Guidance for the Persistence Assessment of UVCB Substances
Cefic-LRI ECO52 project

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Report for European Chemicals Industry Council (Cefic)

ED 13085 | Issue Number 1 | Date 06/05/2022

Customer:

European Chemicals Industry Council (Cefic AISBL)

Customer reference:

ED13085

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06 May 2022

Ricardo Energy & Environment reference:

Ref: ED13085- Issue Number 1

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1 Introduction

Substances of unknown or variable composition, complex reaction products and biological materials (UVCBs) are an important class of chemicals. As a result of their composition, they can present challenges in the regulatory evaluation of their hazards and risks. Environmental persistence is one property of substances that is generally assessed alongside bioaccumulation and toxicity under national, regional or international regulatory frameworks (Moermond, Janssen et al. 2012). Substances that are persistent tend to degrade more slowly in the environment, resulting in a potential for increased and poorly reversible exposure (Scheringer 2002, Mackay, Hughes et al. 2014, McLachlan 2018).

UVCBs pose unique challenges for persistence assessments due to the nature of their composition, as they can contain many individual relevant constituents. Compared with mono-constituent substances, the amount of data needed to address the information requirements can be extremely high and this raises issues of addressing regulatory requirements in a proportionate manner. In addition to their complex composition, many UVCBs also have properties that render them difficult to test and evaluate for their persistence. These aspects have been addressed in a companion report (Hughes, Griffiths et al. 2022).

This report aims to provide guidance to support the persistence assessment of UVCB substances. This has been based on the current regulatory guidance according to REACH and expanded based on a review of the wider literature for case examples, novel approaches and other scientific and policy developments. The document has been structured to first introduce UVCBs as a class of substances, with a number of examples which will provide case studies throughout the document. The challenges of carrying out persistence assessment of UVCBs, in terms of regulatory requirements and data generation challenges, are then outlined. Following this, the UVCB assessment strategy is presented. In line with REACH guidance, this begins with an initial profiling step to characterise the composition, followed by three assessment approaches: known constituents, fraction profiling and whole substance.

Each aspect of the UVCB assessment strategy is discussed in detail, presenting practical approaches to address the regulatory requirements and challenges which may arise, illustrated by the case examples. For the assessment approaches, consideration is provided for when each of these may be appropriate, considering the compositional characteristics and properties of the UVCB in question. Further, novel approaches and developments which could be utilised to support the implementation of regulatory requirements are presented and discussed, reflecting on potential barriers to adoption. Finally, further critical challenges for the persistence assessment of UVCBs are highlighted, including the requirements to assess transformation products and non-extractable residues, as well as the implications of the proposed introduction of a PMT/vPvM hazard class under REACH.

This document is part of a series of reports produced as part of the Cefic-LRI ECO52 project: 'Expanding the conceptual principles and applicability domain of persistence screening and prioritization frameworks, including single constituents, polymers, and UVCBs.'

2 UVCB definition and examples

2.1 Definition

The term 'UVCB' is used to denote substances of unknown or variable composition, complex reaction products and biological materials (OECD 2017a). These substances generally have a complex composition that may be variable, poorly predictable and/or partially unknown. As such, UVCBs present significant challenges in their identification and subsequent assessment.

The range of different types of UVCB is very wide and the specific properties can be diverse (OECD 2017a). UVCBs can be derived from biological or chemical/mineral sources and generally undergo further chemical processing and/or refinement steps, with the resulting material being considered a new substance. Therefore, the complex and variable nature of the composition can arise from both the source material and/or the processing steps.

OECD (2017a) distinguishes UVCBs as generally possessing the following common characteristics:

- Containing numerous chemicals and cannot be represented by a simple chemical structure or defined by a specific molecular formula.
- Not intentional mixtures of chemicals.
- Many are of natural origin (e.g., crude oil, coal, plant extracts, reaction products) and cannot be completely separated into their constituent chemical species.
- The concept of "impurities" typically does not apply.
- Often produced according to a performance specification related to their physical-chemical properties.

UVCBs are distinguished from so-called 'well-defined substances' under REACH, which includes both mono-constituent and multi-constituent substances (ECHA 2017a). These substances can generally be characterised more precisely, based on the identification parameters of REACH Annex VI section 2. However, UVCBs may not be sufficiently identified by these parameters and require additional information, such as information on their source material and manufacturing process.

Mono-constituent substances are defined as those substances with ≥ 80% (w/w) of their composition represented by one constituent. Multi-constituent substances are defined as substances with more than one main constituent present in the concentration range 10 – 80% (w/w). The ranges and typical values of concentrations of the constituents in a multi-constituent substance are predictable. The remaining mass in these substances are considered impurities and should be identified to the extent possible.

Based on the definitions of mono- and multi-constituent substances, it is easily possible that these substances can have complex compositions and impurity profiles. Therefore the guidance presented in this document, whilst specifically aimed to address UVCBs may also be relevant for the persistence assessment of well-defined substances. Indeed, it is stipulated in ECHA guidance that persistence assessment must address all relevant constituents, impurities, additives and transformation and/or degradation products (ECHA 2017b), which can equally be challenging for well-defined substances.

In 2017 the number of UVCB substances registered under REACH was reported to be 21%, with a further 10% of multi-constituent substances (ECHA 2017c). It is not clear how this figure may have changed in the years since. In particular, this period includes the third and final phase-in deadline of

REACH in May 2018, when all substances manufactured or imported at between 1 and 100 tonnes per annum were required to be registered. However, it can be said with confidence that UVCBs and other complex substances represent a significant proportion of substances registered under REACH.

2.2 Examples

A number of UVCB examples have been identified during the course of this project. These examples provide an illustration of the diversity of UVCBs as a class of substances, both in terms of their compositional characteristics and in the approaches used to address them. These examples will be used as case studies for the guidance and referenced throughout the document.

Petroleum substances

Petroleum substances can be considered an archetypal UVCB (Walters 2020). Derived from crude oil and produced through various fractionation and chemical/catalytic processes, petroleum substances are highly complex mixtures of hydrocarbon constituents. They are used in a wide range of applications including as fuels, lubricants, solvents and as precursors for other chemical products.

The constituents of petroleum substances include linear, branched, naphthenic (aliphatic cyclic) and aromatic components of varying carbon chain lengths. The compositional complexity of petroleum substances increases exponentially with carbon number. The composition varies depending on the source and refinery (processing) conditions (ECHA 2017b). Petroleum substances are typically separated by distillation and technical specifications generally include a boiling range. Other processing may take place to suit particular applications, and this further influences the composition.

Natural complex substances (NCS)

Natural complex substances (NCS) are a diverse family of substances of botanical origin commonly used as ingredients in fragrance products as well as consumer and cosmetic products (IFRA 2015). NCS as a group of substances includes essential oils, concretes and absolutes, oleoresins and resinoids, CO_2 extracts and infusions and alcoholic extracts. The composition of a NCS from one unique genus and species is subject to natural variation due to the region and climatic conditions under which the plant was grown, as well as the part of the plant used as source material. Variation will also arise due to the processing steps, which can include drying, cutting, expression, extraction, distillation, fractionation, concentration, precipitation, etc (IFRA 2015).

The composition of NCS can vary widely, from relatively simple, to very complex substances. They can qualify as mono- and multi-constituent substances or UVCBs, according to REACH substance identity definitions. However, they have historically been considered as UVCBs, and their profiles of minor constituents as inherent to the substance as a whole, rather than as 'impurities' (IFRA 2015).

Surfactants

Surfactants are a diverse group of substances whose constituents generally consist of a polar head group and a nonpolar hydrocarbon tail group, and therefore possess surface active properties. They are most commonly used in detergents and other cleaning products (Scott and Jones 2000, Ivanković and Hrenović 2010). Surfactant classes include nonionic, anionic, cationic and amphoteric varieties.

Not all surfactants are UVCBs, and their composition will depend on the manufacturing process and precursors. For example, alkyl sulphate surfactants can be mono-constituent or UVCB depending on the nature of the hydrophobic 'alkyl' component used to produce the surfactant. In the case of

ethoxylated surfactants, the polar 'ethoxy' component of the molecule also contributes to the complex nature as molecules will typically have a range of degrees of ethoxylation.

Chlorinated paraffins

Chlorinated paraffins (CPs) are generally linear hydrocarbons with varying degrees of chlorination. They vary in terms of their carbon chain length and degrees of chlorination, and this leads to thousands of potential congeners (Leonards 2017). CPs are used in a variety of applications including as lubricants, flame retardants, plasticisers, rubbers, paints and sealants.

The CPs are divided into three broad groups: short chain CPs (SCCPs) (C8-C13), median-chain CPs (MCCPs) (C14-C17) and long-chain CPs (LCCPs) (>C18), with chlorination degree varying between 30 and 75% (Leonards 2017). SCCPs have been listed under the Stockholm Convention as Persistent Organic Pollutants (POPs), and MCCPs were recently included on the EU REACH Candidate List of substances of very high concern (SVHC) for Authorisation.

Naphthenic acids

Naphthenic acids are mixtures of cycloaliphatic carboxylic acids (Brient, Wessner et al. 2000). They occur naturally in crude oil and are generally recovered from petroleum distillates by caustic extraction. Naphthenic acids are used commercially in a range of applications including in concrete, drilling oils, jet fuel and as a wood preservative. Interest in naphthenic acids has increased due to of high concentrations occurring in tailings ponds from the oil sands extraction industry in Canada (Clemente and Fedorak 2005).

Fischer-Tropsch (aka 'Gas-to-liquid') hydrocarbon substances

The Fischer-Tropsch process is used to convert a mixture of carbon monoxide and hydrogen (aka "syn-gas") into liquid hydrocarbons (Whale, Dawick et al. 2018). The potential feedstocks for this process include natural gas, or biomass. Due to the different manufacturing process, Fischer-Tropsch hydrocarbon substances have different compositional characteristics than their petroleum derived alternatives. They are reported as containing low amounts of aromatics, unsaturated, heteroatom-containing hydrocarbons, and with differing profiles of aliphatic (linear, branched and cyclic) constituents (Whale, Dawick et al. 2018).

Oleochemicals

Oleochemicals are typically derived from vegetable and animal oils and fats. They can also be derived from synthetic (e.g. petrochemical) feedstocks. They are produced by splitting natural triglycerides into glycerine and fatty acids by hydrolysis. The carboxylic acid functional group can then be converted into various other functional groups, e.g. amine, ester, ammonium, sulphate (OECD 2017b). The fatty acids vary in terms of their chain length and degree of saturation depending on the initial fat or oil used as a feedstock. The chain lengths mainly appear between C6-C24, with the majority between C12 and C18.

3 Challenges for the persistence assessment of UVCBs

There are numerous challenges associated with the testing and assessment of UVCBs. These stem from the nature of their composition, which is complex, variable and (to an extent) unknown. Many UVCBs also have the issue of difficult testing properties, which further hampers their testing and assessment (Figure 1). However, this document will focus on challenges arising from compositional aspects (i.e. those which lead to substances being identified as UVCB). The challenges around difficult test properties are discussed in a separate guidance document developed as part of this project (Hughes, Griffiths et al. 2022). The challenges and guidance on difficult test properties should be considered alongside the information in this document.
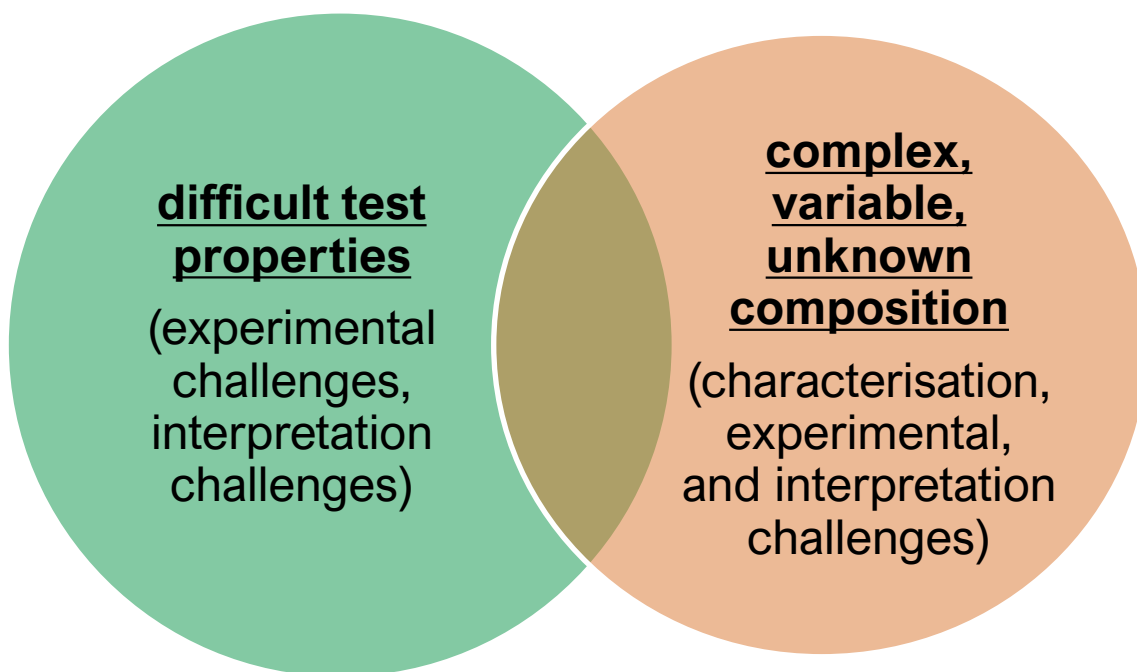

Figure 1 Conceptual diagram of UVCB testing and assessment challenges. Many UVCBs have properties which render them difficult to test, in addition to problems arising from their complex, variable and unknown composition. This document will focus on aspects relating to composition (right hand circle), whereas difficult test properties (of individual constituents) are address in Hughes et al. (2022).

The challenges discussed in this section are divided between those related to:

- **Regulatory requirements** to assess persistence of UVCB substances as part of the PBT/vPvB assessment of REACH Annex XIII (and associated ECHA guidance), and
- **Data generation**, i.e. providing the information (through testing or otherwise) required to assess persistence of the substance.

3.1 PBT assessment requirements according to REACH

The persistence assessment under REACH is carried out as part of the Annex XIII requirement to perform a PBT/vPvB assessment of registered substances (EC 2006, ECHA 2017b). This applies to all substances for which a chemical safety assessment must be performed (i.e. those registered at ≥ 10 tonnes/annum), where the substance is organic, including organo-metals.

The regulatory guidance for PBT/vPvB assessment requires that the assessment is performed on all relevant constituents, impurities and additives. It shall also take account of relevant transformation and/or degradation products (ECHA 2017b). The principles for assessing multi-constituent and UVCB substances are therefore the same as for mono-constituent substances. The main difference between these scenarios is the number of constituents requiring assessment, and therefore the amount of effort required to carry out the assessment satisfactorily. In addition, in the case of UVCBs, a significant proportion of the composition may be unknown or difficult to characterise. It should be noted here that even the persistence assessment of mono-constituent substances can be highly detailed and complex, with the requirement to consider a wide variety of information in a weight-of-evidence determination (Hughes, 2021).

A concentration limit of ≥ 0.1% (w/w) is defined for those relevant constituents requiring assessment, which is reported to be based on "a well-established practice recognised in European Union legislation to use this limit as a generic limit" (ECHA 2017b). In some cases, this threshold may be increased "for the sake of proportionality of assessment efforts". However, the conditions under which this may be modified are not well defined. According to guidance, account should be taken of the use pattern and potential emissions of the substance. In any case the threshold should not exceed 10% (ECHA 2017). It is understood that 1% threshold is more commonly applied to the assessment of natural complex substances (IFRA 2016).

Although the ≥ 0.1% (w/w) threshold is applicable to relevant constituents, in the case of UVCBs the composition may be so complex that few individual constituents are present in concentrations exceeding this threshold. In these cases, the structural similarity of constituents within fractions of the substance needs to be considered (ECHA 2017b). For example, where constituents have similar carbon number, chain length, structure and functional groups etc, and can as such be grouped together and their total mass compared to the ≥ 0.1% (w/w) threshold. This grouping should be based on read-across principles. Hence, the grouping should be informed by whether the constituents are expected to have similar persistence properties. Ultimately, the assessment needs to cover the full composition of the UVCB substance.

If a relevant constituent or fraction of a UVCB is identified as PBT or vPvB, then the whole UVCB substance is considered to be PBT or vPvB. Where the composition of a UVCB substance is expected to be variable, a suitable analytical method may be needed to determine the presence of the constituent/fraction at ≥ 0.1% (w/w) in individual instances of 'batches' of the substance.

The regulatory guidance stresses the need for assessment efforts of UVCB substances to remain feasible and proportionate (ECHA 2017b). However, the details of what constitutes a proportionate assessment are not well defined. In fact, it is very difficult to define this precisely, since proportionality depends on many aspects, including socio-economic elements. Feasibility and proportionality aspects are difficult to balance against the assessment requirements as they are written. What is feasible and proportionate therefore needs to be considered and justified case-by-case.

The regulatory requirements for persistence assessment raise several important questions when considering UVCB substances:

- How can assessment efforts remain feasible and proportionate when assessment requirements are in principle the same as for mono-constituent substances, and the number of relevant constituents/fractions is high?

- How to perform a robust assessment when a proportion of the composition is variable and/or unknown?

- Under what circumstances can the ≥ 0.1% (w/w) threshold for relevant constituents be increased?

- How should constituents present at < 0.1% (w/w) be grouped into fractions for assessment, based on structural similarity?

- Where fractions of (theoretical) constituents are grouped for assessment, how can an associated analytical method be developed so that their presence in the UVCB substance at ≥ 0.1% (w/w) can be verified?

- How can the requirements to consider relevant transformation and/or degradation products be taken into account for UVCBs, when each individual constituent will have its own set of degradation pathways and transformation products?

3.2 Data generation

Data generation relates to all aspects of deriving the information required to assess persistence of a UVCB substance. This information will generally relate to biodegradation of the substance in screening and simulation tests, but may include other information, such as abiotic degradation testing, quantitative structure activity relationships (QSARs), etc.

Substance identity

The first thing to consider is the identity, i.e. composition, of the substance. Having an understanding of the substance identity is crucial to the design of a successful testing and assessment strategy. If the composition of a substance is not known, it will not be possible to know which constituents are of greatest interest from a persistence perspective. It will also not be possible to determine that the UVCB substance as a whole is representative as a test substance (to account for the expected batch-to-batch variability with respect to PBT/vPvB properties), where this is the intended assessment strategy. Many UVCB substances are produced to a specification of physicochemical properties, rather than composition (Salvito, Fernandez et al. 2020). Therefore, they will rarely be characterised to the degree to which all relevant constituents for the PBT/vPvB assessment have been identified. Even with extensive characterisation efforts, a proportion of the composition may remain unknown.

UVCB substances are inherently variable in their composition. This means that the composition will vary from one 'batch' to the next. The extent to which the composition may vary, and the impact this may have on the persistence of the constituents and substance as a whole, will differ from one UVCB to the next. It is necessary to understand and consider this variability to ensure that test substances are representative and adequate to assess the persistence.

Screening testing

Biodegradation screening tests such as the OECD 301 series measure ultimate degradation using non-specific parameters such as oxygen consumption, CO_2 production and dissolved organic carbon (DOC) removal. These parameters do not provide information on the constituents that have been degraded. As a result, there is an uncertainty when these tests are carried out on UVCB substances, which contain different constituents with potentially varying biodegradability. This issue has been

highlighted in regulatory guidance, and biodegradation screening data on UVCB substances are generally not accepted for PBT/vPvB assessment, unless it can be justified that constituents are sufficiently similar. This is potentially very problematic as it severely reduces the options for screening the persistence of UVCB substances. Options are generally limited to instead performing screening testing on individual constituents/fractions or using QSAR tools. However, the latter are generally not considered sufficient on their own to conclude a persistence assessment (ECHA 2017b).

Simulation testing

Unlike biodegradation screening tests, simulation tests measure the primary degradation of a test substance at low concentrations in environmental media using specific chemical analysis (OECD 2006). The use of radiolabelled test substances is usually required in order to establish the fate of the test substance (ECHA 2017b, ECHA 2017d, PFA 2021). This is necessary to determine the formation of CO_2, transformation/degradation products and the quantification and characterisation of non-extractable residues (NER). It is also necessary to determine the mass balance, so that the total amount of test material introduced into the system can be accounted for. Finally, the use of radiolabelled test substances allows much greater analytical sensitivity, facilitating testing at low concentrations.

Due to the complex nature of UVCB substances, it is generally not technically feasible to obtain the whole substance radiolabelled to use as a test material for simulation tests. This effectively means that testing must be carried out with individual constituents or using non-labelled material. If non-labelled material is used it is not possible to track the radioactivity throughout the experiment, and therefore to measure CO_2, NER or mass balance. In such cases, the identification of transformation and/or degradation products would also be expected to be significantly more challenging, if not impossible. The use of non-labelled test material would necessitate testing at higher concentrations. The complex nature of the substance also generally necessitates testing at higher concentrations, as the analytical signal is spread over many individual peaks, affecting the limit of quantitation. Testing at higher concentrations is undesirable in simulation testing and can affect observed kinetics and degradation rates (Alexander 1999, OECD 2006). These are serious issues that affect the reliability and relevance of the data generated and hamper the persistence assessment of UVCB substances according to the regulatory guidance.

Whole substance vs constituents?

Testing UVCB substances as whole substances compared with as individual constituents can lead to potentially differing results. For example, when tested together, multiple constituents may be observed to degrade sequentially in biodegradation screening tests. For this reason the 10-day window is generally not applied (OECD 2006). Also, the co-metabolism of test substances has been reported, whereby substances degrade in the presence of other substances but not when tested alone (ECHA 2017d, Hammershoj, Birch et al. 2019).

Where a UVCB substance is tested at concentrations in excess of its solubility in the test medium, the bioavailability of individual constituents may be impacted by the properties of the bulk substance (e.g. viscosity) (Ribicic, McFarlin et al. 2018, Yu, Duan et al. 2018, Davenport, Curtis-Jackson et al. 2022). Substances that exist as crystalline solids in pure form may show increased solubility when dosed into aqueous media via a UVCB non-aqueous phase liquid, as has been observed in the case of PAHs present in oil (Mackay, Shiu et al. 2006, Liu, Wu et al. 2013).

The testing of UVCB substances as whole substances, rather than as individual constituents in isolation, is arguably the more environmentally relevant scenario as these constituents will be emitted to the environment in the presence of each other, although they may be subject to different fate and transport processes once having been emitted. Furthermore, individual substances co-occur in the environment as dynamic and highly complex mixtures as a result of diverse human activities and natural processes (Kienzler, Bopp et al. 2016, Bopp, Barouki et al. 2018). Therefore it can be argued that the testing of individual substances or constituents in isolation is inherently unrealistic compared to conditions in the field (Alexander 1985).

There may also be significant practical challenges in obtaining specific constituents of interest for use as test substances. These may not be commercially available in an isolated form, and instead require synthesis, or separation from the UVCB substance. Where radiolabelled test substances are required, this may add significantly to the technical challenges and costs.

Ultimately, where UVCB constituents are required to be tested individually, this can increase the overall cost of persistence assessment several-fold compared with the assessment of mono-constituent substances. It is generally recognised that it is neither practical nor feasible to test all constituents of a UVCB individually. Bespoke approaches are generally needed that are tailored to the specific composition and properties of the UVCB substance in question.

4 UVCB assessment strategy

The following sections detail a strategy for the persistence assessment of UVCB substances. This is based on and building upon the approach detailed in the ECHA guidance for for PBT/vPvB assessment under REACH (Figure 2) (ECHA 2017b). The approach therefore carries with it some hallmarks of REACH persistence assessment, such as the concentration cut-off for relevant constituents (≥0.1%), which may not necessarily be applicable under other regulatory schemes. However, the general principles of the assessment approaches described under REACH provide a logical framework to approach the assessment of a UVCB substance. Guidance on persistence assessment of UVCBs from other regulatory regimes is currently lacking, although the topic has been recently addressed in reviews (Leonards 2017, Salvito, Fernandez et al. 2020). The topic of developing categories for assessing UVCBs was also recently covered by OECD (OECD 2017a).

In this approach an initial profiling step is carried out to characterise the substance composition. This information on the composition of the substance is then used to determine the most appropriate assessment approach for the UVCB. Three assessment approaches are detailed: 'known constituent', 'fraction profiling' or 'whole substance'. These assessment approaches can be used alone or in combination to assess the persistence of the substance.

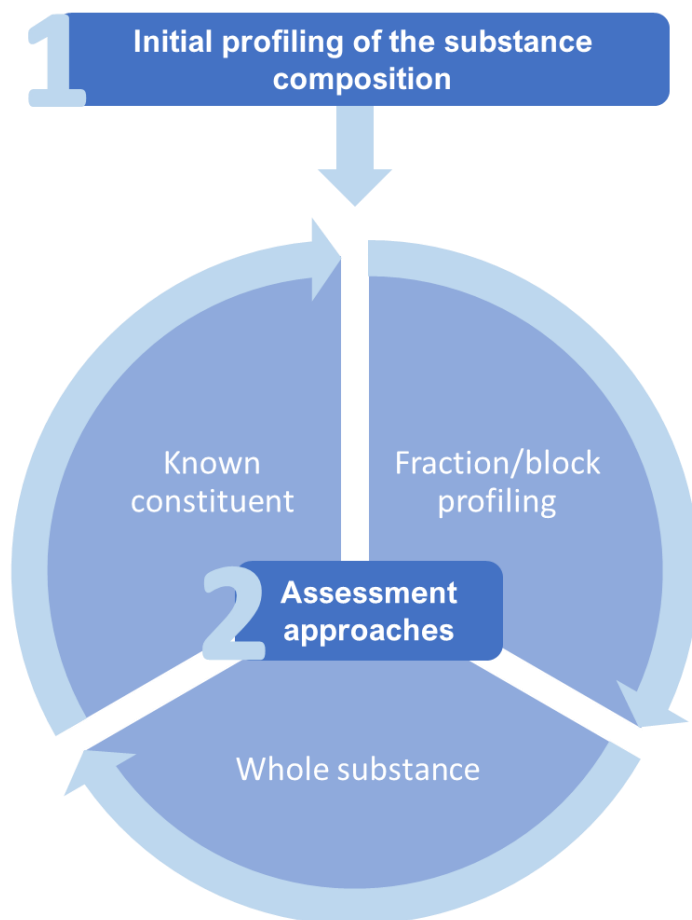**Figure 2 Illustration of the suggested PBT/vPvB assessment approach currently detailed in REACH guidance, upon which this guidance document is based. An initial profiling step followed by three potential assessment approaches: 'known constituent', 'fraction profiling' or 'whole substance'. These assessment approaches can be used alone in combination to assess the persistence of the substance.**

4.1 Initial profiling

UVCB types and compositional diversity

UVCBs can generally be broken down into three common types (Salvito, Fernandez et al. 2020):

1. Extracts from plant or animal products
2. Reaction masses
3. Complex chemical or mineral precursors subject to further processing (e.g. petroleum substances)

UVCBs can exhibit a wide diversity of structural characteristics and complexity. Depending on the nature of the UVCB, the composition may be relatively simple, or extremely complex. The nature of differences between constituents (structure and concentration) may be homologous, logical and predictable, exhibiting incremental differences and falling into discreet classes which can be readily grouped. In other cases, prediction and grouping of constituents for persistence assessment may be more challenging. The extent to which substance composition can vary from one batch to the next, and the influence that source material and processing conditions has on this, can also differ substantially between substances.

All of these characteristics can be influenced by the type of UVCB. For example, reaction masses may have reasonably well-defined boundaries of composition, defined by known parameters of the precursors and the reaction(s) taking place. This can enable the composition to be defined within more limited boundaries. Petroleum UVCBs on the other hand can be extremely complex, with the possible number of constituents increasing exponentially with carbon number, and overall composition depending on the source material and processing conditions. However, even in the face of this complexity, the composition may still exhibit a regular pattern in terms of carbon number and hydrocarbon class distribution. By contrast, UVCBs of biological origin tend to be less predictable in terms of the unique structures present and their concentration ranges, which present challenges in characterising and grouping constituents, and addressing unknown fractions.

Identifying relevant constituents

At the initial profiling stage, the substance needs to be characterised to the extent possible to enable assessment of all relevant constituents, impurities and additives for their persistence. This may require going beyond the requirements for identification and naming of the substance under REACH (ECHA 2017a). Normally the composition should be identified down to 0.1%. However, REACH guidance describes that it may be appropriate to increase this threshold in some cases (e.g. low tonnage), but should not exceed 10%. In the case of natural complex substances (NCS), such as essential oils, a cut-off of 1% is generally applied (IFRA 2016). In the case of very complex UVCBs, where many constituents are expected to be present at < 0.1%, it is necessary to determine whether these constituents can be grouped for assessment such that the sum of their concentrations is ≥ 0.1% (ECHA 2017b).

Compositional descriptors

As well as identification of individual constituents, it may be useful to describe the composition in terms of structural characteristics or groups of related constituents. In practice, it may essential to do this depending on the level of detail to which the substance can be characterised. Structural characteristics may include:

- Carbon range/chain length
- Functional groups
- Structural features/groups (e.g. "sesquiterpene hydrocarbons, C15H24")
- Degrees of cyclisation/branching
- Degree and nature of halogenation

These characteristics/descriptors are distinct from other descriptors, such as physical properties (molecular weight, boiling range, polarity) and source material/process conditions, which on their own do not provide information on composition. However, these descriptors may also provide useful lines of evidence to infer the compositional descriptors of substances and should be included in the evaluation. All effort should be made to describe the boundaries of compositional complexity that is expected to occur within the UVCB substance, and hence what is relevant for persistence assessment.

The compositional descriptors defined at this stage may be useful for further defining fractions or groupings for persistence assessment. However, these may need to be further revised if the constituents within are not expected to have similar persistence, which is the basis of the fraction profiling approach.

Variability

It can be assumed that a persistence assessment of a UVCB needs to be representative of the substance. This means that compositional variability should be understood and taken into account, particularly where this may affect the outcome of a persistence assessment. This can be addressed, for example, by considering the composition of multiple examples or batches of the UVCB. However, this may be challenging where the characterisation efforts for a UVCB substance are particularly onerous.

Addressing the 'unknown fraction(s)'

It is recognised that a proportion of the composition of a UVCB substance is likely to be unknown (ECHA 2017b). Also, that the composition of the UVCB substance may vary from one batch to the next. Information on unknown fractions of a UVCB substance needs to be compiled in order to build an understanding of these fractions to facilitate an assessment of persistence of all relevant constituents. This information can be comprised of multiple lines of evidence, which collectively increase confidence and reduce uncertainty. For example, the UVCB substance may be known to be composed of certain chemical groups or classes with certain structural features, and this reduces the number of potential structures that may be present. It may also be possible to infer compositional characteristics based on the source material and/or manufacturing process. In doing so, 'generic structures' on which to base an assessment of less well-characterised fractions of the substance could be defined (Salvito, Fernandez et al. 2020).

Analytical techniques

Detailed guidance on analytical characterisation strategies for UVCBs is beyond the scope of this document. UVCBs generally require bespoke approaches to be suitable for their specific compositional characteristics and complexity. However, some examples are discussed in the document which may prove useful or be adaptable to other UVCBs requiring assessment.

On a general note, significant advances have been made recently in the development of analytical techniques for characterising organic substances. In particular, in mass spectrometry (MS), high resolution instruments, such as Time-of-flight (TOFMS) and Orbitrap MS, have enhanced the accurate determination of the mass of organic molecules. These have been combined with chromatographic techniques and advanced software to produce powerful techniques for the identification of unknown substances (Leonards 2017). Furthermore, the field of nontarget analysis in particular, allowing rapid screening of chemical mixtures in various matrices, has made great strides (Salvito, Fernandez et al. 2020).

4.1.1   Case examples on initial profiling of UVCB substances

The following case examples for substances identified in Section 2.2 are provided as illustration of how UVCB initial profiling may be approached.

Petroleum substances

Petroleum substances typically have a highly complex composition (Walters 2020). The constituents include linear, branched, and single- and multi-ring naphthenic (saturated) and aromatic components of varying carbon chain lengths. The compositional complexity of petroleum substances increases exponentially with carbon number. As such the complete chemical characterisation of complex petroleum UVCBs still largely unattainable (Onel, Beykal et al. 2019).

The technical function of petroleum substances is generally determined more by their physical properties than composition, and as a result they have historically been identified on the basis of their refinery processing steps and boiling range, rather than by their composition. However, more recently for the more highly refined and narrow cut hydrocarbon solvents, a naming convention has been adopted which includes information on carbon range as well as content of aliphatic linear, branched, cyclic and aromatic hydrocarbon structural descriptors (OECD 2017c). Generally, each carbon number and aliphatic hydrocarbon structural descriptor is included in the name if it is present at > 10% w/w. Aromatic content is indicated as being above or below 2% w/w. Analytical approaches were also recommended to provide the information to inform this naming, and include ultra violet (UV), infra-red (IR), nuclear magnetic resonance (NMR) spectroscopy and/or mass spectrometry (MS), and gas or high performance liquid chromatography (GC or HPLC). This provided a significant step forward in the identification of hydrocarbon solvents, which has been adopted for purposes of their naming under EU REACH (HSPA 2017). However, this approach does not provide information on the more detailed structural characteristics of hydrocarbon solvent UVCBs, and therefore may not be sufficient to facilitate the persistence assessment.

A further approach that has been applied for the characterisation of petroleum UVCBs is two-dimensional gas chromatography (GCxGC). Here samples are separated into two serially joined GC columns, with each column having different properties. This technique is able to achieve effective separation of hydrocarbons based on both volatility and polarity (Concawe 2019). The resulting chromatograms provide effective separation of petroleum substance constituents, particularly in the middle distillate (C8-C30) range. These chromatograms can be processed using blocking schemes to assign chromatographic peaks to carbon numbers and structural classes. Due to the nature of the separation of hydrocarbon constituents by GCxGC, the resulting groups of constituents generally also have similarity in terms of their environmental fate, toxicity and partitioning properties (Concawe 2019). GCxGC has therefore formed a powerful and complementary tool to the environmental hazard and risk assessment of petroleum substances.

GCxGC has also been applied to the analysis of total petroleum hydrocarbon (TPH) composition of refinery effluents, and applied to computational predictions of removal and toxicity (Hjort, Den Haan et al. 2021). Further, the technique has been used for characterising non-polar complex mixtures in environmental matrices and predicting a range of environmental properties including partitioning, baseline toxicity and diffusion coefficients (Arey, Nelson et al. 2005, Nabi, Gros et al. 2014, Nabi and Arey 2017). Further, GCxGC has been coupled with high-resolution time-of-flight MS (HRTOFMS) to achieve even more exhaustive characterisation of test items (Sauer, Barter et al. 2019). This approach has, for example, been used for the identification of components that accumulate in engine oil during use, and elucidation of their properties (Zushi, Yamatori et al. 2019). These applications demonstrate GCxGC as a useful technique with a range of utility beyond substance characterisation, and with potential applicability to other substance types beyond petroleum substances.

More recently, ion mobility spectrometry (IMS)-MS has been utilised as a suitable technique for evaluating the detailed chemical composition and variability of petroleum substances in from three manufacturing categories (heavy aromatic, hydrotreated light paraffinic, and hydrotreated heavy paraffinic) (Roman-Hubers, Cordova et al. 2022). The authors suggest that this technique can provide the information necessary for hazard and risk characterization, in terms of quantifying the variability of the products.

Natural complex substances (NCS)

The use of a 1% cut-off or unequivocal identification and PBT/vPvB assessment of NCS is justified based on the fact that these substances are generally of lower tonnage, and the difficulty of identifying unequivocally the identity of certain common constituents, such as sesquiterpenes (IFRA 2016). This generally requires a pure sample being co-injected to confirm by GC-MS. The natural variation of NCS often necessitates multiple analyses to define constituent ranges. If a constituent is well-known and present as a reference in analytical spectra library, it may be reported at < 1% (IFRA 2016). NCS constituents are generally related as a result of plant biochemistry, which supports that constituents present at < 1% are likely to have similar PBT properties to the known constituents.

Essential oils and natural extracts are typically composed of monoterpenes and sesquiterpenes. A number of these are also registered under REACH as mono-constituent substances and are therefore available to be used as representative substances in persistence assessment, where this is appropriate (IFRA 2016). It is recommended to assemble constituents into blocks on the basis of similarity in the property being assessed (i.e. persistence). Biodegradation is associated with the presence of certain functional groups, such as esters. The carbon skeleton of the constituents, including degree of branching, position of alkyl groups and number of rings may restrict biodegradability.

Terpenes have been classically identified based on the number of isoprene units in their carbon skeleton. Each isoprene unit is composed of 5 carbon atoms, and are named hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenoids, and so on. These classes of terpene are further subdivided into secondary classes (Figure 3). These can relate to the carbon skeleton (acyclic, monocyclic, bicyclic, etc), or further by biogenetic skeleton, following established nomenclature schemes (IFRA 2016).
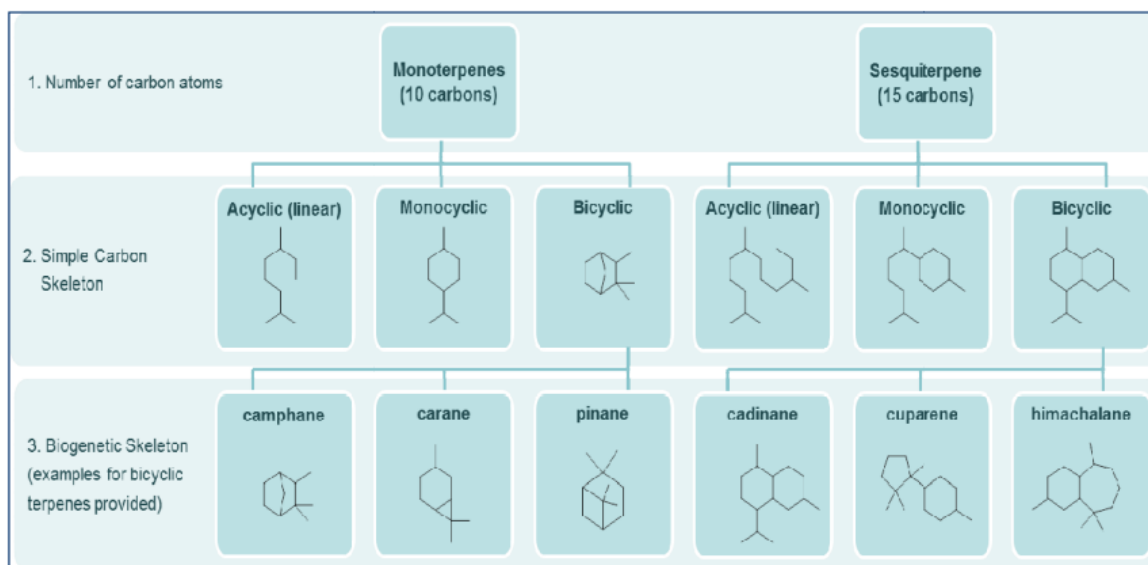
Figure 3 Tiered classification scheme for terpene skeletons, from IFRA (2016).

A grouping scheme for terpenoids is suggested based on number of carbons and chemical functionality (Table 1). However, it recognises that this does not consider the carbon skeleton, and therefore may not be sufficient for assessing biodegradation and persistence. For these endpoints a modified grouping scheme may be required.

Table 1 Criteria for grouping terpenoids, from IFRA (2016).

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

Chemometric analysis has been described as a means of rapid screening of the quality of lavender oil (Beale, Morrison et al. 2017). In this study an untargeted chemometric predictive model was developed to rapidly assess and characterise lavender oils for geographical and environmental adulterations that impact quality. Of 170 compounds that were identified by GC-MS, 15 unique compounds that greatly influence oil quality were identified. The predictive multivariate chemometric model was shown to be capable of rapidly screening and assessing the quality of lavender oil samples with high accuracy. It was acknowledged that initial validation and implementation of the approach was challenging, but this was rewarded by rapid and reliable screening once the methodology was developed. Such an approach could potentially be adapted to characterise and compare samples of UVCB substances for persistence assessment, for example for the non-target detection of potential, relevant constituents requiring investigation, or where identified constituents or compositional profiles are associated with particular hazardous properties. Methodologies for characterisation of specific NCS are generally published in literature, for example for orange oil, rose oil and jasmine (VORA, MATTHEWS et al. 1983, Magiatis, Paraschos et al. 2008, Temraz, Cioni et al. 2009). However, these methodologies may need to be further developed to provide the level of information required for purposes of persistence assessment.

Chlorinated paraffins

Chlorinated paraffins (CPs) are complex mixtures of linear paraffins with varying degrees of chlorination. Typically there are thousands of congeners present (Leonards 2017). It is generally not possible to identify individual constituents of CPs. The structures can be separated and grouped into individual chain lengths and degrees of halogenation using multi-dimensional separation techniques.

It is also challenging to quantify CPs, due to the lack of available analytical standards. Available analytical standards do not have the same chlorine configuration as present in CP technical mixtures and only a limited number of technical CP mixtures are commercially available for use in analysis. Therefore only semi-quantitative analysis of CPs is possible (Leonards 2017).

Short chain CPs can be analysed by GC-MS, with the mass spectrometer operating in the electron capture negative ionization (ECNI-MS) mode. Sensitivity of ECNI-MS decreases when analysing longer chain CPs, and also for detecting lower chlorinated ($<Cl_5$) paraffins. GCxGC with electron capture detection (ECD) is able to separate and analyse CPs, however the optimisation and quantification are complicated and time-consuming, limiting its adoption. GCxGC with ECNI- time of flight (TOF)-MS has also been shown to be successful, although this approach also suffers from poor detection of lower chlorinated paraffins (Leonards 2017). The routine analysis of CPs appears to still be a challenge and subject to further research (van Mourik, Leonards et al. 2015, van Mourik, van der Veen et al. 2018, Van Mourik, Janssen et al. 2021, Fernandes, Vetter et al. 2022).

The high-resolution fractionation of chlorinated paraffins by GC with a 96-well plate was recently successfully applied to separate, purify and enrich isomers from a whole GC chromatogram, and subsequently analysed by NMR (Van Mourik, Janssen et al. 2021). Research is ongoing for full characterisation of the CP isomers using multivariate statistical analysis. This approach is promising for the isolation of individual isomers for use as standards and in subsequent testing of environmental properties.

As a result of the analytical challenges, CPs are generally characterised based on their distributions of carbon chain length and degree of chlorination. This approach was used for the recent REACH substance evaluation of MCCPs (ECHA 2021). The substance was defined as having chain length C14-C17 and chlorine content of 40-63% by weight. However, it was recognised that chlorine content only represents an average, and that a wider range of constituents may be present. The assessment of the persistence therefore considered a broader compositional profile which included lower and higher degrees of chlorination than were included in this range.

Surfactants

An example of surfactant substances that are UVCBs is alcohol ethoxylates. These are generally described by the nature and chain length of the fatty alcohols used to produce them, as well as the degree of ethoxylation. The degree of ethoxylation is normally expressed as an average value, but it should be recognised that this will represent a distribution of ethoxymers. Alcohol ethoxylates can be characterised by LC-MS to determine the ethylene oxide adduct distribution. It was found that an effective approach was to convert alcohol ethoxylates to alcohol ethoxy sulfates and then to analyse by LC-MS with electrospray ionization in the negative ion mode (Cassani, Pratesi et al. 2004).

An analytical method has also been developed for the assessment of alcohol ethoxylates (AE) and fatty alcohols (FA) in sediments (Dyer, Sanderson et al. 2006). 38 of 114 possible AE ethoxymers and FAs found in commercially important products could be measured simultaneously. The methodology involved derivatisation of extracts with 2-fluoro-N-methylpyridinium p-toluenesulfonate (Pyr+), followed

by analysis of derivatised extracts with liquid chromatography/mass spectrometry (LC/MS) operating in the positive ion electrospray mode.

In a regulatory evaluation of the PBT properties of the surfactant UVCB, Sulphonic acids, C10-21-alkane, Ph esters, the composition of the substance was described in detail (ECB 2008). The details captured included the distribution of chain lengths and extent of branching of the alkyl chain, and the presence of mono-, di- and triesters. The nature of alkyl chains could be determined reliably based on the content of the starting material. This level of detail of the composition enabled the assessors to evaluate the PBT/vPvB properties of the different (groups of) constituents and concluded that only the minor impurities in the branched fraction were considered to have potential PBT/vPvB properties, and that these were not considered to be a concern due to low overall presence in the substance. Concerning these minor impurities, it is worth noting that this assessment pre-dates the latest guidance for persistence assessment under REACH.

Naphthenic acids

Naphthenic acids (NA) are generally complex mixtures of alkyl-substituted acyclic and cycloaliphatic carboxylic acids. These can be described with the general formula $C_nH_{2n+Z}O_2$, where n represents the carbon number and Z is zero or a negative, even integer that specifies the hydrogen deficiency resulting from ring formation. The number of rings in the compound can be determined by dividing the absolute value of Z by 2 (Clemente and Fedorak 2005). Mass spectrometry has typically been used to identify individual constituents by assigning ions to the general formula, based on carbon and Z number. However this has not provided satisfactory resolution of complex naphthenic acid mixtures (Clemente and Fedorak 2005).

Subsequently, an advanced analytical method based on 'dilute-and-shoot' reversed-phase capillary HPLC/QTOF-MS has been developed (Bataineh, Scott et al. 2006). This approach was able to separate naphthenic acids based on carbon number, degree of cyclisation and extent of branching, whilst significantly increasing analytical sensitivity compared to infusion techniques. This technique has been used to support subsequent biodegradation assessment of NAs and shows promise for effective initial profiling of commercial NA UVCBs for subsequent persistence assessment (Han, Scott et al. 2008, Toor, Han et al. 2013).

Oleochemicals

Guidance on the description of the composition of oleochemicals has been specified by OECD (OECD 2017b). This class of chemicals have particular characteristics that enable them to be described in a relatively simple and consistent manner. They generally contain one or more alkyl groups with variable chain length, attached to a functional group. The alkyl chain groups have the following characteristics which may vary: chain length, saturation, structure (linear or branched) and position of functional group.

The following descriptors are generally used to characterise the constituents:
- Alkyl descriptor
- Functionality descriptor
- Salt descriptor

The alkyl descriptor describes the number of carbon atoms in the carbon chain. Generally the chain is assumed to be saturated and linear, unless indicated. The chain will be assumed to include even and odd numbered chain lengths, unless indicated. Oleochemicals derived from animal and vegetable oils are normally only even numbered. The functionality descriptor identifies the functional group (e.g.

amines, ester, ammonium, sulphate, etc). The salt descriptor identifies the cation/anion of any salt present, e.g. sodium (Na+), carbonate $(CO_3)^{2-}$. In addition to the structural description, typical, minimum and maximum concentration values for given constituents should be established. It is recommended to analyse a number of separate batches over a period of time (typically five). The above guidance is considered sufficient to adequately describe the composition of oleochemicals (OECD 2017b). Generally degrees of branching are not discriminated in this approach. However, it should be noted that this may be important for the biodegradation (and hence persistence) of the constituent. Therefore, it is recommended to consider additional investigation of branching for the purposes of persistence assessment.

Other examples of substance characterisation

Detailed compositional characteristics and specific methods for characterisation have also been previously described for other substance types such as creosote (Gallacher, Thomas et al. 2017), tall oils (Magee and Zinkel 1992), turpentine (Masten and Haneke 2002), resin acids (Luchnikova, Ivanova et al. 2019) and chondroitin sulfate, a natural polymer isolated from animals and used in the pharmaceutical and neutraceutical industries (Burns, Walker et al. 2018).

4.2 Assessment approaches

Following the initial profiling of a substance, an assessment approach should be selected based on the nature and composition of the substance under investigation. Three assessment approaches are presented: 'known constituents', 'fraction profiling' or 'whole substance'. The choice of assessment approach should be clearly documented and justified. It is recognised that feasibility and proportionality of efforts may play a role in the choice (ECHA 2017b).

Although this document is specifically focusing on the persistence assessment, the guidance for UVCB substances under REACH is provided for the PBT/vPvB assessment. It must be recognised that an approach developed for persistence assessment only may not be sufficient to fully address the requirements as, depending on the specific regulatory purpose, the bioaccumulation potential and toxicity of specific constituents or fractions may need to be considered also. It will therefore not necessarily be sufficient to conclude that a UVCB is P/vP based on a limited part of the substance composition without also considering other applicable hazard properties. Otherwise, the P, B and T assessment conclusions may correspond to different constituents or fractions and therefore fail to provide a coherent PBT/vPvB assessment. It is therefore important to consider the whole substance composition, and ensure that persistence assessments overlay and address the same chemical 'space' as that of bioaccumulation (B/vB) and toxicity (T) assessments conducted on a substance.

4.2.1 Known constituents approach

What is it?

This approach can be used when specific constituents in a substance are *"a priori"* known represent a worst case of P, B and T properties for all constituents in the substance, and that these can be isolated, synthesised or acquired for purposes of testing (ECHA 2017b). It is suggested that constituents for use as 'known constituents' in assessment can be identified via screening approaches, such as read-across, QSARs and experimental testing. Such approaches are discussed in detail in the section on the 'fraction profiling' approach (See 4.2.2).

All constituents present at > 0.1% should normally be considered as relevant initially. Not all relevant constituents have to go through full testing and assessment, but a step-wise assessment is essential to focus on the known constituents which represent the worst case in terms of PBT/vPvB properties. If any of the known constituents are found to fulfil the PBT or vPvB criteria, then the whole substance is concluded as fulfilling the PBT and/or vPvB criteria and the constituent(s) causing this conclusion must be specified in the dossier (ECHA 2017b).

When is it appropriate?

The known constituents approach is most appropriate where the UVCB is well-defined and with a reasonable number of constituents (IFRA 2016, Salvito, Fernandez et al. 2020). It may be more appropriate where UVCBs follow a regular pattern in their composition, such that a reasonable worst-case constituent can be reliably identified, or when the UVCB constituents are structurally very different from each other and don't readily warrant grouping into fractions. Care needs to be taken that none of the non-evaluated (e.g. minor) constituents are expected to pose a greater hazard than those that are being evaluated (Salvito, Fernandez et al. 2020).

Advantages

This approach is seen as advantageous as it allows assessments to be based on direct testing of pure mono-constituent substances, which do not have the significant technical challenges of testing whole UVCB substances. It can also potentially simplify assessment efforts in limiting the number of UVCB constituents requiring detailed testing and/or assessment.

Challenges

In practice, this approach may be better suited for the positive identification of PBT/vPvB substances than the reverse. The reason for this is that it can often be difficult to identify a finite number of constituents that can unequivocally represent the worst-case for P, B and T properties. Each constituent will need to be tested and any one can lead to a positive conclusion for a substance. Further, it can be difficult to define a point when enough constituents have been tested to address the complete composition, and the assessment can stop. This is especially the case for very complex UVCB substances with a wide variety of structural classes present.

Identifying worst-case constituent(s) from screening data can be difficult. For example, in most cases the only screening data that will likely be available are those from QSARs. However, the applicability of constituent structures to QSARs (where available) will have to be assessed, and could vary between constituents. Moreover, different QSARs have been known to give conflicting results (Wassenaar and Verbruggen 2021). In rare cases where experimental biodegradation screening data are available, these are widely recognised as being subject to high variability and therefore difficult to compare (Kowalczyk, Martin et al. 2015).

The known constituents approach relies on identifying worst case constituents for each of P, B and T properties. However, as these different properties are typically independent of each other, this pursuit may be problematic. For example, one constituent may have a higher log Kow and thus screen as more likely to fulfil bioaccumulation (B/vB) criteria, but another constituent may have a higher degree of branching suggesting greater persistence. This situation is made even more complicated by the potential introduction of mobility (M/vM) criteria (see section 5.3). As a result, there may be several constituents that arise as potential candidates for testing under the known constituents approach. Where considering only the property of persistence, a known constituents approach may be more

straightforward. However, as discussed it can be difficult to identify worst-case constituent(s) for persistence assessment with adequate confidence.

Due to the practical limitations of the known constituents approach, and the fact that REACH mandates an assessment of all relevant constituents, additives and impurities, it is anticipated that this approach is likely to be appropriate only in few cases to adequately address this requirement. In reality, most UVCB substances would require some form of grouping of their constituents in order to assess the persistence of all their relevant constituents, additives and impurities, and such an approach is more akin to the 'fraction profiling' approach by definition. Indeed, this approach will likely be necessary to identify those constituents/fractions representing the worst-case. This does not preclude only a subset of these identified fractions (i.e. the worst-case) ultimately being taken forward for detailed evaluation. Therefore, the 'known constituent' approach is arguably not a distinct approach on its own, but rather a means of evaluating relatively simple UVCB substances, after having performed an initial screen and fraction profiling of their constituents. This characterisation of the approach is reflected in other recent reviews of UVCB evaluation approaches (Leonards 2017, Salvito, Fernandez et al. 2020). Of course, the approach can still be applied for the positive identification of individual P/vP constituents, without considering other parts of the composition, which in itself may be sufficient to conclude the PBT/vPvB properties of the UVCB substance.

4.2.2   Fraction profiling approach

What is it?

The fraction profiling (or "block profiling") approach involves the dividing of a UVCB substance composition into fractions/blocks of structurally similar constituents. As such, constituents within these fractions are grouped and expected to have similar PBT/vPvB properties, or for these to follow a regular, predictable pattern (ECHA 2017b). In this case the fraction can be considered to behave as if it were a single constituent, or in a predictable manner. As such the process of fraction profiling of a UVCB is similar to carrying out read-across between substances (only in this case it is read-across between similar constituents within the same substance). The concentration cut-off for relevant fractions is typically ≥ 0.1% in analogy to individual relevant constituents.

For testing and assessment of the fractions, this may be undertaken using representative single mono-constituent test substances. Alternatively, a physical fractionation of the test substance may be carried out, or a similar multi-constituent or UVCB substance may be obtained. Several examples from literature have been described of fractionation techniques being applied for the separation of UVCB samples for further assessment (Leonards 2017, Van Mourik, Janssen et al. 2021). However, the successful physical separation of a UVCB substance into fractions for PBT/vPvB assessment seems yet to have been demonstrated.

As with the known constituents approach, the fraction profiling approach is generally intended to consider P, B and T properties collectively, although it can also be applied to persistence on its own. Where possible, fractions should be defined encompassing P, B and T properties. However, it's also possible to build fractions for each of the properties separately, and then to recombine assessments to arrive at the overall PBT and/or vPvB conclusions for a substance.

When is it appropriate?

The fraction profiling approach can be considered universally applicable to any UVCB substance. This is because the approach can be applied to any example, no matter how simple or complex. However, it might generally be considered more appropriate for more complex UVCB substances that are not

amenable to the whole substance approach. Also, where UVCB substances contain structurally similar constituents that lend themselves to being grouped together. The fraction profiling approach might therefore be considered the default assessment approach, with the other assessment approaches being considered case by case if the specific UVCB substance composition permits.

Advantages

The fraction profiling approach has advantages over the known constituent approach in that it enables a complete assessment of the UVCB composition. It also provides a more targeted and refined assessment than the whole substance approach. As discussed, it may be the only practical option for more complex UVCBs (ECHA 2017b).

Challenges

Depending on the complexity of the UVCB composition and the number of fractions identified, a large amount of data can be required to adequately assess the substance. Especially considering the highinformation requirements for assessing persistence even for mono-constituent substances. This includes the requirement to assess persistence for five different environmental compartments, consideration of non-extractable residues (NER) and the formation of transformation and/or degradation products (see Sections 5.1 and 5.2). It can readily be seen how assessment of potentially many individual constituents or fractions to that level of detail to address the persistence of a single UVCB substance may not be feasible or proportionate (Davenport, Curtis-Jackson et al. 2022). Thankfully, many UVCBs originate from families of similar substances, and the investigations for one may be relevant to others, particularly when applying the fraction profiling approach.

Additional challenges are that it may be onerous to comprehensively generate representative constituents and define appropriate fractions/blocks. Also, it may be analytically challenging to confirm the presence of identified constituents and/or quantify fractions. Further, it may be technically challenging to obtain suitable fractions or representative constituents for testing, e.g. through physical separation, synthesis or commercial availability (Davenport, Curtis-Jackson et al. 2022).

Description of the process

The fraction profiling approach can be described in a series of steps (Figure 4). In reality, the process will likely be iterative, and involve sequential refinements to the generated constituents, screening of the properties and grouping into fractions. These iterations will likely be needed before the constituents/fractions requiring detailed assessment can be identified, and any testing required to conclude the assessment adequately can be carried out.

Figure 4 Sequence of steps involved in the fraction profiling approach.

Following initial profiling, substances will likely have undergone a tentative grouping of constituents based on structural similarity and/or generic compositional descriptors. This may form the basis for the subsequent approach by fraction profiling. However, this may be further refined following screening of the persistence of the constituents.

If the substance constituents have not yet been grouped into fractions, this should be the first step in the process, and follow the principles described in the initial profiling section based on structural similarity (Section 4.1). Following this, the persistence of each defined fraction should be initially assessed. This will normally be done based on screening approaches, such as QSARs, read-across and screening biodegradation testing of the constituents contained within the fractions. If these constituents are not yet analytically confirmed, there may be a step required to identify relevant representative constituents that can subsequently be assessed. This may involve generating theoretical constituents based on an understanding of the substance composition (see below).

The results of this screening process will hopefully confirm that the initial grouping is appropriate. However, it may be the case that fractions should be revised to better account for the distribution of persistence properties across the various constituents present. These revisions may constitute redrawing boundaries of specific fractions or grouping the constituents in a different way. Computational approaches to grouping may also prove useful in arriving at the optimum fraction profiling of the substance (see below).

It should be borne in mind when carrying out fraction profiling that the determined fractions may be required to be separable and isolable from the UVCB substance (if testing of fractions is required), and/or quantifiable by chemical analysis (if comparison to the ≥0.1% w/w concentration cut-off is necessary).

Following the finalisation of fraction profiling and screening of the persistence of the fractions, it should be possible to identify those fractions that are potentially requiring further assessment of persistence. This may be based purely on the persistence, or take into account other properties, such as bioaccumulation and toxicity, in order to arrive at a set of fractions which require further, detailed evaluation.

Of those fractions that require evaluation, it should be decided whether this is to be based on testing of the isolated fraction or on constituents representative of the fraction. In the latter case, representative structures can be either theoretical, or analytically confirmed. It is important that the constituent is indeed representative of the fraction in question. If persistence properties of the constituents within a fraction are expected to be relatively narrowly distributed, this may not be an issue. However, in other cases it may be necessary to select a reasonable worst-case constituent, or further analyse, reorganise or subdivide the fractions. Other considerations in constituent selection are whether the constituent is commercially available, or whether it can be synthesised to a reasonable purity with proportionate effort (Davenport, Curtis-Jackson et al. 2022).

Once the representative fraction(s) or constituents have been selected, these can be taken forward in a testing programme similar to that of a mono-constituent substance to determine their persistence. The testing of an isolated fraction of a UVCB substance may be expected to have similar challenges as to the whole substance approach to UVCB assessment (see Section 4.2.3).

Further guidance on the various steps described and examples of the process in practice are provided in the following sections.

Generating representative constituents

Unless analytically confirmed, constituents will need to be defined on a theoretical basis, based on the understanding of the UVCB substance composition. This can be informed by the initial profiling step, in which 'generic' constituents may have been defined based on limited information from chemical analysis, physicochemical properties, source material, process conditions, etc. The most obvious

option for generating constituents is to produce these manually, by drawing example structures that meet the specific criteria of what is likely to be present. However, it should be recognised from the outset that this approach is unlikely to be comprehensive, and could result in an imbalance or bias in the distribution of generated constituents.

Another option is to use computational approaches to generate a more comprehensive list of structures that are theoretically plausible and therefore assumed to be relevant for the UVCB substance. Dimitrov et al. (2015) describes the challenges in describing the structural composition of UVCBs and sets out a methodology for the coding, generation and selection of representative UVCB constituents. Generic structural information for a UVCB is encoded in an extension of SMILES code named Generic SMILES (G SMILES). Further, a second-level notation named Generic Graph (G Graph) is used to select representative constituents based on physicochemical properties, process of formation and options for constituent generation (Dimitrov, Georgieva et al. 2015). The approach has been used for the generation of representative constituents of petroleum UVCBs for subsequent environmental fate and hazard assessment. Similar *in silico* approaches have also been described for the generation of potential constituents of surfactants (Sauer, Barter et al. 2019).

Kutsarova et al. (2019) expanded on the methodology described by Dimitrov et al. (2015), which may result in a large number of potential representative constituents requiring assessment. A statistical selection approach was developed whereby a sample containing the minimum number of generated constituents required to address the structural variability and properties of concern within a predefined tolerable error could be identified (Kutsarova, Yordanova et al. 2019). This approach may allow the number of identified constituents of a UVCB requiring assessment to be reduced to a more manageable number.

In generating theoretical representative constituents of UVCBs for assessment of persistence, it should be borne in mind that there is an inherent uncertainty concerning the actual presence and concentration of these constituents in the UVCB substance. It is recommended to use multiple lines of evidence to inform the generation of UVCB constituents, to increase confidence that they are indeed relevant to the UVCB in question. This may be based on similar principles to initial profiling of the substance. Analytical confirmation of the presence of representative constituents is preferred, where possible.

Constituent grouping approaches

The concept of grouping constituents of petroleum substances into blocks for environmental risk assessment has been established for some time (Concawe 1996). The so-called 'hydrocarbon blocks' are typically defined based on carbon number and structural class (Table 2). These blocking schemes can be effectively informed by the results of complementary GCxGC analysis. This approach has been implemented in a number toxicity and risk assessment tools, as well as for the PBT/vPvB assessment of petroleum substances (Redman, Parkerton et al. 2012, Redman, Parkerton et al. 2014, ECHA 2017b, Camenzuli 2019, Concawe 2019).

Table 2 Example representation of hydrocarbon block method to describe petroleum substance composition. Mass fractions (% w/w) are specified for each block, defined by carbon number and hydrocarbon structural class. n-P: normal paraffin; i-P: isoparaffin; MonoN: mono-Naphthenics; Di-N: di-Naphthenics; Poly-N: poly-Naphthenics; MoAr: Mono-aromatics; NMAr: Naphthenic mono-aromatics; DiAr: di-aromatics; NDiAr: Naphthenic di-aromatics; TriAr: tri-aromatics; NTriAr: Naphthenic tri-aromatics; PolyAr: polyaromatics. Adapted from Leonards (2017).

Block	Carbon no.	n-P	i-P	Mono N	Di-N	Poly-N	MoAr	NMAr	DiAr	NDiAr	TriAr	NTri Ar	Poly Ar
1	10												
2	11												
3	12												
4	13												
5	14												
…	…												

Hydrocarbon blocks have been populated with constituents generated by the approaches described in Dimitrov et al. (2015), and based on knowledge of the source, manufacturing processes and occurrence of hydrocarbons in petroleum substances (Camenzuli 2019). The properties of these constituents have been screened using the BioHCwin QSAR, which has previously been demonstrated to be suitable to predict the degradation half-lives of hydrocarbons (Prosser, Redman et al. 2016). The matrix has been further populated with experimental biodegradation data from various non-standard experiments on individual hydrocarbons and dispersed oil (Brakstad, Nordtug et al. 2015, Birch, Andersen et al. 2017, Birch, Hammershøj et al. 2018, Ribicic, McFarlin et al. 2018, Hammershoj, Birch et al. 2019).

Strategies for grouping per- and polyfluoroalkyl substances (PFAS) have recently been discussed (Cousins, Dewitt et al. 2020). This large family of substances includes at least 4700 known structures, and grouping approaches are needed to support their prioritisation and evaluation. Grouping strategies were subdivided into those based on intrinsic properties (e.g. persistence, bioaccumulation potential, toxicity, mobility, molecular size) and those that inform risk assessment through estimation of cumulative exposure/effects. Grouping based on (known) intrinsic properties is a useful approach for UVCB constituents as a means of screening out those which may not require persistence assessment, as they do not fulfil other properties of concern that are prerequisites (in combination with persistence) for further regulatory action. The 'arrowhead' concept, whereby structures have common degradation products, may also be of interest in some cases as an approach for the grouping of UVCB constituents.

The principles of grouping UVCB constituents into fractions can be considered similar to those of read-across between mono-constituent substances (Cronin 2013, OECD 2017a). This is generally performed on the basis of structural similarity, although a scientific basis to justify the read-across is also important. Read-across can be supported by a range of evidence, including *in silico* predictions (Cronin 2013). For human health endpoints there has been considerable effort to explore the use of so-called 'new approach methodologies' (NAMs), such as *in vitro* tests and toxicokinetic models (Grimm, Iwata et al. 2016, Stuard and Heinonen 2018, Escher, Kamp et al. 2019, Sauer, Barter et al. 2019, House 2020). The utility of such approaches to support persistence assessments is unclear. However, the studies may provide sources of inspiration for the development of similar strategies for persistence assessment.

The principles of acceptable read-across under REACH are outlined in the ECHA read-across assessment framework (RAAF) and can be used as a basis to establish, justify and refine the fraction profiling of UVCB substances (ECHA 2017e). However, it should be noted that the full requirements of the RAAF are typically more onerous than would be feasible to undertake for the complete UVCB composition. A specific RAAF document was also prepared for UVCBs. However it was

acknowledged that this did not constitute a full extension of the RAAF to UVCBs due to the complexity of such approaches, and that more work was needed (ECHA 2017c).

In a related example, Wassenaar & Verbruggen (2021) have described a PBT assessment of 3-ring PAHs as an assessment entity. This assessment entity consists of phenanthrene, anthracene and alkylated varieties. The assessment uses the SVHC classifications of phenanthrene and anthracene as a basis and then proposes to apply grouping/read-across principles to extend these determinations to the alkylated varieties (Wassenaar and Verbruggen 2021). In the case of persistence, this has been justified on the basis of QSAR predictions (Biowin3 & BioHCwin) and non-standard experimental aqueous biodegradation data. These experimental studies were considered not relevant for direct comparison of derived half-lives to Annex XIII criteria, but were used in a trend analysis as evidence that degradation half-life increases with carbon number. The persistence classification of phenanthrene has been disputed (Hughes, Brown et al. 2020).

Read-across principles have been applied in the biodegradability assessment of structurally related fatty acid amides and *N*-alkyl polypropylene polyamines (NAPPs) (Geerts, Kuijer et al. 2014, Geerts, van Ginkel et al. 2017). In both cases, degrading organisms were found to have broad substrate specificity and this facilitated read-across of results to a broader range of related compounds. In the case of NAPPs, degradation was independent of alkyl chain length. Similar findings were observed in a study on biodegradation of linear alkylbenzene sulfonates with C10-C14 alkyl chains in sediments and river water (Larson 1990). These findings may support better-informed fraction profiling of related UVCBs.

In the determination of fractions/blocks for persistence assessment and the assignment of constituents to those fractions, various computational approaches are available and may be of use. For example, Shah et al. (2016) describe a Generalised Read-Across approach (GenRA), whereby an algorithmic approach is used to make read-across predictions of toxicity using a similarity weighted average based on chemical or bioactivity descriptors (Shah, Liu et al. 2016). The approach was also expanded to analyse the impact of physico-chemical property information, as a surrogate for bioavailability, on the read-across performance (Helman, Shah et al. 2018). In a similar study, RepDose and ELINCS databases were combined to form a common database for the identification of categories, containing physicochemical and toxicological data (Batke, Gütlein et al. 2016). This database was refined and curated for cluster analyses, applying the Predictive Clustering Tree (PCT) approach to detect groups of chemicals with similar toxic profiles and pathways/mechanisms of toxicity.

A hierarchical chemical clustering approach has been developed to support the assessment of structurally similar fragrance ingredients (Date, O'Brien et al. 2020). In this approach, discreet organic chemicals used as fragrance materials have been clustered into groups to support read-across approaches. A tiered system of classification based on (1) organic functional group, (2) structural similarity and reactivity of hydrocarbon skeletons, (3) predicted or experimental Phase I and Phase II metabolism, and (4) expert pruning, has been applied in the context of specific toxicity endpoints. The result is clusters that can be visualised in a top-down hierarchical clustering tree. This approach shows promise for the rapid screening and grouping of UVCB constituents into fractions for further evaluation.

Similarly, Yordanova et al., (2021) described a hierarchical clustering methodology to group individual chemical constituents into small sets that are similar to each other with respect to more than one criterion (Yordanova, Patterson et al. 2021). Selection of constituents can be based on either the most 'conservative' constituent, or on identifying a small representative set of constituents that cover the structural and metabolic diversity of the whole UVCB.

Screening constituent properties and data generation

QSARs are the most likely tools to be used for initial screening of the persistence of UVCB constituents. Commonly used QSAR tools include Biowin, BioHCwin and CATALOGIC (Boethling, Lynch et al. 2003, Howard, Meylan et al. 2005, Dimitrov, Pavlov et al. 2011). More recently, novel QSARs have been developed. For instance, two novel QSARs were developed for predicting ready biodegradability of fragrance substances (Ceriani, Papa et al. 2015). The models in this study were based on two classification methods: classification and regression tree (CART) and k-nearest neighbors (kNN). The resulting QSARs were found to perform better that BIOWIN6 for correctly classifying non-readily biodegradable fragrance materials. A separate novel QSAR has also been developed for assessing the primary degradation of hydrocarbons in aquatic systems (Davis, Camenzuli et al. 2022). This employs a novel machine-learning System-Integrated Model (HC-BioSIM), which integrates chemical structure and test system variability in order to improve the quality of predictions. The PROMETHEUS software tool also contains novel QSARs for the identification of PBT substances (Roncaglioni, Lombardo et al. 2022).

In addition to the use of QSARs, experimental studies may be performed to inform the persistence assessment. This could be at the screening level, e.g. to identify those constituents/blocks of potential concern and requiring more detailed assessment; or at the definitive level, to derive degradation half-life data that can be compared directly with persistence thresholds.

Novel and non-standard approaches

In the case of screening, the most obvious approach may be to carry out OECD screening biodegradation studies according to the Integrated Testing and Assessment Strategy (ITS) for persistence on individual constituents/fractions (ECHA 2017b). However, novel methods have also been developed, which enable biodegradation data to be generated at lower cost or in greater volume. This can be very advantageous for the assessment of UVCBs, where a large amount of data may be required to reach conclusions for the various constituents/blocks of interest. Indeed, novel methods may be essential to adequately address certain UVCBs with difficult test properties (Whale, Parsons et al. 2021). Existing challenges and question remain as to the relevance, and therefore regulatory acceptance of such approaches (Wassenaar and Verbruggen 2021). Some of these potential approaches are discussed below.

High throughput screening methods for biodegradation testing have been developed. Two miniaturised systems based on dyes were evaluated (Cregut, Jouanneau et al. 2014). One system employed an invasive fluorescent dye that serves as a cellular activity marker and the other used a non-invasive fluorescent oxygen optosensor dye. Both systems were found to be feasible for evaluating and screening chemicals for ready biodegradability. In a separate study, a further high-throughput biodegradation system employing 96-well plates was developed for aromatic test compounds (Martin, Goodhead et al. 2017). This system measured biodegradation based on detection of azo-dyes produced by reaction of the test compounds with 4-nitrobenezediazonium tetrafluoroborate (4-NBTFB). The system was utilised to assess the biodegradabilities of a range of structurally diverse aromatic compounds and was found to produce analogous results to conventional ready biodegradability tests. The system was proposed as a means to rapidly screen large numbers of chemicals.

Testing multiple constituents simultaneously

One obvious approach to increasing the amount of data generated in biodegradation testing is to assess more than one constituent in a single experiment. If this can be performed reliably and the degradation of the individual constituents measured, then this would provide data for more substances for the same number of biodegradation tests. In situations where substances do not have challenging test properties, mixtures of test compounds could be introduced to the test system individually, or as a mixture via a pre-prepared stock solution.

In the case of poorly soluble substances, a passive dosing method has been developed to prepare solutions containing mixtures of constituents at low concentrations and assess their biodegradation. The approach has been successfully applied for both hydrocarbons and fragrance substances (Birch, Andersen et al. 2017, Birch, Hammershøj et al. 2017, Birch, Hammershøj et al. 2018, Hammershoj, Birch et al. 2019, Hammershøj, Sjøholm et al. 2020). In these experiments, passive dosing was used to generate aqueous stock solutions of composed hydrocarbon mixtures. These stock solutions were then added to environmental surface water samples in 20 mL vials which were incubated on a roller mixer. Vials sacrificed at various timepoints were analysed using automated headspace solid-phase microextraction (HS-SPME) and primary degradation rates of the test analytes was determined based on their disappearance relative to solutions in ultrapure water. This methodology has proved effective at measuring primary degradation rates of hydrophobic and volatile test chemicals in environmental surface waters at low, environmentally relevant concentrations. It offers several benefits over the standard OECD 309 method, including increased sample numbers (greater data density), minimised sample handling and processing, and avoiding the use of organic solvents in dosing.

It is also possible to derive data for use in the fraction profiling approach from experiments conducted on whole UVCBs or fractions of UVCBs. If the degradation of individual constituents can be tracked analytically then results can be used to derive degradation data for multiple constituents in a single test, under conditions where these constituents are present with other constituents of the UVCB. These conditions are potentially more relevant for UVCB assessment than testing individual constituents in isolation. This approach has been applied in various biodegradation experiments (Prince, Parkerton et al. 2007, Brakstad, Nordtug et al. 2015, Hammershøj, Sjøholm et al. 2020, Møller, Birch et al. 2021). These types of experiments are discussed in more detail in the section on the whole substance approach.

Regulatory concerns with testing multiple constituents simultaneously

Experiments where multiple constituents are tested at once have clear advantages. However, there are concerns related to the regulatory acceptability of such data. One of the main concerns is that the degradation of tested constituents may be influenced by the presence of other constituents (ECHA 2017d). It is suggested that the potential influence on degradation rates could be either positive or negative (Wassenaar and Verbruggen 2021). Overall, there does not appear to be a consensus in the literature on this issue and data are limited, especially under conditions commonly used in determining degradation half-lives for persistence assessment (i.e. at low, environmentally relevant concentrations) (Hammershoj, Birch et al. 2019). However, a recent study comparing degradation rates of single chemicals with those same chemicals in composed mixtures did not show significant differences, suggesting that it may be possible to carry out reliable biodegradation testing of composed mixtures using this approach (Hammershoj, Birch et al. 2019).

It has also been suggested that testing UVCB constituents together may not be relevant due to the partitioning and separation of constituents that takes place once they are emitted into the environment. To address this concern, it may be useful to utilise multimedia fate modelling to assess the likelihood

that such a separation would indeed take place. This could be addressed with the use of fugacity models (Redman, Bietz et al. 2021). For example, a simple equilibrium distribution calculation using a Level I model would allow the partitioning behaviour of different UVCB constituents to be compared. More sophisticated Level II or Level III modelling could be incorporated to take emission routes and degradation processes into account (Hahn and Klein 2022). It could be suggested that if the distributions of constituents within the tested environmental compartments indicate that constituents would be expected to co-occur, then it should be acceptable to test the biodegradation of these constituents in the same test system for purposes of the assessment of the UVCB.

A further issue affecting regulatory acceptance is the fact that many of these approaches use non-standard experimental systems and an assessment as to their relevance to the particular question of persistence must be made. In particular, aspects such as test substance loading and dosing methods, use of adjuvants, addition of nutrients, test system geometry, numbers of time points and replicates, data treatment and endpoints measured may all affect the perceived reliability and relevance of the resulting data (Wassenaar and Verbruggen 2021). The importance of these factors may vary depending on whether the data are to be used for screening the various fractions to identify constituents/fractions requiring further assessment, or for deriving half-lives for direct, definitive comparisons to regulatory persistence cut-offs. As a general precaution it is prudent to explore the regulatory acceptability of test methods, at least in cases where they are to be used for the latter purpose.

Further examples

In the case of essential oils, sesquiterpenes have been identified as a potential constituent of interest for persistence assessment due to their relatively high log K_{ow} values (>4.5) (Jenner, Kreutzer et al. 2011). A screening assessment using standard and prolonged OECD 301F testing of 11 cyclic sesquiterpenes from 10 different families of sesquiterpenes was carried out. Biodegradation QSARs were found to be of limited use as the substances were outside their applicability domains. The OECD 301F testing revealed that most exceeded the pass level of 60% degradation. Of those remaining, two achieved >50% degradation and a third was concluded to have undergone complete primary degradation through specific analysis.

A separate study by Seyfried and Boschung (2014) was carried out to investigate the biodegradability of fragrance substances containing quaternary carbons, which have been associated with poor biodegradability (Boethling, Sommer et al. 2007). In this study, data from OECD 301F screening tests were compared to Biowin and Catalogic QSAR predictions for 27 test compounds. 37% of these substances met the pass criteria after 28 days with a further 26% indicating partial breakdown (≥20% biodegradation). A comparison of structural analogues found that substances with lower water solubility due to the presence of acetate esters or absence of oxygen were found to degrade slower than related structures that did not have these features (Seyfried and Boschung 2014). This likely reflected the difference in bioavailability, which is a known confounding factor in the OECD 301F test (OECD 1992, ECHA 2017d). It was found that both of the employed QSARs failed to accurately represent the potential biodegradability of fragrance compounds containing quaternary carbons. This highlights potential limitations in the over-reliance on QSAR predictions for the screening of UVCB constituent properties, particularly where these constituents are outside the applicability domains of the models.

The effect of ethoxylate number and alkyl chain length on the biodegradation of linear alcohol ethoxylates has been investigated in a manner similar to the fraction profiling approach (Itrich and Federle 2004). Individual homologues were tested separately in batch activated-sludge die-away tests. These included ^{14}C-(ethoxylate) $C_{14}E_1$, $C_{14}E_3$, $C_{14}E_6$, and $C_{14}E_9$ homologues to investigate the

effect of ethoxylate number, and ^{14}C-(ethoxylate) $C_{12}E_6$, $C_{14}E_6$, and $C_{16}E_6$ homologues to assess the effect of chain length. This selection of homologues provided reasonable coverage for the typical range of constituents found in detergent-range linear alcohol ethoxtylates, reported to be C_{12-18} alkyl chain lengths and ethoxylate range of 1 to 22 units. The study provided detailed information on degradation kinetics, metabolite formation, mineralisation and uptake into solids. Ethoxylate number was found to have little effect on primary degradation. However, alkyl chain length had more of an effect, with C16 homologue degrading more slowly than C12 and C14 homologues. Degradation pathways were observed to vary depending on ethoxylate number, with higher ethoxylate number leading to a greater dominance of fission of the central ether bond to yield fatty alcohol and (poly)ethylene glycol degradation products. In a follow-up study, biodegradation of linear alcohols and alcohol ethoxylate substances in batch-activated sludge die away systems was evaluated (Federle and Itrich 2006). Pure homologues of [1-^{14}C] C_{12}, C_{14}, and C_{16} alcohols and the linear alcohol ethoxylates, AE [1-^{14}C alkyl] $C_{13}E_9$ and $C_{16}E_9$ were used to study degradation kinetics and formation of free alcohol in treated effluent. In this study, lower than expected levels of fatty alcohols were formed as degradation products of the alcohol ethoxylate homologues, and instead major metabolites were polar materials resulting from *omega* fission of the alkyl chain prior to or concurrent with central cleavage. Different degradation kinetics were observed between the two studies, which could potentially be explained by the different homologues used, or the natural environmental variation (Federle and Itrich 2006).

Schwarz et al., (2014) conducted an evaluation of semipolar polycyclic aromatic compounds (sPAC), which are often found in association with PAHs in UVCB substances. These compounds are structurally similar to PAHs but contain heteroatoms such as nitrogen, sulphur and oxygen. A total of 15 priority sPACs were identified based on a screening assessment of their properties and frequency of occurrence in UVCBs or environmental samples. However, available data was not sufficient to conclude the substances as PBT or vPvB. The authors concluded on the need for further data to confirm the hazardous properties of the substances, and that current regulation on PAHs bay not implicitly protect the environment from effects by sPACs (Schwarz, Behnke et al. 2014).

4.2.3   Whole substance approach

What is it?

In the whole substance approach, the UVCB substance itself undergoes testing and is assessed for its PBT/vPvB properties. This approach contrasts with the known constituents and fraction profiling approaches, where individual constituents or fractions are tested as representative/worst case candidates to assess the persistence of the substance.

When is it appropriate?

This approach is generally considered acceptable where all constituents are similar with regard to their PBT/vPvB properties (ECHA 2017b). This means that they can be assessed using non-specific endpoints, such as oxygen consumption or DOC removal in biodegradation screening tests. This may not be the only situation where the approach can be used however, if it can be justified to use whole substance testing for more complex or structurally diverse UVCBs. This would likely require more detailed information on the degradation of individual constituents, or additional lines of evidence to provide justification that the data generated are suitable.

Advantages

The approach is the most straightforward, given that the UVCB itself is tested, and there is no need to derive individual constituents or fractions for use as test items. It will typically also require less experimental work overall. As a result it is generally the preferred approach and typical starting point for a persistence assessment by actors responsible for producing and/or placing the substance on the market.

The approach may also be considered more relevant than approaches based on testing individual constituents separately, as this better reflects how the constituents enter the environment (IFRA 2016). However, it should be recognised that individual constituents may degrade and partition differently once emitted and this can influence the overall exposure profile of the UVCB. Multimedia fate modelling approaches may be useful to better understand this aspect (discussed in the fraction profiling approach section).

Challenges

From a regulatory perspective, the uncertainty surrounding the properties of individual constituents of UVCB substances means that the whole substance approach is the most open to challenge, and the known constituents or fraction profiling approaches are generally preferred as they are considered to be more transparent and less uncertain (ECHA 2017b).

Physical properties of the UVCB can also present challenges to applying the whole substance approach. For instance, many UVCB substances are of low water solubility. If the UVCB is not completely miscible in the test medium then bioavailability constraints can prevent or reduce the degradation of certain constituents. The issues of difficult substance properties in biodegradation testing and persistence assessments are discussed in more detail in the separate guidance document on this topic (Hughes, Griffiths et al. 2022).

It is acknowledged that a whole substance approach may be the only option if it is technically not feasible "within reasonable efforts" to establish the identity of the constituents to the level needed (ECHA 2017b).

Ready biodegradability testing on UVCB substances

The ECHA guidance discusses the specific case of applicability of the ready biodegradability test for UVCB substances (ECHA 2017d). By extension, this discussion can also be considered relevant to other regulatory screening tests, such as inherent tests and enhanced ready biodegradability tests. The guidance refers to OECD in determining whether the test can be considered suitable (OECD 2006):

> *"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 (surfactants)... Testing of each individual component may be costly and impractical. If a test on the mixture is performed 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. A case by case evaluation should however take place on whether a biodegradability test on such a complex mixture would give valuable information regarding the biodegradability of the mixture as such (i.e. regarding the degradability of all the constituents) or whether instead an investigation of the degradability of carefully selected individual components of the mixture is required."*

This statement would seem to support the use of ready biodegradability testing for the assessment of some UVCB substances. However, the boundaries or limits of when ready biodegradability testing is not acceptable are not well outlined.

In the evaluation of medium chain chlorinated paraffins (MCCPs), the regulatory position was clarified concerning ready biodegradation data that was available for a series of commercial MCCP substances (ECHA 2021):

> *"Several ready biodegradation screening studies under conditions of enhanced bioavailability have been performed with commercial MCCP product types. Based on the results of the screening tests, it seems that the overall level of degradation appears to decline with increasing levels of chlorination (see Table 24). Overall, these screening studies are not considered appropriate for assessing and concluding on the persistence properties of UVCB substances such as MCCP and their constituents. Indeed, based on the outcome of the screening tests and in absence of information on the degree of degradation of the individual congener groups in the tests, it can be reasonably assumed that the substances tested (see Table 24) contain potentially persistent congeners. For UVCB substances, there are uncertainties related to the screening tests where the contribution of the different congeners of MCCP to the overall degradation is unknown. Therefore screening tests without further supplementary information enabling the possibility for the dossier submitter to verify the claims made with regard to the composition of the test substance, i.e. the identity of the individual congener groups and their concentration in the substance as well as on the degree of degradation of the individual congener groups in a test, are normally not sufficient to draw conclusions on the persistence of MCCP as a substance and in particular on the persistence of its individual constituents, respectively different congener groups. That is why the outcomes of the screening tests for MCCP have been given a low weight in the weight-of-evidence assessment."*

In this case, a rather large number of ready biodegradability tests were available, covering commercially available chlorinated paraffins with narrow carbon number ranges and varying degrees of average chlorination. The main source of variability in the composition would therefore seem to arise from the degree of chlorination and position of chlorine atoms on the various congeners. This statement therefore appears to set a rather high bar for the use of ready biodegradability tests to assess UVCB substances, without further supporting information. In essence, it must be shown that there are no constituents present at > 0.1% w/w that would fulfil the persistence criteria. This should be borne in mind when considering the results of biodegradation screening tests on whole UVCB substances.

Biodegradation simulation testing on whole UVCB substances

In principle, biodegradation simulation tests can be performed on whole UVCB substances. However, there are some unique challenges to doing this, which may make it impractical. The major issue is that UVCB substances are rarely available in radiolabelled form, which means that only non-radiolabelled studies are generally possible. This raises several problems. First, several important aspects of the study cannot be measured, including the mass balance, mineralisation, transformation products and non-extractable residue (NER) formation. These elements are essential for the determination of persistence, and are therefore normally required whenever feasible (ECHA 2017b).

UVCBs that are a result of reaction masses may have a greater opportunity for radiochemical synthesis. This is because the materials can be synthesised using radiolabelled precursors, which should produce similar constituent distributions if prepared under identical conditions. Such a case

was possible in the assessment of degradation of nonylphenol ethoxylates in sediment slurries, where the ^{14}C label was situated on the aromatic ring (all six carbons) of the nonylphenol moiety (Lee Ferguson and Brownawell 2003).

A further issue is that, without radiolabelled test material, test concentrations will likely need to be higher due to analytical sensitivity. The complex nature of UVCBs also inevitably means that test concentrations need to be even higher, and even then it may not be possible to track the degradation of individual peaks via specific chemical analysis. Where test concentrations become very high these conditions no longer resemble those intended for simulation tests (i.e. low, environmentally relevant concentrations). These conditions may affect the kinetics of degradation due to the shift in the ratio of substrate to degrader (Alexander 1999). There may also be other experimental artefacts such as bioavailability limitations and microbial toxicity. Potential exceptions to this situation may be where constituents are sufficiently similar so that degradation can be expressed as an aggregate of all constituents. Or where a UVCB composition is relatively simple so that testing can be carried out at appropriate concentrations with the required analytical sensitivity. These circumstances appear to have applied in a study where linear alkylbenzene sulphonates (LAS) were tested under anaerobic conditions according to OECD 308 guidelines (Corada-Fernández, González-Mazo et al. 2018).

In short, carrying out simulation testing on whole UVCB substances is expected to be highly problematic and unlikely to lead to reliable results for use in persistence assessments. It may therefore be essential to consider constituent-based approaches if assessments are required to progress to standard OECD simulation testing.

Selection of representative test substances

UVCB substances have a composition that is inherently variable. This means that the identity and concentrations of individual constituents may vary from one instance of the substance to the next. As a result, the persistence properties of the UVCB, and the results of an experimental testing are also subject to variation. In order to address this concern, some consideration of the selection of test substance(s) is appropriate when applying the whole substance approach.

The extent to which the substance composition can vary should be well understood. This may involve considering detailed compositional information on multiple batches of the substance, which may already have been performed as part of substance identification or initial profiling activities (IFRA 2016). A screening of constituent persistence properties, similar to that described under the fraction profiling approach, may be useful to understand those which are of greatest concern with respect to persistence. A test substance can then be selected which includes these constituents in greater proportions in order to provide a more conservative/worst-case test substance. If the worst-case is demonstrated to be persistent, it may be of interest to test further batches of the substance, or individual constituents, to understand the extent to which the persistence varies between batches, and whether this is driven by particular constituents.

The evaluation of whether data from the testing of a particular UVCB substance are representative of other instances of the substance can be based on read-across principles. It should be noted here that requirements for read-across between UVCB substances were recently updated in REACH Annex XI (EC 2021):

> *"Structural similarity for UVCB substances shall be established on the basis of similarities in the structures of the constituents, together with the concentration of these constituents and variability in the concentration of these constituents. If it can be demonstrated that the identification of all individual constituents is not technically possible or impractical, the*

structural similarity may be demonstrated by other means, to enable a quantitative and qualitative comparison of the actual composition between substances."

Further, advanced data analysis and visualisation approaches are being developed for assessing similarity and grouping UVCB substances. Onel et al., (2019) proposed a framework for grouping similar complex substances using advanced analytical methods and streamlined data analysis and visualization techniques. Unsupervised and supervised analyses are used for optimal grouping of complex substances based on their analytical features. Two datasets for petroleum UVCBs were tested: 1) GC-MS analysis of 20 standard crude oil and petroleum substance reference materials, and 2) 15 gas oil samples analysed by GC-MS, GCxGC-flame ionization detection (FID), and ion mobility spectrometry-mass spectrometry (IM-MS). The unsupervised analysis used hierarchical clustering using Pearson correlation as the similarity metric. Classification models were built using the Random Forest algorithm for the supervised analysis. Quantitative comparative assessment of clustering results were presented by Fowlkes–Mallows index, and classification results by model accuracies in predicting the group of an unknown complex substance. The effect of the following aspects on the grouping quality were demonstrated: (i) different grouping methodologies, (ii) data set size, and (iii) dimensionality reduction. The effect of different analytical techniques on the characterization of the complex substances was also demonstrated (Onel, Beykal et al. 2019). This work demonstrates how the choice of data analysis and visualisation methods can impact communication of UVCB substance characteristics and evaluation of their similarity. In a separate study, Onel et al., 2018 used similar approaches for the grouping of unknown mixtures with "known" chemicals to enable read-across. In this case analytical data by IM-MS were combined with biological effects data based on *in vitro* testing of dimethyl sulfoxide (DMSO)-soluble extracts of petroleum UVCB substances. The approaches were also used to guide the design of experiments intended to identify novel enterosorbents for reducing the bioavailability of these mixtures (Onel, Beykal et al. 2018).

In a similar exercise on four petroleum substance manufacturing streams, ion mobility mass spectrometry was applied in conjunction with cheminformatics-based data integration and visualization (Grimm, Russell et al. 2017). Substance-specific signatures were derived based on the distribution and abundance of various heteroatom classes. Chemical composition similarity was evaluated based on high-dimensional substance-specific quantitative parameters including m/z distribution, drift time, carbon number range, and associated double bond equivalents and hydrogen-to-carbon ratios. The data integration and visualisation revealed group-specific similarities and batch- or manufacturer-dependent variation. Further, 'new approach methodologies' (NAM) such as *in vitro* tests have been posited as a means for supporting the grouping of complex UVCB substances (Grimm, Iwata et al. 2016, Sauer, Barter et al. 2019, House 2020). However, the potential utility of such approaches to support persistence assessment would need to be explored.

Supporting the use of the whole substance approach

Where the whole substance approach is used, it should normally be evidenced that constituents contained in the substance are expected to have similar persistence properties, and that testing the UVCB substance as a whole is therefore justified. Such evidence could be, for example, analytical data, QSAR or other experimental data informing the fate or properties of constituents.

A good example of where this has been applied is in the biodegradability assessment of a series of gas-to-liquid (GTL) fuels and solvents (Brown, Lyon et al. 2020). These UVCB substances were composed predominantly of linear and branched aliphatic hydrocarbons, and differentiated into their specifications according to boiling point range, leading to different 'cuts' of varying hydrocarbon chain length. Hydrocarbon chain lengths ranged from C_4-C_{26} across the full range of products. The branching in these GTL substances was also relatively simple, with single methyl-branched

constituents predominating up to around C_{11}, with increasing degrees of branching in higher carbon chain length constituents. The substances are therefore complex, but also constrained within reasonably narrow boundaries of the types of structures expected to be present (hydrocarbon chain lengths, structural classes, degrees of branching). Detailed analytical characterisation of each of the substances by GCxGC was available, which enabled the carbon number range and structure (including degrees of branching) of individual hydrocarbon constituents to be elucidated. This included analytical characterisation of over 60 batches of GTL gas oil, used as the feedstock to produce the various solvents, over a years' production, demonstrating a high consistency.

The various UVCB substances were subjected to biodegradation screening testing according to OECD 301B, 301F, 302B and 306. During this experimental programme various challenges were encountered and addressed related to the volatility and poor water solubility of some of the substances (discussed in Hughes et al. 2022). In order to supplement the results of biodegradation testing, test vessels were extracted and analysed to quantify the remaining test material in the test medium. This type of information can provide an indication of primary degradation, or abiotic losses in the test, which can support the interpretation of non-specific biodegradation measurements for complex substances, e.g. oxygen consumption, CO_2 production. In the case of GTL substances, varying abiotic losses were observed depending on the test system, as confirmed using abiotic, sterilised controls (Brown, Hughes et al. 2018, Brown, Lyon et al. 2020). Abiotic losses and recovered test material were combined with biodegradation measurements to produce a mass balance to identify the amount of initially added test material that remained unaccounted for (another potential loss mechanism was conversion into microbial biomass). Finally, biodegradation data on individual hydrocarbon constituents were compiled and additional predictions were performed using QSARs BIOWIN and BioHCwin. BIOWIN 2, 3 and 6 predictions were assessed according to PBT guidance to screen for potential persistence. BioHCwin half-life predictions were compared directly to persistence half-life cut-offs in water.

The study by Brown et al. (2020) provides a very detailed case study of assessing the persistence of a UVCB substances using a whole substance approach. Various lines of evidence have been combined to support this assessment, including data for constituents, which would normally fall into the category of a known constituents or fraction profiling approach. The key elements of this study are:

- UVCB substances with detailed analytical characterisation, including evidence of variation between batches
- Compositional complexity is within boundaries that are sufficiently well defined and narrow (hydrocarbon chain lengths, structural classes, degrees of branching)
- Whole substance biodegradation screening testing is supported by additional mass balance measurements to understand primary degradation and abiotic losses
- Suitability of experimental apparatus is well understood and demonstrated
- Consistency of results across multiple substances and repeated tests
- Assessments supported by additional experimental and QSAR data for known and representative constituents

The mass balance concept was also applied to UVCB substance biodegradation screening by Brillet et al. (2018). An ultimately transformed organic carbon (UTOC) concept was developed as alternative to the standard dissolved organic carbon (DOC) removal, which includes inorganic carbon resulting from respiration and carbon assimilated into biomass. A total organic carbon (TOC) analyser was used to quantify dissolved inorganic carbon, dissolved organic carbon and carbon converted to biomass. The method was demonstrated to be effective to measure a carbon balance in

biodegradation screening tests with soluble test substances (Brillet, Cregut et al. 2018). This approach can be applied to mixtures and UVCB substances to account for a greater proportion of the initially added carbon. This method therefore shows promise to support biodegradation screening testing of UVCBs, as it provides the possibility to evidence complete ultimate degradation of the substance.

In the case of the substance evaluation of medium chain chlorinated paraffins, available data included QSAR predictions of individual CP congeners, ready biodegradability tests on MCCP UVCB substances with varying carbon number range and degrees of chlorination, and an OECD 308 simulation test on C_{14} chlorinated n-alkane, 50% Cl. wt. using a non-radiolabelled test substance (ECHA 2021). In the OECD 308 test the substance was introduced via sand at a relatively high concentration (5 mg/kg dw; water solubility: 0.0061 mg/L), presumably to enable quantification by chemical analysis. The substance was not observed to degrade in the study. In the end the OECD 308 study was decisive in arriving at a conclusion of vP for the test substance, supported by the QSAR calculations and monitoring data, and reading across this outcome to C_{15-17} congener groups. The ready biodegradability tests were considered of low weight (see discussion above on use of ready biodegradability testing on UVCB substances).

Use of specific chemical analysis in whole substance testing

Testing on whole substances can be supported by analytical methods, which provide insight as to the degradation of specific constituents. This overcomes the uncertainty surrounding the degradation of individual constituents where degradation is measured based on non-specific properties, such as oxygen consumption.

The benefits of such approaches would include more cost-effective and rapid generation of data. Generating information on multiple or all constituents of a UVCB simultaneously would dramatically improve the ease at which a UVCB could be assessed, and contrast markedly with constituent-by-constituent approaches for data generation. The data generated could arguably also be considered more relevant to the UVCB, since the conditions better match the exposure profile of the substance in the environment (IFRA 2016).

One example of this approach applied droplet generators to produce dispersions of oil in seawater, which are then incubated in completely filled and sealed vessels on a rotating carousel system. Total oil loadings were kept below a level at which oxygen and available nutrients would be depleted. Primary degradation was followed by specific chemical analysis and could be optionally corrected for abiotic losses using sterilised controls and normalisation to conserved internal marker substances. Using this approach it was demonstrated that degradation was impacted by droplet size (Brakstad, Nordtug et al. 2015), but not significantly affected by the use of dispersants (Brakstad, Ribicic et al. 2018). In separate experiments the GCxGC technique has been used as an analytical support in non-standard biodegradation testing of petroleum UVCBs to provide information on the compositional changes of test substances during the course of testing (Arey, Gros et al. 2020).

Passive dosing has recently successfully been applied in the biodegradation testing of whole UVCB substances (Hammershøj, Sjøholm et al. 2020). In this study diesel and lavender oil were tested at two concentrations, one just below the solubility limit. Test vessels were analysed using headspace solid phase microextraction (HS-SPME) coupled to GC-MS. Constituent peaks were quantified and tentatively identified using appropriate software. Primary degradation kinetics of individual identified constituents could therefore be quantified using this approach. The diesel constituents degraded at comparable rates at both concentrations, but the lavender oil was found to be inhibitory at the higher test concentration. This highlights the need to test at sufficiently low concentrations, although fewer

UVCB constituents could be quantified at the lower concentrations. The study provides an important proof-of-concept and application to whole UVCBs a method that has previously been applied successfully to single chemicals and composed mixtures.

In a study by Møller et al. (2021), cedarwood oil was tested at low concentrations, with wastewater as the inoculum. In this case microvolume solvent spiking with methanol was used to dose the test substance, taking care that the amount of co-solvent applied would not lead to anaerobic conditions in the test vessels. The biodegradation of UVCB constituents was determined with automated solid-phase microextraction coupled to GC-MS/MS. Targeted analysis was used for main constituents and non-targeted analysis for minor constituents and non-polar degradation products. The targeted constituents accounted for 73% w/w of the mixture and primary degradation kinetics were successfully determined for these. Minor degradation products were also observed to degrade, and no non-polar degradation products were detected. The bioaccumulation potential and baseline toxicity potential of the mixture were calculated based on degradation of the major constituents and demonstrated to reduce substantially over the course of the experiment (Møller, Birch et al. 2021).

Passive dosing of UVCBs via silicone rods has also been compared with passive dosing via headspace and found that the exposure profiles are similar between the two approaches (Trac, Sjoholm et al. 2021). In both approaches, constituents will partition between the donor and aqueous phases according to their relative abundance and water solubility. This compares with solvent spiking methods, which introduces the UVCB substance directly into the aqueous phase, and therefore leads to a higher proportion of more poorly soluble components in the solution. Available evidence does not suggest that this is likely to have an impact on the degradation rates of individual constituents, unless the more poorly soluble constituents are at concentrations sufficient to induce toxicity to the microbial inoculum (Hammershoj, Birch et al. 2019, Hammershøj, Sjøholm et al. 2020).

Quantitative structure−persistence relationships and kinetics have been determined for commercial naphthenic acids and naphthenic acids in oil sands process water (OSPW) during aerobic microbial degradation (Han, Scott et al. 2008). A highly specific HPLC−high resolution mass spectrometry method was applied, which is able to separate naphthenic acids based on carbon number, degree of cyclisation and extent of branching. Commercial naphthenic acids were found to contain a substantial labile fraction, and a recalcitrant fraction containing highly branched compounds, whereas naphthenic acids in OSPW were mostly recalcitrant. Carbon number was found to have little effect on the rate of degradation, whereas increased cyclisation (Z) decreased biodegradation rate. In a further study, the selective degradation of naphthenic acids in two OSPWs was investigated in wetland microcosms under different hydraulic retention times over 52 weeks (Toor, Han et al. 2013). In this case, more rapid degradation was observed for naphthenic acids with lower carbon numbers and fewer degrees of cyclisation. The two most persistent groups of homologues were those with carbon numbers 17-20 and *Z* series -6 to -12.

Similarly to the examples discussed on the fraction profiling approach, many of the examples discussed here employ non-standard experimental approaches. Therefore there remains some uncertainty around their regulatory acceptability (for discussion of these aspects, see Section 4.2.2 on 'Regulatory concerns with testing multiple constituents simultaneously').

4.2.4 Further considerations on assessment approaches

Influence of UVCB characteristics on assessment approach(es)

The nature of UVCBs will have an important bearing on the assessment approach(es) suitable for assessing them. For example, if a substance has a narrowly defined composition it may be

appropriate and justifiable to use a whole substance approach. If a substance is more complex, a constituent-based approach may be more applicable. A conceptual diagram of the potential compositional characteristics of a UVCB that may inform assessment approaches is presented in Figure 5.
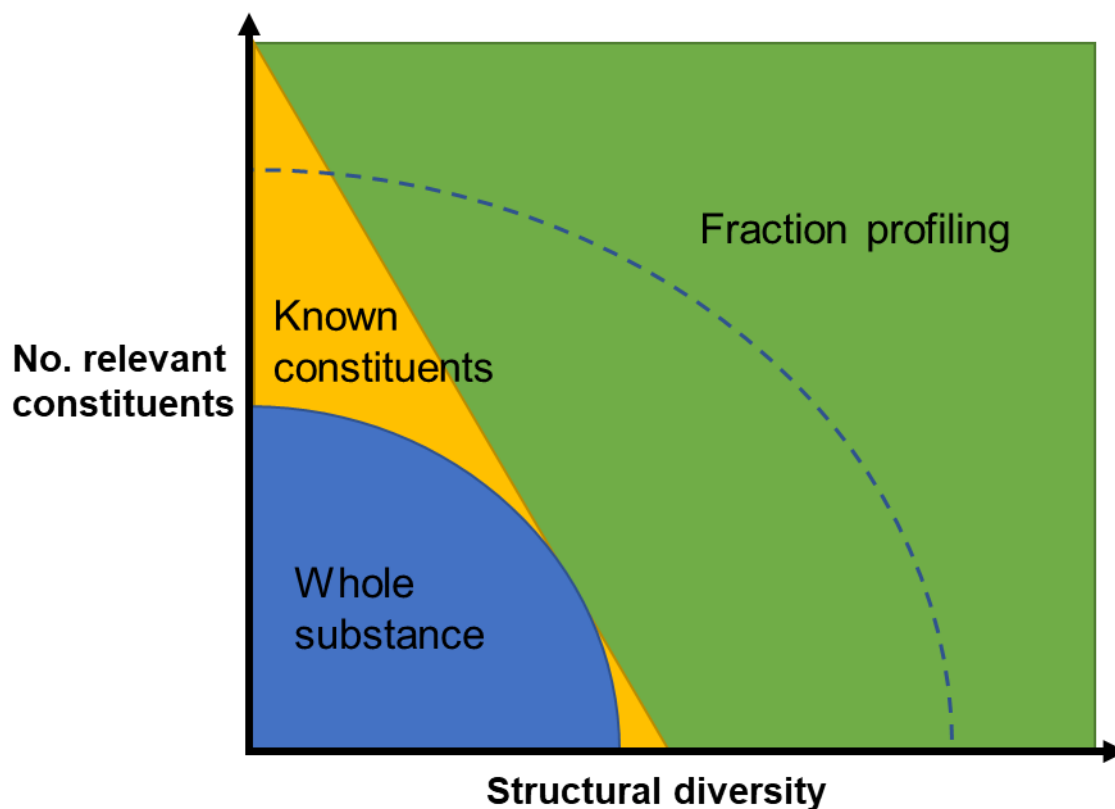

Figure 5 Conceptual illustration of how UVCB compositional complexity may inform assessment approaches. Structural diversity represents the degrees of freedom in which individual structures may vary within the substance, thus presenting distinct classes or groups of constituents requiring individual assessment. The domain of applicability of the whole substance approach may potentially be expanded, as indicated by the dashed line. Intended as a guide to inform approach development. Not to be applied rigidly.

The choice between the known constituents and the fraction profiling approach will mostly depend on the nature of the compositional complexity. If the number of relevant constituents is reasonably low, or the structural variation falls within a simple and relatively homologous series, it may be possible to identify worst case constituent(s) to apply the known constituents approach. However, more often than not the compositional complexity will be greater, for example varying across multiple degrees of freedom (e.g. chain length and structural class). This will invariably warrant a fraction profiling approach, either to group and screen the properties of the constituents (to identify worst-cases), or for the definitive assessment of those fractions. A hypothetical exception could be where the number of relevant constituents is limited, but that structural characteristics are so diverse that grouping of individual constituents is not feasible. However, such examples have not been encountered as part of this review, and some form of grouping is generally expected to be necessary to facilitate further evaluation and satisfy the requirement to assess all relevant constituents, additives and impurities.

In the case of the whole substance approach, the domain of applicability of this approach can potentially be expanded as discussed in Section 4.2.3 (Figure 5). This could be achieved in a number of ways, such as:

- Determining degradation at the constituent level during testing.
- Providing additional lines of evidence to support the approach.
- Using the approach in combination with constituent-based approaches.

Whilst the concept outlined in Figure 5 is intended to support the development of assessment approaches for UVCBs, it is important to recognised that this is only intended as a guide, and not to be adhered to rigidly. UVCB assessment invariably requires a flexible, pragmatic and bespoke approach to address the unique characteristics of the case at hand, including the properties and complexity of the UVCB in question and the compositional information available.

Combination approaches

As indicated at several points throughout this document, UVCB persistence assessment may require a combination of approaches in order to satisfactorily address the regulatory requirements. The three assessment approaches described are therefore not necessarily to be applied in isolation, but aspects of each may be used to address the requirements of a particular substance. Several examples of where such combination approaches have been used have been described in this document (Camenzuli 2019, Brown, Lyon et al. 2020, ECHA 2021). A combination approach has further been advocated in recent reviews by Leonards (2017) and Salvito et al. (2020). A flow chart for the assessment of individual UVCB substances was developed by Leonards (2017) and further adapted by Salvito et al. (2020), and is presented below (Figure 6).
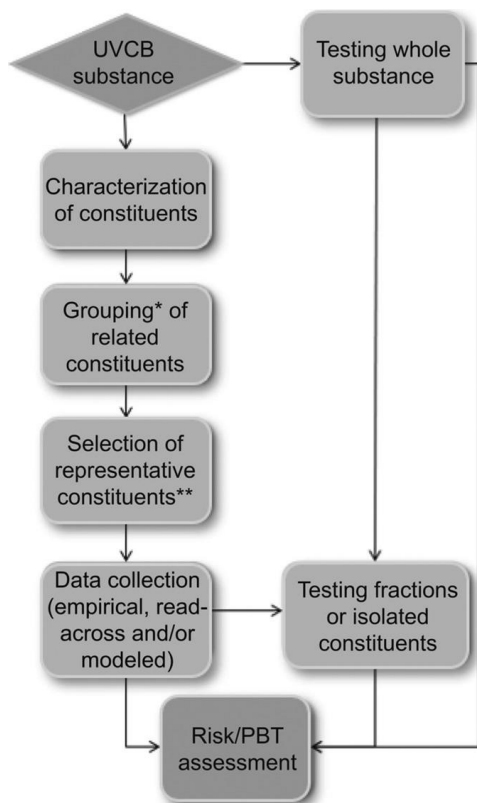

Figure 6 Potential approach for the testing and PBT assessment of a UVCB substance, from Salvito et al. (2020). *Grouping could be based on similarity in chemical class and/or mode of toxicological action. **Selection of representative constituents may be based on those that best or conservatively represent a subclass of similar constituents present in the UVCB.

Supporting further development of approaches

The information presented in this document is based on publicly available information. However, it is anticipated that significant additional insights might be drawn from the experiences of different stakeholders with the persistence assessment of individual UVCB substances. Much of this information is likely to be unpublished, or contained in parts of REACH registration dossiers that are currently not disseminated or searchable. One such example is for grease thickener substances, for which it is understood that significant efforts have been undertaken to develop approaches for the assessment of UVCB substances, and that these have been discussed with regulatory authorities (G. Whale, personal communication). It would seem beneficial that further steps were taken by all stakeholders to facilitate discussion and exchange of knowledge on UVCB substance persistence assessments, so that guidance and approaches could be further developed and disseminated.

5 Other considerations

In this section some further critical issues concerning the persistence assessments of UVCBs are highlighted.

5.1 Transformation and/or degradation products

Degradation of a substance will typically follow one or more pathways, consisting of individual transformation steps, on the path to ultimate degradation (mineralisation). These transformation and/or degradation products (TP) may also be of concern with respect to their persistence and other hazardous properties (Boxall, Sinclair et al. 2004, Ng, Scheringer et al. 2011, Fenner, Canonica et al. 2013, Fenner, Elsner et al. 2021).

According to REACH guidance, persistence assessment requires the assessment of relevant transformation and/or degradation products, and similar arguments apply as for relevant constituents, additives and impurities (ECHA 2017b). The guidance quotes the C.24 test guidelines of the EU test methods regulation (equivalent to OECD 308) as providing the transformation and/or degradation products that are relevant for PBT/vPvB assessment in the situation where simulation tests are being/have been performed:

> *"…in general transformation products detected at ≥ 10% of the applied radioactivity in the total water-sediment system at any sampling time should be identified unless reasonably justified otherwise. Transformation products for which concentrations are continuously increasing during the study should also be considered for identification, even if their concentrations do not exceed the limits given above, as this may indicate persistence. The latter should be considered on a case by case basis...."*

However, the authorities reserve the right to apply other concentration or formation rate limits on a case-by-case basis (ECHA 2017b).

The use of radiolabelled test substances is generally needed to enable TPs to be detected and identified in simulation testing (ECHA 2017d, PFA 2021). This poses a particular problem for UVCBs, as discussed in section 4.2.3 (whole substance approach). Specifically, radiolabelled UVCB substances are generally not available. Also, their complex composition raises the issue that the TPs of many individual constituents would need to be measured at once, which raises analytical challenges and the applicability of the ≥10% w/w cut-off applied for TPs of single substances. The only viable option to address the issue of TPs via simulation testing for UVCBs is to conduct testing on individual constituents. However, the subsequent evaluation of any relevant TPs formed in such simulation testing adds to the list of regulatory demands affecting the proportionality of UVCB persistence assessments, and the question of whether any relevant TPs of concern would arise from constituents that have not undergone simulation testing remains relatively unanswered.

As an alternative to simulation testing of individual constituents to identify relevant TPs, prediction of the degradation pathways and likely TPs of individual structures is possible. Several options are presented in guidance, including the EAWAG-BBD Pathway Prediction System (Ellis and Wackett 2012), the OECD QSAR Toolbox (Yordanova, Kuseva et al. 2019) and the commercial CATABOL software (Jaworska, Dimitrov et al. 2002). A further potentially useful tool is enviPath – a complete redesign and reimplementation of the Eawag BBD Pathway Prediction System, utilising relative reasoning to refine predictions and machine learning models (Wicker, Lorsbach et al. 2015). TPs that are predicted using these approaches could be further evaluated for their persistence using available

QSARs. Such an approach may be unlikely to be satisfactory on its own in terms of uncertainties surrounding the predicted TPs or their persistence properties. However, this may help to direct the assessor towards constituents and their TPs that are of interest for further assessment. The approach would provide some basis to address the explicit regulatory requirement to address TPs in the persistence assessment of UVCBs.

It should be noted that even desk studies such as this could potentially become quite demanding in terms of resource. The 'combinatorial explosion' of potential TPs arising from prediction methods, even for single substances, has been described previously (Ng, Scheringer et al. 2011). Introduce to this the large number of relevant constituents in UVCBs requiring assessment and the problem very rapidly becomes unworkable. Again, bespoke and pragmatic approaches will invariably be needed to group constituents for reliable and proportionate evaluation of their relevant TPs.

5.2 Non-extractable residues

A further challenge related to the implementation of the persistence assessment to UVCBs is the requirements concerning non-extractable residues (NER). The observation of NER is a phenomenon of simulation tests with radiolabelled test substances, in which a proportion of the applied radioactivity invariably remains in the test matrix and non-extractable. The nature and risk posed by these residues (e.g. 'safe sink or hidden hazard?') has been a topic of scientific interest and debate for some time (Nowak, Miltner et al. 2011, Kaestner, Nowak et al. 2014, Ortega-Calvo, Harmsen et al. 2015, Kästner, Trapp et al. 2018, Harmsen, Hennecke et al. 2019).

NER guidance has undergone several updates recently (ECHA 2017b, ECHA 2019). The basis for the regulatory assessment of NERs in persistence is to assume that they are a 'hidden hazard', and as such should be included in the calculated degradation half-life of substances, which is ultimately compared to compartmental cut-offs for persistence assessment. A unified approach has been developed to further divide NER into three types: Type I (physically entrapped parent substance and TPs), Type II (covalently bound residues) and Type III (biogenic residues or microbial biomass) (Schäffer, Kästner et al. 2018). Types II and III are considered as not posing a risk and can be discounted from the persistence assessment, whereas Type I should be included in the determination of the degradation half-life. Methods to carry out such characterisation of NERs remain under development. These aspects were discussed in a recent workshop by the German Environment Agency, and detailed proposals for both the methods to characterise NER types and the subsequent interpretation in persistence assessment are expected in 2022 (UBA 2021).

A clear prerequisite to implementing the NER guidance in persistence assessment is the availability of radiolabelled test substances with which to conduct simulation testing. Without these it is impossible to measure the NER fraction. As already discussed, this is generally not feasible for whole UVCBs. Therefore, it appears that the only way to account for NERs in the persistence assessment of UVCBs is to carry out simulation testing on individual radiolabelled representative constituents of the substance. This is because any attempt to derive degradation half-lives using non-radiolabelled (cold) experiments, whether they be on the whole substance or on representative constituents, will be considered an underestimate of the true half-life, had the NER have been taken into account.

The developing regulatory guidance on NER would therefore appear to be severely problematic for UVCB substances, and the efforts to improve approaches for their assessment. A literal interpretation of the position would appear to rule out definitive persistence assessment of UVCB substances by any means other than constituent-based approaches. Recall also that biodegradation screening testing of whole UVCB substances is also generally not accepted. This issue therefore raises serious

concerns for the successful and proportionate implementation of the requirements of REACH for UVCBs.

Potential solutions

It can be envisaged that there may be possibilities to apply weight of evidence approaches to account for NER in UVCB persistence assessment. For example, if primary degradation half-lives derived without considering the NER fraction are below persistence thresholds with a margin of safety it could potentially be justified that these are unlikely to be exceeded if the NER Type I fraction had been measured and taken into account. Another possibility is to determine the NER 'profile' (e.g. Types I-III, relative to parent degradation) for a representative constituent, and then extrapolate that to other constituents of the UVCB, for which only primary degradation half-lives from non-labelled experiments are available. In both these cases, considerable justification of the approach is likely to be needed. It is unlikely that this could be justified based on current knowledge of the mechanisms of formation of Type I NER across different substance chemistries. Time is needed for the required data to be generated through the implementation of the new guidance and regulatory assessment of substances.

5.3 Introduction of a PMT/vPvM hazard classes

A very important development concerning UVCB persistence assessments is the planned introduction of persistent, mobile and toxic (PMT) and very persistent, very mobile (vPvM) hazard classes under REACH as part of the EU Chemicals Strategy for Sustainability (EC 2020, Hale, Arp et al. 2020). This has been driven predominantly by a concern for persistent and polar substances to contaminate drinking water. To address this concern, criteria have been proposed for 'mobility' (M/vM) based on log K_{oc}. The persistence criteria are proposed to remain unchanged as for PBT/vPvB.

Perhaps an unforeseen and unintended consequence of the introduction of PMT and vPvM hazard classes under REACH is the impact on evaluation of UVCB substances. The current proposed approaches for PBT/vPvB assessment of UVCBs rely on the evaluation of P, B and T properties *in combination*, rather than individually (ECHA 2017b). The absence of one or more of these properties can be used deductively to remove the need to further evaluate that particular constituent or fraction. This allows the assessment to eliminate portions of the UVCB composition from further assessment, allowing efforts to focus on that which is remaining. This was an important aspect allowing UVCB assessments to remain proportionate. However, the introduction of PMT/vPvM assessment has effectively negated this possibility. The reason for this is because a substance that is not B/vB will likely be a candidate for M/vM, since both of these properties are related to the polarity of substances.

Further, assessment approaches based on grouping of related constituents into fractions, or identifying worst case constituents for assessment, will now need to consider the M property, as well as P, B and T, which will likely lead to a larger number of constituents or fractions requiring assessment. Ultimately the introduction of PMT/vPvM is likely to be very problematic for the already challenging task of assessing the hazards of UVCB substances.

6 Conclusions

UVCB substances have a complex composition that may be variable, poorly predictable and/or partially unknown. They present significant challenges in their identification and subsequent regulatory assessment of hazards and risks. In particular, they pose unique challenges for persistence assessment, which generally requires the evaluation of all relevant constituents, additives, impurities and transformation products. Compared with mono-constituent substances, the amount of data needed to address the information requirements can be extremely high and this raises issues of addressing regulatory requirements in a proportionate manner. In addition to their complex composition, many UVCBs also have properties that render them difficult to test and evaluate for their persistence. These aspects are addressed in a companion report (Hughes et al. 2022).

This report has aimed to provide guidance to support the persistence assessment of UVCB substances. This has been based on the current regulatory guidance for PBT/vPvB assessment according to REACH, and expanded based on a review of the wider literature for case examples, novel approaches and other scientific and policy developments. The document has been structured to first introduce UVCBs as a class of substances, with a number of examples which will provide case studies throughout the document. The challenges of carrying out persistence assessments of UVCBs, in terms of regulatory requirements and data generation challenges, are briefly outlined. Following this, the UVCB assessment strategy is presented. In line with REACH guidance, this begins with an initial profiling step to characterise the composition, followed by three assessment approaches: known constituents, fraction profiling and whole substance.

Each aspect of the UVCB persistence assessment strategy is discussed in detail, presenting practical approaches to address the regulatory requirements and challenges which may arise, illustrated by the case examples. For the assessment approaches, consideration is provided for when each of these may be appropriate, considering the compositional characteristics and properties of the UVCB in question. Novel approaches and developments which could be utilised to support the implementation of regulatory requirements are presented and discussed, reflecting on potential barriers to adoption. Finally, further critical challenges for the persistence assessment of UVCBs are discussed, including the requirements to assess transformation products and non-extractable residues, as well as the implications of the proposed introduction of a PMT/vPvM hazard class under REACH.

Persistence assessment of UVCB substances invariably requires a flexible, pragmatic and bespoke approach to address the unique characteristics of the case at hand, considering the properties and complexity of the UVCB in question and the compositional information available. It is hoped that this report will provide useful practical advice and sources of inspiration to enable practitioners to successfully carry out persistence assessments for UVCBs that meet regulatory requirements, whilst remaining feasible and proportionate. Also, the key ongoing challenges of conducting persistence assessments for UVCBs have been highlighted with the intention of stimulating further collaborative discussions and activities, so that regulatory guidance and the implementation of persistence assessment frameworks can continue to be improved in the future.

This document is part of a series of reports produced as part of the Cefic-LRI ECO52 project: 'Expanding the conceptual principles and applicability domain of persistence screening and prioritization frameworks, including single constituents, polymers, and UVCBs.'

7 Acknowledgements

We would like to thank members of the project research team Graham Whale (Whale Environmental Consultancy Ltd), Stefan Hahn (Fraunhofer ITEM), Dieter Hennecke, Michael Klein and Judith Klein (Fraunhofer IME), David Brown and Eleonore Delouvrier (Ricardo Energy & Environment) for their valuable contributions to this work. We would also like to thank the monitoring team for their oversight and helpful input to the project.

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