

Critical Review

Improving the Environmental Risk Assessment of Substances of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials

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Abstract: Substances of unknown or variable composition, complex reaction products, or biological materials (UVCBs) pose unique risk assessment challenges to regulators and to product registrants. These substances can contain many constituents, sometimes partially unknown and/or variable, depending on fluctuations in their source material and/or manufacturing process. International regulatory agencies have highlighted the difficulties in characterizing UVCBs and assessing their toxicity and environmental fate. Several industrial sectors have attempted to address these issues by developing frameworks and characterization methods. Based on the output of a 2016 workshop, this critical review examines current practices for UVCB risk assessment and reveals a need for a multipronged and transparent approach integrating whole-substance and constituent-based information. In silico tools or empirical measurements can provide information on discrete and/or blocks of UVCB constituents with similar hazard properties. Read-across and/or whole-substance toxicity and fate testing using adapted emerging methods can provide whole-substance information. Continued collaboration of stakeholders representing government, industry, and academia will facilitate the development of practical testing strategies and guidelines for addressing regulatory requirements for UVCBs. *Environ Toxicol Chem* 2020;39: 2097–2108. © 2020 Health and Environmental Sciences Institute. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

Substances of unknown or variable composition, complex reaction products, or biological materials (UVCBs; throughout this

article, the term “UVCB” will represent all complex chemical mixtures, and encompass the various denominations adopted by different regulatory frameworks) are difficult to characterize and thus present challenges to regulatory agencies tasked with assessing the potential risks they may pose. These substances, such as petroleum products, resins and rosins, and natural complex substances, can contain a large number of constituents (although the word “constituent” is used throughout this article, the term “component” could also be used and is sometimes preferred by certain regulatory agencies), and their composition can be partially unknown and/or variable, depending on fluctuations in their

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source material (e.g., seasonal and temporal variations in sourcing of raw materials) and/or manufacturing processes. By contrast, multiconstituent substances are better defined, with main constituent concentrations between 10 and 80% (w/w) of the total and impurities counting for <10%. Therefore, variations in a UVCB source or production process could result in compositional changes; although they may not affect the function of the substance, they may warrant a new assessment to ensure that this variation in composition remains environmentally safe. In addition, it may be technically challenging, or in some cases impossible, to identify and test the toxicity and environmental behavior and fate of each individual constituent present in a UVCB, and hence to conduct risk assessments, determine the appropriate classification and labeling needs, or perform persistence, bioaccumulation, and toxicity evaluations. The testing of the whole substance (e.g., using whole mixture testing approaches) and the interpretation of the resulting data can be equally challenging because physicochemical properties, environmental fate, or ecotoxicological endpoints characterized in laboratory settings may be different from what they could be in the real environment.

International regulatory frameworks, specifically the European Chemicals Agency's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; European Chemicals Agency 2019), Environment and Climate Change Canada's Canadian Environmental Protection Act (Environment and Climate Change Canada 2016, 2017a), and the US Environmental Protection Agency's (USEPA's) Toxic Substances Control Act (US Environmental Protection Agency 2016), have been faced with the complexities of substance identification; characterizing fate, exposure, and hazard; and ultimately assessing the potential risks associated with UVCBs. Several industrial sectors (e.g., petrochemicals, personal care products, pine chemicals) have attempted to address the problem by developing frameworks and methodologies for the characterization and analysis of complex substances, including UVCBs (King et al. 1996; Redman et al. 2014; European Federation of Essential Oils/International Fragrance Association 2015).

This review is based on the output of a 2016 workshop sponsored by the Research Institute for Fragrance Materials (RIFM) and the European Centre for Ecotoxicology and Toxicology of Chemicals, entitled "Developing a Strategy to Improve the Environmental Risk Assessment of Difficult to Test Multi-Constituent Substances," and continuing efforts of a tripartite technical committee (i.e., comprising government, industry, and academic experts) organized by the Health and Environmental Sciences Institute (HESI) to develop a risk-assessment framework for UVCBs. These ongoing efforts aim to identify best practices and key research needs to support environmental risk assessment of UVCBs (Déglin et al. 2018, 2019; Salvito et al. 2018). Despite the progress made in assessing chemical transport in food webs and risks to air-breathing organisms, basic risk-assessment paradigms are still needed in most industry sectors for chemical mixtures as complex as UVCBs (Kelly et al. 2007). Therefore, this project will initially be restricted to ecological risk assessment in aquatic organisms. In the present review, we present the state of the science and identify critical research needs.

CHARACTERIZATION OF UVCB CHEMICAL COMPOSITION

Normally, UVCBs are produced to a specification relating to their physical–chemical properties or other performance characteristics and not strictly based on a set proportion of known constituents. As a result, they can contain a large number of constituents that in many cases cannot be practically chemically identified in an analytical chemistry sense but may still meet a strict set of product performance criteria that may relate to the underlying composition. The term "large number," in the present review, refers to a "countable" number of discrete chemicals that might be separable as chromatographic peaks. In addition, composition variability (V) often results from differences in the source material and/or variations in the manufacturing process.

Given the uncertainty and variability in the composition of UVCBs, constituent identification and substance naming are difficult, which presents a major challenge for risk assessment because the chemical structure of the individual constituents determines their fate, exposure, and toxicity. In the last decade, analytical techniques for the identification of organic chemicals, and especially mass spectrometry, have significantly improved. For example, the great strides made in the field of nontargeted analysis, which allows for the rapid screening of chemical mixtures in various matrices such as water, dust, food, and so forth, are promising for the assessment of UVCBs (Escher et al. 2020). Although these techniques can generally be used to determine generic structures of UVCB constituents, they can also be time-consuming; and they require the constituents or representative constituents to be present in mass spectral databases, commercially available, or isolated from the mixture.

Existing identification approaches

Generic structures can be used for a first-tier assessment and can help direct empirical testing and in silico modeling of UVCBs. Some general approaches to uniquely identify UVCB substances have resulted in the following nomenclature rules for 3 common types of UVCBs that have been recognized by various regulatory authorities: 1) UVCBs that are extracts from plant or animal products (e.g., biologicals) are normally identified by genus and species, as well as other common names relating to the source (e.g., rose, *Rosa canina*, ext., Chemical Abstracts Service Registry Number [CAS RN] 84696-47-9). 2) Unknown or variable composition, complex reaction products, or biological materials that are the result of a chemical reaction are identified by the immediate precursor chemicals and the nature of the reaction (e.g., benzenediazonium, 2-methoxy-4-(phenylamino)-, salt with 3,5-dimethylbenzenemethanesulfonic acid [1:1], reaction products with 1-[methoxymethyl]-4-[4-methylphenoxy]benzene and 1,1'-oxybis[4-(methoxymethyl)benzene], CAS RN 121754-48-1). 3) Unknown or variable composition, complex reaction products, or biological materials that are products from industrial processes are identified with a

description of the process from which they originate and, in some cases, physical properties (e.g., fatty acids, tall-oil, low-boiling [CAS RN 65997-03-7]). Many petroleum substances fall under this type (e.g., kerosene [CAS RN 8008-20-6], light hydrocracked petroleum distillates [CAS RN 64741-77-1], or cracked steam cracked petroleum distillates [CAS RN 68477-38-3]).

Some UVCBs have been in use since the Industrial Revolution, and many are so-called existing substances in regulatory chemical inventories. Identity information for these “old substances” may be lacking, such that to do any meaningful assessment, regulatory authorities may require additional details, including unambiguous chemical identity of the major or most hazardous substance constituents (e.g., International Union of Pure and Applied Chemistry name, CAS RN, structure), compositional information (i.e., percentage of weight/weight of constituents or fractions of the UVCB), and, possibly, supporting analytical details (i.e., method of determination of substance identity and composition; Environment Canada 2005; European Chemicals Agency 2014). However, nomenclature approaches are currently not specific enough or applied with sufficient consistency to uniquely identify substances, resulting in identification issues. Examples are as follows: 1) Multiple CAS RNs (or other identifiers such as the European Inventory of Existing Commercial Chemical Substances number) that truly designate one substance (i.e., multiple registration of the same substance). 2) Two or more clearly distinct substances that fall under a single CAS RN (i.e., the original CAS RN nomenclature was too vague/broad) or other identifier. 3) Different substance names and CAS RNs or other identifiers in different jurisdictions.

The Organisation for Economic Co-operation and Development (OECD) correspondence group on the characterization of UVCB substances of the Working Party on Hazard Assessment has recently made efforts to improve the accuracy and consistency of the nomenclature used for UVCBs. This could enable regulatory authorities to increase cooperation in the field of substance assessment and could help industry address regulatory requirements from multiple jurisdictions. However, thus far, guidance has only been developed for 2 specific subcategories (i.e., chemical classes) of UVCBs: oleochemicals (plant and animal fat derivatives) and hydrocarbon solvents (Organisation for Economic Co-operation and Development 2014a, 2015). In addition, nomenclature rules and systems of characterization alone will not help reduce the uncertainty for the large number of UVCBs that still lack sufficient chemical characterization data to inform such nomenclature rules. Other initiatives seek to increase the compositional information available for UVCBs within model platforms and assessment tools such as the OECD QSAR Toolbox (Organisation for Economic Co-operation and Development 2019b) and through open-source data portals such as the USEPA's CompTox Dashboard and NORMAN MassBank (MassBank Project 2011; US Environmental Protection Agency 2017). These databases may assist in reducing the compositional uncertainty of many UVCBs of regulatory significance and provide more consistent characterization.

Characterization of UVCBs using representative constituents

Characterization of UVCB constituents can be extremely important. The most developed approach for any type of UVCB assessment is that which has been advanced for petroleum substances. It uses the hydrocarbon block methodology, which characterizes petroleum UVCBs based on “blocks” defined according to constituents' carbon chain length and chemistry. Each of these groups of structurally related hydrocarbon constituents, with similar physical–chemical, fate, and toxicity properties, is then assigned property values representing all constituents in that group (King et al. 1996; Redman et al. 2014). The idea that the fate, behavior, and toxicity exhibited by the whole substance released to the environment can be predicted using representative (in the context of risk assessment) constituents of the UVCB is taken from the hydrocarbon block methodology that may be applicable to UVCBs other than petroleum products. For example, methods for assembling constituent blocks of natural complex substances for environmental assessment are discussed in a European Federation of Essential Oils/International Fragrance Association (EFEO/IFRA) guidance (European Federation of Essential Oils/International Fragrance Association 2016). This approach allows for more refinement and consideration of additional constituents as more detailed chemical characterization information becomes available. Selection of representative structures can be based on analytical data availability, production process/sources, literature, in silico predictions, and/or experimental determination.

Characterization of UVCBs for the use of read-across

Some chemicals with similar structures or properties show consistent or predictable trends in effects. When looking at a limited number of analogous substances, effects can be “read across” from one substance to another, with some uncertainty; and thus, conservativeness is applied in some contexts (European Centre for Ecotoxicology and Toxicology of Chemicals 2012; European Chemicals Agency 2017c). These relationships also provide an argument for establishing expected chemical properties and effects for groups of analogous chemicals, termed “chemical categories.” Savings in time, cost, and animals have been the main arguments for using analogues or chemical categories for defined substances. Grouping into chemical categories and read-across from analogues are potentially powerful strategies in the hazard assessment of UVCBs because they accommodate flexibility in substance composition. For example, substances that are produced via the same process, such as distillation of solvents, can be expected to have similar compositions and similar effects. The OECD has published a guidance document on how to create chemical categories, with specific guidance for complex substances (e.g., UVCBs; Organisation for Economic Co-operation and Development 2014b).

Two situations that might be foreseen for the use of read-across for UVCBs include reading across information from a discrete substance to one or more constituents of a UVCB for which data are lacking and reading across information from a UVCB substance with data to a UVCB without data. Constituent-to-constituent extrapolation would follow the same approach as outlined for read-across between discrete substances.

A prerequisite for read-across is structural similarity, in that UVCB-to-UVCB extrapolation requires verification that both substances contain similar general classes of constituents (e.g., fatty acids, monoterpenes, isoalkanes, etc.). In addition, the relative difference in proportions of each known risk driver (blocks or individual constituents) should not vary significantly between the 2 substances, to avoid any significant difference in fate, ecotoxicity, or other properties being read across. Finally, it is important to justify why potential differences in the chemical structures should not influence the fate and toxicity of the UVCB or should do so in a regular pattern. Because of the uncertainty inherent to this approach, it may be difficult to know when similarity between 2 UVCBs is sufficient for read-across. For example, potential “matrix” effects could alter expected substance properties (e.g., synergism/antagonism, etc.). These effects are difficult to predict and can limit the accuracy of the read-across, unless the contribution of each class of constituents within the matrix to the property being read across is consistent and predictable. Some of the emerging issues that need to be addressed when applying read-across to UVCBs have been recently identified by the European Chemicals Agency (2017c). Some research has also been conducted in the area of ecotoxicology to investigate when and to what degree synergistic interactions lead to significantly higher mixture toxicity than the normally used “concentration addition” and “similar mode of action” approaches could predict (Backhaus and Faust 2012). This work, and others, could be expanded to validate principles for UVCB-to-UVCB read-across.

IDENTIFICATION OF THE FATE AND HAZARD PROPERTIES

Environmental fate and bioaccumulation

The approach chosen to assess the environmental fate of UVCBs depends on the level of understanding of their chemistry. It may be based on the whole substance, groups/blocks of constituents with similar environmental fate and toxicity properties, or individual constituents that contribute most to the potential hazard and risk (risk drivers). The fate parameters influencing exposure estimates, such as substance physicochemical properties and biodegradability, are important in assessing UVCBs. Bioaccumulation is also considered because it is vital for understanding the substance's behavior in the environment and in the food chain, including, for example, secondary poisoning in predators when biomagnification occurs. Biodegradation and bioaccumulation properties are also relevant for persistence, bioaccumulation, and toxicity/very

persistent, very bioaccumulative assessments. As stated earlier, the full characterization of UVCBs of this nature can present analytical challenges both in identification of all constituents and in achieving detection down to certain established regulatory thresholds. Some low thresholds (e.g., <0.1% w/w) can be impractical from an analytical standpoint or because of the nature of the analyte, as explained in the EFEO/IFRA guidelines on the environmental assessment of natural complex substances (European Federation of Essential Oils/International Fragrance Association 2016).

Environmental fate

In theory, the “environmental fate” of UVCBs refers to the distribution and transformation of each constituent of a substance in the environment by various biotic and abiotic processes after release into the environment. Fate depends on the specific characteristics of a chemical compound, how it degrades and partitions in environmental compartments, and how it bioaccumulates in organisms and in the food chain. In the context of fate and exposure assessment, substance physicochemical and partitioning properties (e.g., vapor pressure, water solubility, log octanol–water partition coefficient [K_{OW}], log organic–carbon partition coefficient, log octanol–air partition coefficient, etc.) along with information on biodegradation are needed to estimate the distribution of chemicals among environmental compartments and to calculate predicted environmental concentrations for each compartment, both of which may be used in the overall assessment of ecological risk.

These substances (UVCBs) are composed of individual constituents that may possess different physicochemical and fate properties. Therefore, the environmental fate assessment should ideally be based on a constituent approach—that is, on individual or groups of constituents with similar environmental fate properties that contribute most to the potential hazard and risk of the mixture. This approach is challenging for substances that are not well characterized. Another challenge relevant to UVCBs arises when a substance is composed of constituents that significantly differ in their distribution and fate properties (e.g., vapor pressure, water solubility, adsorption behavior, and biodegradability), leading to fractionation of the substance and a different exposure pattern in the environment compared with the original composition of the substance (Figure 1). Fractionation of UVCB constituents can be modeled using chemical fate and transport mass balance models, as illustrated by MacLeod et al. (2004) in an analysis of 24 hydrocarbon blocks representing constituents of gasoline. The authors concluded that such model-based methodologies may be applicable for fate assessment used in screening-level ecological risk assessments of mixtures that have constituents of similar modes of toxic action. The transformation of constituents during use or in environmental media may also result in exposure to degradation products with different intrinsic properties, which could make the fate assessment of UVCBs even more complex.

It is still unclear whether standard tests used to generate environmental fate information for single substances might be

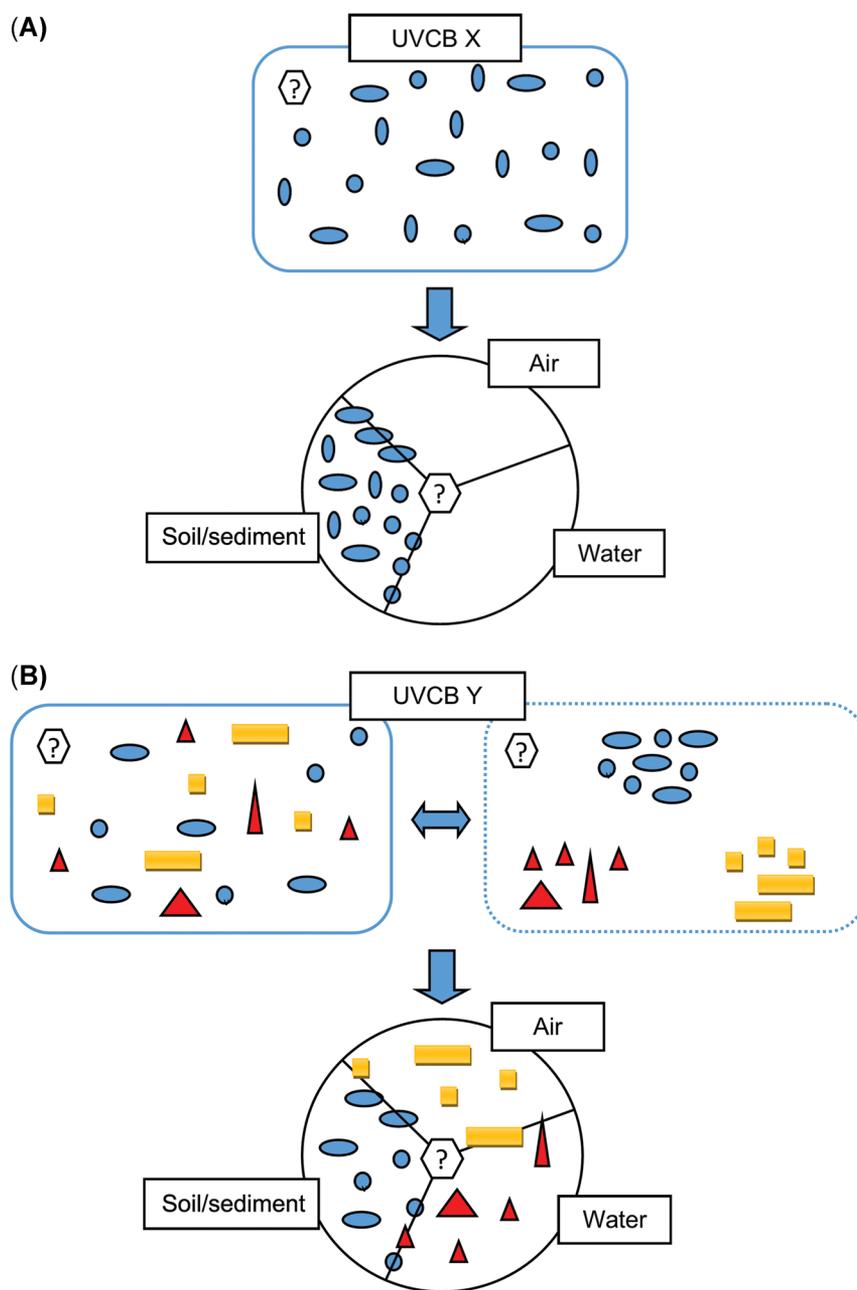


FIGURE 1: Environmental distribution of (A) a substance of unknown or variable composition, complex reaction product, or biological material (UVCB) substance X containing constituents with similar physicochemical properties and (B) a UVCB substance Y containing constituents with different physicochemical properties determining their partitioning in various environmental compartments. In both cases, different shapes represent constituents that could be grouped according to other properties, such as mode of toxic action.

appropriate for testing UVCBs and how such systems might be modified to provide relevant information on UVCB constituents or on chemicals that represent the UVCB mixture. Modifications to standard screening biodegradation tests are being explored to 1) apply such tests to UVCBs, and 2) improve the understanding of biodegradation for the constituents present in or representing those mixtures. These include the use of additional measurements, such as chemical analysis and measurement of biomass to aid interpretation, and passive dosing to establish suitable exposures of hydrophobic organic mixtures (Birch et al. 2018; Brillet et al. 2018; Brown

et al. 2018). It is recognized that further research is needed and that the influence of competitive effects, sequential degradation, cometabolism, and the conditions of screening tests on the results and their interpretation should be considered. A recent study of mixture effects on biodegradation kinetics showed that the number of mixture constituents had no or only a limited effect on biodegradation half-lives when tested at environmentally relevant concentrations (Hammershøj et al. 2019). Biodegradation testing at low concentrations is thus expected to be more realistic, but it can also pose considerable analytical challenges for UVCBs.

Testing the whole substance may be relevant for substances that are composed of structurally similar constituents that are expected to have similar degradation potential. It may also be useful as a first-tier assessment to obtain a biodegradability profile when limited or no data are available for the individual or representative constituents and/or when the identity of a significant part of the complex mixture is unknown. A challenge is the dosing and exposure assessment of a whole substance when its constituents display a wide range of physicochemical properties. The application of specific quantitative structure–activity relationships (QSARs) to substance constituents can help identify whether one or more constituents may be persistent in certain environmental compartments and thus guide testing. Other methods (e.g., chemical specific analysis) can help establish whether some constituents may have similar biodegradation profiles or whether some of them more easily degrade than others. The key question is then whether any remaining constituents possess properties of concern (i.e., persistence, bioaccumulation, and toxicity or very persistent, very bioaccumulative) and whether persistent metabolites might be formed. Thus, from an ecological relevance perspective, it is acknowledged that fate-directed toxicity testing and risk assessment may be the way forward for certain classes of UVCBs that are within the applicability domain of the method (e.g., neutral and poor to moderately water soluble) and may be expected to show large changes in toxicity following fate processes (e.g., biodegradation, sorption, etc.) such as petroleum and essential oils, where it could be expected. In this area, an ongoing project under the European Chemical Industry Council/Long-range Research Institute/RIFM program aims to 1) develop and test new approaches for fate-directed ecotoxicity assessment of UVCBs based on new combinations of analytical methods, dosing methods, fate-directed fractionation, toxicity testing, and models; 2) conduct a case study on selected UVCBs and develop a generic risk-assessment strategy for UVCBs; and 3) cross-fertilize and partially align the ongoing research activities related to UVCBs at 3 European research institutes (European Chemical Industry Council/Long-range Research Institute 2019).

Bioaccumulation

Bioaccumulation factors (BAFs), bioconcentration factors (BCFs), biomagnification factors (BMFs), and possibly trophic magnification factors are key parameters to assess the accumulation potential of chemicals in organisms. Whereas the BAF, a field measurement, accounts for the accumulation of chemicals within organisms through diet and absorption from their environment, the BCF, determined in the laboratory setting, characterizes uptake from the surrounding environment only (often water), excluding ingestion, and the BMF quantifies the concentration increase of a given contaminant with increasing trophic level (Arnot and Gobas 2004). However, because UVCBs can be composed of constituents with varying lipophilicity, susceptibility to metabolism, and excretion, a single BAF value may not account for the accumulation potential of the whole substance. Therefore, if representative

chemical structures can be assigned groups of related constituents of potential concern, then further assessment using BCF/BAF prediction models that can address important toxicokinetic processes such as metabolism may be suitable.

In risk assessment, bioaccumulation information is often used for environmental hazard prediction and profiling as well as wildlife and human food chain exposure modeling. In the absence of experimentally derived bioaccumulation data, the $\log K_{OW}$ of the representative constituents in a UVCB could be estimated using QSAR methods, provided the molecular structures are known, or based on measured data if the individual/representative constituents could be measured separately (i.e., if available in a pure form for testing). Alternatively, the high-performance liquid chromatography (HPLC) method (OECD Test No. 117) allows whole-substance testing and the determination of a range of $\log K_{OW}$ values representing the constituents present in the mixture (Organisation for Economic Co-operation and Development 2004). This could be particularly valuable when the mixture contains unknown constituents. For example, if all peaks present in the HPLC chromatogram were determined to have a $\log K_{OW} \leq 4.5$ ([REACH bioaccumulation screening criterion for persistence, bioaccumulation, and toxicity and very persistent, very bioaccumulative assessment [European Chemicals Agency 2017b]), it may be estimated that the mixture does not contain any constituents that have a significant bioaccumulation potential. However, if some peaks had a $\log K_{OW} > 4.5$, then the constituents corresponding to those peaks would need further investigation.

A caveat of this approach is that $\log K_{OW}$ will not accurately represent a substance's bioaccumulation potential if it is driven by mechanisms other than lipophilicity (e.g., protein or phospholipid binding). This could be the case for substances such as organometallics, ionizable substances, and surfactants (European Centre for Ecotoxicology and Toxicology of Chemicals 2013). With such substances, other parameters such as the membrane–water or the protein–water partition coefficients may be better suited than the $\log K_{OW}$. Furthermore, the bioaccumulation potential of certain substances can be largely controlled by their susceptibility to metabolism and excretion, which could lead to lower bioaccumulation than that estimated from only the partitioning properties of chemicals. Thus, screening assessments based simply on $\log K_{OW}$ must be used with caution.

Many predictive BCF models have been developed, particularly for fish, and can be used in the absence of experimental data. Because of the considerable impact biotransformation can have on bioaccumulation and bioconcentration, some of these models seek to assess and incorporate biotransformation rates (Arnot and Gobas 2003, 2004; Dimitrov et al. 2003; Arnot et al. 2009).

Biotransformation can also be measured by *in vitro* methods. The OECD recently adopted 2 new test guidelines (Test Nos. 319a and 319b) on *in vitro* methods for fish hepatic clearance (Organisation for Economic Co-operation and Development 2018b, 2018c). These methods can be used to improve *in silico* predictions of test chemical bioaccumulation in fish, and with few exceptions, they have only been applied to

single chemical substances or isomeric mixtures. Despite this, in principle, these methods could be used to determine *in vitro* intrinsic clearance rates of UVCBs. The OECD guidance document on rainbow trout hepatocytes and rainbow trout liver S9 subcellular fractions discusses considerations such as possible inhibitory effects associated with mixtures and the fact that the extraction method, analytical method, and test concentrations must be suitable for all constituents of the mixture (Organisation for Economic Co-operation and Development 2018a). The Organisation for Economic Co-operation and Development (2018a) recommends testing the biotransformation of the individual constituents of mixtures if they are available and highlights that the testing of UVCBs may be challenging and can only be performed on constituents for which an analytical method is available. That said, these *in vitro* methods provide a valuable second-tier screening test that bridges the gap between $\log K_{OW}$ and *in vivo* BCF studies. Therefore, their suitability to the bioaccumulation assessment of UVCBs would be worthy of further exploration.

The feasibility of measuring BCFs of chemicals in mixtures *in vivo* using a single dietary exposure and chemical benchmarking has been demonstrated by Chen et al. (2018) for 8 test fragrance chemicals and 3 benchmark chemicals. Dietary exposure to the whole substance delivers comparable exposure to all constituents of the mixture, which would be difficult to achieve using aqueous exposure given the range of physical–chemical properties exhibited by the chemicals. The method relies on the ability to analytically quantify each chemical in the mixture and fish samples.

Environmental toxicity

Data on the ecotoxicity of UVCBs can be generated by testing or modeling of known constituent structures present or can be predicted based on similar or analogous chemicals or structures (i.e., using read-across or modeling). Ecotoxicity testing of individual constituents relies heavily on an understanding of the substance composition, its associated physicochemical properties, and the ability to ascertain the concentration of that substance in the testing media. Much of the attention has been focused on aquatic toxicity testing, which represents a priority for many chemicals, and is often used in the first tier of risk assessment. For example, OECD Guidance No. 23 provides extensive information on best practices for aquatic toxicity testing of difficult-to-test chemicals (e.g., UVCBs; Organisation for Economic Co-operation and Development 2019a). However, as is the case for monoconstituent substances, the physicochemical properties and uses of a substance will dictate the most relevant exposure medium, and fate-directed toxicity testing will improve the utility of the risk assessment.

The physicochemical properties and effects of a chemical can be predicted based on its structural characteristics. This is the basis for QSARs. In the case of UVCBs with sufficient compositional information (modeled or measured), the hazards of individual constituents can be combined using a mixture approach to predict the hazard of the whole substance. The

“concentration addition” method can arguably be considered the most practical and accurate, although in most cases remaining conservative. For example, when evaluating the toxicity of a mixture, evidence shows that this approach is protective enough even when the mode of action is not the same for all classes of constituents (Cedergreen 2014). More specifically, this approach is well adapted to petroleum mixtures, which consist largely of narcotic chemicals known to produce additive concentration effects. Therefore, for petroleum substances, the PetroTox model was developed to predict aquatic and sediment toxicity based on concentration addition (Redman et al. 2012). For natural complex substances like essential oils, the iSafeRat calculation method was generated to predict substance aquatic toxicity. The iSafeRat calculation method uses available composition information and a bioavailability factor to estimate hazard (Bichere et al. 2014).

Because UVCBs by definition are difficult to analyze (Organisation for Economic Co-operation and Development 2019a), ecotoxicity has to be assessed carefully to account for the lack of substance composition information. Two approaches to the hazard assessment of UVCBs consist of a representative chemical constituent-based approach and a whole substance-based approach.

Representative chemical constituent-based approach. For all multiconstituent substances and many UVCBs, some substance composition information is available or can be determined. A simplistic approach to UVCB hazard prediction is to focus on a few constituents that are expected to drive the hazard of the substance. This approach works well if only a few constituents make up the bulk of the substance, and none of the nonevaluated constituents can be expected to be more hazardous than the dominant constituents (absence of problematic elements or moieties).

Whole substance-based approach. The whole substance-based approach can be challenging when assessing substances for which the composition is undefined or uncharacterized. In addition, other substance properties (e.g., poor solubility, high volatility, etc.) often confound testing and analytical confirmation (Organisation for Economic Co-operation and Development 2019a). Whole-substance testing is a presumably more realistic reflection of the overall substance toxicity when considering direct discharge to the environment. However, it does not provide information on the toxicity of the individual constituents or how those constituents contribute to the overall toxicity observed.

The main challenge specific to UVCBs is achieving confidence that the material is being dosed in a way that combines maximum bioavailability with a stable concentration. Guidance No. 23 suggests modifications or additions to other OECD testing protocols to accommodate UVCBs (Organisation for Economic Co-operation and Development 2019a). Feedback from contract research organizations and academic/industry toxicology laboratories emphasizes difficulties with maintaining functional solubility, which is the test substance concentration achieved in an aqueous testing system as opposed to the measured water solubility. Several factors contribute to

disparities in the 2 values, including test substance degradation (hydrolysis and photolysis), test substance volatility, and adsorption to the testing vessel and organisms. These factors make it difficult to maintain stable test concentrations and lead to analytical variability. An additional challenge with whole-substance testing is the requirement to maintain a “stable exposure concentration”—that is, to keep the test substance concentration in the testing medium between 80 and 120% of nominal or mean measured values over the entire exposure period (Organisation for Economic Co-operation and Development 2019a). This can be difficult to achieve when the ability to analyze and identify the test substance is limited. Finally, testing the whole substance may be of limited usefulness for persistence, bioaccumulation, and toxicity and risk assessments because it would not allow the specific property of each constituent or fraction of constituents to be estimated, even though they may often be needed, considering the modified exposure pattern in the environment due to differences in fate compared with the original composition of the substance (see section *Environmental fate*). On the other hand, the great advantages of whole-substance toxicity testing are that the toxicity of the entire mixture is determined and that it does not rely on assumptions including the absence of synergistic interactions.

To promote fit-for-purpose assessments, any of the approaches to determining environmental toxicity of a UVCB detailed in the present review or their combination can be utilized depending on how much of the test substance composition, physicochemical properties, and potential exposure routes can be characterized.

APPROACHES FOR UVCB HAZARD AND RISK ASSESSMENT

The overriding issue with performing hazard assessments on UVCBs is determining the most appropriate methodology(ies) to apply to assessing their toxicity, considering that environmental risk assessments are more commonly based on mono-constituent substances. Because of this, risk assessments on UVCBs may require modifications/additions to existing risk-assessment methodologies, which were traditionally developed for assessing monoconstituent substances. It has become clear that UVCB risk assessment requires informed and flexible approaches. To some extent, UVCBs are considered in endpoint-specific guidance (Organisation for Economic Co-operation and Development 2000; European Chemicals Agency 2014, 2016, 2017a) and in guidance pertaining to ecological assessment approaches (Organisation for Economic Co-operation and Development 2000). Further, UVCBs have been considered in several articles on the ecological assessment of mixtures (e.g., Dimitrov et al. 2015; Kutsarova et al. 2019).

Substance grouping

Grouping of UVCBs into assessment categories, groups, or substance classes is covered in guidance on the grouping of

substances and on the use of read-across approaches (Organisation for Economic Co-operation and Development 2014b; European Chemicals Agency 2017c). Two of the main factors taken into account for the efficient and accurate grouping of UVCBs are the sharing of the same or related representative constituents (i.e., related chemical classes or groups of constituents within the same mode of toxicological action; see section *Characterization of UVCBs using representative constituents*, for information on the selection of representative constituents) and the applicability of read-across between members of the same group (see section *Characterization of UVCBs for the use of read-across*). In addition, consideration of UVCBs of related or the same industrial sector(s) and types of use will help simplify the already complex exposure analysis usually required for UVCBs.

Current and prospective regulatory approaches, frameworks, and risk-assessment methodologies

Currently, UVCB risk assessments are being undertaken using a combination of approaches. These include weight of evidence (WoE) approaches, which can be very effective for these types of substances. Meaningful assessments require flexibility regarding the way dosing is achieved (e.g., water accommodated fraction [WAF], passive dosing, direct addition, etc.), the perceived need for traditional whole-substance aquatic toxicity data, and the metrics for reporting the outcomes (e.g., toxicity thresholds of concern and other indicators of other hazards). The metric for reporting the hazard assessment outcome will vary depending on the chosen approach (e.g., representative chemical constituent or whole substance). For example, the whole-substance approach on a UVCB in which the test substance is difficult to analyze may require a nominal loading rate, as is the case with WAFs. However, this does not reflect the actual exposure, for which measured concentrations are preferred. If there is a single constituent driving the hazard, it may be more meaningful to report the concentration of that constituent.

Despite the prevalence of WAFs used in UVCB toxicity assessment, there is a consensus among regulators that WAFs are not always useful for persistence, bioaccumulation, and toxicity or risk assessments because they do not give adequate effect levels (e.g., no observed effect concentrations) for deriving toxicity screening values (e.g., predicted-no-effect concentrations), which are used for chemical regulatory purposes. Fate-directed toxicity testing may provide the information necessary for these assessments as this research develops. Novel dosing methodologies such as passive dosing (Smith et al. 2010) are promising methods to control the concentration of each mixture constituent by partitioning from a preloaded polymer donor (Jahnke et al. 2016; Organisation for Economic Co-operation and Development 2019a; Hammershøj et al. 2020). Alternatively, WAF accuracy, precision, and relevance could be improved to make it more fit-for-purpose in environmental risk assessments.

Environment and Climate Change Canada uses a WoE approach and looks for consistency and strength in the data and

lines of evidence (LoEs) evaluated in an assessment (Health Canada 2017). For example, for petroleum substances, Environment and Climate Change Canada has relied on whole-substance toxicity testing, with PetroTox (Redman et al. 2014) used as supporting evidence or to fill data gaps. For non-petroleum organics such as resins and rosins, Environment and Climate Change Canada has used a combination of whole-substance and constituent-based approaches relying on an in-house selection tool that ranks intraclass bioavailability, persistence, and ecotoxicity for the selection of known or predicted representative UVCB constituents (example assessments can be found by consulting Environment and Climate Change Canada [2017b]). The European Chemicals Agency oversees REACH registrations and adheres to the “one substance, one registration” principle. There is an explicit need for a well-defined substance, especially because persistence, bioaccumulation, and toxicity assessments are required for all constituents >0.1% (w/w). Despite this, UVCBs can be registered as single substances (i.e., one registration) under REACH, provided that the variability in composition does not create significant differences in the hazard concerns that would change the classification. In comparison, a multiconstituent substance can be registered as a series of its separate constituents. In both instances, the results of environmental toxicity testing using the WAF approach can be used for classification, labeling, and packaging; but careful interpretation of the test results is necessary.

The USEPA has not yet laid out any explicit guidelines for characterizing and assessing UVCBs. In the context of the Toxic Substances Control Act, the agency takes a case-by-case approach to determine appropriate fate and hazard-assessment methods for UVCBs. For example, at times, the agency considers a fate and exposure-driven approach for hazard assessment of UVCBs. This consists of studying the behavior of the test substance in environmental media as well as relevant routes of exposure prior to choosing the type and method of hazard assessment. Basic criteria needed include, but are not limited to, K_{OW} or other partition coefficients, water solubility, or other physical–chemical properties or fate parameters to inform initial testing. This approach is also the basis for a project (ECO42) on fate-directed hazard assessment of UVCBs (European Chemical Industry Council/Long-range Research Institute 2019).

CONCLUSIONS AND RECOMMENDATIONS

Need for a multipronged and transparent approach

A general approach for UVCB risk assessment should (after determining the primary focus of the assessment) integrate whole-substance information with representative constituent-based information in a WoE approach, to minimize data gaps associated with reliance on either the whole substance-based approach or the representative chemical constituent-based approach. This could be done with a tiered strategy requiring the testing of either the whole UVCB, fractions of it, or isolated

constituents, depending on considerations from the regulating authority about potential data gaps as identified by characterization and screening of constituent information (see Figure 2).

The grouping of constituents with similar properties and the selection of representative structures can be based on *in silico* predictions and/or experimental work. A recent approach using computational methods was specifically developed to generate representative structures for UVCBs and their constituents (Dimitrov et al. 2015). Data collection for representative structures based on (Q)SAR or persistence, bioaccumulation, and toxicity models can be used for the persistence, bioaccumulation, and toxicity assessment. This information can further guide the experimental work on representative structures and therefore reduce animal testing.

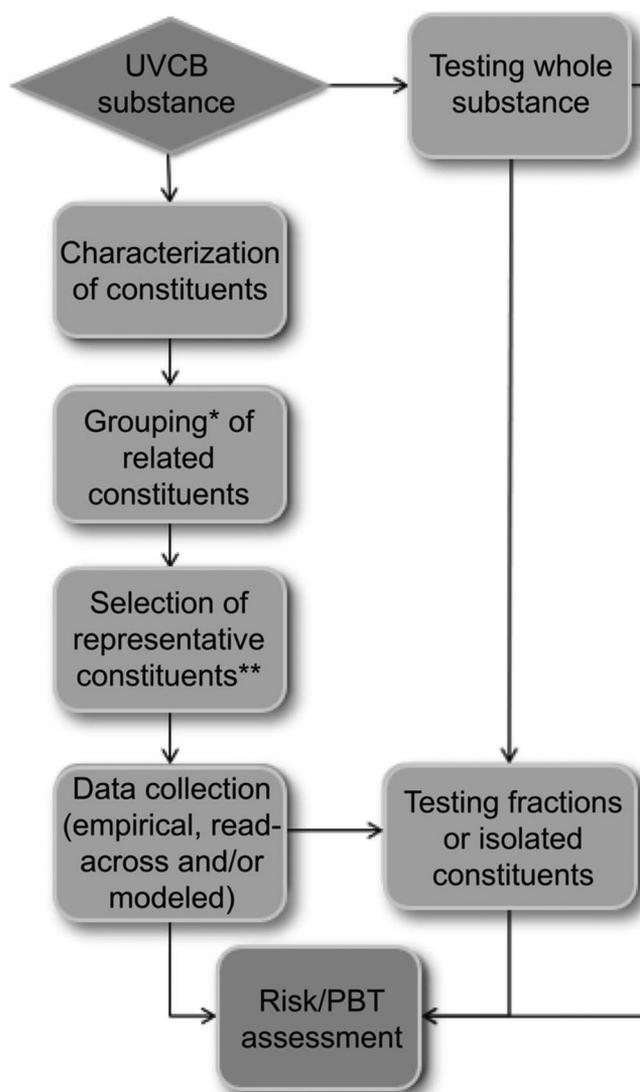


FIGURE 2: Potential approach for the testing and risk assessment of a substance of unknown or variable composition, complex reaction product, or biological material (UVCB) in a regulatory context. *Grouping could be based on similarity in chemical class and/or mode of toxicological action. **Selection of representative constituents may be based on those that best or conservatively represent a subclass of similar constituents present in the UVCB. See *Substance grouping* for more details on grouping. PBT = persistent, bioaccumulative, toxic.

Matching of constituent-based information (i.e., a modeled composition of the substance) to ensure ground-truthing of the information presents a great challenge. Integrating increasingly diverse LoEs in regulatory risk assessments may make the resultant conclusions less clear and difficult to communicate. Transparent and open approaches to the presentation of LoEs, WoE, and associated uncertainties, including the impact and sensitivity of these uncertainties on the ultimate risk conclusion, are a must.

Overcoming the challenges

Recommendations. Constituent identification and quantification are key factors in the assessment of UVCBs. Even if the exact chemical structure of each constituent cannot be defined or quantified, current analytical techniques can often determine generic structures and some of their physical–chemical properties (e.g., boiling point). However, case studies are needed to investigate whether generic structures can be used as a first-tier assessment. Blocking of constituents with similar generic properties can be used to guide experimental studies, predict hazard properties, address data gaps, and characterize a UVCB for the purposes of risk assessments. Blocking could likely be applied to a wider range of substances than currently (e.g., the hydrocarbon block methodology for petroleum substances). Presently, methods are being developed in which toxicity testing of multiconstituent substances and UVCBs is made more relevant for both hazard and risk assessments. Such testing is done by considering the environmental fate processes acting on constituents of these substances based on new combinations of analytical methods, dosing methods, fate-directed fractionation, toxicity testing, and models (ECO42 project; European Chemical Industry Council/Long-range Research Institute 2019). Novel testing strategies that do not exclusively rely on the aqueous solubility of multiconstituent substances and UVCBs should be developed because many multiconstituent substances and UVCBs may poorly dissolve in water. The development and validation of models with extended application domains are needed to predict the physicochemical, fate, bioaccumulation, and toxicity properties of the constituents.

Ongoing efforts. Since the 2016 workshop (whose conclusions are summarized in this review), several initiatives have commenced to advance the state of the science on UVCB risk assessment. Several workshop participants and a subteam of this article's authors and their respective agencies have contributed to the revision of OECD Guideline No. 23 as well as the ECO42 project (European Chemical Industry Council/Long-range Research Institute 2019) initiated in April 2018, which are already producing valuable results informing fate-directed toxicity testing and assessment.

In early 2018, HESI also launched a new initiative to address the needs for new multisectoral approaches for UVCB risk assessment. Through the engagement of stakeholders facing challenges associated with these substances, this project fosters the exchange of knowledge about UVCBs to develop and

optimize a cross-sectoral framework for the classification, identification, characterization, and assessment of these substances. In December 2018, HESI organized an international workshop involving experts from regulatory agencies, academia, and multiple industry sectors, which resulted in the development of a tiered approach for substance identification and characterization. This draft framework presents an initial effort to determine key substance identity information to perform robust and fit-for-purpose ecological risk assessments for UVCB substances, along with criteria that could be used in a WoE approach for substance identification and characterization, and exposure assessment. Case studies were developed to ground-truth this approach, and the findings will be summarized in a peer-reviewed publication. The second phase of this project has been initiated and will focus on exposure evaluation, including fate and transport modeling, fate-directed assessments, and a general evaluation of existing exposure assessment tools and methods.

The continued collaboration of stakeholders from multiple sectors (government, industry, and academia) is paramount to overcome the complexities associated with the risk assessment of UVCBs because their combined expertise and experience will be an asset to the development of practical guidelines that are fit-for-purpose and will address regulatory requirements for preparing ecological risk assessments on these substances.

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