EFEO/IFRA
Guidelines on the Environmental Assessment of Natural Complex Substances (NCS)

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- Version 01. 26 May 2016 –

TABLE OF CONTENTS

Overview of REACH and CLP requirements for NCS environmental assessment

<1t

Classification and labelling [1]

No need to generate data for classification purposes.

Based only on available data on the NCS itself and if not available, on the constituents
From for example :

- IFRA Labelling Manual
- CLP Inventory and/or harmonised classification (Annex VI of CLP)
- ECHA website

Section II.3

≥ 1 t

Classification and labelling [2]

ACUTE TOXICITY

-> Calculation approach

- constituents
  - •measured toxicity
  - •predicted by models or by read across)
- block approach
- *predictions of mixtures toxicity* #

Section II.3.1.2

-> Measured results from tests on oil itself

Section II.3.2.1.1

CHRONIC – LONG TERM ADVERSE EFFECTS

BIODEGRADATION	BIOACCUMULATION	AQUATIC TOXICITY
If per constituents (or block approach) •measured biodegradation • predicted by models or read across Section II.3.1.2 If per essential oil (NCS itself) • prediction of the biodegradation of NCS itself# • measure of biodegradation $ Section II.3.2.1.2	-Model predictions or indirect /direct measure of log Kow > 4; or BCF >500 Section II.3.1.2 Section II.3.2.1.2	Similar to acute toxicity approach, but : models are less suitable, and measured data for chronic toxicity are more difficult to generate (technical challenges) Section II.3.1.2 Section II.3.2.1.2

specific mixture models available ; $ only when the constituents have similar structure

≥10t

Persistence, Bioaccumulation and Toxicity Assessment [3]

- Use of data generated under Annex VII and VIII of REACH,
- Weight of evidence and any robust scientific evidence as per revised Annex XIII
- For criteria, See Appendix 2

Section II.5

Chemical Safety Assessment [4]
Environmental Risk Assessment [5] : RCR*: PEC/PNEC to be < 1

Exposure - Predicted Environmental Concentration (PEC)	Effects- Predicted No Effect Concentration (PNEC)
Weighed PEC on constituent Section II.6.2	• Constituent approach: Critical Component Approach, Lead Component Identification; • Block approach • Whole substance ** Section II.6.3

* Risk Characterisation Ratio; **caution to derive PNEC

1) no new information needed
2) dataset required according to Annex VII,VIII or IX depending tonnage band
3) based on available data and weight of evidence
4) based on tonnages and uses of essential oils
5) for hazardous substances

Guidelines on the Environmental Assessment of Natural Complex Substances (NCS) for 2018 REACH deadline

PART I. INTRODUCTION AND BACKGROUND

I.1. Introduction

The purpose of this document is to provide guidance to member companies on the regulatory requirements for the environmental assessment of natural complex substances ("NCS"), including for the purposes of their classification and labelling, REACH[1] registration and for the assessment of their possible persistent, bioaccumulative and toxic ('PBT') and very persistent and very bioaccumulative ('vPvB') properties.

The current document is a complement to the ECHA Guidance Document on Substance identification (the "ECHA SID Guidance") and the EFEO/IFRA Guidelines on Substance Identification of NCS[2] (the "NCS SID Guidelines"). It also updates the environmental part of the Protocol for REACH Registration of Natural Complex Substances (revision 2, January 7, 2009).

These Guidelines have been prepared in close cooperation with the European Chemicals Agency (ECHA) and are based on ECHA Guidance Documents that are currently in the process of being updated. Therefore, users of these Guidelines should consult the ECHA website (www.echa.europa.eu) for any updated Guidance in the future.

The information in these Guidelines does not constitute legal advice and does not engage the responsibility of its authors. It is the responsibility of the users of this document to ensure compliance with REACH and the CLP Regulation[3] when registering and placing NCS on the market.

These Guidelines focus on the requirements for the environmental assessment of NCS under REACH as these have proved to be very challenging to implement since they address in most cases potential effects of multi-constituent substances or UVCB[4] substances on ecosystems in any environmental compartment and require consideration for the environmental fate and behaviour of those substances.

Because of their biological nature, the composition of NCS may vary significantly, from simple compositions of a few constituents, up to very complex substances comprising more

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

[2] EFEO/IFRA Guidelines On Substance Identification And Sameness Of Natural Complex Substances (NCS) under REACH and CLP (http://efeo-org.org/wp-content/uploads/2015/08/EFEO-IFRA-Guidelines-NCS-SID-REACH-CLP-Version-5-August-2015.pdf)

[3] Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures

[4] Unknown or Variable composition, Complex reaction products or Biological materials

than 100 constituents which cannot be fully characterised. Further, constituents of an NCS may exhibit different physico-chemical properties that are relevant for their environmental assessment (e.g. water solubility, volatility, lipophilicity and ability to adsorb onto particles and surfaces), but also for their classification and labelling, leading to several issues of characterisation, testing and difficulties in achieving the environmental assessment.

For all these reasons, it is important that the registrants know first how to correctly identify and characterise their NCS. This will also help determine the specific tonnage to be used for the registration of each characterised substance, and thus the type of data needed for registration. More details on how to identify NCS and how to characterise their composition can be found in the NCS SID Guidelines.

The first part of the present Guidelines provides a summary of the regulatory requirements applicable to NCS under REACH and the CLP Regulation[5] that are connected to the environmental effects of such substance, as well as the concepts and approaches that can be used to meet such requirements. It includes chapters on the collection and generation of environmental data, on the assessment of such data including the classification and labelling and PNEC derivation, the PBT/vPvB assessment and finally risk characterisation. These chapters should be considered together and be addressed in a holistic way when determining the strategy to assess the environmental effects of NCS as requirements in one stage may influence the level of data required at another stage in the overall assessment.

Also, this Part introduces the various assessment approaches for NCS and the identification of their "relevant constituents".

Please note, however, that the "whole substance approach" addressed in these Guidelines as an option to assess UVCBs or multi-constituent substances could in practice be difficult to implement to NCS for the purpose of classification and labelling, the PBT/vPvB assessment and the environmental risk assessment in general, including testing requirements. For further details on the assessment approaches, see Sections I.4. and II.2.1. of these Guidelines.

Further detailed guidance for each of these issues, including illustrative examples, is provided in the second part of these Guidelines.

I.2. Regulatory background

I.2.1. General Considerations

One of the basic principles of REACH is that manufacturers, importers and downstream users have to ensure that the substances they manufacture, place on the market or use do not adversely affect human health or the environment.

For manufacturers and importers, this requires the submission of a registration dossier for all substances they manufacture or import in quantities of 1 ton or more per year. It is the tonnage manufactured or imported per year that will determine the level of data that will be required for registration purposes as per Annexes VI to X of REACH.

[5] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

Substances manufactured and imported below 1 ton per year are only subject to classification and labelling requirements under the CLP Regulation (i.e. obligation to self-classify based on relevant available data and to notify this information to the classification and labelling inventory). For substances above 1 ton per year, all available data should be considered first before new tests are being conducted to meet the standard information requirements for each tonnage threshold (1 to 10 tons, 10 to 100 tons, 100 to 1000 tons and above 1000 tons). In some cases, adaptations to the standard information requirements may also be possible.

When a substance is manufactured or imported at 10 tons or more per year, a chemical safety assessment (CSA) should also be conducted and documented in the registration dossier in the form of a chemical safety report (CSR). The hazard information collected or generated in the context of the CSA is in turn used for classification and labelling, for the PBT assessment and for the derivation of threshold or non-threshold levels for human health and the environment.

It is only when the result of the hazard assessment shows that the substance is classified according to certain hazard classes, or that the substance is assessed to be a PBT or vPvB, that an exposure assessment and a risk characterisation have to be conducted within the CSR.

I.2.2. Identification of NCS

The ECHA SID Guidance generally considers that NCS fit the sub-category of "UVCB sub-type 3". However they may also be characterised as mono- or multi-constituent substances[6] based on their composition. Further details on the naming conventions for NCS can be found in the NCS SID Guidelines.

Characterisation of NCS is based on the botanical source, the manufacturing process and the chemical composition but the chemical composition is the key determinant to decide whether a given NCS can qualify as a UVCB or as a mono- or multi-constituent substance.

The NCS characterisation as a UVCB or as a mono- or multi-constituent substance will affect the type of data required for the registration dossier and the ability to use non testing approaches such as read-across, grouping and/or the use of (Q)SAR (Quantitative Structure-Activity Relationship) model predictions on relevant known or suspected constituents, as further explained below in Section I.2.3.4.

The NCS composition can also affect the hazard classification of the substance under the CLP Regulation and therefore the PBT assessment and hazard assessment in general.

According to the ECHA SID Guidance Section 4.3.1.1, compositional information for UVCBs should not differentiate between constituents and impurities but the chemical composition and the identity of the constituents should still be given as far as known.

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[6] **Mono-constituent substances** are defined as substances in which one constituent is present at a concentration of at least 80% (w/w)

Multi-constituent substances are defined as substances consisting of several main constituents present at concentrations generally above or equal to 10% and below 80% (w/w)

UVCB substances are defined as substances of Unknown or Variable composition, Complex reaction products or Biological materials. These substances cannot be sufficiently identified by the above parameters.

All known constituents and all constituents present at concentrations ≥ 10% should be reported by an IUPAC name and possibly a CAS N° and the typical concentrations and concentrations ranges should be given as well. Unknown constituents should be identified as far as possible by a generic description of their chemical nature.

When several qualities[7] are covered in the same registration dossier, composition ranges of the various qualities should also be provided.

However, Section 4.3.1.1 of the ECHA SID Guidance also provides that constituents, additives and impurities (in case of mono- or multi-constituent substances) that are relevant for the classification and/or PBT assessment of the substance shall always be identified independent from their concentration[8].

I.2.3. REACH Information Requirements

Article 12 of REACH specifies the information required to be submitted depending on tonnage. It first confirms that "all physico-chemical, toxicological and ecotoxicological information that is relevant and available to the registrant needs to be provided".

I.2.3.1. Standard Information Requirements

Article 12 provides the minimum or "standard" information requirements that must be provided per tonnage threshold while Article 23 introduces the relevant deadlines for phase-in substances as follows:

Table 1: Standard information requirements per tonnage bands

Tonnage threshold	Sub-groups	Standard information Requirement
< 1 ton		None (only classification and labelling under the CLP Regulation)
1 < 10 tons	Phase in substances not meeting Annex III criteria[9]	REACH Annex VI (Administrative data, Substance identification, Classification and labelling, Exposure data) + Section 7 of REACH Annex VII (information on physico-chemical properties)
	Phase-in substances meeting Annex III criteria	Same as in line above + REACH Annex VII
10 < 100 tons		Same as in line above + REACH Annex VIII + CSA
100 < 1000 tons		Same as in line above + REACH Annex IX
> 1000 tons		Same as in line above + REACH Annex X

[7] See Question 2 on page 7 of the EFEO/IFRA Guidelines on Substance Identification and Sameness of Natural Complex Substances at: http://efeo-org.org/wp-content/uploads/2015/08/EFEO-IFRA-Guidelines-NCS-SID-REACH-CLP-Version-5-August-2015.pdf

[8] This is derived from (i) the CLP Regulation which provides for reasons of practicability cut-off values for substances containing hazardous substances (impurities, additives and constituents) that should be considered for classification as well as from (ii) REACH Annex XIII which provides that "The identification shall also take account of the PBT/vPvB-properties of relevant constituents of a substance and relevant transformation and/or degradation products".

[9] Criteria for substances registered between 1 and 10 tons, with reference to Article 12(1)(a) and (b):
(a) substances for which it is predicted (i.e. by the application of (Q)SARs or other evidence) that they are likely to meet the criteria for category 1A or 1B classification in the hazard classes carcinogenicity, germ cell mutagenicity or reproductive toxicity or the criteria in Annex XIII;
(b) substances:
(i) with dispersive or diffuse use(s) particularly where such substances are used in consumer mixtures or incorporated into consumer articles; and
(ii) for which it is predicted (i.e. by application of (Q)SARs or other evidence) that they are likely to meet the classification criteria for any health or environmental hazard classes or differentiations under Regulation (EC) No 1272/2008.

Therefore, the standard information requirements of Annexes IX and X will normally not apply to 2018 registration dossiers.

I.2.3.2. Standard ecotoxicological and environmental fate information

As these guidelines are aimed for registration submissions by the 2018 REACH deadline, the standard ecotoxicological and environmental fate information requirements for Annex VII and VIII substances will apply and these are summarised below[10]:

Table 2: Standard environmental information requirements for Annex VII and VIII substances

REACH Annex	Information requirements	VII	VIII
Volume (t/y)		**>1**	**>10**
9.1.1	Short-term toxicity testing in *Daphnia*	x	x
9.1.2	Growth inhibition study on algae	x	x
9.1.3	Short-term toxicity testing on fish		x
9.1.4	Activated sludge respiration inhibition study		x
9.2.1.1	Biotic degradation (ready test)	x	x
9.2.2	Abiotic degradation (hydrolysis= f(pH))		x
9.3.1	Adsorption/desorption screening study		x

I.2.3.3. Chemical safety assessment (for > 10 t/y substances)

When substances are manufactured/imported in volumes of 10 tons or more, a CSA covering (1) an environmental hazard assessment and (2) a PBT/vPvB assessment is required.

In general, as described in Article 14 of REACH, a CSA consists of the following steps:

- Collection and generation of available and required information on intrinsic properties
- Physicochemical hazard assessment; including classification
- Human health hazard assessment; including classification and derivation of derived no effect levels (DNELs) or derived minimal effect levels (DMELs)
- Environmental hazard assessment; including classification and derivation of predicted no effect concentrations (PNECs)
- PBT and vPvB assessment

It is only when the result of the hazard assessment shows that the substance is classified according to any of the hazard classes or categories of Article 14(4)[11] or that the substance is

[10] Physico-chemical information requirements are not covered by this document. However, the octanol-water partitioning coefficient (Log Kow) is relevant as a screening assessment of bioaccumulation for the purposes of environmental classification and labelling and the PBT assessment. Thus methods such as the use of the OECD 117 to determine the range of log kow for an NCS are discussed in Part II of the document. The determination of other pertinent physico-chemical properties (i.e. vapour pressure and water solubility) was discussed in the Protocol for REACH Registration of Natural Complex Substances (revision 2, January 7, 2009).

[11] Hazard classes outlined in Article 14(4) : explosives (2.1), flammable gases (2.2), flammable aerosols (2.3), oxidising gases (2.4), flammable liquids (2.6), flammable solids (2.7), self-reactive substances and mixtures types A and B (2.8), pyrophoric liquids (2.9), pyrophoric solids (2.10), substances and mixtures which in contact with water emit flammable gases (2.12), oxidising liquids categories 1 and 2 (2.13), oxidising solids categories 1 and 2 (2.14), organic peroxides types A to F (2.15); acute toxicity (3.1), skin corrosion/irritation (3.2), serious eye damage/eye irritation (3.3), respiratory or skin sensitisation (3.4), germ cell mutagenicity (3.5), carcinogenicity (3.6), adverse effects on sexual function and fertility or on development (3.7), effects other than narcotic effects (3.8), STOT RE (3.9), aspiration hazard (3.10); hazardous to the aquatic environment (4.1); hazardous to the ozone layer (5.1)

assessed to be a PBT or vPvB, that an exposure assessment and a risk characterisation have to be conducted.

I.2.3.4. Alternatives to testing

Standard environmental hazard information requirements as listed in Annex VI to X of REACH do not necessarily need to be fulfilled by conducting new experimental testing. Available existing information should be considered first, including the use of "non-testing approaches" such as the use of *in vitro* methods, (Q)SAR predictions, read-across and grouping approaches, as well as adaptations to the standard information requirements as per Annex XI.

Indeed, generation of new ecotoxicity data to fulfil the information requirements including the PBT assessment should be considered only as a last resort option (Annex VI, Step 4 of REACH) in particular when vertebrate animals are involved and the use of non-testing approaches should be used whenever possible for filling data gaps.[12]

I.2.3.5. Practical Guidance

Practical Guidance on how to assess the relevance and reliability of available information is provided in Section R.7.8.4.1 of the ECHA Guidance on Information Requirements and Chemical Safety Assessment (the "ECHA IR & CSA Guidance").

Once all available data has been gathered, a data gap analysis should be conducted by comparison of the identified information needs for the substance with the available information considered to be relevant and reliable.

When, as a result of the data gap analysis, it appears that the information requirements cannot be fulfilled, new information may need to be generated. For information requirements of Annexes VII or VIII, any new test should be conducted in accordance with Article 13 while for Annex IX or X substances, proposals for testing should be submitted to ECHA before any test is being conducted.

Integrated testing strategies (ITS) are available in part B of the ECHA IR & CSA Guidance and provide endpoint specific guidance on how to gather and assess available information, and consider new data needs and testing strategies. Part II of these Guidelines will also illustrate possible approaches for assessing aquatic hazard and environmental fate information for NCS.

I.3. Environmental hazard assessment

The environmental hazard assessment consists of an evaluation of all available information on the hazards for ecosystems in any environmental compartment (water, air, sediment or soil). Hazards for predators in the food chain (secondary poisoning), as well as hazards to the microbiological activity of sewage treatment systems, should also be covered. Environmental fate data (degradability and bioaccumulation) are also required.

[12] Industry Guidance on the data requirements and methods of data collection for registration of NCS used as fragrance ingredients can also be found in the "Protocol for REACH Registration of Natural Complex Substances" (revision 2, January 7, 2009)

The results from this assessment will determine the classification and labelling of the substance in accordance with the CLP Regulation (see Section I.3.1. below) as well as the derivation of Predicted No-Effect Concentration (PNEC) for each compartment (see Section I.5. below) and need to be considered for the PBT assessment.

I.3.1. Classification and labelling

Classification under the CLP Regulation is a two-step process requiring the identification of all relevant available information on substances and mixtures and its subsequent evaluation to decide on the classification and labelling according to the criteria set forth in Annex I to the CLP Regulation.

For environmental hazard classification, the criteria are set forth in Section 4.1.2 (for substances) and Section 4.1.3 (for mixtures) of Annex I to the CLP Regulation.

The environmental hazard classification is based on aquatic toxicity data for substances or mixtures, and information on the degradation and bioaccumulation behaviour. Indeed, for acute aquatic hazards, the classification will be based on acute aquatic toxicity data only. However, for long term hazards, classification will be based on both data on aquatic chronic toxicity and degradation. If adequate information on chronic toxicity is missing, acute aquatic toxicity data and environmental fate data (including the $LogK_{ow}$ when no bioaccumulation data is available) will be used to derive the classification as outlined in table 4.1.0 of Annex I to the CLP Regulation.

There is no requirement under the CLP Regulation to generate new data for the purpose of classification only. Hence, when the criteria cannot be applied directly to available identified information, (as may be the case for substances < 10 tons) the evaluation should be conducted by applying a weight of evidence determination, using expert judgement.

I.3.1.1. "Relevant constituents" for the purpose of classification

According to the CLP Regulation, substances containing identified hazardous substances, whether in the form of an impurity, additive or individual constituent, should be taken into account for the purposes of classification, if the concentration of the hazardous substance is equal or greater than the applicable cut-off values set out in Section 1.1.2.2 of Annex I of the CLP Regulation.

This means that identification and evaluation of available data for the purpose of classification should cover information on the substance itself as well as "relevant constituents" which, according to Section 4.1.3.1. of Annex I to the CLP Regulation, are *"those which are classified 'Acute 1' or 'Chronic 1' and present in a concentration of 0,1 % (w/w) or greater, and those which are classified 'Chronic 2', 'Chronic 3' or 'Chronic 4' and present in a concentration of 1 % (w/w) or greater […]. Generally, for substances classified as 'Acute 1' or 'Chronic 1' the concentration to be taken into account is (0,1/M)% ".*

For highly toxic substances (Aquatic Acute 1 and/or Aquatic Chronic 1), multiplying factors (M-factors)[13] will have to be applied to account for the fact that these substances, even at lower concentrations, could contribute to the classification of the mixture.

[13] According to Article 10(1) of the CLP, "*Specific concentration limits (SCLs) and generic concentration limits (GCLs) are limits assigned to a substance indicating a threshold at or above which the presence of that*

I.3.1.2. Identification and evaluation of relevant available data

According to the ECHA Guidance on the Application of the CLP criteria (Version 4.1 – June 2015) (the "CLP Guidance"), the harmonised criteria for classification of substances as hazardous for the aquatic environment focus on single substances but there are exceptions for complex substances, such as multi-constituent substances and UVCBs: although they are considered to be "substances" under REACH, the approach for their classification requires consideration of the relevant constituents they contain and therefore, the rules for "mixtures" may apply.

For the classification of mixtures, the identification of the relevant information can be either based on the mixture itself or on individual substances contained in the mixture depending on the type of information available and the hazard class/category to be considered.

The evaluation of the information should be based on data from the mixture itself, if there is valid, adequate and reliable information available on that mixture. This is not applicable, however, for the evaluation of the biodegradation and bioaccumulation properties where only data for the individual constituents in the mixture should be used, whenever possible, as provided for in Article 6(4) of the CLP Regulation.

The approach for classification of aquatic environmental hazards is thus a tiered approach which takes into account the availability of the information for the mixture itself and for its constituents.

However, according to Annex I Section 4.1.3.6.1., if no adequate information is available for all relevant constituents of the mixture, "*the mixture shall be classified based on the known components only, with the additional statement on the label and in the SDS that: 'Contains x % of components with unknown hazards to the aquatic environment'.*"

The tiered approach for the classification of mixtures is provided in Figure 4.1.2 of Section 4.1.3.2. in Annex I to the CLP Regulation and is reproduced in Appendix 1 of the present document.

I.3.1.3. Specifications of multi-constituent substances and UVCBs for the purpose of classification

Multi-constituent substances and UVCBs require specific considerations when it comes to addressing the adequacy of available data for these substances. Indeed, since these substances may not be dissolved into homogenous solutions, as provided for in Section 4.1.3.2.2. of the CLP Guidance on substances difficult to test, the applicability of standard test methods and, thus, interpretation of results may not be warranted.

As a matter of example, when it relates to aquatic toxicity, the CLP Guidance provides that *"for organics, consideration therefore needs to be given to using the data derived from the*

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substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous ". The concept of multiplying factors (M-factors) under the CLP has been introduced for substances that are very toxic to the aquatic environment as specific concentration limits (SCLs) cannot be applied to this hazard class in order to give them an increased weight when classifying the mixture. M-factors have therefore to be applied to the concentration of the substance(s) classified as Aquatic Acute 1 and/or Aquatic Chronic 1 substances in the mixture when classifying the mixture using the summation method.

testing of water-accommodated fractions (WAFs) for aquatic toxicity, and the use of such data in the classification scheme."

Further details on WAF principles and methodologies, as per the OECD Monograph No. 23 (2000) when testing the whole NCS for aquatic toxicity are provided in Part II of this document.

Specific considerations when addressing other endpoints such as biodegradation and bioaccumulation are also detailed in Part II of this document.

I.3.1.4. Classification approaches for NCS

Classification of NCS is, therefore, a very complex process with adapted rules that may have to be applied on a case-by-case basis, however, on the basis of the above, it derives that two approaches will be relevant for the classification of NCS:

- Classification based on calculations using data on known constituents or blocks of constituents, including the use of read-across and/or validated (Q)SAR results.
- Classification based on data on the NCS itself

Detailed guidance on how to apply these approaches for the purpose of classifying NCS with practical examples illustrating possible approaches are provided in Part II of this document.

I.3.2. PBT/vPvB Assessment

PBT substances are substances that are persistent, bioaccumulative and toxic, while vPvB substances are substances that are characterised by a particular high persistence in combination with a high tendency to bioaccumulate.

When substances are manufactured/imported in volumes of 10 tons or more, a CSA covering a PBT/vPvB assessment is required. This assessment will thus not be needed for most NCS to be registered by 2018 as they are in the 1-10 ton range.

When needed, a PBT/vPvB assessment requires as a first step that the available data generated in the context of the CSA are used and compared with the PBT/vPvB criteria set-out under Section 1 of Annex XIII. If the substance meets the PBT/vPvB criteria (or it is considered as if it is a PBT or vPvB in the registration dossier), an emission characterisation as provided in Annex I, Section 4 of REACH and risk characterisation as specified in Annex I, Section 6.5 have to be conducted.

I.3.2.1. Criteria to identify PBT and vPvB Substances

The criteria for the identification of PBT and vPvB substances are set-out in Section 1 of Annex XIII and are reproduced in Appendix 2 to this document. Further details are provided in Section II.5. of this document.

REACH Annex XIII provides that for the identification of PBT/vPvB substances, all relevant information shall be used in an integrated manner applying a weight-of-evidence approach and using expert judgement when comparing the information to the criteria of Annex XIII.

This means that all available information relevant for the identification of a PBT or a vPvB substance has to be considered together, including results of monitoring and modelling, *in*

vitro and animal data, information from grouping and use of read-across, (Q)SAR results, occupational data as well as epidemiological and clinical studies.

Annex XIII provides that *"the available results regardless of their individual conclusions shall be assembled together in a single weight-of-evidence determination."*

In such case, a stepwise approach as outlined in Section 2 of Annex XIII has to be followed:

The relevant available information is first compared with the criteria provided in Section 1 of Annex XIII and if the substance fulfils those criteria or is considered as if it is a PBT/vPvB, then an emission characterisation has to be conducted. The outcome of the comparison step is further summarised below.[14]

I.3.2.2. Possible Outcome of the PBT Assessment

Three conclusions may be derived from the comparison step as follows:

- If the substance is not identified as a PBT/vPvB, the PBT/vPvB assessment will stop at this level.

- When the available information indicates that the substance is a PBT or a vPvB, the next step will be to conduct an emission characterisation describing all sources of emissions to the different environmental compartments during all activities carried out by the registrant and all identified uses. The results of the emission characterisation will finally be used in order to determine effective measures to minimise emissions resulting from manufacture or identified uses for the whole life-cycle.

- If, however, during the comparison step, the available data does not allow to conclude on the PBT/vPvB properties, further information will have to be generated (or a testing proposal submitted for Annex IX and X information requirements) until an unequivocal conclusion can be reached[15].

For NCS registration submissions due by the 2018 deadline, the technical dossier will contain only Annexes VII and VIII information. In such cases the registrant shall use the screening information requirements provided for in Annex XIII Section 3.1. (reproduced in Appendix 3 of the present Guidelines) and derive a conclusion on the basis of this screening information as well as other available information in a weight-of-evidence determination.

I.3.2.3. Relevant Constituents for PBT Assessment purposes

As mentioned in Section I.3.2.1. above, Annex XIII of REACH specifies that: " *The identification shall also take account of the PBT/vPvB-properties of relevant constituents of a substance and relevant transformation and/or degradation products".*

[14] See also Figure R.11-2 of chapter R.11(PBT/vPvB assessment) of the ECHA Guidance on IR & CSA (version 2.0-November 2014) and reproduced in Appendix 3 of these Guidelines

[15] Unless exposure-based adaptations as per Annex XI Section 3.2(b) or (c) can be claimed. In such case, the substance is considered "as if it is a PBT or vPvB" in the registration dossier.

The term "constituents", as described in the ECHA SID Guidance, "*refers to constituents and impurities of well-defined substances, constituents of UVCB substances, and additives to all substances"*.

There is no definition of the terms "relevant constituents" but Section R.11.4.1. of the ECHA IR & CSA Guidance provides that *"Constituents, impurities and additives are relevant for the PBT/vPvB assessment when they are present in concentration of ≥ 0.1% (w/w). This limit of 0.1% (w/w) is set based on a well-established practice rooted in a principle recognised in European Union legislation. Individual concentrations < 0.1 % (w/w) normally need not be considered."*

The ECHA Guidance also provides that *"regardless of whether substance identification is possible or not, the registrant should carry out a PBT/vPvB assessment for all constituents above 0.1% (w/w)".* (ECHA Guidance R.11.4.1.). Otherwise, he should provide a justification in the CSR explaining why he has considered certain constituents, impurities or additives present in concentration of ≥ 0.1% (w/w) not relevant for the PBT/vPvB assessment.

But, as explained in the ECHA Guidance, some flexibility is allowed in the threshold value when *"for the sake of proportionality of assessment efforts and the level of risk being considered"*, i.e., when the use pattern and potential emissions of constituents, impurities or additives having PBT/vPvB properties justify so, it may be raised to above 0.1% provided that the new threshold does not exceed 10% (w/w) for the total amount of all constituents with PBT/vPvB properties and that the total amount of these constituents within the manufactured/imported substance does not exceed 1 ton/year.[16]

I.3.2.4. Possible Need to Generate Additional Data

As indicated above, the standard information requirements for substances produced/imported below 100 tons/year (Annex VII and VIII) may not be sufficient to allow a PBT/vPvB assessment and complex substances may be difficult to characterise to a level allowing the identification of relevant constituents for the PBT assessment. In that case, additional data for each PBT inherent properties of the relevant constituents for which the information is insufficient or not available may have to be generated.

The ECHA Guidance recommends that "*care is taken when deciding which information is required to assess the PBT properties with regards to studies involving vertebrate animals and that the strategy involves, when information is needed for several properties, that the assessment focuses on the potential persistence property first before generating information on bioaccumulation or ecotoxicity data since absence of persistence property will allow to conclude that the substance is neither a PBT or a vPvB*".

Therefore, if it can be shown that a substance and its degradation products are not persistent, it is not necessary to assess further whether they fulfil the 'B' or 'T' properties. Data for bioaccumulation or (eco)toxicity may however be necessary for the risk assessment and be part of the standard requirements of a higher tonnage band.

[16] The 0.1% threshold may also be reduced according to the guidance e.g., for very toxic substances, the information on the toxicity derived for the classification and labelling purposes could be used for defining such a lower concentration limit for PBT/vPvB assessment.

Strategies to assess each of the 'P', 'B', or 'T' properties of UVCBs are to be developed on a case by case basis as described in the ECHA IR & CSA Guidance under Chapter R.11.4.2.2.

I.3.2.5. Terminology

Finally, the ECHA IR & CSA Guidance has determined a terminology to be applied in the registration dossier for substances subject to the PBT/vPvB assessment in order to reflect their PBT status based on relevant constituents and/or transformation and degradation products. A distinction is made between:

- ***PBT or vPvB substance****: A substance having a constituent with PBT or vPvB properties, which is present at a concentration of 80 % or more;*

- ***Substance containing maximum X % (or X% - Y%) PBTs or vPvBs****: A substance having one or more constituents or impurities with PBT or vPvB properties in individual amounts equal or above 0.1 % (but less than 80%). The percentage can be a maximum percentage (X) or a range (X-Y), whatever is applicable.*

- ***Substance forming PBTs or vPvBs:*** *If any constituent, impurity or additive of a substance degrades or is transformed into substances which fulfil the PBT or vPvB criteria and if these transformation or degradation products are formed in "relevant" amounts. The term "relevant" has been defined for the registrant's substance in Section R.11.4.1. For the purpose of the REACH Article 59 process for identification of Substances of Very High Concern, the assessment of what are "relevant" transformation/degradation products may be done case-by-case. The percentage of degradation or transformation products may be indicated as for impurities or constituents with PBT- or vPvB- properties, if applicable (more guidance on degradation/transformation products is given in Section R.11.4.2.2).*

I.3.2.6. Specificities for UVCBs and NCS in the PBT/vPvB Assessment

Because of their nature, UVCBs characterisation for the purpose of the PBT/vPvB assessment has led industry to tremendous challenges and several approaches have been used to address issues of identification of UVCBs.

I.3.2.6.1. *"Relevant constituents" for the PBT assessment of NCS*

As explained above, the threshold value for "relevant constituents" is in principle 0.1% but for the sake of proportionality of assessment efforts and the level of risk being considered, this threshold may be elevated.

In terms of assessment effort, it is impractical for natural complex substances to identify constituents down to 0.1% w/w. Many constituents, notably sesquiterpenes, are notoriously difficult to unequivocally identify by GCMS (i.e. ideally requiring a pure sample for confirmation by co-injection) and quantification of individual constituents may be complicated by co-elution. Furthermore, due to the natural variation in the chemical composition of botanical products, multiple analyses are required to define constituent ranges. Therefore, the fragrance industry typically applies a 1% cut-off value for unequivocal identification (in line with requirements for substance identification under REACH and the CLP Regulation). Sometimes, if a constituent is well-known and present as a reference in analytical spectra library, a constituent present at < 1% will be reported.

In terms of the level of risk, for any given NCS the constituents are generally related as a consequence of the plant's biochemistry (see Section II.3.2. for further details). Therefore, any unidentified constituents present at < 1% are expected to have similar PBT properties to the known constituents. Thus applying a block approach or a whole substance approach to assess the PBT properties of an NCS can be applied (see Section II.5) alleviating the need to define a specific constituent threshold for the assessment.

However, if the NCS is known to contain specific constituent(s) that are suspected to have (v)P, v(B) and T properties, the "known constituent approach" (see Section I.4.) should be applied along with the 0.1% threshold.

I.3.2.6.2. *Identification of "relevant constituents" in multi-constituent substances and UVCBs*

Multi-constituent substances and UVCBs share the particularity that their composition may not allow an easy characterisation of their constituents to a level that is sufficient to fulfil the PBT/vPvB assessment requirements.

When an NCS is characterised as a multi-constituent substance, the assessment should in principle be less difficult since its composition is well-defined and all relevant constituents including impurities and additives must be identified with their approximate concentrations[17]. Every constituent of a multi-constituent substance present in concentrations relevant for the PBT assessment can thus be compared with the PBT criteria. Based on the consistency of properties of constituents in a multi-constituent substance, it may be possible to set up blocks that may allow read-across, or grouping and/or use of (Q)SAR model predictions to fill data gaps and/or generate new information on those constituents.

However, for UVCBs, because their composition is variable, the number of constituents is relatively large and the fraction of unknown constituents may be significant, adapted approaches for identification and assessment of UVCBs have been proposed in the ECHA IR & CSA Guidance.

Indeed, as per Section 4.3.1.1 of the ECHA SID Guidance, characterisation of UVCB requires only that all known constituents present in concentrations ≥ 10 % are specified by the IUPAC name and possibly a CAS number; and that the typical concentrations and concentrations ranges are provided.

"Minor constituents" are not regarded as impurities for UVCBs and unknown constituents are to be identified as far as possible by a generic description of their chemical nature. However, constituents relevant for classification and/or for the PBT/vPvB assessment must also be identified when their concentration is ≥ 0.1 % (w/w) (but as explained under Section 1.3.2.6.1, due to impracticability issues specific to natural complex substances, the fragrance industry typically applies a 1% cut-off value for unequivocal identification).

[17] Please note that in principle, constituents of a multi-constituent substance should be reported when present at a concentration between 10-80%. Components present at lower concentrations would typically be reported as impurities. When NCS can be characterised as multi-constituent substances, the concept of impurity cannot apply and constituents below 10% should be reported under the 'Constituents' heading. An explanation for the deviation from the multi-constituent rule should be added in the field 'Remarks' of each such constituent.

To overcome the identification issues of relevant constituents in multi-constituent substances and UVCBs for the purpose of classification and/or the PBT assessment, the concept of "fraction or block approach" has thus been introduced. This approach allows the assessment of unknown constituents for their PBT/vPvB properties on the basis of groups or fractions of constituents sharing similar structural properties (see also Section I.4. below and Part II of this document for assessment approaches of UVCBs), when scientifically practical according to the Guidance.

This approach requires an assessment of all the available data collected to characterise the composition of the UVCB in order to determine the type of chemical structures that may be present in the UVCB.

The next step will be to identify key structural classes (or blocks) of the unknown constituent fraction and to determine when feasible, within each class, the approximate concentrations of the fraction they represent in the UVCB.

Fractions below the threshold relevant for classification and/or the PBT assessment would not require to be characterised on the basis of representative structures (ie below 1% for most Annex VIII NCS as explained above). It may however be difficult for UVCBs to demonstrate that the concentration of the fraction of any representative is always below the threshold since their composition is variable.

I.3.2.6.3. *PBT Identification and Assessment criteria*

For most NCS subject to registration, information requirements relate to Annex VIII endpoints (<100 tons/year) and thus the available data may not be sufficient to decide unequivocally whether the NCS meets the PBT/vPvB criteria. Indeed, for Annex VIII registrations, there is no standard requirement to provide bioaccumulation data, long term aquatic studies or degradation half-life values. Generation of data beyond the tonnage band requirements through non-testing or testing approaches may therefore have to be considered until a conclusion can be drawn. Alternatively, (and as indicated above in Section I.3.2.2.), the registrant may decide not to conduct the PBT assessment and consider the substance as if it were a PBT/vPvB substance. These aspects are further detailed in Part II of these Guidelines.

To overcome issues related to the assessment of UVCBs, several assessment approaches may be contemplated as provided in the next Section.

I.4. Assessment approaches for UVCBs

As described above, when assessing adequacy of data or when testing is required for classification purposes and/or to determine persistence, bioaccumulation and toxicity in the PBT/vPvB assessment, the ecotoxicity and environmental fate and behaviour data should in principle be considered for each relevant constituent of the substance (see Sections I.3.1.1. and I.3.2.3.).

This may, however, become a challenging task for NCS as they may comprise a high number of constituents including unknown constituents. Further, for some endpoints, only data on the whole substance is available (e.g. mammalian studies). Finally, constituents of NCS may exhibit different physico-chemical properties and lead to technical challenges and problems

for the interpretation of results of the aquatic toxicity, biodegradation, bioaccumulation, partitioning behaviour and water solubility endpoints.

The ECHA PBT Expert Group has addressed the specific issue of UVCBs in a discussion paper (hereinafter the "ECHA PBT Discussion Paper" and still under revision[18]) and according to this document, depending on the knowledge of the substance, its raw materials and manufacturing process, its constituents and their predicted properties, several assessment approaches may be adopted as per the following:

(1) The "known constituents approach" : This approach can be used when a substance is known to contain specific constituents at relevant concentrations that are suspected to have (v)P, (v)B and T properties.

(2) The "block approach" (fraction profiling): The substance is divided into fractions/blocks of structurally similar constituents or which follow a regular predictable pattern of structures.

(3) The "whole substance" approach: The UVCB substance is considered to be a single chemical substance for the purpose of the assessment and testing.

Further details and illustrations on how to apply the assessment approaches to NCS for the PBT assessment and classification purposes are provided in Part II of this document.

I.5. PNEC derivation and risk characterisation

For substances manufactured or imported in quantities of 10 tons or more, if as a result of the hazard or PBT assessment the substance shows to be hazardous or a PBT/vPvB, then an exposure assessment and risk characterization has to be conducted.

The exposure assessment shall be based on the generation of exposure scenario(s) as well as exposure estimations (predicted environmental concentrations or PEC).

The exposure scenario should describe the conditions of manufacture and use, including operational conditions (OC) and risk management measures (RMM) required to demonstrate that the risks to human health and the environment are adequately controlled.

During the risk characterisation stage, the predicted environmental concentrations for each environmental sphere should be compared with the PNECs identified during the hazard assessment stage. The risk characterisation will have to be carried out for each exposure scenario covered in the CSA. The objective is to demonstrate that when the conditions of the exposure scenarios are implemented, risks will be controlled.

The risk will be considered to be adequately controlled, throughout the lifecycle of the substance if the exposure levels estimates do not exceed the PNEC.

It is important to note however that the assessment approaches used for the NCS as described in point I.4. above (i.e. key constituent(s) identified as lead indicator(s)/risk

[18] ECHA PBT expert group draft discussion paper on Assessment approaches for UVCBs -PBT/vPvB assessment - EG_20150710

determining substance(s), block approach or whole substance) may influence the derivation of PNECs for the NCS.

When PNECs could not be derived for some constituents, a qualitative assessment to demonstrate that potential effects are avoided when implementing the exposure scenario should be carried out.

This is the case for PBT/vPvB substances where it is not possible to assess the potential long term risks of these substances and therefore no PNEC can be derived for any environmental compartment. Instead of exposure estimates, an emission characterisation is required to be able to show that emissions are already minimised by the implemented risk management measures on site and those recommended to downstream users.

An illustration of approaches for assessing potential risks from NCS identified as hazardous is presented under Part II of this document.

PART II. ENVIRONMENTAL ASSESSMENT APPROACHES FOR NCS

As outlined in Part I. of this document, the environmental hazard and risk assessment of NCS is a difficult task that requires special consideration, due to the nature of NCS and the need to address in principle the physico-chemical, fate and ecotoxicity of all constituents of the substance.

Different methodologies may be used to characterise and assess NCS in order to comply with the REACH and the CLP Regulation requirements. The NCS may be assessed:

- by assessing specific constituents (the "known constituent approach"); or,
- on the basis of fractions/blocks of constituents (the "block approach"); or,
- on the basis of information on the NCS itself ("whole substance approach").

The following Sections of these Guidelines discuss these assessment approaches and provide illustrations and examples on how to apply them when conducting the environmental assessment and classification and labelling of NCS.

II.1. NCS characterisation and specific considerations

As mentioned in Part 1, the ECHA SID Guidance generally considers that NCS fit the sub-category of "UVCB sub-type 3". However they may also be characterised as mono- or multi-constituent substances based on their composition.

NCS are generally composed of a large number of constituents, of which some are known and can be characterised. However there are situations when constituents are unknown or poorly characterised.

As detailed in the NCS SID Guidance, multi-constituent substances are treated as "well-defined substances" containing a few constituents present between 10% and 80%. ECHA Guidance calls for the identification of other constituents between 1% and 10% as "impurities". Previously under ELINCS, the 1 – 10% constituents were notified only to the extent they make a significant contribution to the overall substance classification. Many NCS of concern to EFEO/IFRA fall into the multi-constituent category and are thus "well-defined" substances.

Further, unknown constituents of UVCBs have to be identified as far as possible by a generic description of their chemical nature. For the UVCB constituents of interest to the fragrance industry, these generic descriptors typically are "monoterpene" and "sesquiterpene", modified by the appropriate functional descriptors "hydrocarbon", "alcohol", "ketone", etc.

Terpenoid structures can be further subdivided as acyclic, monocyclic, bicyclic etc. Mass spectral molecular ion fragmentation patterns often allow this level of description even when an exact molecular structure cannot be deducted. With this method, NCS can be characterised as a "mixture" of known substances and one or more generic terpenoid blocks.

The structural nature of the blocks limits many of their physical parameters, such as vapour pressure, water solubility, octanol-water partition coefficient, etc., to a fairly narrow range allowing one to treat them as quasi-substances with properties sufficiently similar to known substances serving as surrogates for risk assessment purposes.

For example the ultimate fate of these quasi-substances may be predicted by analogy to known substances, the biodegradation pathways for which are generally well understood [Marmulla, 2014], [Mikami, 1988], [Alvarez, 1999]**.**

From a "commercial perspective" a "well-defined NCS", be it a multi-constituent substance or a UVCB, is an NCS meeting analytical and compositional norms established by ISO/TC 54 or other quasi-official bodies.

ISO/TC 54 has published 100 standards for essential oils and other NCS available in the global market and 10 additional standards are in various stages of development. For each NCS the standard sets ranges for the following parameters that have counterparts in REACH Annex VI Section 2:

- Appearance
- Colour
- Odour
- Density
- Refractive index
- Acid value
- Typical gas chromatograms (polar and non-polar support)
- Flashpoint

Standards also generally include in addition to the chromatograms a table with concentration ranges of key constituents determined to be important for sensory and physical uniformity.

Minimum concentrations of concern can be as low as 0.1% but, typically, the minimum average concentration of a profiled substance is ≥ 1%. Importantly, not all substances present at 1% or more in the NCS are included in the specification; the sum of the average values for listed constituents typically exceeds 80%.

II.2. Approaches for assessing NCS

II.2.1. Assessment approaches and strategies for NCS

When assessing NCS for their environmental effects under REACH, including for purposes of classification and labelling, the choice of the approach will depend on several factors such as knowledge of constituents and/or fractions in the whole substance, differences in properties amongst them and the ability to characterise these.

Also, technical limitations in testing and feasibility to generate new data will influence the choice of the approach. In some cases, the strategy will require a step-wise approach starting with one approach and improving the assessment by using or combining other approaches to different constituents or groups of constituents.

Sections II.2.1.1 to II. 2.1.3 below describe the various approaches that may be used for assessing NCS for their environmental effects and for classification and labelling purposes. The three approaches described were proposed by the ECHA PBT Expert Group to address specific issues of UVCBs in the ECHA PBT Discussion Paper. However, they could equally be applicable to complex multi-constituent substances and to address other environmental assessments requirements such as classification and labelling, and risk assessment. Advantages and drawbacks to the use of each of these approaches are also summarised below:

II.2.1.1. The "known constituents approach"

The "known constituents approach" can apply when a substance is well characterised and/or is known to contain specific constituents that are relevant for classification and for the PBT/vPvB assessment when they are suspected, based on screening level information, to represent the worst case of the (v)P, (v)B and T properties.

The approach can also be used if the specific constituents can be isolated or separately manufactured for testing or if there is existing available data for the individual constituents. A number of the constituents found in NCS have similar uses in their own right and have or will be registered for REACH as substances for fragrances (e.g. the constituents highlighted in bold in Appendix 4). The screening assessment is based on the individual known constituents, using available data on that constituent (or on read across–substances, if justified).

Similar to the fraction/block approach, there is no need to test all identified constituents. If, for the PBT/vPvB assessment, at least one of the relevant constituent meets the combination of P, B and T or vP and vB properties, the entire complex multi-constituent substance/UVCB substance must be concluded to contain PBT/vPvB constituents.

The pros and cons of the "known constituents approach" are summarized below:

Pros	Cons
• Actual tests are performed on a purer substance, and so easier to perform and interpret • May be the only scientifically defensible option for substances with diverse known constituents • The specific constituents may already be known for their properties and hence assessment effort can be reduced	• Requires greater analytical ability to characterise whole substance at start of PBT assessment than whole substance approach • May require generation of specific material for testing • May require more than one test for each endpoint, which might raise vertebrate testing issues (e.g. for bioaccumulation or mammalian toxicity testing) • Requires demonstration that any representative constituent of the fraction chosen for testing is a reasonable worst case

II.2.1.2. The "block approach" (or "fraction profiling")

In the fraction/block approach, constituents that are structurally similar or that follow a regular predictable pattern of structures are grouped in fractions that are normally considered as if they were single constituents.
The ECHA PBT Discussion Paper outlines several methods to implement the fraction/blocks approach in the PBT assessment for each of the 'P', 'B', 'T' properties but the approach is also applicable for classification and labelling and for the whole environmental assessment generally:

(1) The substance is divided into fractions containing similar constituents based on structural descriptors. The assessment and/or testing are conducted on the fraction itself, not on individual (or surrogate) constituents. The properties may in this fraction either be very similar or follow a regular pattern related to the variation of the structural descriptors.

(2) The substance is divided into fractions containing constituents which are expected to have the same degradation behaviour (e.g. based on ready biodegradation data).

(3) The "hydrocarbon block method": this method was developed for petroleum substances and is applied when the complex multi-constituent substance/UVCB substance can be divided into fractions containing constituents which are very similar with regard to the properties to be assessed. For each of the fractions one or more representative chemical substance(s) (constituent(s), or surrogate constituent(s)), is/are chosen for which testing and assessment is carried out.

A method to assemble relevant fractions/blocks of constituents for NCS is described in Section II.2.2. below.

Accordingly, a stepwise approach may be adopted for the assessment and optimisation of the testing strategy in the environmental assessment, including for classification and labelling purposes so that there is no need to test all fractions.

Focus should therefore be given to the worst case fractions.

The pro's and con's of the "block approach" are summarised below:

Pros	Cons
• More targeted and refined assessment compared to the whole substance approach • Assessment of a complex chemical fraction-wise allows efficient targeting of the testing • May be the only practical option for some very complex UVCBs • Provides a refinement option if "known constituents" approach is not feasible	• Requires greater analytical ability to characterise whole substance at start of the environmental assessment than whole substance approach • May require generation of specific material for testing • May require more than one test for each endpoint, which might raise vertebrate testing issues (e.g. for bioaccumulation or

Pros	Cons
	mammalian toxicity testing) • Requires demonstration that any representative constituent of the fraction chosen for testing is a reasonable worst case • May lead to an over estimation of the endpoint

II.2.1.3. The "whole substance" approach

When all the constituents are expected to have very similar properties, standard test methods may be applicable (ECHA Guidance, Chapter R.7b for aquatic toxicity); in this case, the NCS can be considered as a single chemical substance for the purpose of the assessment and testing.

However, even when the NCS is composed of constituents with dissimilar properties, the use of the whole substance approach may still be applicable (OECD Monograph No.23, 2000; ECHA Guidance; Chapter R.7b for aquatic toxicity; ECHA PBT Discussion Paper). In these cases, care has to be taken in the interpretation of the results.

In any case, when using the whole substance approach for NCS, justification regarding the choice and the applicability of the approach should be provided.

The pros and cons of the "whole substance approach" are summarized below:

Pros	Cons
• Whole substance data may be more ecologically relevant • Use of water accommodated fraction (WAF) for aquatic toxicity testing is well described • May be the only option if suitable test substance analysis is infeasible • Not required to provide data on each constituent (some of which would not be available in a pure form or easily isolated/prepared) • Reduced data generation requirements (including vertebrate testing)	• Test results may not provide information on the behaviour and properties of the individual constituents • Available whole substance test data may be difficult to interpret (either due to physico-chemical issues or because the composition of test items may vary) • Some whole substance testing may not be feasible (e.g. if constituent physico-chemical properties vary significantly)

Finally, the ECHA PBT Discussion Paper provides that a combination of one or more of the three approaches above is also possible and different approaches may be applied at the

different stages of the assessment, e.g. if information and knowledge on the substance increases during the assessment.

II.2.2. Methods for assembling constituents blocks of NCS

Essential Oils and natural extracts used in the fragrance industry are typically composed of monoterpenes and sesquiterpenes. Some small organic molecules may also be present. An illustrative list of constituents present in fragrance NCS is provided in Appendix 4 of these Guidelines.

In NCS, the terpenoid constituents are often related as a consequence of the plant's biochemistry. This provides an opportunity to group related constituents and to treat each "block" as a single substance. Within each constituent block, data for one or a few constituents may be used to represent the whole block thus avoiding the need to generate data for all known constituents within an NCS. For complex NCS where it is not feasible to unequivocally identify all constituents, it may be possible to include unidentified constituents within a block of structurally similar constituents. For example, sesquiterpenes are notoriously difficult to unequivocally identify when present in a complex mixture and ideally require a pure sample for confirmation by chromatographic co-injection.

Furthermore isolation is often not feasible and/or impractical for constituents present at low levels. However, as a consequence of the plant's biochemistry, unknown terpenoids are typically related to known constituents in a given essential oil and thus may be included in a suitable constituent block for assessment.

Each block of constituents should be assembled on the basis of similarity with regard to the properties to be assessed. For the environmental assessment, the key properties are aquatic toxicity, bioaccumulation potential and biodegradation. These are environmental information endpoint requirements for REACH and influence the outcome of the hazard classification, PEC/PNEC calculations and PBT assessment.

Aquatic toxicity is driven by the mode of toxic action, which in turn depends on the chemical functionality present. For example, neutral organic molecules such as alcohols, ketones, ethers and hydrocarbons act via a simple non-polar narcosis mechanism whereas a specific mode of action may be associated with more reactive chemicals such as aldehydes or alpha-beta unsaturated carbonyl compounds that have the ability to bind to proteins. Lipophilicity (as modelled by log Kow) is also known to be a determinant of the toxicity in aquatic organisms. A trend of increasing aquatic toxicity with increasing log Kow is usually observed within a given mode-of-action class up to a log Kow cut-off value of approximately 5.0-6.4 [EPA, 2012].

The potential for bioaccumulation is normally screened by using the octanol/water partition coefficient. The relationship between the log Kow of an organic substance and its bioconcentration as measured by the concentration factor (BCF) in fish has considerable scientific literature support for chemicals that bioaccumulate by passive diffusion and are not biotransformed. The capability of fish to metabolise a substance to more polar constituents, leads to lower BCF values. The potential for metabolism will depend on the chemical structure of the substance.

Biodegradation is the transformation by micro-organisms by enzymatic reactions. Thus the ability of a substance to biodegrade depends upon its chemical structure. The presence of certain functional groups, such as ester groups that can be easily cleaved, will have a positive effect on the structure's biodegradability. The carbon skeleton is particularly important as the degree of branching, position of alkyl groups and number of rings can prevent common mechanisms and biodegradation pathways from occurring.

The above key structural and physicochemical properties relevant for ecotoxicity and environmental fate are summarised in Table 3.

Table 3: Key structural and physico-chemical properties relevant for ecotoxicity and environmental fate

Endpoint	Chemical Properties		
	Carbon Skeleton	Functional Group	Log Kow
Aquatic toxicity		✓	✓
Bioaccumulation	May be important for biotransformation		✓
Biodegradability	✓	✓	

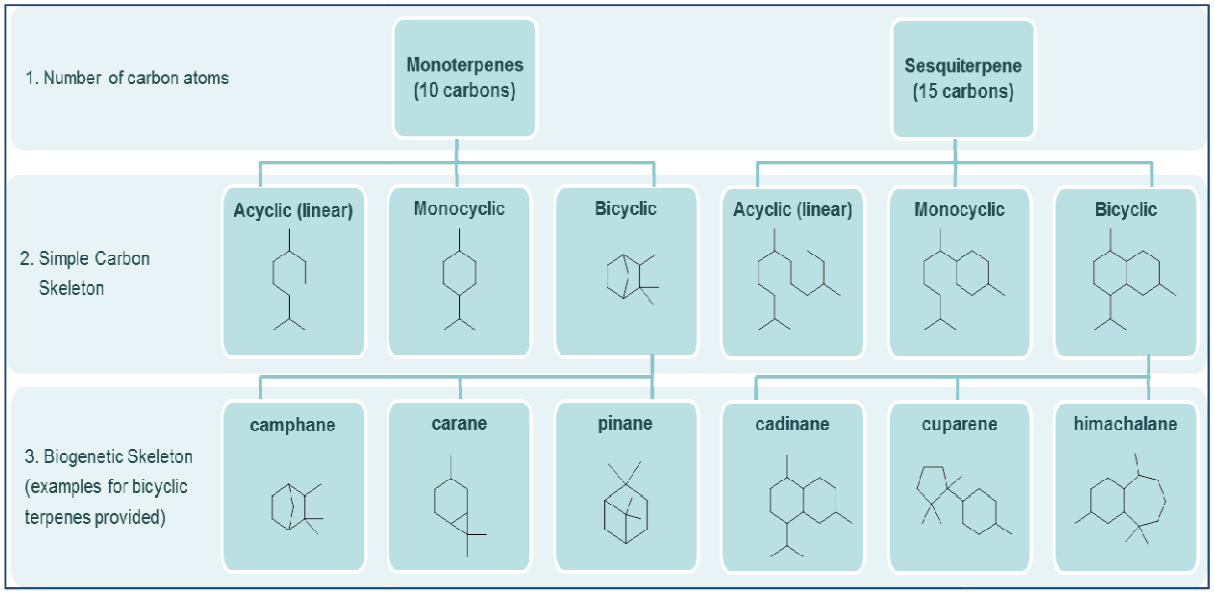
Terpenes have been classically identified through the recognition of an "isoprene" pattern in their carbon skeleton. The number of these significant C5 units in a compound has given rise to a simple primary classification system (Table 4).

Table 4: Primary Classification System for Terpenes

Name	No. of isoprene units	No. of carbon atoms
Hemiterpenoids	1	5
Monoterpenoids	2	10
Sesquiterpenoids	3	15
Diterpenoids	4	20
Etc……..		

The organisation of the isoprenyl carbon skeleton within each primary class then gives rise to various secondary classes or sub-classes (Figure 1). For example, the carbon skeleton could be classified simply based on whether it is acyclic, monocyclic, bicyclic or tricyclic. If further sub-categorisation is required then biogenetic relationships or skeletons may be used. Over the years, as the knowledge of the terpene chemistry developed, various biogenetic classification or nomenclature schemes have been proposed. The most well-known are those of Devon and Scott (1972), Roberts (1981) and Fraga (2013).

Figure 1: Tiered Classification Scheme for Terpene Skeletons



A terpene classification scheme as described above in conjunction with chemical functionality, may provide a suitable approach for assembling NCS constituent blocks for environmental assessment. The size of the molecule (i.e. number of isoprene units / number of carbon atoms) and presence of polar functional groups will determine the lipophilicity (log Kow) of each constituent (Table 5), which as mentioned previously is an important determinant for both aquatic toxicity and bioaccumulation. The chemical functionality is important for assigning aquatic toxicity mode-of-action while the carbon skeleton is key for biodegradation.

Table 5. Criteria for grouping terpenoids

Constituent Block	Assembly Criteria		Log Kow range
	Number of carbon atoms	**Chemical functionality**	
1	Monoterpene	Hydrocarbon	3.9-5.7
2	Monoterpene	Oxygenated	2.6-4.4
3	Sesquiterpene	Hydrocarbon	5.7-7.0
4	Sesquiterpene	Oxygenated	3.4-5.6

An alternative way of grouping terpenoids, particularly when the structure of constituents cannot be unequivocally identified, may be by correlations between mass spectral fragments and/or chromatographic retention indexes.

If an NCS being assessed also contains some non-terpenoid constituents, these may be considered either individual single constituents or if appropriate also grouped into structurally related families, such as linear aliphatic aldehydes, benzyl esters or phenols.

II.3. Classification and labelling

As described in Section I.3.1.4 of these guidelines, classification of NCS can be based on data on the NCS itself or on calculations using data on known constituents or blocks of constituents.

It is important to note that data obtained from the use of non-testing methods can also be used to derive the classification as provided in Section 4.1.1.2.2 of Annex I to the CLP Regulation provided they fulfil the requirements specified in Section 1 of Annex XI to REACH (see Section II.4.2. below).

II.3.1. Classification based on calculation using data on relevant constituents or block of constituents

II.3.1.1. Principle

As indicated in Section I.3.1.4., the classification is based on the classification and the percentage of each relevant constituent or block of constituents of the NCS.

II.3.1.2. Classification of constituents

Classification of constituents may be either based on their existing classification or on data available for these constituents where classification can be derived:

(a) Using existing classification on constituents

Classification of constituents can be obtained directly through sources such as the IFRA/IOFI Labelling manual (see Appendix 5), the C&L inventory (available through: http://echa.europa.eu/information-on-chemicals/cl-inventory-database) or the REACH dossier of the substance available on the ECHA dissemination website for example.

In case a relevant constituent has a harmonised classification as provided in Annex VI to the CLP Regulation, this classification shall be used by the manufacturer, importer or downstream user. In the absence of a harmonised classification, when for a same substance, the classification provided by the various sources are different, the reliability of each available classification needs to be assessed with care. Preference should be given to the most reliable and relevant classification.

Examples:

Substance: 1,8-cineole - CAS: 470-82-6	
Sources	**Classification for environmental hazards according to the CLP Regulation**
IFRA/IOFI labelling manual 2014	Not classified
C&L inventory	12 entries: - One with 782 notifiers (corresponding to the REACH registration joint submission classification): not classified - One with 1 notifier: classified as Aquatic chronic 3, with a check indicating that an impurity or an additive impacts the notified classification
REACH dossier (ECHA dissemination website)	Not classified

Cineole is not classified as hazardous for the aquatic environment according to its REACH registration dossier, 11 entries (corresponding to 1190 notifiers) in the C&L inventory and the IFRA labelling manual. Only 1 notifier of the C&L inventory classified cineole as hazardous for the aquatic environment, but no supporting data is provided to confirm this classification. As a conclusion, cineole can be considered as not classified as hazardous for the aquatic environment.

Substance: linalool - CAS: 78-70-6	
Sources	**Classification for environmental hazards according to the CLP Regulation**
IFRA/IOFI labelling manual 2014	Not classified
C&L inventory	25 entries : - One with 1245 notifiers (corresponding to the REACH registration joint submission classification): not classified - 1 entry with one notifier: Aquatic Chronic 2 - 1 entry with one notifier: Aquatic Chronic 3
REACH dossier (ECHA dissemination website)	Not classified

Linalool is not classified as hazardous for the aquatic environment according to its REACH registration dossier, 23 entries (corresponding to 1714 notifiers) in the C&L inventory and the IFRA/IOFI labelling manual. Only 2 notifiers of the C&L inventory classified linalool as hazardous for the aquatic environment, but no supporting data are provided to confirm this classification. As a conclusion, linalool can be considered as not classified as hazardous for the aquatic environment.

(b) Classifying according to available data on relevant constituents

Classification can be obtained using available data on relevant constituents and applying the classification criteria for substances, as specified in Annex I 4.1.2 of the CLP Regulation.

- For acute (short-term) hazard classification:

The acute aquatic toxicity available for each relevant constituent and each trophic level (fish, crustacea, algae) will be identified.

As set out in the CLP Regulation, the lowest of the available toxicity values between the different trophic levels will be used to define the appropriate acute hazard category according to Table 4.1.0.

- For long-term hazard classification:

If chronic toxicity data are available for relevant constituents, they will be used in priority.

In the absence of chronic toxicity data, long-term aquatic hazards will be assessed by considering also environmental fate data (degradability and bioaccumulation).

The classification of each constituent will be based on the acute toxicity data, the degradability and the octanol/water partition coefficient (log Kow) or the bioconcentration factor (BCF) (see Section 4.1.2 and Annex I: table 4.1.0 of the CLP Regulation).

Example: "Essential oil of thyme (containing thymol), Spanish type"
(EC : 284-535-7, CAS: 84929-51-1)

Composition:

(NF ISO 14715 – November 1999)

Constituents	CAS#	EC#	% min	% max
thymol	**89-83-8**	**201-944-8**	**37**	**55**
cymene para	**99-87-6**	**202-796-7**	**14**	**28**
terpinene gamma	**99-85-4**		**4**	**11**
linalool	**78-70-6**	**201-134-4**	**3**	**6,5**
carvacrol	499-75-2		0.50	5.50
myrcene	**123-35-3**	**204-622-5**	**1**	**2.8**
terpinene alpha	99-86-5		0.9	2.6
pinene alpha	80-56-8	201-291-9	0.5	2.5
terpinene-1- ol-4	562-74-3		0.1	2.5
caryophyllène beta	87-44-5		0.50	2.00
carvacrol methyl ether	6379-73-3	228-959-2	0.10	1.50
α-thujene	3917-48-4		0.2	1.5
trans-sabinene hydrate	15537-55-0		traces	0.5

Thyme oil (containing thymol), Spanish type is mostly composed of monoterpenes alcohols and monoterpenes hydrocarbons. Generally more than 90% of constituents of essential oil of the substance can be analytically identified (constituents in bold are those present in minimum at above 1% in the oil).

Available data (aquatic toxicity, biodegradation and bioaccumulation)

No valid test data for the substance as a whole is available. As a consequence, classification is considered based on individual constituents using the summation method (see following table).

- Aquatic toxicity

Information for aquatic toxicity was obtained for all relevant constituents. However they were not easily available for all constituents: some data are owned by a private company; some others, especially for minor constituents, such as carvacrol methyl ether, alpha thuyene, trans-sabine hydrate, were obtained through QSAR predictions as no other option was available.

- Biodegradation

Results of ready biodegradation test data were available for major constituents.

For minor constituents, read-across was performed which allowed the use of available biodegradation data for carvacrol and sabinene for carvacrol methyl ether and α-thujene/ trans-sabinene hydrate, respectively. For detailed explanation for the use of the read-across approach, see Section II.4.2.2.

- Bioaccumulation:

An experimentally determined BCF value was only available for one constituent. Octanol/water partition coefficients were obtained for all constituents. For those constituents with log Kow close to the cut-off value of 4, bioconcentration factors were also calculated using QSAR models. The QSAR predictions gave outcomes of the same order of magnitude. As a consequence, we can consider without too much uncertainty that these predicted BCF values are relevant to assess the potential of the related constituent to bioaccumulate. In the case, of carvacrol methyl ether (log Kow 4.08), the predicted BCF values are less than the cut-off value of 500.

The use of QSARs and the conditions that must be met is discussed in Section II.4.2.1.

Available data :

Constituents	LC 50 Acute fish (mg/L)	EC 50 *Daphnia* (mg/L)	EC50 Algae(mg/L)	Source	Degradability[19]	Source	Log Kow	Source	BCF (kg/L wet weight)	Source
thymol	4.7	3.2	no data	A	inherently biodegradable (94,6% 5d ; 302B)	A	3.30	a		
cymene para	48	6.5	4	b'';b",b*	rapidly degradable (>60% in 301F but failed 10d window)	C	4.50	c		
terpinene gamma		> limit of solubility #		b#	not rapidly degradable (29% 28d, 48% 70d (301F); 61% 70d (302C))	C	4.75	d		
linalool	27.8	59	88.3	A	rapidly degradable (64.2%, 28 d; 301D)	A	2.84	a		
carvacrol	10.8760	4.0920	7.9260	d*	rapidly degradable	b#	2.50	b#		
myrcene	> limit of solubility [a]	> limit of solubility [a]	> limit of solubility [a]	A	rapidly degradable (76% in 28 d; 301D)	A	4.17	a	262 621-733	d" d**
terpinene alpha		> limit of solubility # read-across from terpinene gamma constituent			not rapidly degradable (40% 28d, 62% 60d (301F))	C	4.75 5.3	d c		
pinene alpha	> limit of solubility [a]	> limit of solubility [a]	> limit of solubility [a]		rapidly degradable (>60% in 301B but failed 10d window)	A	4.48	a	1248	a
terpinene-1- ol-4		6.3		b*	rapidly degradable	B	3.33	d		
caryophyllene beta		> limit of solubility #		B	rapidly degradable	b#	6.30	d		
carvacrol methyl ether	1.8390	1.2650	2.0820	d*	rapidly degradable (read-across from Carvacrol constituent)	b# d#	4.08	d	228 392-395	d" d**
α-thujene	0.6620	0.4730	0.9080	d*	rapidly degradable (read-across from Sabinene (CAS: 3387-41-5))	b#	4.48	d#	420 1137-1237	d" d**
trans-sabinene hydrate	10.7370	6.8070	7.9970	d*	rapidly degradable (read-across from Sabinene (CAS: 3387-41-5))	b# d #	3.19	d#		

a: ECHA dissemination web site
b: RIFM data base, available to members, data owned by RIFM * or member company # or publication~
c: proprietary data
d: QSAR, ECOSAR* or OASIS# or BCFWIN via equation " or BCFWIN BCF Arnot model including biotransformation rate estimates, depending on fish trophic level **

[19] The CLP Regulation criteria for biodegradability uses the term "rapidly degradable". See CLP Regulation Section 4.1.2.9.5.

For each constituent, it was checked if a harmonised classification exists for acute and chronic toxicity hazards in Annex VI of the CLP regulation. Only thymol was identified to have a harmonised classification for chronic toxicity hazard: Aquatic chronic 2.

For the other constituents, on the basis of the above gathered information, each constituent was classified for acute and long-term aquatic hazards as illustrated in the following table below.

Classification of constituents for acute and chronic hazard:

Constituents	% max (used in summation method)	Lowest LC50	Acute aquatic classification	M Factor for acute classification	Chronic aquatic classification	M Factor for chronic classification	Constituent classification reasoning
thymol	**55**	3.20	Not classified		**Chronic 2**		LC50>1mg/L→ not classified for acute toxicity **Harmonised classification as Chronic 2**
cymene para	**28**	4.00	Not classified		**Chronic 2**		LC50>1mg/L→ not classified for acute toxicity lowest EC/LC50 between 1 and 10 mg/l and log Kow>4 → **Chronic 2**
terpinene gamma	**11**	> limit of solubility #	Not classified		**Chronic 4**		LC50>1mg/L→ not classified for acute toxicity EC/LC50>limit of solubility, and log Kow>4 and not rapidly degradable → potential concern → **fulfils the criteria for chronic 4 as defined in table 4.10 of Annex I of CLP**
linalool	**6.5**	27.80	Not classified		Not classified		LC50>1mg/L→ not classified for acute toxicity log Kow<4 and rapidly degradable → not classified for chronic toxicity
carvacrol	5.50	4.09	Not classified		Not classified		LC50>1mg/L→ not classified for acute toxicity log Kow<4 and rapidly degradable → not classified for chronic toxicity
myrcene	2.8	> limit of solubility a	Not classified		Not classified		LC50>1mg/L→ not classified for acute toxicity log Kow close to the cut-off value. BCF obtained through QSAR borderline 262-733 →borderline potential to bioaccumulate. However, EC/LC50>limit of solubility and rapidly biodegradable → not classified for chronic toxicity
terpinene alpha	2.6	> limit of solubility # read-across from terpinene gamma constituent	Not classified		**Chronic 4**		LC50>1mg/L→ not classified for acute toxicity EC/LC50>limit of solubility, log Kow>4 and not rapidly degradable → potential concern → **fulfils the criteria for chronic 4 as defined in table 4.10 of Annex I of CLP**
pinene alpha	2.5	> limit of solubility a	Not classified		Not classified		LC50>1mg/L→ not classified for acute toxicity EC50/LC50 >limit of solubility, BCF > 500, log Kow>4 and rapidly degradable → not classified for chronic toxicity
terpinene-1- ol-4	2.5	6.30	Not classified		Not classified		LC50>1mg/L→ not classified for acute toxicity log Kow<4 and rapidly degradable → not classified for chronic toxicity
caryophyllène beta	2.00	> limit of solubility #	Not classified		Not classified		LC50>1mg/L→ not classified for acute toxicity EC50>limit of solubility, log Kow>4 and rapidly degradable→ not classified for chronic toxicity
carvacrol methyl ether	1.50	1.27	Not classified		Not classified		LC50>1mg/L→ not classified for acute toxicity log Kow obtained through QSAR close to the cut-off value. BCF obtained through QSAR <500 →low potential to bioaccumulate and rapidly degradable → Not classified for chronic toxicity
α-thujene	1.5	0.47	**Acute 1**	1.00	**Chronic 1**	1.00	**LC50<1mg/L→ classified as Acute 1** **lowest EC/LC 50 <1 mg/l and log Kow >4 → chronic 1**
trans-sabinene hydrate	0.5	6.81	Not classified		Not classified		LC50>1mg/L→ not classified for acute toxicity log Kow < 4 and rapidly degradable → not classified for chronic toxicity

Classification for acute toxicity hazard :

Only one constituent, α-thujene, fulfils the requirements for acute toxicity hazard *(EC/LC50<1mg/L).*

According to the summation method, classify for acute hazard if: Σ (Acute 1 × M) ≥ 25%

Using the classification of the essential oil constituents: (1.5% × 1) = 1.5% (which is < 25%).

Therefore, the substance is not classified for acute aquatic hazard.

Classification for chronic toxicity hazard:

According to the summation method,

Step 1: Classify as Chronic 1 if: Σ (Chronic 1 × M) ≥ 25% (if not, then go to Step 2).

Step 2: Classify as Chronic 2 if: Σ (10 × Chronic 1 × M) + Σ (Chronic 2) ≥ 25% (if not, then go to Step 3).

Step 3: Classify as Chronic 3 if: Σ (100 × Chronic 1 × M) + Σ (10 × Chronic 2) + Σ (Chronic 3) ≥ 25% (if not, then go to Step 4).

Step 4: Classify as Chronic 4 if: Σ (Chronic 1) + Σ (Chronic 2) + Σ (Chronic 3) + Σ (Chronic 4) ≥ 25%

Using the classification of the essential oil constituents:

Step 1: (1.5% × 1) = 1.5% (which is < 25% → Step 2).

Step 2: (10 × 1.5% × 1) + 28% + 55% = 98% (which is > 25%).

Therefore, **the substance fulfils the criteria for chronic 2**.

II.3.2. Classification derived using data on the NCS itself

II.3.2.1. Principle

II.3.2.1.1. *For acute (short-term) hazard classification*

As indicated above under Section I.3.1., there is no requirement under the CLP Regulation to generate new data for the purpose of classification. Classification should be based on available information (measured data or predictions).

However, generation of acute toxicity data may be required to fulfil the REACH requirements and can be obtained performing a test on the NCS itself provided that the conditions described in Section II.4.1.2 are fulfilled. The result based on lethal loading level, E(L)L50, is then compared to the criteria for acute toxicity as set up in table 4.1.0 of the CLP Regulation. When adequate toxicity data are available for relevant constituents or representatives of a block of constituents, the acute toxicity, E(L)C50 of the NCS may be calculated using the constituent additivity formula (see Section II.4.1.1.).

The lowest acute effect, L(E)C50 between the available trophic levels are used to assess the acute (short-term) aquatic hazard.

II.3.2.1.2. *For long-term hazard classification*

If adequate chronic aquatic test results are available for the NCS, they will be used in priority and classification will be made according to Table 4.1.0 of the CLP Regulation. When adequate chronic toxicity data is available for relevant constituents or representatives of a block of constituents, the NOEC of the NCS may be calculated using additivity formula and used for long-term hazard classification.

For the majority of essential oils and their constituents, it is likely that no chronic test data will be available. In this case, the acute toxicity data for the whole substance in combination with the biodegradability and bioaccumulation data for the individual constituents may be considered. For example, if all the constituents are rapidly degradable and the log Kow are less than 4, the NCS will not be classified for long term hazards.

Testing the NCS as a whole substance in a biodegradability test would only be applicable in very specific cases (i.e. in case of structurally similar constituents with similar chain-lengths, degree and/or site of branching or stereoisomers), since biodegradation tests are intended for testing pure substances. If the NCS tested, composed of structurally similar constituents, achieves a biodegradation level of greater than 60% in a ready biodegradability screening test, it would be considered as being readily (bio)degradable and subsequently rapidly degradable in the environment.

If a test on such a complex substance is performed and it is anticipated that a sequential biodegradation of the individual constituents is taking place, then the 10-day window should not be applied to interpret the results of the test. A case-by-case evaluation should however take place on whether a biodegradability test on such a substance would give valuable information regarding its biodegradability as such i.e. regarding the degradability of all the constituents, or whether instead an investigation of the degradability of carefully selected individual constituents of the complex substance is required (OECD, 2006).

http://www.oecd-ilibrary.org/docserver/download/9730001e.pdf?expires=1463646549&id=id&accname=guest&checksum=FCF1DC897E65F54F374868A11DF06296

In addition, in case of a borderline degradation, where some of the constituents are rapidly degradable while others are not, a more detailed assessment of the degradability of the individual constituents in the complex substance is required. For example, in cases where the NCS contains only one or a few relevant constituents that are not rapidly biodegradable and/or have a log Kow > 4, biodegradation and bioaccumulation properties would need to be further investigated in order to conclude on classification based on data on the NCS itself (see Sections II.4.1.2.2 and II.4.1.2.3. for detailed description on the environmental fate properties assessment).

II.4. Generation of Data for the Environmental Assessment

II.4.1. Information requirements under Annex VII and VIII of REACH

As described in Section I.2.3.2. of these Guidelines, the set of standard (eco)toxicological and environmental fate information requirements for Annex VII and VIII substances include short term aquatic toxicity data (*Daphnia*, algae, fish and an activated sludge respiration inhibition study, as well as degradation studies (biotic and abiotic) and an adsorption/desorption screening study. In addition, data on physico-chemical properties (vapour pressure, water solubility, and octanol-water partitioning coefficient) are needed in order to conduct the environmental hazard assessment.

However, when it relates to testing on vertebrate animals, i.e., aquatic fish toxicity studies, alternatives to testing should be considered first and the use of all available data, including "non-testing approaches" should be used to fill data gaps. Testing on vertebrate animals should therefore be considered only as a last resort option.

II.4.1.1. The constituents /"block of constituents" approach

II.4.1.1.1. *Aquatic toxicity*

Principle

When adequate toxicity data are available for relevant constituents or representatives of a block of constituents, the aquatic toxicity of the NCS may be calculated using the constituent additivity formula that is commonly applied to mixtures of substances.

Separate calculations of additivity are required for each acute endpoint:

- Toxicity to *Daphnia*
- Algal growth inhibition
- Toxicity on fish (if manufactured and imported material tonnage exceeds 10 tons/year)

Methods that predict the toxicity of mixtures, taking into consideration the relative partitioning behaviour of the constituents, are becoming available which may be useful in assessing the acute mixture toxicity of NCS.

Component additivity formula for acute toxicity:

$$\frac{\sum Ci}{L(E)C_{50m}} = \sum_{n} \frac{Ci}{L(E)C_{50i}}$$

where:

C_i	=	concentration of component i (weight percentage);
$L(E)C_{50i}$	=	(mg/l) LC_{50} or EC_{50} for component i;
η	=	number of components, and i is running from 1 to n;
$L(E)C_{50m}$	=	L(E) C_{50} of the part of the mixture with test data.

It should be noted that the component additivity formula assumes that all constituents will completely dissolve and contribute to the overall toxicity of the mixture.

Data on constituents

Reliable measured data on constituents are preferred when available.

a) However, data from (Q)SARs or read-across can also be used as described in Section II.4.2.

Pros and cons of the approach:

Pros	Cons
• Makes use of existing data on constituents or block constituent representatives • Is consistent with the constituent approach for PBT/vPvB assessment • More flexible for applying worst-case assumptions when the substance composition is uncertain and/or variable • Avoiding testing the substance	• Often lead to an over-estimation of the aquatic toxicity of the NCS • Data availability for some constituents • Uncertainty of result when using data on constituents based on QSAR • Synergistic or antagonistic effects and other mixture effects are disregarded • Requires analytical ability to identify major constituents • Data sharing issues/ expensive approach if numerous constituents are considered • Data reporting and processing • Do not take into account the effect of the unknown constituents

When to use this approach:

- Composition of the NCS well-defined
- Reasonable number of constituents
- Data are available for most constituents
- To be used at a first step
- Limitation to be used for chronic aquatic toxicity

Example : "Essential oil of thyme (containing thymol), Spanish type"
EC : 284-535-7, CAS: 84929-51-1

Constituents	LC 50 Acute fish (mg/L)	EC 50 *Daphnia* (mg/L)	EC50 Algae(mg/L)	% min	% max
thymol	4.7	3.2	no data	**37**	**55**
Cymene para	48	6.5	4	**14**	**28**
terpinene gamma		> limit of solubility #		**4**	**11**
linalool	27.8	59	88.3	**3**	**6.5**
carvacrol	10.8760	4.0920	7.9260	0.50	5.50
myrcene	> limit of solubility [a]	> limit of solubility [a]	> limit of solubility [a]	1	2.8
terpinene alpha		> limit of solubility # read-across from terpinene gamma constituent		0.9	2.6
pinene alpha	> limit of solubility [a]	> limit of solubility [a]	> limit of solubility [a]	0.5	2.5
Terpinene-1- ol-4		6.3		0.1	2.5
caryophyllène beta		> limit of solubility #		0.5	2
carvacrol methyl ether	1.839	1.265	2.082	0.1	1.5
α-Thujene	0.662	0.473	0.908	0.2	1.5
trans-sabinene hydrate	10.737	6.807	7.997	traces	0.5

The additivity formula is applied to each trophic level using the upper limit of the provided range as percentage for each constituent. When no data is available for a constituent, the percentage of this constituent is not taken into account in the additivity formula.

Acute fish LC50 =
(55+28+6.5+5.5+1.5+1.5+0.5)/(55/4.7+28/48+6.5/27.8+5.5/10.8760+1.5/1.8390+1.5/0.6620+0.5/10.7370)= 6.0979 mg/L

Daphnia EC50 =
(55+28+6.5+5.5+2.5+1.5+1.5+0.5)/(55/3.2+28/6.5+6.5/59+5.5/4.0920+2.5/6.3000+1.5/1.2650+1.5/0.4730+0.5/6.8070)= 3.5975 mg/L

Algae EC50 =
(28+6.5+5.5+1.5+1.5+0.5)/(28/4.000+6.5/88.3+5.5/7.9260+1.5/2.0820+1.5/0.9080+0.5/7.9970)= 4.2637 mg/L

II.4.1.1.2. *Biodegradation*

The assumption is that if the relevant constituents of the NCS are readily biodegradable, the NCS itself can be considered as readily biodegradable and consequently rapidly degradable for classification purposes.

II.4.1.2. The whole substance approach (Testing the NCS itself)

When information has to be generated through testing the NCS itself, it is important to select a test item which is representative of the NCS qualities covered by the REACH registration dossier (See NCS SID Guidance for more details on NCS qualities in a registration dossier).

It is also important to note that selecting the whole substance approach for testing NCS/multi-constituent substances will require a justification to demonstrate that the approach is applicable and appropriate to generate adequate data for the purpose of the environmental assessment.

II.4.1.2.1. *Aquatic toxicity*

Aquatic toxicity testing of the NCS itself (whole-substance approach) may be the preferred or only option when the composition of the NCS is not fully known and/or when data on the constituents are not sufficiently available to assess the NCS as a whole. Aquatic toxicity testing of multi-constituent substances will depend on the physico-chemical properties (especially the water solubility) of the constituents. Various situations are possible:

- all constituents are fully soluble: test methods described for water soluble substances;
- all constituents are highly insoluble in water or the constituents will unlikely cross biological membranes (i.e. when MW > 700, log P >6, diameter > 17Å, eg in Dimitrov *et al.* 2003);
- since NCS are most often containing constituents of varying solubility, testing the NCS itself by preparing Water Accommodated Fractions (WAF) could be considered according to the principles outlined in OECD Monograph No.23 (2000).

In cases where constituents have specific individual properties (e.g. degradability, volatility, etc.) additional steps should be taken to control possible losses.

II.4.1.2.1.1. <u>WAF Principle and methodology</u>

A Water Accommodated Fraction ("WAF") is defined as an aqueous fraction containing the dissolved and/or stable suspended and/or emulsified fraction of the NCS. The method used to prepare the WAF needs to be fully described in the test report (OECD Monograph No. 23 (2000) and CONCAWE, 1992), with evidence provided of attainment of equilibrium and its compositional (or overall) stability over time.

Preparation of representative test media - When preparing the test media, various factors can affect the composition of the aqueous phase:

(a) <u>the stirring /shaking rate,</u> which will determine whether equilibrium is reached. A too vigorous stirring may lead to the formation of an emulsion, the micelles causing physical effects to algae and *Daphnia*, that are not reflecting the intrinsic toxicity of the dissolved constituents.

(b) <u>the mixing time:</u> Increasing the duration of the mixing time will increase the probability of reaching equilibrium, but also increase losses of constituents that are volatile

or prone to oxidation. It is therefore recommended to mix for sufficient time to reach the equilibrium.

In order to ensure that test organisms are exposed to clear test medium, separation techniques such as centrifugation, or filtration (less recommended) may be applied to remove non-dissolved test substance from the test medium.

As stated in Betton (1997) and OECD Monograph No. 23 (2000), when testing a complex mixture of poorly soluble constituents, serial dilutions of a stock solution (as it is commonly done for single water-soluble chemicals) should not be applied, instead WAFs should be prepared individually. This is because the composition of the aqueous phase changes depending on the loading rate. This is reflected in table 6 below, showing that the concentration of the least soluble constituent does not change when the loading rate of the NCS increases.

Table 6 : Composition of aqueous phase according to the NCS loading for soluble and poorly soluble constituents

NCS loading rate (mg/L)	Concentration of constituent A present at 10% in NCS (with solubility of 1 mg/L)	Concentration of constituent B present at 10% in NCS (with solubility of 1000 mg/L)
10	1.0	1.0
100	1.0	10
1000	1.0	100

Adapted from Betton (1997).

Test data obtained with WAFs apply to the NCS as an entity and the exposure is generally expressed as the 'loading rate' as opposed to measured concentrations. The acute effect or lethal loading level (typically expressed as the E(L)L50) is comparable to L(E)C50 values determined for pure substances tested within their solubility range. It may therefore be used directly for classification. However, it is questionable whether to use it to derive a PNEC for environmental risk assessment, since partitioning in the environment will make the comparison with a PEC meaningless.

Analytical determination should be conducted to confirm exposure concentrations. Since NCS are generally composed of various constituents, it is evident that not all relevant constituents can be analysed. In addition it is likely that the composition will evolve with time and is dependent on the loading rate and solubility of each constituent.

In order to address this situation, two methods can be used as per the following:

(a) analyse the dissolved total organic carbon (TOC) content in the aqueous phase. This will provide an integrated figure of the exposure concentration and will be used as a tracer. The ecotoxicological results will be given as LR or EL50.

(b) quantify at least one relevant constituent representative of the NCS as a tracer. The ecotoxicological values will be also expressed as LR or E(L)50, not based on the measured concentration of the tracer constituent

According to Guidance on the application of the CLP criteria (Version 4.1., June 2015)), the validity of the WAF results depends on the demonstration that "*the tested organisms have been exposed to the toxic components of the mixture in proportion to the composition of the*

mixture" and that only in those cases will WAF results be used for classification purposes. However, it must be recalled that a WAF by definition reflects equilibrium dissolved concentrations of the mixture constituents at a given loading rate and for poorly water-soluble complex mixtures, the product/water ratios may exceed water solubilities of some constituents where the concentration of the less soluble constituents will vary with time. Therefore, maintaining the constituent proportion between the WAF preparation and the original NCS would be challenging. As a consequence, a WAF test performed according to OECD Monograph No. 23 (2000) and for which the exposure has been maintained during the duration of the test would be considered valid for classification purposes.

When to use WAF preparations

- When the NCS is composed of constituents with different physico-chemical properties, especially poorly soluble constituents.
- When the NCS is composed of a significant number of unknown constituents in the NCS (see above).
- When the NCS is composed of many constituents with none or unreliable data.
- As last resort to generate meaningful data for classification purposes.
- To support /refine the outcome of other approaches.

Pros and cons of WAF testing

Pros	Cons
• Takes into account possible mixture effects • The loading rate for the WAF test can be used directly for classification and labelling purposes • More ecologically relevant approach, reflects the behaviour of the various constituents in the aquatic environment (take into account the toxicity of the soluble constituents)	• Preparation methods of the test solution can be challenging for some NCS • Results may be difficult to use for ERA[20] purposes

II.4.1.2.2. *Biodegradation*

Ready biodegradation tests have been developed for single substances and measure ultimate biodegradation as a function of either the CO2 evolved or O2 consumed. These standard tests if applied to an NCS do not provide information on the biodegradability of individual constituents.

Although these tests are intended for pure chemicals, it is sometimes relevant to examine the ready biodegradability of mixtures of structurally similar chemicals like oils and surface-active substances (OECD, 2006). The OECD Guidelines states that if a substance consists of *"constituents with different chain-lengths, degree and/or site of branching or stereo-isomers, even in their most purified commercial forms" and "it is anticipated that a sequential biodegradation of the individual structures is taking place, then the 10-day window should not be applied to interpret the results of the test."* Some NCS may also be regarded as consisting of structurally similar constituents that are expected to have similar degradation

[20] Environmental Risk Assessment

potential. In such cases the NCS itself may be tested in a suitable standard ready biodegradation test. When the composition of the NCS is not fully known and therefore data on the constituents is not sufficiently available, then testing the NCS itself may be the only option.

The most suitable ready biodegradability screening tests for a fragrance NCS are those that are designed for poorly soluble and volatile substances, e.g. OECD 301C, 301D, 301F, 310. The percentage of carbon or oxygen in the NCS will need to be determined (for example, by elemental analysis) for the calculation respectively of the theoretical maximum CO2 production (ThCO2, required for OECD310 test) or Theoretical Oxygen Demand (ThOD, required for OECD 301C, 301D and 301F). The 10 day window may be waived for a complex, multi-constituent substance with structurally similar constituents and only the pass level of 60% at 28 days applied for a readily biodegradability classification (OECD, 2006).

If the NCS meets the stringent ultimate ready biodegradation test criterion (NB: the 10d-window does not need to be met for substances with structurally similar constituents) the substance can be considered rapidly degradable for classification and labelling purposes and it can be concluded that the underlying constituents that comprise the NCS are not expected to be persistent for the PBT assessment. For environmental risk assessment purposes, data on lead constituent(s) or representative structures may be required.

II.4.1.2.3. *Bioaccumulation*

For this endpoint, the classification criteria are based on the bioconcentration factor (BCF) or on the octanol/water partition coefficient (log Kow) if no BCF data is available.

However, the CLP Guidance provides that *"Complex substances contain a range of individual substances which can have a great variation in their physico-chemical and toxicological properties. It is generally not recommended to estimate an average or weighted BCF value. It is preferable to identify one or more representative constituents for further consideration."*

Based on the above, the partition coefficient derived for the whole substance for most NCS will be meaningless because of the range of individual substances that may be present and it is generally not recommended to estimate an average or weighted log Kow value.

Instead a range should be given from the calculated or measured values of the constituents or from a multi-constituent log Kow measuring technique, such as HPLC (OECD 117). The bioaccumulation assessment using the log Kow value is clearly a rapid screening procedure to determine whether a substance is lipophilic, where octanol is considered a surrogate for lipids.

When a refined assessment of the bioaccumulation potential is required, the fish as a living organism wherein complex processes are taking place should be considered as a second tier.

However, due to technical difficulties in testing complex mixtures with a fish bioconcentration test (OECD 305), experimental BCF data may often not be available and the use of accepted computer models that can address important toxicokinetic processes such as ADME processes (Absorption, Distribution, Metabolism and Excretion) (e.g. Nichols et al 2009), is allowed. As for the log Kow, the refined BCF values should be given as a range.

II.4.1.3. Generation of data for other relevant endpoints

II.4.1.3.1. *Activated sludge respiration inhibition*

Data from this test is used to derive PNECstp for use in an environmental risk assessment (i.e. required for Annex VIII substances that are classified as hazardous). The test can be waived if:

- a substance is readily biodegradable and predicted environmental concentrations (PECs) are below the test concentration applied
- there are mitigating factors, such as a very low solubility that would limit the exposure.

In theory, the test is technically feasible for complex and poorly water soluble mixtures. However, depending on the approach that will be used for the risk assessment of an NCS, data for the selected lead constituent(s) or block of related constituents will be required.

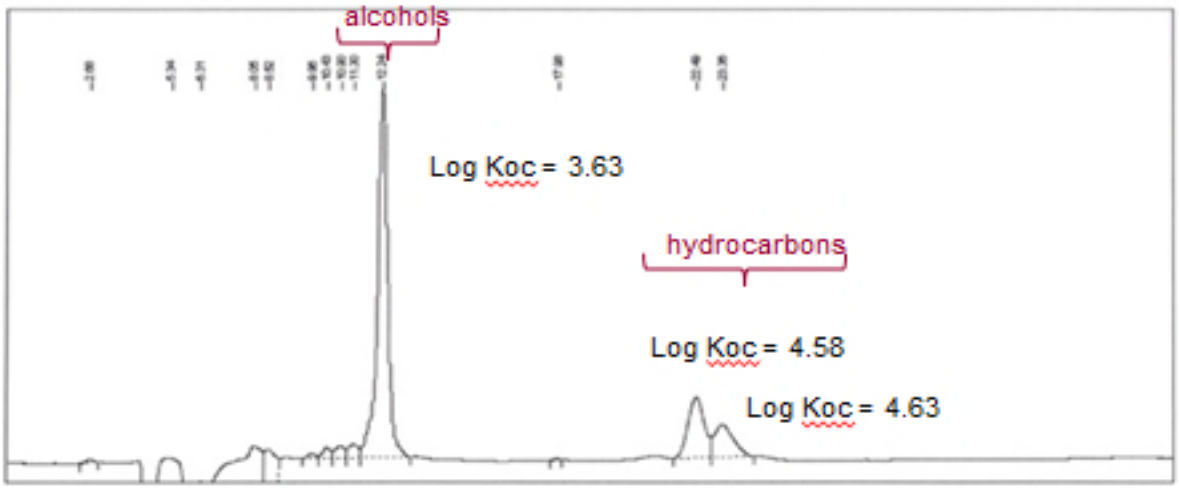
II.4.1.3.2. *Abiotic degradation (hydrolysis)*

This is required for substances greater than 10 tons that are not rapidly degradable. Furthermore, it should be noted that most essential oils used in perfumery are obtained by steam distillation and as such their constituents can be regarded as hydrolytically stable. This test is not adapted to complex mixtures, because of analytical monitoring issues. Thus a constituent approach is preferred.

II.4.1.3.3. *Adsorption/desorption screening*

This information is required for environmental risk assessment purposes (Annex VIII substances that are classified as hazardous). The HPLC estimation method (OECD 121) can be applied to mixtures and can be used to obtain data for blocks of related constituents (Figure 2). Alternatively data for appropriate constituent(s) selected for assessing the environmental risk of the NCS may be estimated using well-established QSAR models.

Figure 2: HPLC Chromatogram for a Koc determination of essential oil composed of sesquiterpene hydrocarbons and sesquiterpene alcohols



II.4.2. Generation of data through non testing methods ((Q)SAR, read-across)

If as a result of the analysis of available information, data gaps exist to fulfill the REACH requirements (i.e., no reliable test results are available for the NCS of interest or individual/groups of constituents), expert judgment should be used in these specific cases, including predictive tools based on in-silico models and/ or the use of data from closely structurally related materials.

Non-testing data can generally be provided by:

- Quantitative structure-activity relationships (QSARs); and
- Read-across using either an analogue or category approach

General guidance on the use of the above approaches is given in ECHA Guidance Sections R.6.1 (QSARs) and R.6.2. (development of chemical categories and analogue read-across). The development and application of all kinds of non-testing methods is based on the *similarity principle*, i.e. hypothesis that similar compounds should have similar biological activities.

These methods can be used for the assessment of aquatic toxicity, biodegradation and bioaccumulation if they provide relevant and reliable data on the chemical of interest. Specific guidance on the use of non-test methods for these endpoints is provided in the individual endpoint specific Sections of the ECHA Guidance documents: Chapter R.7b for aquatic toxicity and biodegradation, Chapter R.7c for aquatic bioaccumulation.

In the following Sections, a brief overview of the use of the above approaches is provided with details of how they might be applied to the assessment of NCS and their constituents. For example, there may be no experimental data available for many constituents or constituent groups of NCS. Such data gaps could be filled by the use of QSARs, where applicable, and/or read-across from a structurally related constituent for which reliable experimental data is available. Equally, it may be appropriate to use read-across in the "whole substance approach" if the NCS being assessed is very similar in terms of its chemical composition to an NCS for which reliable relevant data is available. QSARs and grouping approaches could also be used to identify blocks of constituents with similar predicted properties, allowing the subsequent use of the block approach in the assessment of the NCS if experimental data is available for one or a few constituents in the block.

II.4.2.1. (Q)SARs

SARs and QSARs, collectively referred to as (Q)SARs, are theoretical models that can be used to predict in a qualitative or quantitative manner the physico-chemical, biological (e.g. toxicological) and environmental fate properties of compounds from a knowledge of their chemical structure.

Use of (Q)SAR

Both REACH and the CLP Regulation refer to (Q)SARs as methodologies to be taken into account employing weight of evidence and expert judgment to conclude on the hazardous properties when valid data from testing are not available. ECHA Guidance R.6.1 (2008) discusses the regulatory use of QSARs based on experience and covers use for risk assessment (R.6.1.4.1), classification and labelling (R.6.1.4.2), and PBT (vPvB) assessment (R.6.1.4.3). The use of QSAR in EU classification is illustrated by the use of predicted log Kow

values in the classification of long term aquatic hazard (bioaccumulation). ECHA R.6.1.4.2 also states that "*when valid test data on the preferred predictor of bioaccumulation (fish BCF) are not available, the BCF value can be calculated by using a QSAR or by using a decision rule based on the (experimental or calculated) log Kow value, provided that the QSAR is considered valid for the chemical in question*".

Further, data obtained from the use of non-testing methods can directly be used to derive the classification as provided in Section 4.1.1.2.2 of Annex I to the CLP Regulation and this was already possible in the previous legislation under Section 1.6.1 of Annex VI of Directive 67/548/EEC which provides that data required for classification and labelling may be obtained from various sources, including from results of validated structure-activity relationships.

This has been illustrated in OECD (2007) specifically for the classification of long term aquatic hazard (bioaccumulation), whereby in the absence of measured log Kow and fish experimental BCF, QSAR predictions for log kow can be calculated with valid QSAR (see Section II.4.2.1 on QSAR).

The use of experimental BCF generally trigger a less conservative classification, compared to when using only the log P to assess the bioaccumulation (OECD, 2007). This is not surprising because many organic compounds are biotransformed within fish and this is reflected in Q(S)AR models such as the most recent version of the Arnot-Gobas model and the OASIS BCF model which also accounts for ADME processes (Absorption, Distribution, Metabolism and Elimination) taking place within a living organisms, as well as other related mitigating factors (molecular size, water solubility, ionization, etc).

Regarding the use of (Q)SARs, REACH Annex XI contains the following wording:

Results obtained from valid qualitative or quantitative structure-activity relationship models ((Q)SARs) may indicate the presence or absence of a certain dangerous property. Results of (Q)SARs may be used instead of testing when the following conditions are met:

- *results are derived from a (Q)SAR model whose scientific validity has been established,*
- *the substance falls within the applicability domain of the (Q)SAR model,*
- *results are adequate for the purpose of classification and labelling and/or risk assessment, and,*
- *adequate and reliable documentation of the applied method is provided.*

This wording emphasises the principle that information generated by (Q)SARs may be used instead of experimental data, provided a number of conditions are met. In order to establish the scientific validity, the QSAR model should meet the requirements of the OECD principles:

- defined endpoint;
- an unambiguous algorithm;
- a defined domain of applicability;
- appropriate measures to assess robustness and predictivity;
- A mechanistic interpretation if possible.

To ensure transparency, the endpoint must be clearly defined, as for a given endpoint, there can be several different experimental protocols available to build the model. Specific aquatic

toxicity aspects of the OECD validity criteria are provided in ECHA Guidance, Table R.7.8-1. By way of example, here a clearly defined endpoint is assumed if the QSAR model is based on experimental data with a) a single measured biological endpoint (eg. mortality of a specific fish species) , b) comparable exposure conditions (e.g. exposure duration, same age of test organisms) and c) a single statistically derived endpoint (e.g. LC50).

The applicability domain of the (Q)SAR must be defined in order to check if the prediction for the chemical in question will be sufficiently reliable.

In summary, the evaluation of the reliability of a non-testing result includes two steps:

1. Evaluation of the validity of the model or expert system
2. Evaluation of the reliability of the outcome of a prediction

Both evaluations should be reported in detail. Templates, so called QSAR model reporting formats (QMRFs) and QSAR prediction reporting formats (QPRFs) are provided in ECHA Guidance Section R6.1.9 and Section R.6.1.10 respectively.

According to ECHA Guidance Chapter R.6.1, "*In the ideal situation, (Q)SAR results can be used on their own for regulatory purposes if they are considered relevant, reliable and adequate for the purpose, and if they are documented in an appropriate manner. In practice, there may be uncertainty in one or more of these aspects, but this does not preclude the use of the (Q)SAR estimate in the context of a Weight of Evidence approach*".

The use of a category/analogue approach and QSARs may be used together for the same endpoint, where the QSAR could allow the identification of the structure and the MoA, and the identification of an analogue substance will substantiate the approach.

For the use of QSARs in the assessment of constituents of UVCB's and multi-constituents substances, the same principles apply as for the use of QSARs in the assessment of mono-constituent substances.

A wide variety of publicly available and commercial computational tools have been developed that are suitable for the development and application of (Q)SARs. An indicative list of those most relevant for environmental assessments is provided below:

Examples of tools:

Publicly available (free):

- OECD Toolbox can be used to support the prediction of phys-chem or (eco)toxicological parameters or make a grouping for read across. Several Guidance documents are available at http://www.oecd.org/chemicalsafety/risk-assessment/guidancedocumentsandreportsrelatedtoqsars.htm and include one specifically on "Strategies for grouping chemicals to fill data gaps to assess acute aquatic toxicity endpoints" (OECD, 2013)

- Toxicity Estimation Software Tool (TEST) (acute ecotoxicity, BCF) – Available at https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test

- VEGA/Caesar (ecotoxicity, BCF) – available at http://www.vega-qsar.eu/

- EPIsuite (aquatic toxicity using ECOSAR, BCF using BCFBAF, biodegradation using BIOWIN, physico-chemical properties) available at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

Commercially available tools (for purchase) [21]:

- CATALOGIC (biodegradation, BCF, aquatic toxicity) – available from OASIS –LMC (http://oasis-lmc.org/products/models/environmental-fate-and-ecotoxicity.aspx)

- iSafeRat (phys-chem, aquatic toxicity) – available from Kreatis (http://www.kreatis.eu/en/qsars-products-services.php?endpoint=0)

It is to note that both CATALOGIC and iSafeRat have a submodule that allows to derive, respectively the biodegradation of a mixture, and the aquatic toxicity of a mixture.

Examples of QSAR models and an overview of programs for predicting aquatic toxicity are given in ECHA Guidance Chapter R.10. Several models exist for acute aquatic toxicity. However, currently reliable QSAR models for chronic toxicity are rare and thus reliable QSAR results will be seldom available (ECHA R.7.8.5.3, footnote 9). Models developed to predict biodegradation are given in ECHA Guidance Section R.7.9.3.1. The combined results of the three freely available estimation models, BIOWIN 2, 6 and 3 in the EPI suite, form part of the PBT screening criteria to preliminary identify substances with a potential for persistence (see as well Table 9, Section II.5.1). BCF QSAR models are discussed in ECHA Guidance Section R.7.10.3.2.

Further information on available QSAR models can also be found in the ECETOC report "(Q)SARs: Evaluation of the commercially available software for human health and environmental endpoints with respect to chemical management applications. Technical Report No. 89. p 164 (2003).

A key element in assessing the reliability of a QSAR prediction is whether the chemical is within the applicability domain (predictive space) of the model or not. The importance of this is illustrated in a recent study on the persistence assessment of cyclic sesquiterpenes (Jenner et al, 2011). The biodegradation of 11 sesquiterpenes was predicted using BIOWIN, BioHCwin and CATALOGIC and compared with experimental results.

By way of example, the results for the BIOWIN 2 and CATALOGIC BOD Kinetic 301F model are summarized in table 7 below. The comparison of the biodegradation predictions via BIOWIN 2 and the measured results reveal that BIOWIN 2 is a poor predictor of biodegradability of the sesquiterpenes. On the contrary, CATALOGIC 301F is able to predict the outcomes correctly in 10 out of the 11 substances. This is probably related to the fact that the training set of CATALOGIC 301F contains a greater number of substances with similar structures.

[21] This is based on information available to the authors of these Guidelines at the time of writing. It is possible that other tools are commercially available

Table 7: Examples of applicability domains

Sesquiterpene	BIOWIN v4.10 BIOWIN 2 Non linear model predictions#	CATALOGIC v5.10.8 BOD kinetic 301F Model			Experimental result of OECD 301F % BOD (28d)	Convergence of OASIS prediction and measured biodegradation
		Structural Domain	Metabolic domain	Predicted % BOD (28d)		
α-Bisabolol	0.13 (not RB)	IN	IN	70 (RB)	73 (RB)	YES
α-Cedrene	0.03 (not RB)	IN	IN	66 (RB)	78 (RB)	YES
Cedrol	0.01 (not RB	IN	IN	72 (RB)	88 (RB)	YES
α-Humulene	0.17 (not RB)	OUT	IN	60 (RB)	64 (RB)	YES
(–)-Thujopsene	0.01 (not RB)	OUT	IN	57 (not RB)	36 (not RB)	YES
α-Cadinene	0.53 (not RB)	OUT	OUT	16 (not RB)	50 (not RB)	YES*
α-Gurjunene	0.17 (not RB)	OUT	OUT	0 (not RB)	43 (not RB)	YES*
Longifolene	0.03 (not RB)	OUT	OUT	0 (not RB)	50 (not RB)	YES*
Himachalenes	0.17 (not RB)	OUT	OUT	8 (not RB)	38 (not RB)	YES*
Germacrene D	0.53 (not RB)	OUT	OUT	43 (not RB)	19 (not RB)	YES*
β-Caryophyllene	0.17 (not RB)	OUT	OUT	42 (not RB)	70 (RB)	NOT

from Jenner et al 2011

RB: Readily Biodegradable according to the OECD 301 criteria.

*****This analysis also illustrates the fact that the model, even if the structures are outside both structural and/or metabolic applicability domains, the predictions of non RB seem to be supported by the measured results. But clearly there will be cases (eg β-Caryophyllene) whereby experimental results would refute the predictions.

For BIOWIN models the structural domain can be ascertained manually by checking whether the chemical contains substructures unknown to the model. BIOWIN 2 only contains one fragment used in the prediction of the studied sesquiterpenes, which means that for some sesquiterpenes only molecular weight was used in the prediction and that for others a maximum of three of the 15 sesquiterpene carbon atoms included in the biodegradability estimations. In summary, the domain of BIOWIN 2 is inadequate for sesquiterpenes.

α-Bisabolol, α-cedrene, and cedrol belong to all constituents of the CATALOGIC 301F model applicability domain and for these three substances the predicted percentage biodegradation is in close agreement to the observed values (note: the OECD301 ready test validity criteria for the difference between replicates is <20%). As expected, larger differences are seen for the sesquiterpenes that were out of the model domain. This clearly illustrates that the chemical in question must be within the applicability domain of a QSAR model in order for the outcome of the prediction to be reliable.

II.4.2.2. Read-across

REACH Annex XI offers the possibility of evaluating chemicals by grouping them in categories, instead of evaluating each chemical on a one-by-one basis. The techniques used to group chemicals are referred to as category approach or analogue approach, while read-across is the technique of filling data gaps under REACH. An analogue approach is used for a limited number of chemicals where trends in properties are not apparent while the category approach refers as to when read-across is employed between several substances that have structural similarities. The similarities may be based on the following : common functional groupd (e.g. aldehyde, epoxide, ester, specific metal ion); common constituents or chemical

classes, similar carbon range numbers (which is frequently the case with UVCBs; an incremental and constant change across the category (e.g. a chain-length category), often observed in physico-chemical properties, e.g. boiling point range; the likelihood of common precursors and/or breakdown products, via physical or biological processes, which result in structurally similar chemicals (e.g. the metabolic pathway approach of examining related chemicals such as acid/ester/salt).

Structural similarity between the source and target substances is a pre-requisite but is not sufficient. A read-across hypothesis needs to be provided, as well as a comprehensive scientific justification and thorough documentation for the use of the read-across approach. The information should be provided in a structured way, recognizing the strengths of the read-across as well as identifies possible shortcomings. For that matter, since read-across and grouping are very common alternative approaches to data gap filling under REACH, ECHA developed an internal tool for the systematic examination of predictions based on read across, the Read Across Assessment Framework (RAAF) which has been published in 2015. The RAAF is a framework for a consistent and structured assessment of grouping and read across approaches under REACH for mono-constituent substances, while specifications for multi-constituent substances and UVCBs are still under development. Chapter R.6 ECHA (2008) provides guidance on the grouping of chemicals, whereas in 2012, ECHA publishes a Practical Guide 6 on how to report read-across and categories (ECHA, 2012).

Read-across entails the use of relevant information from analogous substances (the "source" information) to predict properties for the "target" substance(s) under consideration.

Since NCS are a mixture of constituents, read-across for NCS can be potentially applied at the level of constituents within a NCS or at the NCS itself. This will be achievable for the well-defined substances (mono- or multi- constituent's ones). For UVCB-type substances, however since the target and source substances need to be identified without ambiguity, the applicability of read across for NCS is less straightforward.

II.4.2.2.1. Constituents approach

The RAAF for mono-constituents (2015) describes the principles upon which the read-across is based, therefore it is expected that following these indications should increase its acceptability. The assessment follows the following steps:

(i) Preparatory assessment: the pre-conditions to fulfil relate to (a) the substance identity of the registered substance (or constituent) which should be unambiguous for both the target and source substance and (b) the documentation of the read-across, which should contain comprehensive justification, sufficient to allow a scientific assessment.

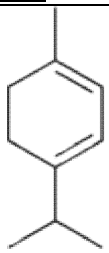
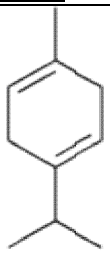
(ii) Description and selection of scenarios, whereby the analogue or category approach should be distinguished. Thereafter, the basis of the read-across hypothesis is identified to determine the correct scenario. The hypothesis may rely on the premise that the source and target substances undergo (bio)-transformation to common compounds or that (b) different compounds have the same type of effects

(iii) During the scientific assessment, the adequacy and scientific robustness of the information provided in the dossier are evaluated. Several assessment options

are possible: acceptability of the approach according to high/medium/just sufficient confidence; not acceptable in current format, or not acceptable).

Example : Read across for aquatic toxicity, whereby terpinene alpha is the target substance and terpinene gamma the source substance

(i) Pre-Assessment : Substance identity and documentation

SUBSTANCE IDENTITY	**TARGET SUBSTANCE**	**SOURCE SUBSTANCE.**
	terpinene alpha	**terpinene gamma**
	1-Isopropyl-4-methyl-1,3-cyclohexadiene	4-Methyl-1-(1-methylethyl)-1,4-cyclohexadiene
	CAS 99-86-5	CAS 99-86-4
		
MW,	136.24 C10H16	136.24 C10H16
SMILES	CC1=CC=C(C(C)C)CC1	CC1=CCC(C(C)C)=CC1
	Similar predictions	
Vp (mm Hg,25 deg C)	1.66	
Log P	4.75 KOWIN v1.67 4.25 (Exper. database match Ref: Griffin,S et al. (1999)	
Water solubility (mg/L)	5.915 WSKOW v1.41	
Measured Aquatic toxicity	**no effect at solubility limit**	no effects at solubility limit in acute *Daphnia* test (RIFM Database, Symrise 2000)

(ii) Description of the Scenario

The read across of the short term toxicity on aquatic invertebrates is supported by the analogue approach, whereby one single source **terpinene gamma** substance is used to provide information on a single target substance **terpinene alpha.**

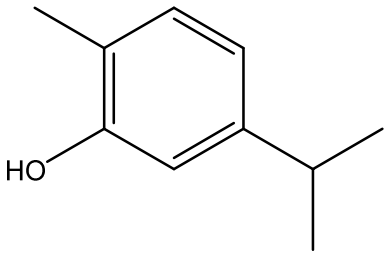
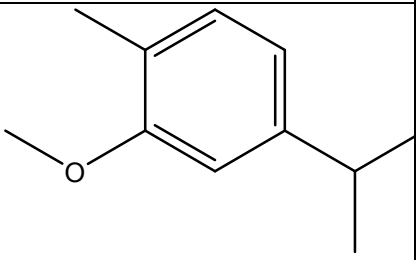
The read-across between terpinene alpha and gamma is based on the hypothesis that the target chemical (terpinene alpha) will have a similar ecotoxicological profile as terpinene gamma (the target chemical). This is because of their similar structure, same hydrocarbon backbone, same groups in same position, same C6 cycle, with same number of double bond. The only difference is that one double bond in a different position. This is not believed to impact the aquatic toxicity.

Available measured ecotoxicity data for the source substance (terpinene gamma) reveal that there is no effect at the level of solubility. Since the physico-chemical properties of both source and target substances are predicted to be similar, it is very likely that the lack of effects at solubility level of terpinene alpha is a reasonable assumption.

The data gap on short term toxicity on aquatic invertebrates for terpinene alpha can be filled as no effect at solubility limit.

Example : Read across for biodegradability, whereby carvacrol is the target substance and carvacrol methyl ether the source substance

(i) Pre-Assessment : Substance identity and documentation

	SOURCE SUBSTANCE	**TARGET SUBSTANCE**
SUBSTANCE IDENTITY	**carvacrol** **5-Isopropyl-2-methylphenol**	**carvacrol methyl ether** **5-Isopropyl-2-methylphenol - methoxymethane (1:1)**
	CAS #499-75-2	CAS #6379-73-3
	 carvacrol	 carvacrol methyl ether
SMILES	Cc1ccc(cc1O)C(C)C	CC(C1=CC=C(C)C(OC)=C1)C
MW	150.218	164.244
Vp	0.0232 MPBPWIN v1.42	0.00476 MPBPWIN v1.42
WS (mg/L)	301.1 WSKOW v1.41 920.37 from fragments 1250 (Exper. database match Ref: YALKOWSKY and DANNENFELSER (1992)	84.26 WSKOW v1.41 301.17 from fragments
logP	3.52 KOWIN v1.67 3.49 (Exper. database match Ref: Griffin,S et al. (1999)	4.06 KOWIN v1.67
Biodegradability OASIS CAtalogic v5.11.17 Kinetic 301F v.13.v.16	82% predicted (84% of the Atom Centered fragments are correctly predicted)	84% predicted (86% of the Atom Centered fragments are correctly predicted)
BIOWIN v4.10	BIOWIN 1 : 0.9012 BIOWIN 2 : 0.9497	BIOWIN 1 : 0.9492 BIOWIN 2 : 0.9650
Measured biodegradability	87% after 28d (301F, 10-day window fulfilled) (Givaudan 2010)	Measured biodegradabilty data on amalogue support the predictions that **carvacrol methyl ether is readily biodegradable**

(ii) Description of the Scenario

The read across on the biodegradation endpoint is supported by the analogue approach, whereby one single source **carvacrol** substance is used to provide information on a single target substance **carvacrol methyl ether.**

The target chemical (carvacrol methyl ether) and the target chemical (carvacrol) share common structural similarities. They both will have a similar hydrocarbon backbone, same C6 cycle, same (methyl and isopropyl) groups in same position, with same number and position of double bonds. The only difference is one of the function (at the same position) is

an alcohol in the source substance and a methoxy group in the target chemical (carvacrol methyl ether).

The physico-chemical properties for the relevant parameters differ slightly and are due to the presence of additional methyl, which increases the log P by a 0.5 log unit and decrease the water solubility. This decrease is solubility is such that it will not affect the bioavailability to bacteria. The read-across between carvacrol and carvacrol methyl ether is based on the hypothesis that the target chemical (carvacrol methyl ether) will have a similar biodegradation profile as carvacrol (the target chemical). Appendix 6 describes the biodegradation pathways as predicted via CATALOGIC model 301F.

The results from a ready biodegradability test (OECD 301 F) on the source substance (carvacrol), whereby 87% biodegradation was observed after 28d and whereby the 10-day window criteria was fulfilled. It can therefore be concluded that the target substance (carvacrol methyl ether) is also readily biodegradable. This conclusion is further supported by the outcome from two different biodegradation models (BIOWIN and CATALOGIC), which converge predicting that both substances are degrading at > 60% after 28 days (See Table 7 above).

II.4.2.2.2. Whole substance approach

The use of read-across for the NCS itself, even more for UVCB type 3 is very relevant since botanical source, manufacture process, etc are factors that influence their chemical composition and it is clearly impractical to test any single batch produced. Read-across should be applicable for NCS of similar compositions. However the basis of applying read-across (identification of the target and source substances without ambiguity) is in contradiction with the nature of NCS themselves with variable composition.

As pointed out, the EFEO/IFRA Guidelines on substance identification and sameness of natural complex substances under REACH and CLP (2015) acknowledge that there is no definition for similar composition and propose to use the definition of "similar mixture" of the US ATSDR : "mixtures having the same chemicals but in slightly different proportions or having most but not all chemicals in common and in highly similar proportions". In those cases, read across of test results and classification may be warranted.

The hypothetical example below shows that in principle it should be possible to read-across the generic outcome on a NCS provided the main constituents are the same and their presence in the NCS is within an acceptable range (eg, 20-30%). It is clear that acceptance of variability within a range is not straightforward and that a simple rule cannot be applied, but rather the overall composition and the nature of the main constituents should be evaluated on a case by case basis.

Table 8 : Read-across example

		SOURCE NCS	TARGET NCS
		Essential oil of thyme (thymol) spanish type *Thymus spp*	Other type of thyme oil, *Thymus*
Constituents	**CAS#**	% min - % max	acceptable ranges
thymol	89-83-8	37-55	20-70
Cymene para	99-87-6	14-28	10-30
terpinene gamma	99-85-4	4-11	Traces-20
linalool	78-70-6	3-6.5	None
carvacrol	499-75-2	0.50-5.5	Traces-10
myrcene	123-35-3	1-2.8	Traces-5
terpinene alpha	99-86-5	0.9-2.6	Traces-5
pinene alpha	80-56-8	0.5-2.5	Traces-5
Terpinene-1- ol-4	562-74-3	0.1-2.5	Traces-5
caryophyllène beta	87-44-5	0.50-2.00	Traces-5
carvacrol methyl ether	6379-73-3	0.10-1.50	Traces-5
α-Thujene	2867-05-2	0.2-1.50	Traces-5
trans-sabinene hydrate	15537-55-0	Traces-0.5	Traces-5

Examples of tools for read-across purposes :

- The RIFM database – available at http://www.rifm.org/rifm-science-database.php
- OECD Toolbox - available at http://www.oecd.org/chemicalsafety/risk-assessment/guidancedocumentsandreportsrelatedtoqsars.htm
- LRI AMBIT Chemoinformatic system – available at http://ambit.sourceforge.net/

Further useful reading on how to apply read-across can also be found in the ECETOC technical report "Category approaches, read-across, (Q)SAR". Technical Report No. 116 (2012).

Concluding remarks

Read across is a powerful technique to data gap filling of NCS, which is especially applicable at the level of single constituents. It is less straightforward when reading across between various NCS where, per definition, the composition and the ranges of each constituent are variable. Therefore caution is required and scientific argumentation and sufficient documentation need to be provided to increase its acceptability.

II.5. Persistence, bioaccumulation and toxicity (PBT) assessment

II.5.1. General requirements

As mentioned in Section I.3.2 of above, there are three lines of evidence that are used to identify PBTs namely, persistence (P), bioaccumulation (B) and toxicity (T). The screening and assessment criteria for PBT properties provided for in Annex XIII of REACH are reproduced in Appendix 3 of these Guidelines and summarised again in the below Table 9. The revision of Annex XIII in Regulation 253/2011 allows the use of additional information provided its suitability and reliability can be reasonably demonstrated. This includes the use of valid (Q)SARs, in-vitro data, information from mammalian studies etc.

Table 9: Criteria for the categorization of compounds as P,B,T under REACH

<table>
<tr><th></th><th colspan="5">Persistence (P)</th><th>Bioaccumulation (B)</th><th>Toxicity (T)</th></tr>
<tr><td rowspan="3">Screening Criteria</td><td>BIOWIN Model</td><td>2</td><td>3</td><td>6</td><td rowspan="3">Result: BIOWIN 2 and 3 OR BIOWIN 2 and 6</td><td rowspan="3">log Kow >4.5</td><td rowspan="3">Acute LC50, EC50, or ErC50 ≤ 0.1 mg/l.</td></tr>
<tr><td>Potential</td><td><0.5</td><td><2.2</td><td><0.5</td></tr>
<tr><td>Borderline</td><td><0.5</td><td><2.7</td><td><0.5</td></tr>
<tr><td>Definitive Criteria

(Persistence half-life in days)</td><td colspan="5">Fresh water t½ >40 (vP >60)

Marine water: t½ >60

Marine sediment: t½ >180

Freshwater sediment: t½ >120 (vP >180)

Soil: t½ >120 (vP >180)</td><td>BCF >2,000 in aquatic species

(vB >5,000)</td><td>Environmental: Chronic NOEC <0.01 mg/L

Human Health: Carcinogen Cat.1A/B, Mutagenic Cat. 1A/B, Toxic for Reproduction Cat. 1A, 1B or 2, or other evidence of toxicity.</td></tr>
</table>

II.5.2. First stage: Screening

Screening information involves the use of readily available data, typically information from Annexes VII and VIII endpoints, and may be used to indicate whether the substance may have PBT or vPvB properties and whether further information is needed to conclude with certainty whether the PBT/vPvB criteria are fulfilled or not.

For NCS and their constituents, only screening data is likely to be available. This is typically information on ready biodegradability (P), log Kow (B) and acute aquatic toxicity (T). It should be noted that generating data that numerically match with the Annex XIII criteria, i.e. degradation half-life values or BCF values, is often not possible for an NCS itself because its complex nature does not permit the respective tests to be conducted.

In addition, in this screening tier, generating new information through non-testing methods (valid (Q)SAR predictions, read-across for the relevant constituent(s) or representative structures for those blocks of concern is an option (see Section II.4.2 for more detail).

II.5.2.1. Persistence

Although the PBT assessment of mixtures should consider the information for individual constituents or blocks of constituents, it may be appropriate to use test data for an NCS itself. The first step in proposed strategies for the P assessment of UVCB substances (R.11.4.2.2, ECHA 2014b and JRC 2014) is that if the UVCB consist of homologous structures and it 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. Enhanced ready biodegradation tests (e.g. prolonged up to 60 days) and specified tests on inherent biodegradability may also be used to conclude that a substance is not persistent (R.7.9.4.1 ECHA 2014a; Table R.11-4 ECHA 2014b). Thus if an NCS passes a ready or enhanced ready biodegradation test and consists of constituents that are expected to have similar biodegradation potential, it may be concluded that all of the underlying constituents (and hence the NCS itself) are not P or vP. In turn this means that the substance does not meet the PBT or vPvB criteria.

For non-homologous structures, judgment should be on a case-by-case basis depending on the relative composition and expected degradability of individual constituents.

Section II.4.2. provides examples how the combination of non-testing methods and data may be used to assess the persistence of NCS.

II.5.2.2. Bioaccumulation

The first tier assessment for bioaccumulation may include a multi-constituent log Kow measuring technique, such as HPLC (OECD 117). If all the peaks in the HPLC chromatogram have a log Kow <4.5, it can be concluded that the underlying constituents comprising the NCS are not B or vB and that these constituents (and hence the NCS itself) do not meet the PBT or vPvB criteria. Alternatively the log Kow for individual known constituents may be used (measured or estimated using QSARs). In addition, in this tier generating new information through valid (Q)SAR predictions) for the relevant constituent(s) or representative structures for those blocks of concern is an option. For bioaccumulation assessment specific models available include BCFBAF within the EPISuite set of models and the BCF Baseline models in CATALOGIC.

II.5.2.3. Toxicity

Short-term aquatic toxicity data exist for a significant number of constituents found in NCS, since many are used in their own right as fragrance substances. There are no examples where the E(L)C50 is < 0.01mg/L (the REACH screening T criterion for a substance to be considered definitively T) and very few with a value of <0.1mg/L (screening criterion for potentially T). Therefore, constituents within NCS are generally not expected to fulfil the T criteria for aquatic toxicity. Nevertheless, for constituents or blocks of constituents which have been screened as potentially P and B, further information on toxicity may be required to complete the PBT assessment

In the absence of data on constituents, aquatic toxicity data generated using the WAF procedure (see Section II.4.1.2.1.) might also be suitable for addressing T within a PBT assessment.

Section II.4.2. provides examples how the combination of non-testing methods and data may be used to assess the toxicity of NCS.

II.5.3. Second tier methodology

When a substance is considered to be potential PBT/vPvB based on screening criteria, it follows that a second tier assessment is required. The second tier assessment proceeds with generating new information for the relevant constituent(s), representative structures for those blocks of concern, or for the whole substance.

II.5.3.1. Persistence

Relevant to NCS, is a recent study on the persistency assessment of cyclic sesquiterpenes (Jenner *et al,* 2011). Most sesquiterpenes have a log Kow > 4.5 and are identified as potentially bioaccumulating according to REACH screening criteria. Thus information on their biodegradability is important for the PBT/vPvB assessment of essential oils. Eleven cyclic sesquiterpenes commonly found in essential oils were selected from 10 different families characterised by their carbon skeleton. Existing (Q)SAR models were found to be of limited use because most sesquiterpenes were outside the structural domain of the models. In addition, sesquiterpene carbon skeletons contain structural fragments, such as quaternary carbon atoms and fused or bridged ring systems, which are usually associated with poor biodegradability. However, the experimental results reported in this article showed that a range of structurally diverse sesquiterpenes are biodegraded suggesting that a large terpene-degrading microbial community exists in nature.

II.5.3.2. Bioaccumulation

The revision to Annex XIII provides for a Weight of Evidence approach to bioaccumulation assessment that can include non-animal testing. In addition to screening based on log Kow and the availability of read-across for individual constituents or blocks, these can include the use of (Q)SAR models, *in vitro* data on aquatic bioaccumulation *in vitro* methods such as fish liver S9 and primary hepatocyte assays and biomimetic extraction procedures (e.g., SPME, SPMD).

Ultimately, if a definitive conclusion is required for specific constituents, an OECD 305 test may need to be considered.

II.5.3.3. Toxicity

Less chronic data is available but again, to the best of our knowledge, no known fragrance substance has met the definitive aquatic toxicity criterion of a long-term NOEC or EC10 < 0.01mg/L. Thus the vast majority of NCS constituents are not expected to meet the aquatic toxicity criterion within a PBT assessment. Other evidence of chronic toxicity (i.e. STOT RE1 or STOT RE2 according to the CLP Regulation) and evidence of CMR toxicity must also be reviewed (see further details on the PBT/vPvB assessment process under Appendix 3).

Again, when the conclusions on the basis of results from the whole substance approach are "not PBT", the assessment should include a robust justification explaining why all constituents are sufficiently similar to support such a conclusion.

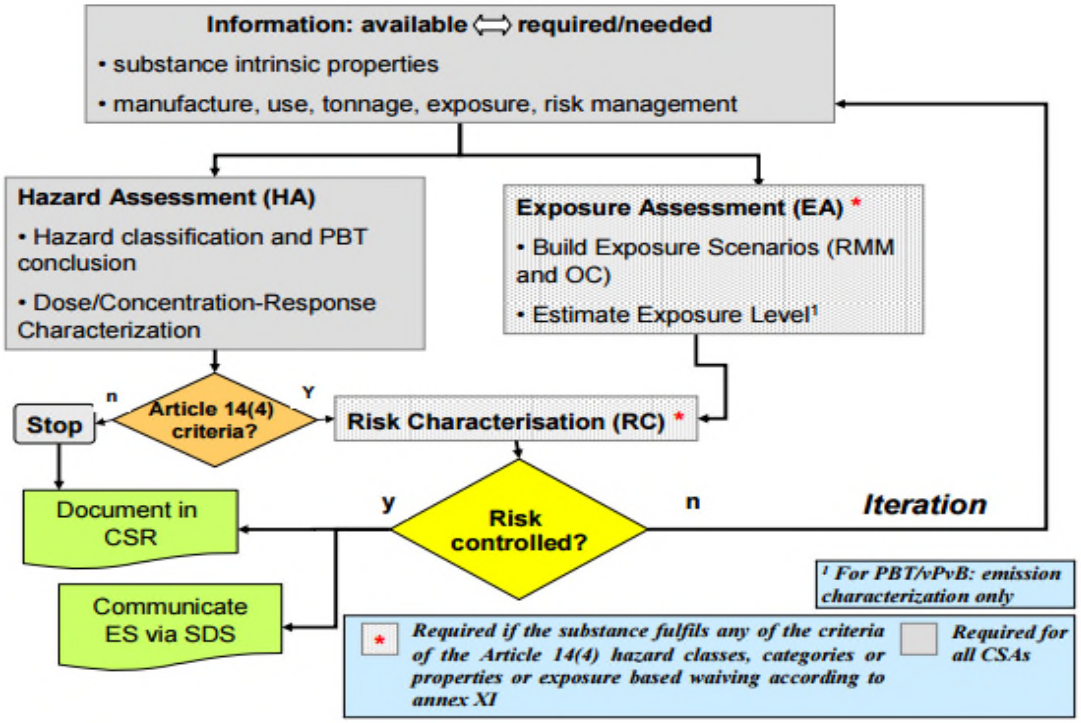
II.5.4. On-going developments

Finally, it is worth noting that the ECHA PBT Discussion Paper which was developed in the frame of the activities of the ECHA PBT expert group will likely lead to the revision of the ECHA Guidance documents in the near future.

II.6. Risk Assessment

An environmental risk assessment, as part of the CSA, is required under REACH for substances ≥ 10tpa when the substance is classified according to any of the hazard classes or categories of Article 14(4) or that the substance is assessed to be a PBT or vPvB. The process of environmental risk assessment includes three steps: hazard assessment, exposure assessment and risk characterization. If the risk characterisation shows that risk is not controlled, an iteration of the CSA is needed. This can be done by generating more refined exposure and/or hazard information or by introducing new RMMs.

Figure 3. Overview of the CSA process (taken from ECHA Guidance Part A)



ECHA provides guidance on how to carry out environmental exposure in the context of REACH (Chapter R.16, 2016) and how to derive PNECs (Predicted No-Effect Concentrations) for the different environmental compartments (Chapter R.10, 2008). The Guidance applies to single substances and does not cover options for approaches that could be applied to a complex mixture, such as a NCS, where the environmental distribution and fate may be different for different constituents / groups of constituents in the NCS.

The information developed as part of this NCS Guidance document for registrants has identified several key points regarding substance composition that are important in considering approaches to both exposure and effects characterization.

II.6.1. Risk assessment approaches for NCS

For well characterized NCS, with data available for constituents (or analogues of constituents) and/or blocks, a constituent by constituent approach or block assessment approach may be appropriate (or some combination).

For less well characterized NCS, a hybrid approach (by constituent, block, or whole substance) may best characterize a substance's environmental risk. Certainly, each material may present unique circumstances, resulting in a "case by case" approach.

Each NCS will need to be evaluated using available information and tools that are appropriate to the registrants understanding of material composition and availability of relevant data.

It is worth noting that the use of the additive approach in the context of the ecotoxicity assessment will not require consideration of synergistic or antagonistic effects since it can be considered in most cases as a worst case approach. As noted elsewhere, and with rare exception, NCS are composed largely of terpene and sesquiterpene hydrocarbons, alcohols, and esters exhibiting narcosis modes of action.

II.6.1.1. Constituents approach

For example, in a "constituent approach" each constituent would be assessed individually by assessing the hazard of constituent x, the exposure of constituent x and the risk of constituent x. The risk for the substance is then assessed by either assuming additivity of risk or based on the worst-case.

Approaches have been proposed to identify substances in a mixture that contribute most to the potential hazard and risk of the mixture . These have been developed to communicate information on safe use of mixtures to downstream users. The mixtures in this context are "formulations/preparations" as defined under REACH. The approaches developed to identify risk-driven substances might be appropriate for identifying constituents within an NCS for which an appropriate environmental risk assessment might be based.

The DPD+ methodology, based on the Dangerous Preparations Directive (DPD), developed by CEFIC whereby the lead substance was based on risk phrases (CEFIC 2009 and 2010). This has been updated by the Lead Component Identification (LCID) methodology (CEFIC, 2016) which is used to derive the applicable OCs and RMMs to determine safe use information for the mixture and can be used for the ERA of NCS. The Lead Component methodology accounts only for the substances present in mixtures classified as hazardous in concentrations above the concentration limits set in Art. 14.2.

ECHA is developing the Critical Component Approach (CCA) for assessing critical constituents based on PNECs. The CCA method will determine the Risk Determining Substance(s) (RDS(s)) for a mixture, processing all hazardous substances with a PNEC value and present in the mixture in a concentration above the most stringent cut-off limit based on the CLP label of the substance.

II.6.1.2. The block approach

The block assessment approach would be used for groups of constituents with similar environmental fate and toxicity properties. For example, an essential oil composed of sesquiterpene alcohols and sesquiterpene hydrocarbons may be regarded as two blocks of constituents based on their water solubility and adsorption properties (see as well figure 2, Section II.4.1.3.3). A constituent (or related structure) can be chosen to represent each block and data required to complete the risk assessment collected (e.g. PNECs, biodegradability, adsorption properties such as logKow, LogKoc). The volumes assessed in the risk assessment may be based on the typical % w/w of each constituent block present in the NCS.

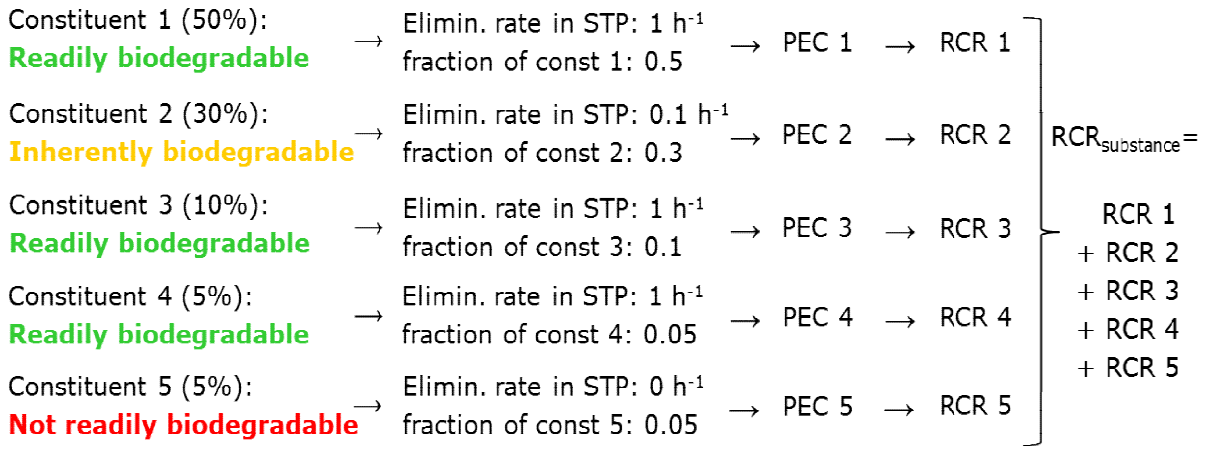
II.6.1.3. The whole substance approach

For an NCS composed of constituents expected to have similar environmental, fate and ecotoxicity properties, the whole substance approach may be appropriate.

In addition, for complex NCS which are either not well characterized or whose constituents cover a wide range of physico chemical properties, the whole substance approach may be a suitable option or it may provide complementary information to the other approaches.

II.6.2. Exposure Assessment (PEC determination)

Different constituents have different properties, therefore different behaviours in the environment. It is thus preferable to assess the exposure of each constituent (or of block of homogeneous constituents) individually. For example, the elimination rate in the sewage treatment plant (STP) is derived for each constituent/block of homogeneous constituents based on the biodegradation information for each constituent/block of constituents. The elimination rate in the STP is needed to calculate the predicted environmental concentration for each constituent/block of constituents. The fraction of the constituent/block of constituents in the substance should be taken into account when calculating individual PEC: e.g. if 10 tons of a substance containing 50% of a constituent is released, only 5 tons of that constituent is released. Other parameters specific for each constituent/block of constituents are also necessary for calculating the distribution of each constituent/block of constituents in the environment: e.g. water solubility, log Kow, vapour pressure. Each PEC is associated with a PNEC, and risk characterization ratios (RCR) can be calculated for each constituent/block of constituents. The RCR for the substance can be calculated as the sum of the RCR for each constituent/block of constituents.



II.6.3. Hazard assessment (PNEC Determination)

II.6.3.1. Block Approach

For blocks of constituents of substances of similar structure and physical chemical properties, QSARs can be applied using a worst-case scenario (i.e., highest log Kow), or if data are available on members of the block, the lowest value for an aquatic toxicity endpoint (NOEC, EC50, LC50) can be utilized and appropriate assessment factors are applied to determine the PNEC. These are then compared against their respective PECs as noted above to determine the overall RCR.

II.6.3.2. Constituent approach

PNECs are determined for individual constituent in the same manner as for discrete compounds. Use of QSAR or measured data for the constituents are used and appropriate assessment factors are applied to determine the PNEC. These are then compared against their respective PECs as noted above to determine the overall RCR.

II.6.3.3. Whole-substance Approach

Due to the complexity of assessing NCS, and the 'case by case' nature of this assessment, a whole substance approach to PNEC assessment may be appropriate as well. This could provide confirmatory or complimentary data to either the constituent or block approach (or both). Furthermore a PNEC derived from a WAF could present a more environmentally realistic PNEC; however, this may depend on the complexity of the NCS and how well characterized the substance is.

II.6.4. Concluding remarks

No definitive methodology for an NCS risk assessment has been presented here. It has been noted by both the regulatory community and industry that due to the complexity and characterization of these substances, that risk assessment can be a 'case by case' effort. The three different approaches presented here (and various combinations of these approaches) can result in several differing PECs and PNECs. The registrant needs to provide justification as to the appropriateness of their decisions for deriving the reported PEC and PNEC, and why they are sufficiently conservative.

II.7. Economic considerations

In addition to the registration fees, the preparation of the dossier including the data required by REACH has a financial impact which, in some cases, in particular for SMEs and VSEs, could be impossible to afford.

In order to help all potential registrants complying with REACH requirements, several initiatives have been taken at European level and additional ones can be considered at National or Regional levels.

II.7.1. European initiatives

Published in the Official Journal on 6th January 2016, the European Commission has adopted a new Implementing Regulation under REACH to clarify the provisions on joint submission of data and data-sharing, in the run-up to the 2018 registration deadline.

The new rules, applicable as of 26 January 2016, specify what is understood by "fair, transparent and non-discriminatory" with regard to ***data cost-sharing*** in the REACH Regulation. The Regulation sets rules to ensure that potential registrants joining a Substance Information Exchange Forum (SIEF) are given the right to request a breakdown of the study and administrative costs that make up the price for the joint registration. Registrants are only required to share the costs for the data they need to submit to the Agency. They don't need to pay for data that goes beyond the requirements of their tonnage band. Practical advice is available on ECHA website.

The requirement of joint submission of data by all registrants of the same substance (***one substance, one registration***) has also been reinforced in order to avoid duplication of tests and to ensure that registration costs are shared appropriately. ECHA will have a stronger role in getting companies to submit a joint registration when there are multiple registrants for the same substance. Since 26 January, REACH-IT does not allow anymore registrations outside the joint submissions. Related Guidance documents and other support material will be updated to reflect the changes.

II.7.2. National and Regional initiatives

In accordance with REACH, Member States have established ***National Helpdesks*** offering services in local languages and having a good understanding of national conditions.

It is also possible to get support through competent authorities. It is actually the case in France where an inter-ministerial committee has been put in place. This committee is designed to facilitate the interpretation and application of the European Regulation for the natural complex substances (in this case - essential oils).

Provided they are complying with European Competition rules, certain forms of financial support can be considered at national or regional levels. National or regional authorities have the possibility to financially support a company up to 200.000 € within a period of three years, if the conditions of Commission Regulation on *de minimis aid*[22] are respected.

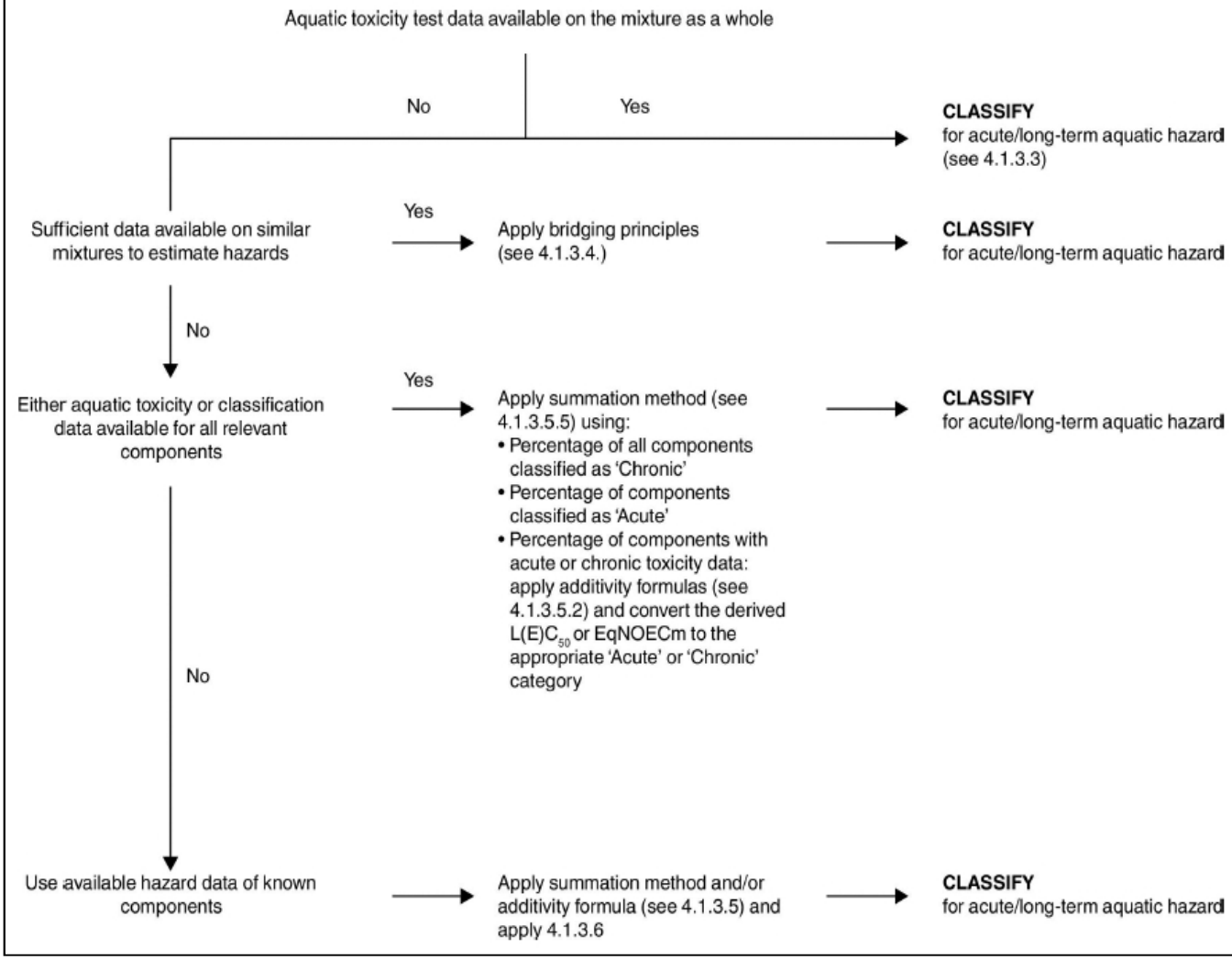
[22] Commission Regulation (EU) No 1407/2013 of 18 December 2013 on the application of Articles 107 and 108 of the Treaty on the Functioning of the European Union to de minimis aid. OJ L 352 p. 1, 24.12.2013.

Appendices

Appendix 1 – Tiered approach for the classification of substances and mixtures

Figure 4.1.2

Tiered approach to classification of mixtures for acute and long-term aquatic environmental hazards



Appendix 2 - Criteria to identify PBT and vPvB Subtances

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

Appendix 3 - Overview of the PBT/vPvB assessment process

Screening and Assessment information for the assessment of PBT/vPvB properties

Annex XIII.Section 3.1- Screening Information

The following information shall be considered for screening for P, vP, B, vB and T properties in the cases referred to in the second paragraph of Section 2.1 and may be considered for screening for P, vP, B, vB and T properties in the context of Section 2.2.

3.1.1. Indication of P and vP properties

(a) Results from tests on ready biodegradation in accordance with Section 9.2.1.1 of Annex VII;

(b) Results from other screening tests (e.g. enhanced ready test, tests on inherent biodegradability);

(c) Results obtained from biodegradation (Q)SAR models in accordance with Section 1.3 of Annex XI;

(d) Other information provided that its suitability and reliability can be reasonably demonstrated.

3.1.2. Indication of B and vB properties

(a) Octanol-water partitioning coefficient experimentally determined in accordance with Section 7.8 of Annex VII or estimated by (Q)SAR models in accordance with Section 1.3 of Annex XI;

(b) Other information provided that its suitability and reliability can be reasonably demonstrated.

3.1.3. Indication of T properties

(a) Short-term aquatic toxicity in accordance with Section 9.1 of Annex VII and Section 9.1.3 of Annex VIII;

(b) Other information provided that its suitability and reliability can be reasonably demonstrated..

Annex I.3.2. Assessment Information

The following information shall be considered for the assessment of P, vP, B, vB and T properties, using a weight-of-evidence approach.

3.2.1. Assessment of P or vP properties

(a) Results from simulation testing on degradation in surface water;

(b) Results from simulation testing on degradation in soil;

(c) Results from simulation testing on degradation in sediment;

(d) Other information, such as information from field studies or monitoring studies, provided that its suitability and reliability can be reasonably demonstrated.

3.2.2. Assessment of B or vB properties

(a) Results from a bioconcentration or bioaccumulation study in aquatic species;

(b) Other information on the bioaccumulation potential provided that its suitability and reliability can be

reasonably demonstrated, such as:

— Results from a bioaccumulation study in terrestrial species;

— Data from scientific analysis of human body fluids or tissues, such as blood, milk, or fat;

— Detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment;

— Results from a chronic toxicity study on animals;

— Assessment of the toxicokinetic behaviour of the substance;

(c) Information on the ability of the substance to biomagnify in the food chain, where possible expressed by biomagnification factors or trophic magnification factors.

3.2.3. Assessment of T properties

(a) Results from long-term toxicity testing on invertebrates as set out in Section 9.1.5 of Annex IX;

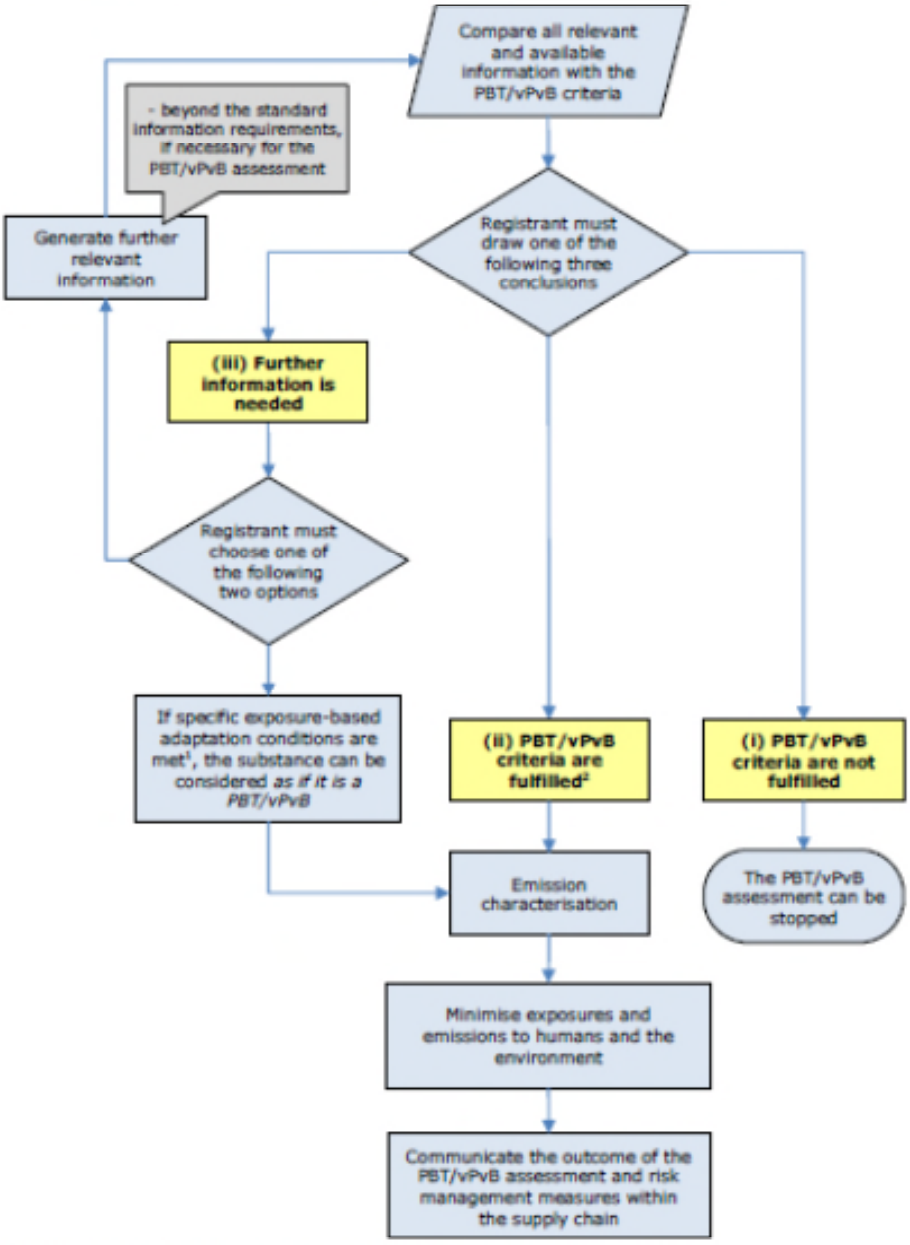
(b) Results from long-term toxicity testing on fish as set out in Section 9.1.6 of Annex IX;

(c) Results from growth inhibition study on aquatic plants as set out in in Section 9.1.2 of Annex VII;

(d) The substance meeting the criteria for classification as carcinogenic in Category 1A or 1B (assigned hazard phrases: H350 or H350i), germ cell mutagenic in Category 1A or 1B (assigned hazard phrase: H340), toxic for reproduction in Category 1A, 1B and/or 2 (assigned hazard phrases: H360, H360F, H360D, H360FD, H360Fd, H360fD, H361, H361f, H361d or H361fd), specific target organ toxic after repeated dose in Category 1 or 2 (assigned hazard phrase: H372 or H373), according to Regulation EC No 1272/2008;

(e) Results from long-term or reproductive toxicity testing with birds as set out in Section 9.6.1 of Annex X;

(f) Other information provided that its suitability and reliability can be reasonably demonstrated.



[1] Please refer to the conditions as specified in Section 3.2(b) or (c) of Annex XI to REACH.
[2] Normally not applicable if only screening information is available.

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

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

Appendix 4 - illustrative list of constituents present in fragrance NCS

Adapted from Appendix 2 of the NCS Protocol, 2009. Constituents listed are present at or > 1 % in NCS that were identified by EFEO/IFRA in 2008 as requiring REACH registration

Constituents in bold are also available as individual fragrance ingredients and will be registered under REACH

CAS	*EC No*	*Name*
98-86-2	202-708-7	**Acetophenone**
		Aciphyllene
1195-32-0		Alpha-paraDimethylstyrene
4180-23-8	224-052-0	**Anethole – trans**
		Aromadandrene
65-85-0	200-618-2	**Benzoic acid**
140-11-4	205-399-7	**Benzyl acetate**
120-51-4	204-402-9	**Benzyl benzoate**
118-58-1	204-262-9	**Benzyl salicylate**
17699-05-7	241-702-9	Bergamotene alpha
495-61-4		Bisabolene beta
507-70-0	208-080-0	**Borneol laevo**
5655-61-8	227-101-4	**Bornyl acetate laevo**
		Bourbonene beta
		Bulnesene
22451-73-6		Bulnesol
483-76-1		Cadinene delta
		Calacorene alpha
79-92-5	201-234-8	**Camphene**
76-22-2	200-945-0	**Camphor**
13466-78-9	236-719-3	**Carene delta-3**
6485-40-1	229-352-5	**Carvone l**
87-44-5	201-746-1	**Caryophyllene beta**
1139-30-6	214-519-7	Caryophyllene epoxide
469-61-4	207-418-4	**Cedrene alpha**
546-28-1	208-898-8	Cedrene beta
77-53-2	201-035-6	**Cedrol**
470-82-6	207-431-5	**Cineole 1,8**
5392-40-5	226-394-6	**Citral (Neral +geranial)**
103-54-8	203-121-9	**Cinnamyl acetate**
106-23-0	203-376-6	**Citronellal**

CAS	*EC No*	*Name*
7540-51-4	231-415-7	**Citronellol- l**
105-85-1	203-338-9	**Citronellyl formate**
		Coniferiyl ethyl ether-trans
		Copahene alpha
122-03-2	204-516-9	**Cuminic aldehyde**
16982-00-6	241-061-5	Cuparene
5989-27-5	227-813-5	**d-Limonene**
5524-05-0	226-872-4	Dihydrocarvone
33880-83-0	251-713-0	Elemene beta
		Elemicine
639-99-6	211-360-5	Elemol
1209-71-8		Epi-gamma Eudesmol
140-67-0	205-427-8	**Estragole (Me.Chavicol)**
97-53-0	202-589-1	**Eugenol**
93-28-7	202-235-6	**Eugenyl acetate**
502-61-4	207-948-6	Farnesene alpha all trans
4602-84-0	225-004-1	**Farnesol (E)-(E)**
29548-30-9	249-689-1	**Farnesyl acetate (E)-(E)**
		Foeniculine- trans
106-24-1	203-377-1	**Geraniol**
105-87-3	203-341-5	**Geranyl acetate**
106-29-6	203-381-3	**Geranyl butyrate**
105-86-2	203-339-4	**Geranyl formate**
7785-33-3	232-078-9	**Geranyl tiglate**
		Germacrene D
		Guaiadiene-6-9
		Guaiene alpha
6753-98-6	229-816-7	Humulene alpha
489-86-1	207-702-8	Guaiol
491-07-6	207-727-4	**Isomenthone**
89-79-2	201-940-6	**Isopulegol**
		Isovalencenol
		Khusimol
58461-27-1	261-264-2	Lavandulol
25905-14-0	247-327-7	Lavandulyl acetate
21747-46-6	244-565-3	Ledene (Viridiflorene)

CAS	*EC No*	*Name*
		Ledol
78-70-6	201-134-4	**Linalol**
115-95-7	204-116-4	**Linalyl acetate**
494-90-6	207-795-5	**Menthofuran**
2216-51-5	218-690-9	**Menthol laevo**
10458-14-7	233-944-9	**Menthone**
2623-23-6	220-076-0	**Menthyl acetate**
93-58-3	202-259-7	**Methyl benzoate**
409-02-9	206-990-2	**Methyl heptenone**
93-16-3	202-224-6	**Methyl isoeugenol**
		Muurolol T
123-35-3	204-622-5	**Myrcene beta**
607-91-0	210-149-6	Myristicin
515-00-4	208-193-5	Myrtenol
20747-49-3		Neomenthol
13877-91-3	237-641-2	**Ocimene beta (cis + tr.)**
589-98-0	209-667-4	**Octanol-3**
106-68-3	203-423-0	**Octanone-3**
104-93-8	203-253-7	**Para-cresol methyl ether**
99-87-6	202-796-7	**Para-cymene**
		Paramenthadienal-1,3,7
		Paramenthadienal-1,4,7
		Patchoulene alpha
514-51-2	208-182-5	Patchoulene beta
		Patchoulene gamma
5986-55-0	227-807-2	Patchouli alcohol
23963-70-4		Phellandral
55719-85-2	259-774-5	Phenylethyl tiglate
80-56-8	201-291-9	**Pinene alpha**
127-91-3	204-872-5	**Pinene beta**
547-61-5	208-927-4	Pinocarveol-trans
89-81-6	201-942-7	Piperitone
		Pogostol
		Pogostone
1191-16-8	214-730-4	**Prenyl acetate**
15932-80-6	240-070-1	**Pulegone**

CAS	*EC No*	*Name*
3033-23-6 16409-43-1	221-217-9 240-457-5	**Rose oxides**
3387-41-5	222-212-4	Sabinene
17699-16-0 15537-55-0	241-703-4 239-584-9	Sabinene hydrate (cis and trans)
94-59-7	202-345-4	Safrole
		Santene
		Seychellene
99-86-5	202-795-1	**Terpinene alpha**
99-85-4	202-794-6	**Terpinene gamma**
562-74-3	209-235-5	**Terpinene-1-ol-4**
98-55-5	202-680-6	**Terpineol alpha**
586-62-9	209-578-0	**Terpinolene**
2867-05-2	220-686-7	Thujene alpha
470-40-6	207-426-8	Thujopsene (Widdrene)
508-32-7	208-083-7	Tricyclene
2408-37-9	219-309-9	Trimethyl-2,2,6 cyclohexanone
121-33-5	204-465-2	**Vanillin**
		Vanillyl methyl ketone
473-67-6	207-470-8	Verbenol
		Vetivenene beta
15764-04-2	239-855-1	Vetivone alpha
18444-79-6		Vetivone beta
552-02-3	209-003-3	Viridiflorol
		Widdrol
16203-25-1	240-332-5	Zizanoic acid

Appendix 5 - IFRA labelling Manual

The so-called IFRA-IOFI Labelling Manual (LM) is a document jointly issued by IFRA and IOFI[23] containing classification and labelling information for substances used by the fragrance and flavour (F&F) industry.

The information provided in this LM is intended to provide guidance to companies aiming to achieve a consistent hazard classification and labelling for F&F substances.

Moreover, the LM serves as a means to ensure an identical approach within the F&F Industry during the classification process and to serve as a reference of supplementary convention and expert interpretation for specific substances. The benefit of this activity is to provide a single global classification of all F&F materials to avoid regional differences as the GHS standards are rolled out globally over time.

The LM is prepared by the IFRA-IOFI GHS Task Force with global representation including Brazil, Europe, the United States and Japan. The TF evaluates the known hazard information of substances used by the F&F, suggest hazard classifications, and provide the LM annually.

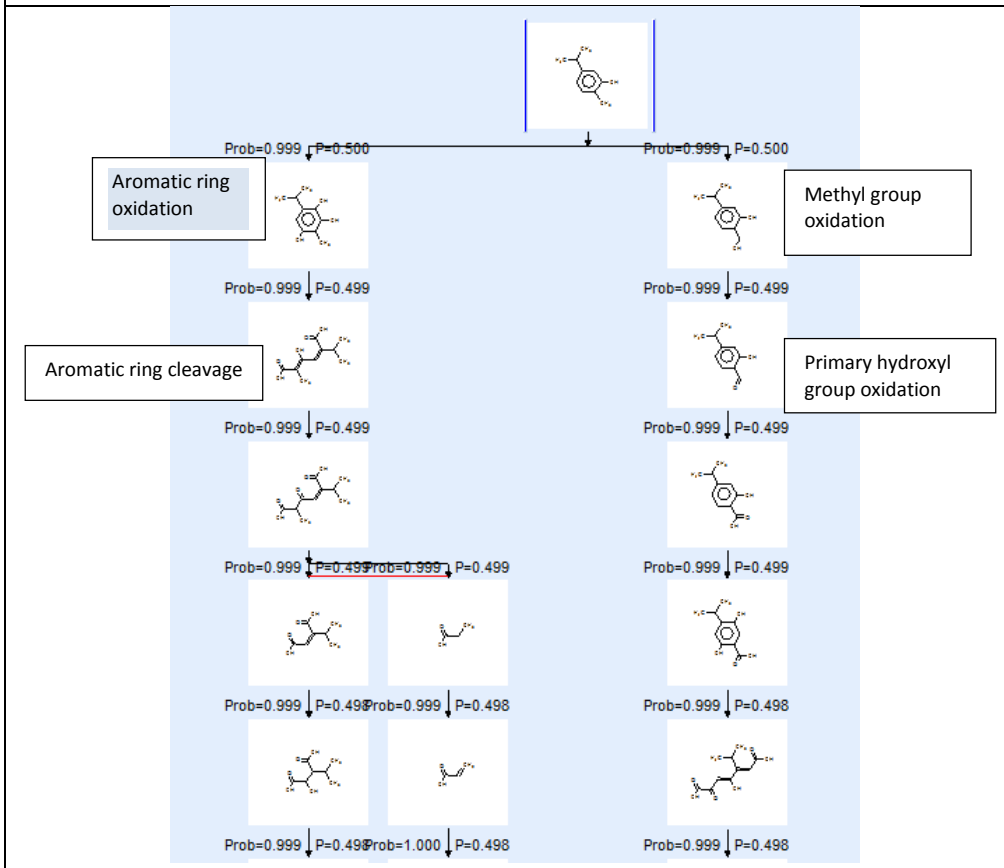
The TF is made up of industry participants that, as a group, is responsible for providing the classifications based on their professional expertise Substances that are to be classified as hazardous, as well as those which are not classifiable based on current knowledge, are included in the LM.

[23] International Organization of the Flavor Industry

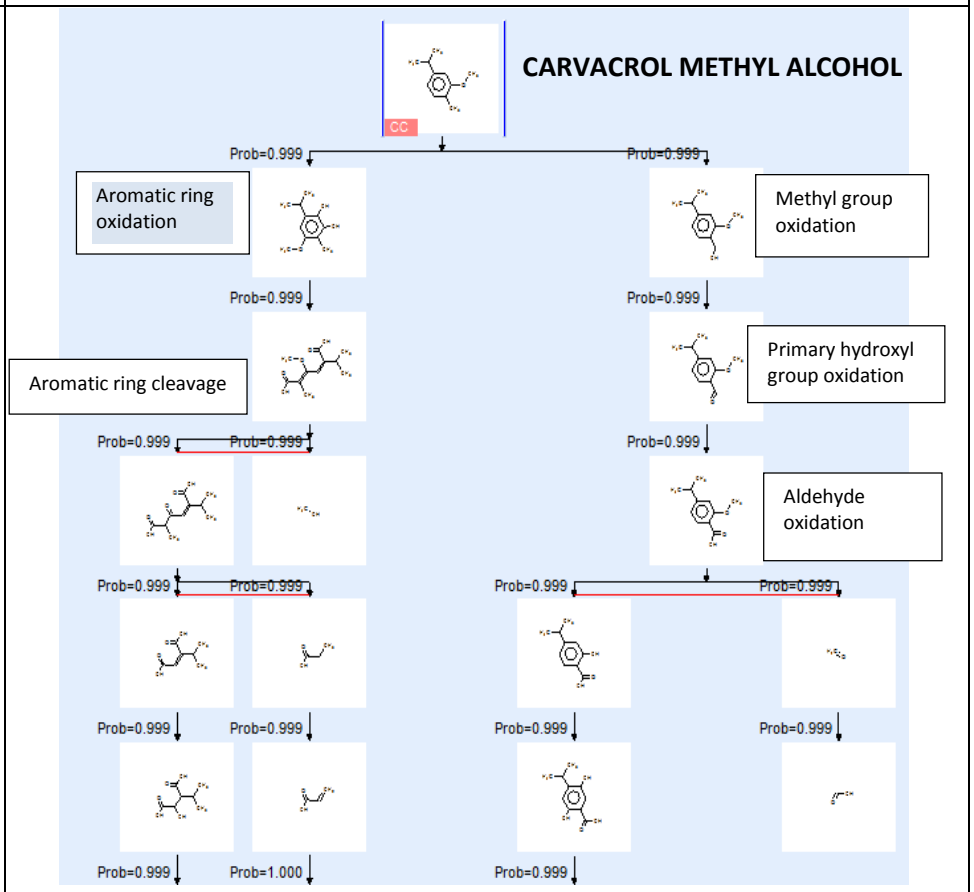
Appendix 6 - Transformation tables proposed by OASIS Catalogic v5.11.17 Kinetic 301F v.13.v.16 for source substance (carvacrol) and target substance (carvacrol methyl ether)

The metabolic pathways show that the 2 substances share the same catabolic reactions, whereby each reaction has a probability of 0.99 to occur. The below is an illustrative example of the predictive biodegradation pathway using the metabolism simulator in CATALOGIC, the latter of which was developed based on known and published biotransformation reactions. All reactions are referenced with the model and other biodegradation reactions can be found at http://eawag-bbd.ethz.ch/.

Biodegradation profile for carvacrol (source substance)



Biodegradation profile for carvacrol methyl ether (target substance)



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