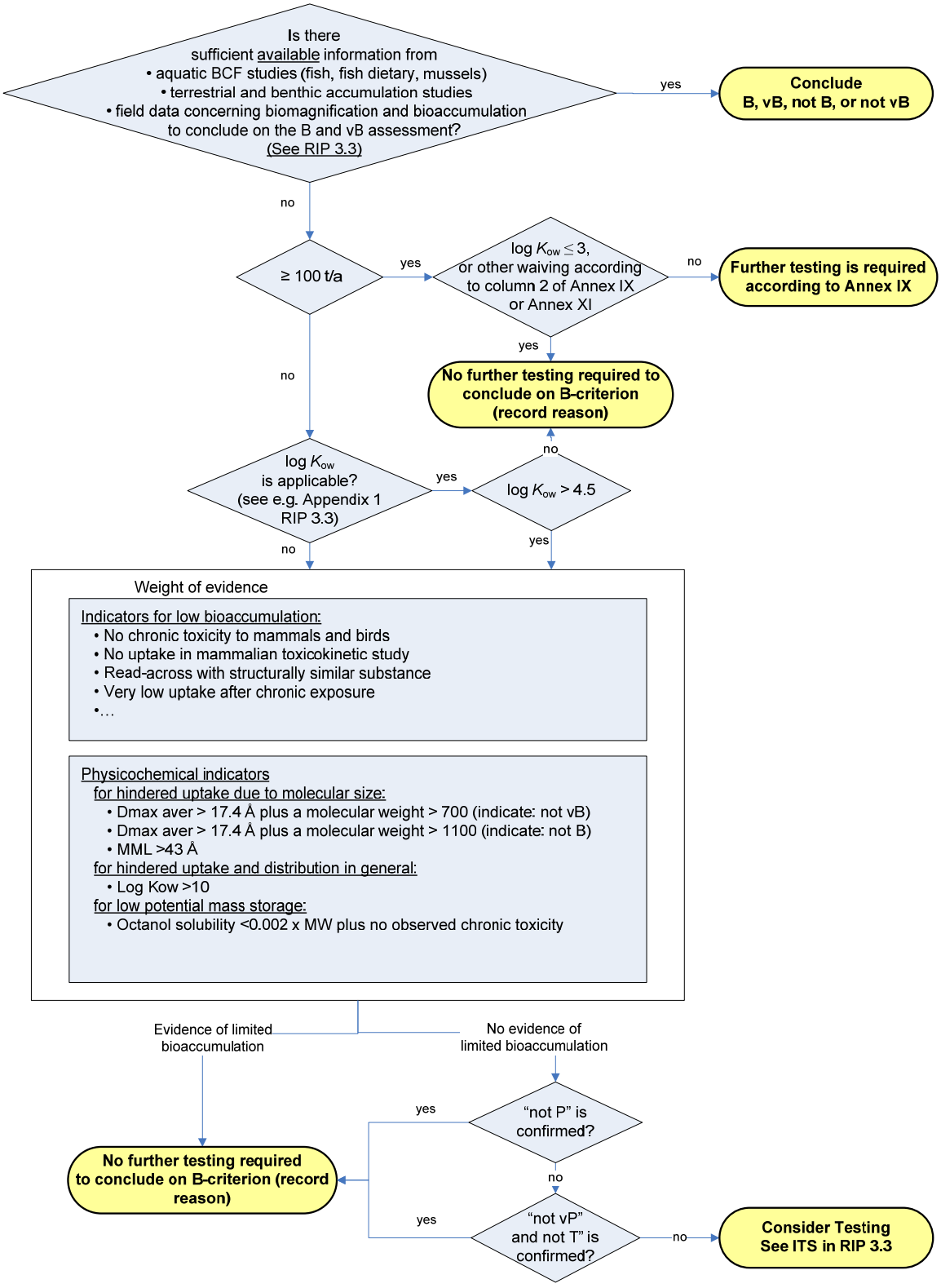


Figure R. 11-2: Integrated testing strategy for B-assessment

R.11.1.3.3 Toxicity assessment (T)

Definitive criteria

According to the REACH legislation (Annex XIII), a substance is considered to fulfil the toxicity criterion (T) when:

- the long-term no-observed effect concentration (NOEC) for marine or freshwater organisms is less than 0.01 mg/l, or
- the substance is classified as carcinogenic (category 1 or 2), mutagenic (category 1 or 2), or toxic for reproduction (category 1, 2 or 3), or
- there is evidence of chronic toxicity, as identified by the classifications: T, R48[7], or Xn, R48 according to Directive 67/548/EEC.

The evidence of CMR and chronic toxicity specified above does not only refer to substances that are already classified accordingly (i.e. R-phrases: R45, R46, R48, R49, R60 – R63) but also to the obligation to check whether the criteria for assigning the respective classifications are fulfilled in accordance with the provisions of Annex I (1.3, Step 3: Classification and Labelling)[8]. If any classification criterion leading to the assignment of the mentioned R-phrases 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 used for classification as T directly but reprotoxicity studies or other chronic data on birds, if existing, should be used as supporting data in conjunction with other evidence of 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 first P, B and then T should be applied and also vertebrate-animal testing should be minimised by first testing non-vertebrate species. For determination of definitive criteria for T, chronic tests must be performed. Under normal circumstances, these criteria are applied based on the methodologies defined in the tonnage triggered testing list (REACH annexes VII to X). Other test methods must be assessed on a case by case basis or are based on the recommendations described in the effects assessment methodology.

As the aquatic T criterion is based on a NOEC for pelagic organisms, the standardised chronic tests on fish, daphnids and algae are preferred to assess the NOEC. 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, especially fish, 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 values related to pelagic toxicity are accounted for in Annex XIII. A possible way to determine whether a substance has equivalent toxicity in sediment as in the water column could be to extrapolate the sediment toxicity value (e.g. NOEC) to a pelagic toxicity value

7 Note that R48 is normally assigned in combination with other Risk phrases and does not appear alone.

8 Guidance on classification of substances is provided in Annex VI to Directive 67/548/EEC, and the classification of preparations subject to Directive 1999/45/EC is also based on the criteria set up in Annex VI. The classification system set out under Directives 67/548/EEC and 19999/45/EC will in the future be replaced by the Global Harmonised System (GHS).

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).

However, it may as well be used to back-calculate a NOEC value of an existing sediment test to a corresponding pelagic NOEC.. The pelagic NOEC 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 value of 0.01 mg/l increases linearly with the suspended matter-water partitioning coefficient ($K_{susp\text{-}water}$) (see Section R.7.8).

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:

$$NOEC_{water} = \frac{RHO_{susp}}{K_{susp-water} \cdot 1000} \cdot NOECsed$$ **Equation 11-1**

$NOEC_{water}$ *(mg.L^{-1})*

RHO_{susp} *(bulk density of wet suspended matter expressed in kg.m^{-3})*

$K_{susp\text{-}water}$ *(m^3.m^{-3})*

$NOEC_{sed}$ *(mg.kg^{-1})*

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 solubilities below 0.01 mg/l, a chronic limit test ($C_{sed,lim}$) can be performed at the spiking 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})*

$K_{susp\text{-}water}$ *(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.7.15.1. 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 seem not to be applicable for the definitive assessment of the T criteria.

Screening criteria

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).

The toxicity criterion (T) for PBT assessment cannot be decided on the basis of acute studies alone. If the screening criterion is met, the substance is referred to definitive T testing and chronic studies are required regardless of the tonnage band unless the E(L)C50 < 0.01 mg/l. Normally, the testing order for conclusion on T based on chronic data is *Daphnia* and then fish[9]. If the T-criterion is fulfilled by the chronic algae or *Daphnia* data, a chronic fish test is not necessary.

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 < 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. (see decision tree for aquatic endpoints, steps 2, 5 & 6 and Figure R. 11-3).

In the absence of definitive 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

9 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.

chronic testing and conclusion on the T-properties[10]. If no conclusive evidence for significant differences in sensitivity between the groups can be found the testing order as mentioned above shall apply.

If the relevant test species is selected in accordance with the suggested approach in the paragraph above, lack of toxicity at or below the definitive T criterion for the tested species is evidence that further studies on T are not necessary. If however a long-term test on *Daphnia* or algae provides a NOEC close to but above 0.01mg/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 23, 2000). Some examples together with recommendations to overcome the technical difficulties are provided in the chapter on assessment of problematic substances (see Guidance on Information requirements

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. 11-4 . 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 to be fulfilled.

If the substance is documented to fulfil the P and B criteria at least acute testing should be performed to determine whether the substance meets the screening criteria for T.

10 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.

Table R. 11-4: Use of acute experimental data and non-testing data for T (screening) assessment

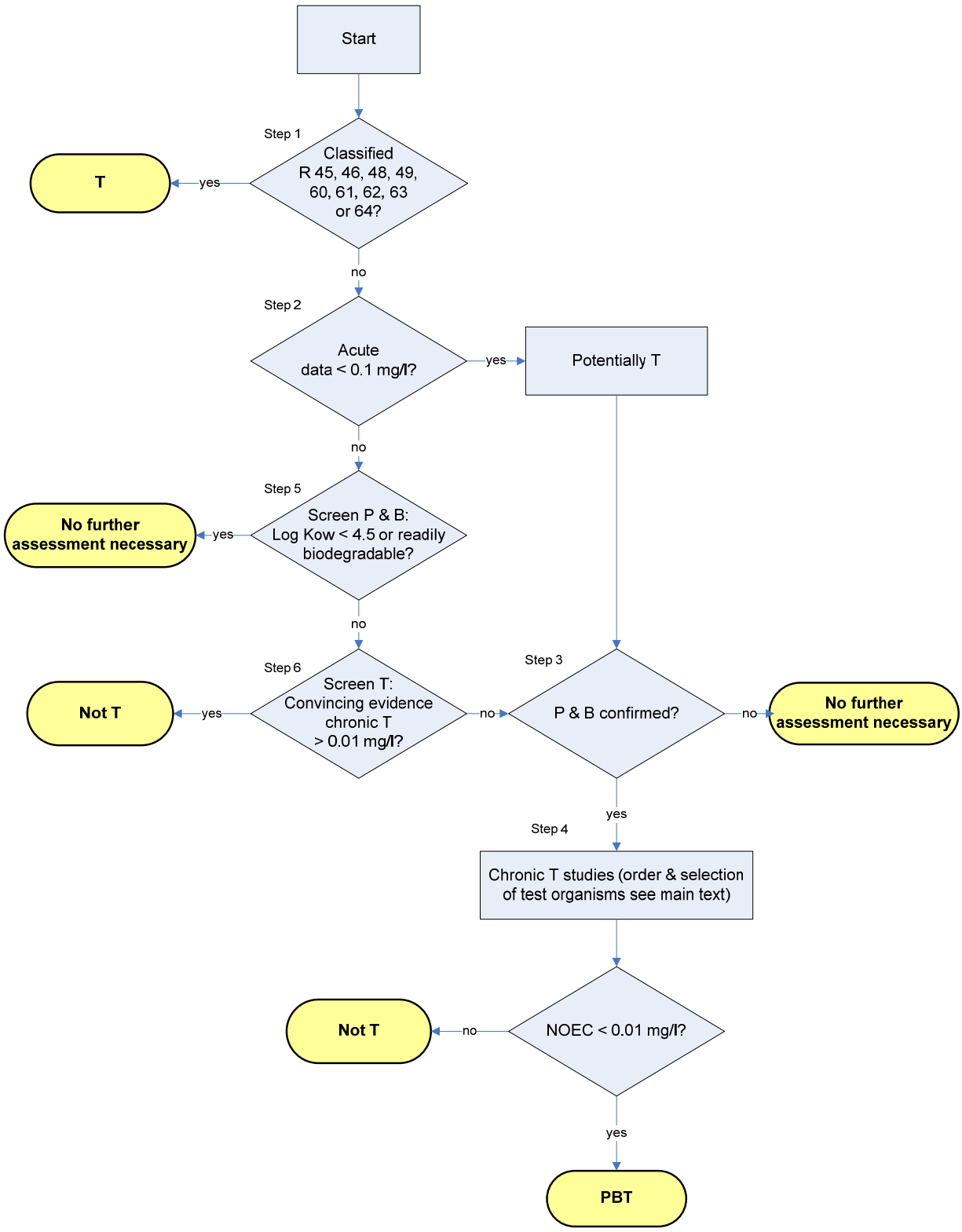
Type of data	Criterion	Screening assignment***	Definitive assignment
Short-term aquatic toxicity*	EC50 or LC50 ≥ 0.1 mg/L	presumably not T	-
Short-term aquatic toxicity*	EC50 or LC50 < 0.1 mg/L	potentially T	-
Short-term aquatic toxicity**	EC50 or LC50 < 0.01 mg/L	-	T

* From acute tests or valid QSARs ** from acute tests *** 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.

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

In this section the guidance on the recommended testing strategy is provided as annotated flow chart.

Figure R. 11-3: T testing in support of PBT assessment for the aquatic environment



According to Article 14, 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 (class 1 or 2), mutagenic (class 1 or 2) or toxic to reproduction (class 1, 2 or 3) or T, R48, or Xn R48, THEN define the substance as T and stop assessment
- IF not classified or likely to be classified as carcinogenic (class 1 or 2), mutagenic (class 1 or 2) or toxic to reproduction (class 1, 2 or 3) or T, R48, or Xn R48, THEN move to step 2.

Step 2: Assessment of acute aquatic toxicity data;

- IF any EC50 < 0.1 mg/l, THEN the substance is a Potential T candidate. Move to step 3.
- IF all EC50 ≥ 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* (nb: 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, then not B and STOP
- IF substance is readily biodegradable, then not P and STOP
- IF log Kow > 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

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* For specific guidance on identifying of P & B, please refer to Section 11.1.3.1 for persistence and Section 11.1.3.2 for bioaccumulation

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

R.11.1.4 Assessment of PBT/vPvB properties – consideration of specific substance properties

R.11.1.4.1 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 persistency, 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. 11-2 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 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 & T should follow the standard approach as described in Section R.11.1.3

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.11.1.3 is warranted.

STEP 3 vPvB Assessment for substances with log K_{ow} > 10

Step 3a Persistency 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. 11-8 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) 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 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 criteria (1. or 2.) mentioned above is fulfilled the substance should not be considered as vPvB, provided that potential metabolites are itself 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.11.1.3.2 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.11.1.3 is warranted.

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

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 for guidance on identification of MoA) 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

$$C_{octanol}\ [mg/L] < 0.002\ [mMol/L] \times Mol\ weight\ (g/Mol) \qquad \textbf{Equation 11-3}$$

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 123 slow stirring method (OECD, 2006a). But 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. 11-5). In order to overcome the difficulties to measure 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. 11-5). 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. 11-12). A measured log $C_o/C_w = 4.5$ would mean that the measured K_{ow} should be < 4.5.

In Table R. 11-5 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 log K_{ow} exceeds the log C_o/C_w between 1 and 8 orders of magnitude (more data see Appendix R. 11-2).

Table R. 11-5: Solubility of some pigments and comparison of their Co/Cw values with estimated Kow's

(US EPA,2000)

Colour Index Name	Mol weight (g/Mol)	Co (µg/L) at ambient temp	Cw (µg/L) at ambient temp	log Co/Cw	Log Kow (KOWWin)
Pigment Yellow 12	630	48* 50	0.8 0.4	**1.8*** **2.1**	**7.1**
Pigment Red 122	340	600	19.6	**1.5**	**2.5**
Pigment Red 168	464	124	10.8	**1.1**	**7.1**
Pigment Violet 23	589	330	25	**1.1**	**9.4**
Pigment Yellow 12: values with * relate to saturated solvents = water saturated octanol, octanol saturated water, this Log Co/Cw corresponds to log Kow					

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 be also 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 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 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 105 methods may be used in adapted form.

Step 2 B & T Assessment

The octanol solubility of the substance is compared with the critical body burden (CBB) according 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 be checked in addition to confirm reduced uptake and low toxicity. In this case the substance has low bioaccumulation potential and low toxicity.

Result 2B: C_o > CBB and log $C_o/C_w \leq 4.5$

If the octanol solubility is above the CBB a build up to a critical concentration of the substance in lipid cannot be excluded and additional information on adsorption is required. If the quotient log Co/Cw of measured solubilities is ≤ 4.5 (if measurable / available) a reduced uptake is expected as well. Animal studies should be assessed in addition 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 & T assessment as described in Section R.11.1.3 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 & 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.11.1.4.2 Assessment of multi constituent substances

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

The process of assessing multi-constituent substances (MCS) and UVCB substances is made up of several stages, including identification of the main constituents (10 – 80% of the substance) and significant impurities (in the range 0.1 – 10% of the substance). 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 that a PBT/vPvB assessment can be conducted. Clear information on the composition of the substance is required within analytical and practical possibilities.

Multi-constituent substances

For MCSs this 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 Section R.6). 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 English IUPAC name and 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 shall 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.

b) Gathering and assessing available information

The next stage of an assessment of a MCS or a UVCB is to gather all the relevant information relating to the constituents defined (in a 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.11.1.1 and R.11.2) 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, 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

The consequence of the statement above means 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 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 making a decision for those blocks.

(iii) Toxicity

Toxicity is defined via a concentration response (Mackay et al, 2001) and is dependant 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 phys-chem 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 23, 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.

(c) 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.11.1.3.2 on B assessment).

(d) 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.

R.11.1.5 Summary and overall conclusions on PBT or vPvB properties

A detailed analysis of the Persistence, Bioaccumulation and Toxicity should be brought together into a clear conclusion on whether the substance should be treated as a PBT/vPvB substance. There are a number of conclusions from this comparison that call for different responses from a registrant.

i) The data show that the properties of the substance meet the specific criteria detailed in Annex XIII, or do not allow a direct comparison with all the criteria in Annex XIII, but nevertheless indicate that the substance would have these properties

ii) The data show that the properties of the substance do not meet the specific criteria detailed in Annex XIII or do not allow a direct comparison with all the criteria in Annex XIII but nevertheless indicate that the substance would not have these properties and the substance is not considered a PBT/vPvB

iii) The data on the properties of the substance do not allow a direct comparison with all the criteria in Annex XIII and further information is needed

iv) Further information would be needed to conclude on the PBT/vPvB properties of the substance. However, the registrant (for several reasons) has decided not to conduct confirmatory testing.

The sub-chapters below provide more details on the circumstances that would lead to each of these decisions and how this should be recorded and dealt with by the registrant.

Where it is concluded that the substance is a PBT/vPvB substance or should be treated as PBT/vPvB substance an emission characterisation should be conducted and appropriate RMM and OCs be developed and indicated in the CSR and SDS to ensure safe handling. Guidance on emission characterisation and risk characterisation is given in Section R.11.2

It should be noted that the identification of a substance as being PBT/vPvB according to the criteria in Annex XIII does not automatically lead to a proposal for inclusion of the substance into Annex XIV and the subsequent requirement for authorisation. PBT/vPvB substances can be proposed by MS competent authorities and the Agency by request from the Commisison to be included into Annex XIV. Prior to a Commission decision to include substances in Annex XIV, the Agency will recommend priority substances taking into account the opinion of the Member State Committee. This prioritisation for inclusion in Annex XIV takes account of the presence of PBT/vPvB properties, wide dispersive use or high volumes. (see *Guidance on inclusion of substances in Annex XIV (substances subject to Authorisation*)**.** Hence, the implementation of appropriate and rigorous RMMs could influence the likelihood of inclusion in Annex XIV.

i) The data show that the properties of the substance meet the specific criteria detailed in Annex XIII, or do not allow a direct comparison with all the criteria in Annex XIII, but nevertheless indicate that the substance would have these properties

This would be the case if, as a result of an analysis of existing data, or of data generated under Annex IX and X, the environmental 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, a substance for which one of the constituents ≥ 0.1 % (w/w) has been shown to meet the Annex XIII criteria, shall itself be treated as if meeting the Annex XIII criteria. This general percentage may however be adapted in relation to both PBT/vPvB assessment efforts (see further Section R.11.1) and to risk assessment and management measures (see further Section R.11.2) in order to ensure a proportionate response to the real risk to man or the environment.

In these circumstances a registrant must complete an emission characterisation according to the principles detailed in Section R.11.2 and propose appropriate control measures in the CSR and SDS to ensure safe manufacture and use (see also Section R.11.2).

In some circumstances, the available data may not allow a direct comparison to Annex XIII for each of the criteria, but there may be other relevant data available. It is necessary for a registrant to consider all the information that is available on the property or properties for which a comparison is not possible to determine whether further information should be generated or whether a conclusion can be drawn.

It may be possible, for example, to make a scientific decision based on the information already available that a test for determining a particular property is not necessary, if the particular criteria would be met if the appropriate test was 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.

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 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 should be treated as if the Annex XIII criteria are met. 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 (cf. further in relation to both PBT/vPvB assessment efforts (Sections R.11.1 and R11.1.1) and to emission and risk characterisation and management measures (Section R.11.2));
- Substances for which it is technically difficult (or impossible) to carry out the necessary testing to confirm whether or not the PBT or vPvB criteria as given in Annex XIII are met but for which there are indications from other data (e.g. screening data). When for these substances it has been shown that they meet the screening criteria for P, B, and T, as a whole, or by analysis of constituents, impurities or transformation/degradation products, the registrant should treat them as if the Annex XIII criteria were met and implement or recommend appropriate and proportionate RMMs and OCs in the CSR and SDS as if the substance was a PBT/vPvB (respectively a substance containing PBTs/vPvBs or a substance forming PBTs/vPvBs; see Section R.11.1.1.2 for terminology). Such a decision should take into account the best available scientific judgement;
- 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 indicate an equivalent level of concern. These data should be examined carefully and a 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. It is a political/policy decision on the level of uncertainty that can be accepted 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 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 with any scientific rigor, then some level of expert interpretation must apply. For practical purposes it is prudent to limit this to ‘borderline cases’, e.g. where one or more of the P, B or T properties are not quite met according to Annex XIII criteria, but there is other information available to indicate concern. A key concern for PBT/vPvB substances is their potential for widespread distribution and where there is evidence that this can occur or has occurred, then this should be taken into account. One example 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 an equivalent level of concern and to consider the substance in question as a PBT or vPvB.

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 monitoring is available and this shows that the levels in environmental media or biota are rising, the substance should be considered as persistent irrespective of the Annex XIII criteria. If the substance also meets the BT or vB criteria, it should 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. Any decision not to consider and treat such a substance as PBT (in case the T criterion is fulfilled as well) or vPvB should be clearly justified in the PBT 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), and this may cover 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, this should be considered as equivalent to the bioaccumulation criterion irrespective of the measured BCF. Further discussion of the use of BMF indicators is included in Section R.11.1.3.2. 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.

ii) The data on the properties of the substance do not meet the specific criteria detailed in Annex XIII or do not allow a direct comparison with all the criteria in Annex XIII but nevertheless indicate that the substance would not have these properties, and the substance is not considered a PBT/vPvB

This would be the case if, as a result of an analysis of existing data, or of data generated under Annex IX and X, any one of the parameters, i.e. environmental 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, subject to an examination of other data as detailed in conclusion (i) above.

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 that the substance is not PBT/vPvB. Such would for instance be the case if the screening criteria were not met for any particular endpoint.

Where however supplementary information is available, such as monitoring data, that indicates a particular property such as persistence may in fact be present, a cautious approach should be adopted and, if necessary, a further assessment should be carried out.

In the case of aquatic toxicity, there will be occasions when the available acute toxicity data will be insufficient to judge whether chronic effects might occur at or below the 0.01 mg/L level. In cases where the water solubility is low and/or the octanol/water partition coefficient is high, the acute test will not give a true measure of toxicity because of the low exposure and/or slow uptake. Where toxicity is a critical parameter, i.e. the substance is considered as potentially or actually persistent and bioaccumulative, it will be necessary to make proposals for further confirmatory testing of either toxicity or other property in line with the strategy detailed in Section R.11.1.3.

Where the conclusion of the PBT assessment is that the substance is not PBT/vPvB, this should be clearly justified in the CSA[11].

iii) The data on the properties of the substance do not allow a direct comparison with all the criteria in Annex XIII and further information is needed

Where an analysis of the data on the PBT properties of a substance do not allow a direct 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 whether further testing according to the information requirements of Annex IX and X 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. Furthermore, such additional testing does not involve the use of animals[12]. It should be noted that when further information is required for the endpoints mentioned in Annexes IX and X it will also be necessary to submit a testing proposal as part of the technical dossier and to record this in the CSR. While waiting for results of further testing, the registrant 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.

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 whether the substance falls under conclusion (i) (i.e. is a PBT/vPvB) or (ii) (is not a PBT/vPvB).

When the available data are not sufficient to conclude whether the Annex XIII criteria are met a registrant has not only the option to generate further information allowing to draw a decision as described above but may choose to treat the substance as if it were a PBT/vPvB substance. This case falls under conclusion (iv) and is described there.

iv) Further information would be needed to conclude on the PBT/vPvB properties of the substance. However, the registrant (for several reasons) has decided not to conduct confirmatory testing.

There may be cases where a clear decision on the properties of a substance cannot be made. For instance, where there is a reason to expect that a substance may contain a known PBT component or impurity (e.g. where such a component is used in manufacture or might reasonably be expected to be a by-product of manufacture) but it is not possible to characterise a substance identity (see

11 NB: This still could mean that a normal exposure assessment and risk characterisation in accordance with sections 5 & 6 of Annex I is required if the substance is dangerous in accordance with the classification criteria of Council Directive 67/548/EEC (see sections R.XY and R.YX of this TGD).

12 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.11.1.

Sections R.11.1.1 and R.11.1.4.2) to an extent that will allow the registrant to state with enough confidence that his substance does not contain PBT/vPvB constituents/impurities or that it does not generate degradation/transformation products with PBT/vPvB properties above the relevant threshold level (i.e. ≥ 0.1% w/w per individual component).

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.

In all cases, the registrant should discuss in detail in the CSR the potential for PBT/vPvB properties of his substance, its constituents, impurities and/or transformation/degradation products. Based on this discussion he should provide a justification why further testing is not conducted.

The difficulty to draw a clear decision does not obviate the requirement on a registrant to identify and implement or recommend appropriate and proportionate RMMs and OCs in the CSR and SDS to ensure that control of exposure and emissions is being achieved commensurate with the perceived risk. In other words, if for example the registrant decides not to perform confirmatory testing but there are strong indications that the substance contains PBT constituents, these RMMs and OCs should be based on the presumption that his substance is a PBT.

When the available data are not sufficient to conclude whether the Annex XIII criteria are met a registrant may still choose to treat the substance as if it were a PBT/vPvB substance, rather than incur the cost of additional testing. This may arise, for example, when only screening data are available for one or more of the end-points. The decision would need to be justified in the CSR and an emission characterisation and risk characterisation should be completed according to the principles detailed in Section R.11.2. Appropriate RMMs and OCs must be recommended in the CSR and SDS to ensure that rigorous control of emissions and exposure is being achieved commensurate with the PBT/vPvB properties of the substance.

R.11.2 Emission characterisation, risk characterisation and risk management measures

If it is concluded that the substance is a PBT or vPvB substance, or that it should be treated as such, the registrant must develop Exposure Scenario(s) (ES(s)) for manufacturing and all identified uses as for any other dangerous substance (Article 14 (4)). However, whereas for dangerous substances meeting the classification criteria 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/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. The subsequent risk characterisation for PBT/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 lifecycle of the substance that results from manufacture or identified uses (Annex I (6.5)). RMMs and OCs are documented in an ES(s).

Generally, if a substance contains one or more constituents with PBT/vPvB properties in individual amounts ≥ 0.1 % (w/w) or if transformation/degradation products with the respective properties in amounts ≥ 0.1 % are being generated (see Section R.11.1.1 for details), the substance must be subjected to PBT/vPvB specific emission characterisation and risk characterisation. However, for the sake of relevance of risk exerted by the amount of a PBT/vPvB substance manufactured/imported by a registrant, and hence with regard to the requirements for risk characterisation and nature of RMM to be implemented, it may be considered to use a threshold value of 10% (w/w) for the total of all constituents or transformation/degradation products having PBT or vPvB properties (see Section R.11.1), if it is possible to estimate with sufficient certainty that the total manufacture/import or supply of PBT/vPvB constituents in that substance and the total amount of degradation/transformation products with PBT/vPvB properties generated by that substance do not exceed 1 t/y[13]. In the considerations as to whether application of this percentage trigger could be appropriate, the use pattern of the substance and the potential emissions of the constituents or transformation/degradation products having PBT or vPvB properties must be accounted for.

R.11.2.1 Emission characterisation

The objective of the emission characterisation is:

- to identify emissions of a PBT/vPvB-substance to the environment; and
- to identify exposure routes by which humans and the environment are exposed to a PBT/vPvB-substance.

13 Please note that the proposed one tonne per year threshold for the total of compounds with PBT/vPvB properties in a substance consisting of more than one component (be it a preparation or a multi-constituent substance) is not an 'allowable release' threshold. It refers instead to the content in a substance that will need to have appropriate risk assessment and management justified in the chemical safety report. 1 t/y is the level at which the registration requirement under REACH normally begins to apply if a substance was supplied alone or in a preparation. 1 t/y is also the trigger for registration in an article. Therefore, this amount is considered to be a suitable threshold level for relevance and hence adaptation of required risk assessment efforts and, depending on the results of risk assessment, possibly risk management measures.

The principal tool to achieve this objective is exposure scenarios. Part D and Chapters R.12 to R.18 provide guidance on how to develop exposure scenarios for substances in general. Parts of the exposure assessment guidance are relevant also for PBT/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/vPvB substances. Below guidance is given on some issues where special considerations are needed for PBT/ vPvB substances.

Throughout the development of an ES for a particular use, the objective of the risk characterisation for PBT/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 should be included in the risk management framework and their effectiveness be assessed. The final ES, or ES(s) in case of different uses, shall be presented under the relevant heading of the chemical safety report, and included in an annex to the SDS. It shall 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 and vPvBs are substances of very high concern, the registrant shall pay attention to the level of detail of his assessment as well as to whether its accuracy and reliability is sufficient for a PBT/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/vPvB-substances. The emission characterisation shall, in particular be specific in the use description and concerning RMMs, and shall 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 should 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 balanced 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 lifecycle, 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/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 16 of the SDS.

R.11.2.2 Risk characterisation and risk management measures for PBT and 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. Annex I (6.5) requests further 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 lifecycle of the substance that results from manufacture or identified uses.

Risk characterisation for PBT/vPvB substances includes, as for other dangerous 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 Sections R.7.1 and R.9).

The estimation of emissions to the environment and exposure of humans performed in the emission characterisation (Section R.11.2) provides the basis for risk characterisation and risk management of PBT/vPvB substances.

R.11.2.2.1 Options and measures to minimise emissions and exposure

A registrant has to generate ES(s) which minimise emissions of and exposures to PBT/vPvB substances. 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[14]. The registrants are advised to consider in early phase 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 considerations.

For the uses the registrant decides to include in his CSA and therefore develops ES(s), supply chain communication can be crucial for getting detailed enough information on conditions of use applied in practise. 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 certain use. He should list 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 under heading 16 of the SDS.

The registrant has to implement the risk management measure 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.

[14] In cases where a CSR is developed for authorisation application purposes, ES(s) are required for those uses for which an applicant decides to apply for. An authorisation applicant can be manufacturer, importer and/or downstream user of the substance. All authorisation applications have to include an analysis of alternatives. However, that will be a separate part of the application and not included in the CSR. See guidance for authorisation application.

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).

Rigorous containment of the substance

The PBT/vPvB 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 lifecycle 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 lifecycle of the matrix including the waste life stage. Note however that residues of the original PBT/vPvB substance in the matrix or impurities with PBT/vPvB properties resulting from side-reactions must as well be considered (see Section R.11.1.1).

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/vPvB 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/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 personal

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

R.11.2.2.2 Risk Characterisation for humans in cases of direct exposure to PBT/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/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) shall 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 shall be applied[15]. 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/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.

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.11.2.2.3 Documentation of the risk characterisation and communication of measures

Given the potential risk exerted by PBT/vPvB substances, the descriptions of the implemented, respectively 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 implemented the recommended RMMs and OCs correctly.

The risk characterisation for all ESs developed for the identified uses of the PBT/vPvB substance has to be documented under heading 10 of the CSR. The registrant is according to REACH Article 14 obliged 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.

[15] 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.

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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)

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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 TEC NES WG on PBTs.

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 plus a molecular weight of greater than 1100
2. a maximum molecular length (MML) of greater than 4.3 nm
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 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.

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 305 bioconcentration test guideline (OECD, 1996). 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 Cf/Cw (Cf: concentration of test chemical in fish at steady state; Cw: concentration of test chemical in the exposure phase (water) or Ku/Kd (Ku: rate constant for uptake and Kd: 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 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 305 test guideline, 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 has 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. 11-6). The table also shows whether P-glycoprotein has been detected and could be a functional efflux protein active in the tissue.

Table R. 11-6: 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). 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.

Not withstanding 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. 11-7 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 S ijm 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. 11-7: 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 (persistency, 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 TEC-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. 11-4, Figure R. 11-5 and Figure R. 11-6 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).

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 L/kg. The 95% confidence interval for this exercise is 9.5 (Figure R. 11-4).

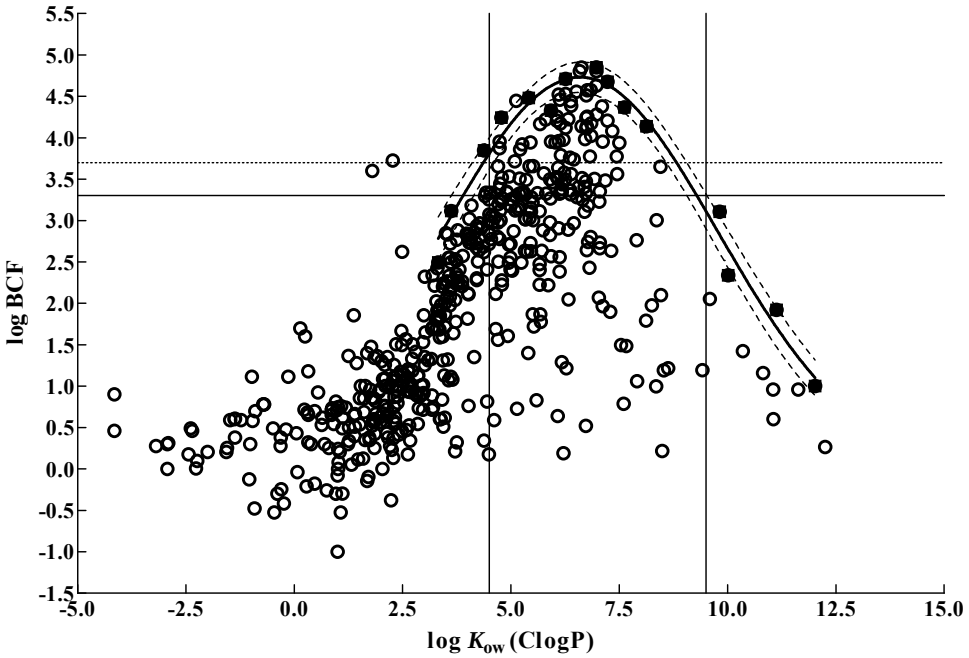


Figure R. 11-4: Log BCF v calculated log K_{ow}

Figure R. 11-5 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).

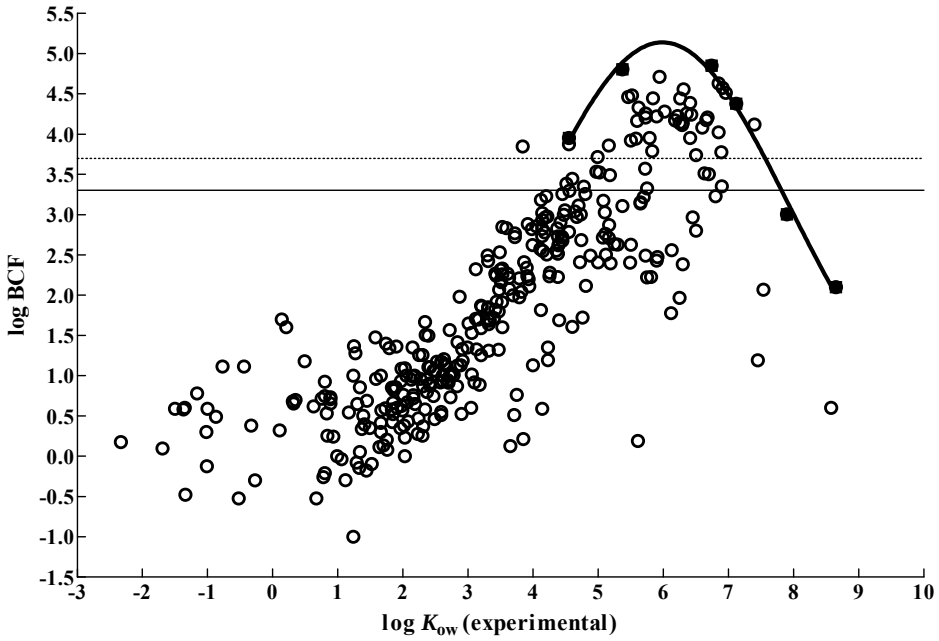


Figure R. 11-5: LogBCF v measured log K_{ow}

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. 11-6). 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).

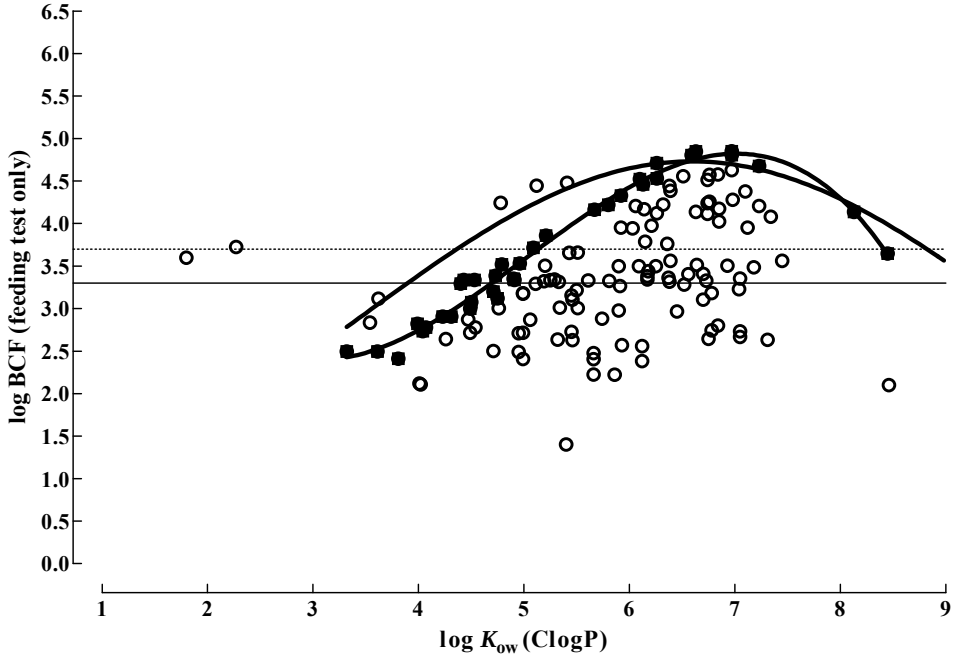


Figure R. 11-6: LogBCF derived from feeding studies versus calculated log K_{ow}

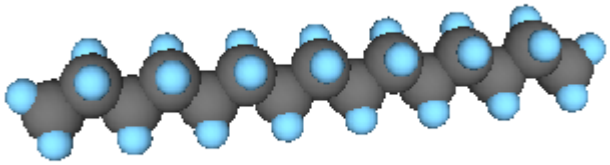
Summarized, the results of Figure R. 11-4 to R.11-6 suggest that the B-criterion is unlikely to be triggered for substances with a log K_{ow} higher than 10. As with the other indicators described in the main paper, a log K_{ow}-value higher than 10 should be used in a weight of evidence in combination with the other indicators.

Appendix R.11-1 Annex 2

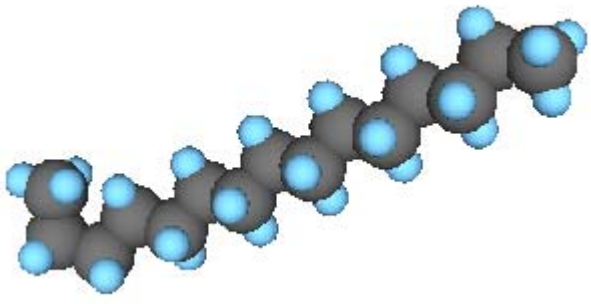
GRAPHIC DEFINITIONS FOR THE MOLECULAR DIMENSIONS USED IN THE MAIN PAPER

- 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.

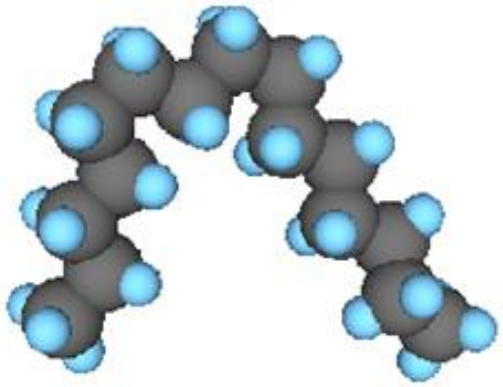
Conformer 1 (ΔHo = -84.5 kcal/mol), Dmax = 21.4; Deff = 4.99; Dmin = 4.92



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 Software

OASIS

To calculate $D_{max\ ave}$ conformational analysis of the molecule needs to be conducted. This is done by estimating D_{max} of each conformers and then the average Dmax values across the conformers. An OASIS software module is used to generate the energetically stable conformers representing conformational space of the molecules. The method is based on genetic algorithm (GA) generating a final number of structurally diverse conformers to best represent conformational space of the molecules (Mekenyan et al 1999 and 2005). For this purpose the algorithm minimizes 3D similarity among the generated conformers. The application of GA makes the problem computationally feasible even for large, flexible molecules, at the cost of non-deterministic character of the algorithm. In contrast to traditional GA, the fitness of a conformer is not quantified individually, but only in conjunction with the population it belongs to. The approach handles the following stereochemical and conformational degrees of freedom:

- rotation around acyclic single and double bonds,
- inversion of stereocenters,
- flip of free corners in saturated rings,
- reflection of pyramids on the junction of two or three saturated rings.

The latter two were introduced to encompass structural diversity of polycyclic structures. When strained conformers are obtained by any of the algorithms the possible violations of imposed geometric constraints are corrected with a strain-relief procedure (pseudo molecular mechanics; PMM) based on a truncated force field energy-like function, where the electrostatic terms are omitted (Ivanov et al, 1994). Geometry optimization is further completed by quantum-chemical methods. MOPAC 93 (Stewart, 1990 and 1993) is employed by making use of the AM1 Hamiltonian. Next, the conformers are screened to eliminate those, whose heat of formation, DHfo, is greater from the DHfo associated with the conformer with absolute energy minimum by user defined threshold - to be within the range of 20 kcal/Mol (or 15 kcal/mol) threshold from the low(est) energy conformers (Wiese and Brooks, 1994). Subsequently, conformational degeneracy, due to molecular symmetry and geometry convergence is detected within a user defined torsion angle resolution.

Calculation of the 3D Dimension of a Molecule

A molecular modelling program, e.g. Molecular Modelling Pro, uses a 2D molecular structure as a starting point for the calculation. In the 1st step the program calculates the least strained 3D conformer using e.g. MOLY Minimizer as built in the Molecular Modelling Pro. Normally this minimizing of strain requires multiple steps. If the strain energy is minimized the program calculates the 2nd step the 3D molecular dimensions (x length, y width, z depth) e.g. in Angstrom. Based on these x,y,z dimensions Molecular Modelling Pro is able to calculate a global maximum and minimum which can be used a Dmax.

OECD QSAR Toolbox

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

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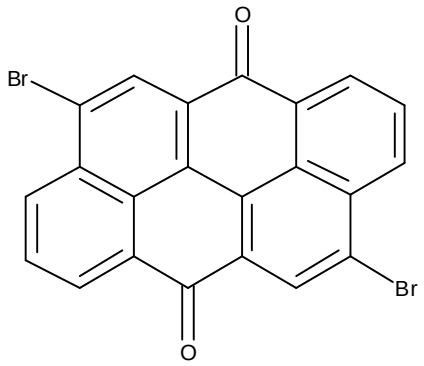
Appendix R.11-1 Annex 3

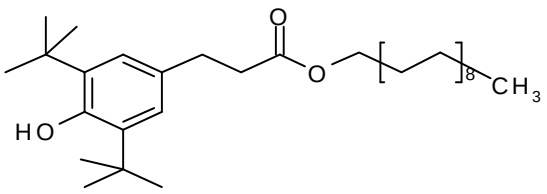
EXAMPLES - USE OF THE INDICATORS FOR LIMITED BIOACCUMULATION

Example R. 11-1

INDICATOR n-Octanol solubility		
Name	Pigment Red 168	
CAS No.	4378-61-4	
Mol weight (g/Mol)	464	
Co (µg/L)	124	
CBB (µg/L)	928	
Co < CBB	YES	
log Co/Cw	1.1	

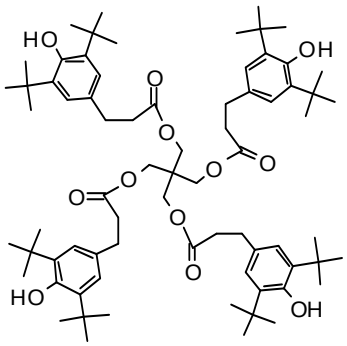
Remark:
The n-octanol solubility Co of Pigment Red 168 is well below the Critical Body Burden (CBB) which is an indicator of low bioaccumulation potential. In addition the log Co/Cw (octanol/water) is 1.1 which means low uptake through biological membrane

Example R. 11-2

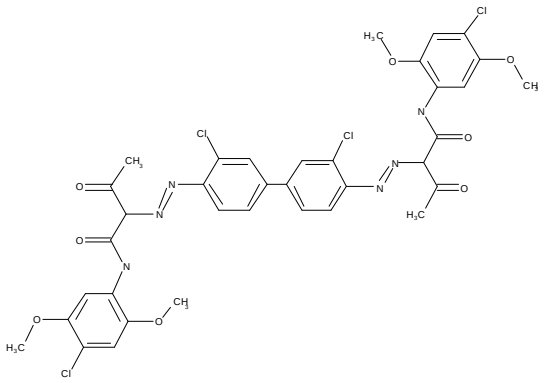
INDICATOR Kow > 10		
Name	ODBPA	
CAS No.	2082-79-3	
Mol weight (g/Mol)	531	
log Kow	13.4	

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

Example R. 11-3

INDICATOR Average Size > 17 A & MW > 1100 g/Mol PLUS log Kow > 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 > 17 A & 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 Annex 3.1-B Example 2.		

Example R. 11-4

INDICATOR Average Size > 17 A & 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**	
Co (µg/L)	**9**	
CBB (µg/L)	**1636**	
Co < 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. 11-8: List of antioxidants (from Ullmann, 1995)

Antioxidant type		CAS No.	MW (g/Mol)	calc. Kow (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}

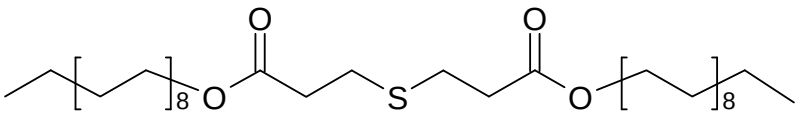
Example R. 11-5

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

Table R. 11-9: Properties of the antioxidant

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

Structure



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

log K_{ow} calc. 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 **Persistency 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 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 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.**

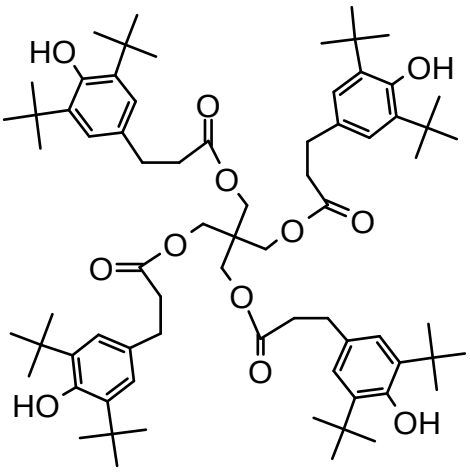
Example R. 11-6

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. 11-10: 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 301B)	No
T criteria fulfilled	No

Structure



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

log K_{ow} calc. 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 ***Persistency 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 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 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.

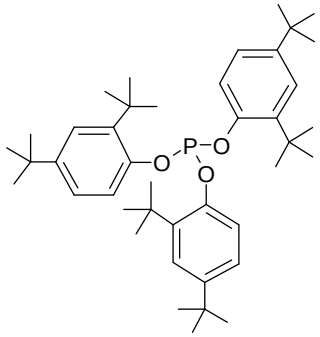
Example R. 11-7

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

Table R. 11-11: 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 301B)	No
T Criteria fulfilled	No

Structure



STEP 1 Calculated / measured log K_{ow}

log K_{ow} calc. 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 Persistency 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 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. 11-12: 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 Co (µg/L)		Critical Body Burden (CBB) (µg/L)	Co < CBB		Water solubility Cw (µg/L)	log Co/Cw
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.11-12 (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 Co (µg/L)		Critical Body Burden (CBB) (µg/L)	Co < CBB		Water solubility Cw (µg/L)	log Co/Cw
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.Black 31	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

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

Example Pigment Yellow 12, CAS No. 6358-85-6

Table R. 11-13: Data for Pigment Yellow 12

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

STEP 1 Solubility measurement of Octanol and Water

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

STEP 2 B & 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, 2006, Data ownership is with ETAD

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

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 11.1.4.2 ((i) Persistence), for homologous substances, where there is convincing evidence of ready biodegradation of the whole substance, e.g. in an OECD 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 half-lives and a third that could be used for assessing potential metabolites.

Howard et al, 2005, describe a model that predicts the half-live 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, 2006, 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, 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. There are two suggested approaches.

Firstly 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.

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

In case data are available from bioconcentration studies on benthic and terrestrial invertebrate species they may be used as indicator for a high bioaccumulation potential. Results of these studies are expressed as biota-to soil/sediment accumulation factor (BSAF). In order to compare BSAF with BCF values care must be taken if a species with a very low lipid content was used because BCF values are normaly reported on a wet weight basis. Lipid normalization (to 5% lipid content) should therefore always be performed, whenever possible for substance that are lipid binding.

The relationship between BSAF and BCF is epressed in the following equation, in which BCF could be replaced by the criterion for B or vB.

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

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 L/kg and higher, based on pore water concentration. Similar for a substance with a log Kow of 4.5 a BSAF value higher than 5 is an indication that the BCF exceeds the value of 5000 L/kg, based on pore water concentration.

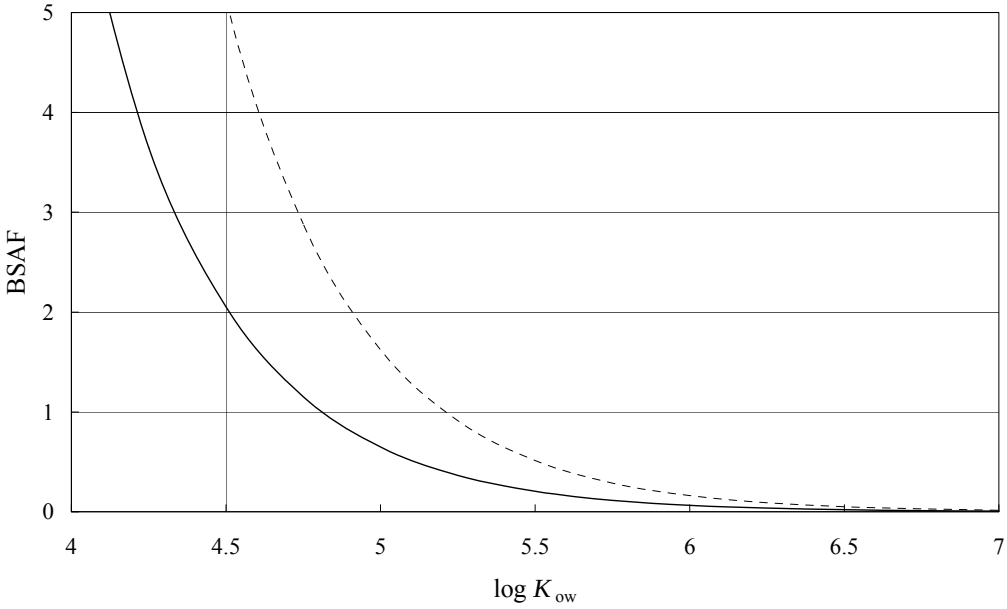

Figure R. 11-7: Relationship between lipid and organic carbon normalised BSAF values and log Kow as indicator for the B and vB criterion.

The solid line is calculated with a BCF value (5% lipids) from pore water of 2000 L/kg, the dotted line is calculated with a BCF value of 5000 L/kg. The log Koc has been calculated according to the equation log Koc = log Kow - 0.21 by Karickhoff et al. (1979).

Due to increasing sorption with log Kow, the BSAF values for calculated BCF values of 2000 L/kg and 5000 L/kg rapidly decrease. Therefore, for a substance exceeding log Kow of 5.5, a BSAF value in the order of 0.5 and above indicates that the substance may be B and vB.

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 Kow 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 L/kg, because of the strongly increased sorption for highly hydrophobic substances. Moreover, sorption might be higher than what is expected from log Kow 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.