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Energy & Environment

# Framework to Improve the Use of Weight of Evidence in Persistence Assessments

Cefic-LRI ECO52 project

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Report for European Chemicals Industry Council (Cefic)

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# 1 Introduction

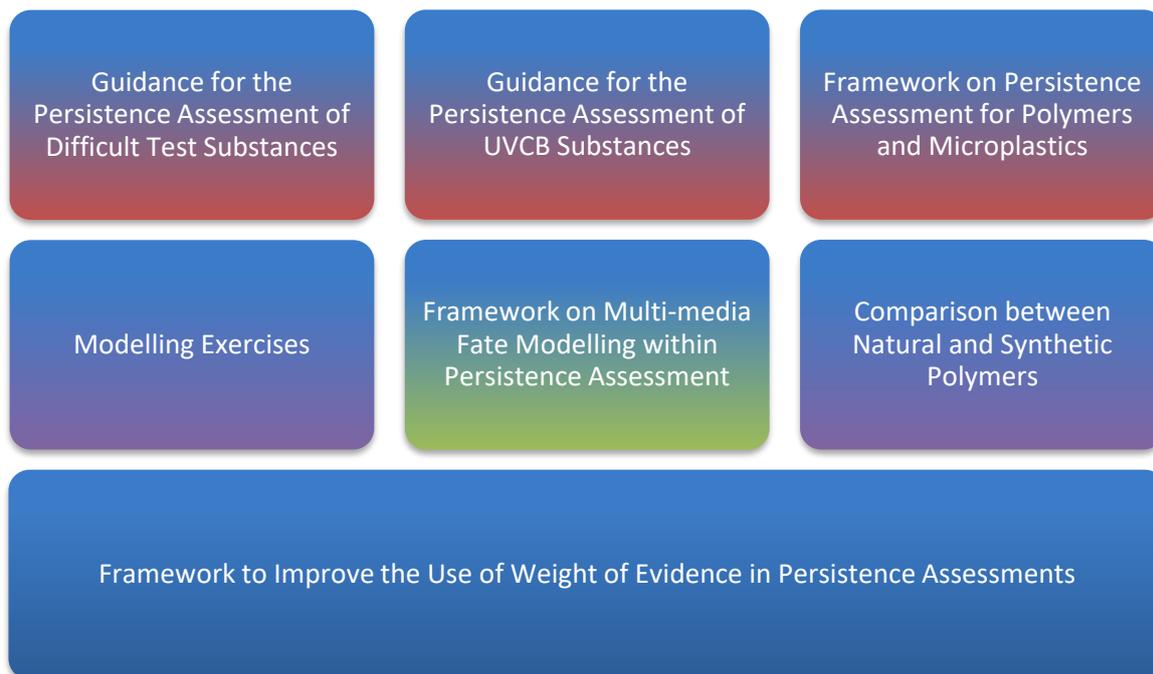
Persistence assessment is a cornerstone of chemical regulatory assessment and management frameworks, whereby the potential of a chemical to remain in the environment after having been released is evaluated (Rodan, Pennington et al. 1999, Boethling, Fenner et al. 2009, Moermond, Janssen et al. 2012, Mackay, Hughes et al. 2014, ECHA 2017a, 2017b). Such assessments are required under numerous regulatory frameworks globally. Persistence is typically assessed alongside bioaccumulation and toxicity as part of “PBT/vPvB” and “POP” frameworks. The assessment itself considers degradation half-lives of chemicals in different environmental compartments, which are compared to set criteria. Other information may be relevant to the persistence assessment, and a weight of evidence approach is generally required (Hughes, Brown et al. 2020).

Persistence assessments have many challenges to their implementation. Degradation data are often highly variable owing to a range of environmental and experimental factors (Boethling, Fenner et al. 2009). Guidance is also needed to support the evaluation of the quality (reliability and relevance) of these data, and in the use of weight of evidence to arrive at conclusions (Ingre-Khans, Ågerstrand et al. 2018, Hughes, Brown et al. 2020). Further, many substance types pose unique challenges for the application of standard approaches and test methods in persistence assessments (Davenport, Curtis-Jackson et al. 2021, Whale, Parsons et al. 2021). Polymers in particular are a class of substances which pose certain challenges for persistence assessments and require specific consideration (ECETOC 2020). In addition, the opportunities of multimedia fate modelling to support more robust and realistic persistence assessments have so far hardly been implemented (Webster, Mackay et al. 1998, Scheringer, Jones et al. 2009, McLachlan 2018, Redman, Bietz et al. 2021).

In this document, the work of the Cefic-LRI ECO52 project has been brought together into a consolidated framework to improve the use of weight of evidence in persistence assessments. The EU REACH persistence assessment is the main focus, although other global frameworks have been considered and the work of the project is also considered relevant to these. This document provides an overview to the project and other reports; addresses the persistence concepts, history, frameworks and developments; provides a means to improve weight of evidence guidance and account for sources of variability in degradation data; and presents a coherent framework and decision tree for incorporating the guidance developed in the project into current and future persistence assessments.

## 1.1 Other reports in this series

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.’ The various reports produced in this project are presented in Figure 1.



**Figure 1. Overview of reports produced in the Cefic-LRI ECO52 project. Three documents concern the persistence assessment of challenging substance types (red hue). One documents concerns the application of multimedia fate modelling in persistence assessments (green hue). Two documents concern the results of literature searches or exercises investigating particular topics (purple hue); one on multimedia fate modelling and one on a comparison of natural and synthetic polymers. Finally, an overarching document (blue hue) detailing a framework for improving the use of weight of evidence in persistence assessments (this report).**

#### **WP2A – Guidance for the Persistence Assessment of Difficult Test Substances**

This document compiles the latest knowledge of how substance properties can render substances difficult to test and subsequently evaluate in a persistence assessment. The document provides guidance as to how these issues can be addressed. It is structured to first establish the importance of considering substance properties in persistence testing and assessment, and how challenging test properties can influence these activities. The properties that have been identified as posing challenges for persistence assessment are poorly soluble, sorbing, volatile, toxic to micro-organisms, ionisable, complexing, unstable and chiral. Finally, each property is discussed in detail as to how it can affect biodegradation testing and persistence assessment, and how these challenges can be overcome.

Throughout the document, information includes general considerations as well as specific guidance related to (bio)degradation testing. In line with the REACH persistence assessment framework, specific considerations have been provided separately for screening tests, simulation tests and other non-standard tests, where possible. The latest developments and novel approaches to address difficult test substance properties from literature have also been compiled and the potential utility of these developments and their regulatory acceptability discussed.

#### **WP2B – Guidance for the Persistence Assessment of UVCB Substances**

This report provides 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 is 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 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 have been 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.

## **WP2C – Framework on persistence assessment for Polymers and Microplastics**

In this report, the potential application of persistence assessment frameworks to polymers has been investigated. Polymers are a group of substances which have previously been considered exempt from registration and evaluation under the EU REACH regulations “*since polymer molecules are generally regarded as representing a low concern due to their high molecular weight*” (ECHA 2012). However, in the future, polymers potentially require registration and thus environmental hazard and fate assessment under REACH. Specific physico-chemical properties of polymers may result in specific technical limitations for the applicability of the standard biodegradation testing guidelines and may affect their fate in the environment. For example, water soluble, poorly soluble, and water insoluble/particulate polymers will show quite different fate behaviour (ECETOC 2020). For this reason, there are considerable challenges when trying to assess the environmental hazard and fate properties of polymers over and above those faced in the assessment of individual substances. A special case is the discussion about persistence assessment of microplastics. Microplastics are a subset of polymers defined by a particular physical form. In the EU the restriction of intentionally added microplastic is proposed unless a polymer can be shown to be biodegradable.

Information on polymers has been gathered and analysed. The focus is on how to provide relevant and credible information to assess the environmental persistence of polymers (not plastic materials or articles). Finally, a proposed framework (including suitable workflows/decision trees) to support the persistence assessment of polymers (and microplastics) in a weight of evidence approach is included. The framework aims to give an insight into how the existing criteria on persistence assessment for single substances in the PBT assessment under REACH can be used, interpreted, or adapted for polymers to avoid long-term presence and accumulation in the environment.

## **WP3A – Modelling Exercises**

This document presents a series of modelling exercises, which aim to explore the use of overall persistence in persistence assessment of a range of substances, including UVCBs, polymers and microplastics. The overall persistence was reviewed as this is important in the EU, since current

assessment of P and vP is based on a set of degradation half-lives in environmental media, obtained from standardized experimental test systems.

The aim was to establish a more comprehensive understanding of the potential environmental fate, including identification of physical and chemical properties most relevant for the prediction (modelling) of environmental partitioning and the compartment of concern. This report documents the methods and results of the modelling work, which formed the basis for the guidance document WP3B.

### **WP3B – Framework on multi-media fate modelling within persistence assessment**

In this document, based on the outcome of literature review and the modelling exercises (WP3A), a methodology was prepared on how to integrate the multi-media fate modelling and overall persistence concept into the persistence assessment framework to improve the robustness of current persistence assessment methodologies. Under consideration of the applicability domain of the models, the approach describes in a clear and simple way how to use the models to get information on sensitive and critical parameters. It demonstrates how the overall persistence can be used to derive evidence on the compartment of concern as well as to decide on remaining concern in untested compartments, and how multi-media fate modelling can help to interpret contradictory results from single compartments. This approach is supplemented with examples from the exercises, and the possibility of how it can be used to support the persistence assessment of difficult test substances, UVCBs and polymers has been discussed.

### **WP4 - Comparison between natural and synthetic polymers**

Polymers make up many of the materials in living organisms. So-called natural polymers are for example, cellulose, chitin, protein, carbohydrates such as starches etc. Natural polymers are regarded as no concern for the environment. They are integral parts of the environment and though they might not meet current regulatory requirements, e.g. with regard to degradability, it is proven that they don't accumulate there over time.

In this report, behaviour of natural polymers under standard laboratory degradation conditions has been evaluated, providing potential benchmarks for man-made polymers. Further, the similarities and differences between natural and synthetic polymers are in the focus. The available information on degradation of some natural and synthetic polymers under different conditions are compared, whereby literature data are preferred using standardized testing methods, and which compares the degradation potential of different polymer sources using the same method.

## 2 Persistence assessment concept, history, and recent developments

Persistence assessment is a regulatory process whereby the intrinsic potential of a chemical to remain in the environment after having been released is evaluated (Rodan, Pennington et al. 1999, Boethling, Fenner et al. 2009, Moermond, Janssen et al. 2012, Mackay, Hughes et al. 2014, ECHA 2017b). The assessment focuses on degradation processes that are able to remove the substance from various compartments in the environment. In the course of this process, chemicals with the greatest persistence can be identified and prioritised for regulatory action. Persistence assessment is a cornerstone of regulatory frameworks, such as EU REACH, where persistence is typically evaluated alongside toxicity and bioaccumulation potential.

The interest in persistence appears to mostly stem from the concern that chemicals with this property present the potential for increased and poorly reversible exposure, and hence risk (Scheringer 2002, Boethling 2016, McLachlan 2018, Cousins, Ng et al. 2019). Persistence might therefore be considered a hazard property (in the sense that it is an intrinsic property of the substance) that is itself an indicator of risk. The property itself is not associated with any particular (eco)toxicological effect. It therefore differs from most other hazard properties but is similar to bioaccumulation potential (Gobas, de Wolf et al. 2009). Under EU REACH, substances can be identified as substances of very high concern purely on the basis of their persistence and bioaccumulation potential. This is reportedly owing to the increased uncertainty in the estimation of risk to human health and the environment when applying quantitative risk assessment methodologies to these substances (ECHA 2017b).

There appears to be consensus on the protection goal of persistence assessment being related to concerns from increased and poorly reversible exposure (Scheringer 2002, Boethling 2016, McLachlan 2018, Cousins, Ng et al. 2019, Redman, Bietz et al. 2021). However, other concerns related to persistence have been raised previously, such as the risk of contamination of remote or pristine regions (ECHA 2017b, Hale, Arp et al. 2020), contamination of land and drinking water resources (Alexander 1973, Cousins, Ng et al. 2019, Hale, Arp et al. 2020, Jin, Huang et al. 2020), and unforeseeable future or planetary boundary threats (Cousins, Ng et al. 2019, Arp, Kühnel et al. 2021, Naidu, Biswas et al. 2021, Persson, Carney Almroth et al. 2022). The issue of persistence is therefore multi-faceted, and may mean different things to different people. In this document we will attempt to chart the course of the development of persistence assessment frameworks, and provide context to the approaches and criteria currently in use. It is hoped that this may facilitate further discussion and development of approaches as regulatory frameworks continue to evolve.

### 2.1 Early awareness of the persistence issue

An awareness of potential issues from substances resistant to degradation in the environment first arose in the 1950s. Around this time a chemical revolution had taken place in the detergent industry, with the replacement of soap in cleansing and laundry formulations with synthetic alkylbenzene sulphonate (ABS) surfactants (Swisher 1987). However, the poorly degradable nature of ABS led to noticeable foaming in wastewaters, treated sewage and receiving waters. After the discovery of this issue, the more degradable linear alkylbenzene sulphonate (LAS) was developed, which had replaced ABS by the 1960s and largely addressed the issue.

Another example of environmental issues caused by persistence relates to the widespread use of chlorinated pesticides. It was predominantly the 1930s to 1950s that saw the influx of numerous

patents and papers for the production of chlorinated hydrocarbons for use as industrial chemicals and pesticides (Britton, Coleman et al. 1933, Kearns, Ingle et al. 1945). By the 1950s, chlorinated hydrocarbons were being used across various industries, from pesticides to coolant and lubricating oils (Rosner and Markowitz 2013). The 1950s saw the steady emergence of studies linking dichlorodiphenyltrichloroethane (DDT), i.e. the most widely distributed insecticide at the time, and some of the other chlorinated hydrocarbons to toxicity in humans. However, it was not until several years after their first production and release to the environment that the seminal book “Silent Spring” by Rachel Carson in 1962 drew public attention to the ecologically hazardous nature of some of these substances (Carson 1962). The hazardous nature of these chemicals was not unknown at this point, however Silent Spring highlighted that the persistence of the chlorinated hydrocarbon leads to exposure, which resulted in bioaccumulation and toxic effects across the food chain. For example, leading to alteration of calcium metabolism in birds resulting in the production of thin eggshells. This work ultimately led to a ban on DDT and other chlorinated hydrocarbons (Dunn 2012).

Following the publication of Silent Spring and other research highlighting the bioaccumulative and toxic potential of chlorinated hydrocarbons, the 1960s and early 1970s saw scientists start to elucidate and quantify the extent of their persistence. It was at this time that evidence emerged of DDT and other pesticides being present in soil years after their application (Nash and Woolson 1967, Lichtenstein, Fuhremann et al. 1971, Stewart and Chisholm 1971). Publications by other scientists around this time, e.g. Alexander (1973), suggested that persistence is needed for biomagnification to occur. Persistence was formally linked to bioaccumulation and toxicity for the first time in 1973 following the use of “PBT” (i.e. Persistent, Bioaccumulative and Toxic) in the Chemical Substances Control Law in Japan (METI 1973).

## 2.2 Development of regulatory criteria

The development of persistence assessment frameworks has taken place over several decades. The evolution of persistence assessment criteria has previously been reviewed by Matthies et al. (2016). This review charts the development of PBT assessment frameworks around the globe, and demonstrates that the persistence assessment criteria in use today largely emerged in the 1990s. Assessment approaches have continued to develop since then, but the fundamental basis for determining persistence (i.e. degradation half-lives in water, sediment and soil) remains unchanged.

While Japan’s Chemical Substances Control Law had introduced the term “PBT”, it was not until 1978 that half-life criteria were first used to define persistent chemicals. The International Joint Commission (IJC) Critical Pollutants List defined persistent chemicals as having a half-life in water of >56 days (eight weeks) under the Great Lakes Water Quality Agreement (GLWQA), revised in 1987 (IJC 1987). No other environmental media were included at this point as the protection goal was to protect and restore the waters of the Great Lakes under the US-Canada GLWQA.

Once the first half-life criteria had been established, work in the late 1980s identified the need to develop expert systems for predicting persistence (Hushon 1987, Boethling, Gregg et al. 1989); this coincided with the development of fugacity models in the 1990s, for example Mackay’s four-compartment level III fugacity model which includes non-diffusive/“one-way” transfer processes (Mackay 1991). Following these discussions about predicting persistence, Ontario’s Ministry of Environment and Energy Candidate Substances List for Bans or Phaseouts introduced a scoring system, in 1992, for persistence half-lives in air, water or sediment, based on data available in the Chemical Evaluation Search and Retrieval System (CESARS) database (OMEE 1993). A score of 7 (51-100 days) or 10 (>100 days) met the selection criteria for persistence. Environment Canada’s List A of the Accelerated Reduction and Elimination of Toxics (ARET) Program used the same

aforementioned scoring system (EC 1995). By 1993, the IJC Critical Pollutants List had applied the same half-lives to other media, in addition to water: 56 days for water, air, soil, sediment and biota (IJC 1993).

The uniform half-life criteria across environmental compartments diverged during the mid to late 1990s. In 1993, Environment Canada and Health Canada (EC/HC) proposed different half-lives for different environmental media when screening for substances for nomination to the Priority Substances List (PSL2) by the Canadian Environmental Protection Act (CEPA). This list of 25 substances utilised persistence half-lives of >50 days in surface water, >100 days in groundwater, and >180 days in sediment, which were based on consultations with stakeholders (EC/HC 1993).

Further changes to the PBT regulations in Canada were encouraged by the publication of a report in June 1995 based on a meeting of the Environment Canada *ad hoc* science group (EC 1995). The science group reviewed persistence data for 68 priority substances and, based on empirical analyses, computer modelling and expert judgement, recommended the following half-lives (EC 1995):

- $\geq 2$  days for air, based on the concern with long-range transport;
- $\geq 182$  days (six months) for water, based on empirical analyses of available data suggesting a critical value between 2 to 8 months, with 6 months capturing substances of most concern;
- $\geq 182$  (six months) for soil, based on empirical analyses of available data suggesting a critical value of 6 months, as well as a simple first order kinetics model showing that 6 months results in a slow build-up of residue to a maximum of  $\sim 30\%$  of the annual application (compared to 1 year which results in an increasing fraction persisting to a concentration maximum equalling the annual application);
- $\geq 365$  days for sediment, based on results of the Quantitative Water Air Sediment Interaction (QWASI) model.

The report also highlighted the wide range of half-lives, even within one medium, and recommended using expert judgement to assess data quality. Further, it was emphasised that there may be a need to revise criteria in the light of scientific progress.

In 1995, the Government of Canada Toxic Substances Management Policy (TSMP) implemented the above persistence criteria for track 1 substances (virtual elimination from the environment), in order to avoid the release of chemicals similar to non-ionic, hydrophobic 'reference chemicals' used to derive the criteria. Notably, the half-life for air drastically decreased from >56 days in previous regulation to >2 days in order to account for the long-range transport of persistent organic pollutants (POPs). In 1998, POPs were formally identified in regulation with the introduction of the United Nations Economic Commission for Europe (UNECE) Aarhus Protocol on POPs, coming into force in 2003. It utilised a reference chemical approach, using the same half-life of >2 days for air, but reducing the other half-lives to >60 days in water and >6 months in soil and sediment (UNECE 1998).

Also in 1998, the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) first came into force, however it was not until 2001 that half-life criteria were defined; as OSPAR focuses on protecting the marine environment, a singular half-life of 50 days was introduced for water (fresh and marine) (OSPAR 2002). As shown in Table 1, various other regulations adopted differing PBT criteria in subsequent years. For example, in 1999 the United States Environmental Protection Agency (US EPA) Toxics Release Inventory adopted P half-lives of >2 days for air and >60 days for water, soil and sediment, as contamination of chemicals with half-lives of <60 days can be significantly and naturally reduced within a year (USEPA 1999). The CEPA 1999 Persistent, Bioaccumulation, inherently Toxic to non-human organisms (PBiT) regulations retained its original

1995 P criteria of  $\geq 2$  days for air,  $\geq 6$  months for water and soil, and  $\geq 365$  days for sediment (CEPA 1999a). The United Nations Environment Program (UNEP) Stockholm Convention on POPs, signed in 2001 and in force in 2004, opted for a lower half-life in water of  $>2$  months instead of the  $>6$  months seen in the Aarhus Protocol on POPs, but kept the  $>2$  day and  $>6$  month half-lives for air and soil/sediment, respectively (UNEP 2001). This 2 month water half-life (and a BCF of 5,000) agreed upon by the Criteria Expert Group was a political compromise between the EU, who supported lower thresholds of 2 months in water and a BCF of 2,000, and others, who supported less conservative values of a 6 month half-life and a BCF of 5,000 (Matthies, Solomon et al. 2016). The Stockholm Convention named 12 persistent chemicals, the so-called “dirty dozen” (UNEP 2001). These were the pesticides aldrin, dieldrin, endrin, chlordane, heptachlor, DDT, mirex and toxaphene, the industrial chemicals hexachlorobenzene (HCB) and polychlorinated biphenyls (PCB), and the combustion by-products polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF).

**Table 1. Overview of persistence criteria in selected regulations.**

	Year in force	Degradation half-lives					
		Air	Freshwater	Marine Water	Soil	Freshwater Sediment	Marine Sediment
<b>GLWQA</b>	1978		56 d				
<b>IJC</b>	1993	56 d	56 d		56 d	56 d	
<b>Canada TSMP</b>	1995	2 d	182 d		182 d	365 d	
<b>US CMA PBT policy</b>	1996	5 d	182 d		365 d		
<b>NAAEC-CEC</b>	1997	2 d	182 d		182 d	365 d	
<b>UNECE Aarhus Protocol on POPs</b>	1998	2 d	60 d		182 d	182 d	
<b>US EPA TSCA PBT-ban pending testing</b>	1998		180 d				
<b>US EPA TSCA PBT-control action pending testing</b>	1998		60 d				
<b>US EPA Toxics Release Inventory</b>	1999	2 d	60 d		60 d	60 d	
<b>CEPA 1999 P and B Regulations</b>	2000	2 d	182 d		182 d	365 d	
<b>OSPAR PBT criteria</b>	2001		50 d				
<b>UNEP Stockholm Convention</b>	2001	2 d	60 d		182 d	182 d	
<b>EU REACH PBT criteria</b>	2007		40 d	60 d	120 d	120 d	180 d
<b>EU REACH vPvB criteria</b>	2007		60 d	60 d	180 d	180 d	180 d

It was not until the 2000s that the term “PBT” and “vPvB” were first mentioned in EU regulation, in the 2003 EU Technical Guidance Document on risk assessment in support of various directives for chemical and biocidal products. A hazard-based approach was taken since it was considered that there was too much uncertainty in quantitative risk assessment to establish ‘safe’ environmental concentrations (ECB 2003). Notably, the document defined separate half-lives for marine and freshwater compartments. The guidance assigned persistent (‘P’) half-life criteria of  $>40$  days for freshwater,  $>60$  days for marine water,  $>120$  days for freshwater sediment and  $>180$  days for marine sediment, and very persistent (‘vP’) criteria of  $>60$  days in water and  $>180$  days in sediment (EC

2003). The criteria were based mainly on the 2001 OSPAR criteria, and hence criteria for soil and air were neglected.

In 2006, the EU Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (EC) No. 1907/2006, effective from 2007, included PBT/vPvB assessment for the identification of substances of very high concern (SVHC) (EC 2006). Annex XIII of this regulation set criteria for identification of PBT and very persistent, very bioaccumulative (vPvB) substances, which included the same half-life criteria as above, but introduced a soil half-life of >120 days for P and >180 days for vP substances. This effectively negated risk assessment as a decision-making tool for PBT/vPvB substances in the EU, and the addition of soil criteria meant that all ecosystems were considered.

To summarise, the current persistence criteria in use have been developed in concert with bioaccumulation (and toxicity) criteria, with the specific aim of addressing PBT/vPvB/POP substances. This should be borne in mind when assessing persistence of substances in isolation, or when adopting these criteria for other purposes. The criteria themselves are a product of not just science but also of policy goals, meaning that there is no objective 'correct' criterion for persistence. Rather, the persistence criteria should be considered a product of prevailing policy aims. In that sense, they should arguably be periodically reviewed to ensure that they are appropriately calibrated to positively identify the correct substances and deliver the policy goals in question. Rodan et al. (1999) perhaps put it aptly that criteria should be "reasonable and tend to isolate a limited number of clearly hazardous POPs from the majority of organic chemicals, while not being so stringent that the ability to respond to as yet unidentified risks is seriously compromised".

## 2.3 Recent developments

There have been several notable developments concerning persistence assessments in recent years. One of these concerns the weight of evidence approach, which is discussed in detail in Section 3. Several other developments are presented and discussed below. In addition to these developments, general assessment approaches, including tiered testing strategies, continue to be developed and refined in regulatory guidance (e.g. ECHA 2017b). Further, novel experimental and computational methods continue to be developed, reflecting our improving understanding of the processes that impact chemical persistence in the environment, and seeking to address particular challenges such as difficult properties of test substances. These additional aspects are not discussed further here, but are addressed at several points throughout this and other documents in this series.

### Overall persistence ( $P_{ov}$ )

As seen above, the numerous regulations that use persistence criteria up to the mid-2000s had only considered half-lives in individual environmental compartments (air, soil, waters and sediments), neglecting the dynamic multimedia exchanges, partitioning and degradation processes that may have an important bearing on the overall persistence of a substance in the environment. Overall persistence, or  $P_{ov}$ , may be estimated using multimedia mass balance models. It is most commonly calculated as the steady-state residence time, i.e. the turn-over time of the substance in a flow-through system, or the ratio of the mass present in the system to the emission mass flux (Scheringer, Jones et al. 2009).

This more holistic approach was first introduced by Mackay (1979), but started gaining attention in the 1990s and early 2000s with the development of multimedia mass balance models and, later, other tools to screen for persistence, such as the US EPA PBT profiler in 2002 and the OECD  $P_{ov}$  and L RTP screening tool in 2009 (OECD 2009, Wegmann, Cavin et al. 2009). Since then, a number of

publications have evaluated and promoted the use of  $P_{ov}$  (OECD 2002a, Fenner, Scheringer et al. 2004, OECD 2004a, Klasmeier, Matthies et al. 2006, Scheringer, McLeod et al. 2006, Scheringer, Jones et al. 2009). The OECD “Workshop on the Use of Multimedia Models for the Estimation of Environmental Persistence and Long-Range Transport in the Context of POPs/PBTs Assessment”, held in Ottawa in 2001, generated a report recommending the evaluation of the uncertainty of  $P_{ov}$  in different multimedia models, before making a core set of models freely available for public use (OECD 2002a). ECETOC (2003) suggested the use of half-life ranges rather than individual critical values, and recognised that  $P_{ov}$  is a useful metric *in addition to* single media half-lives. By 2011, ECETOC suggested the use of  $P_{ov}$  *instead of* single media half-lives (ECETOC 2011). Although Environment and Climate Change Canada (ECCC) use the metric to form exposure profiles as part of their Ecological Risk Classification (ERC) of Organic Substances (ECCC 2016), the use of  $P_{ov}$  in regulation has been limited thus far.

The application of multimedia fate modelling and  $P_{ov}$  concepts in the persistence assessment have been explored in this project. For details, see the relevant documents on ‘Framework on Multi-media Fate Modelling within Persistence Assessment’ and ‘Modelling Exercises’ (Hahn, Klein et al. 2022, Klein, Klein et al. 2022).

### **Influence of temperature**

The influence of temperature on degradation rates has become relevant for persistence assessments in the EU (Matthies and Beulke 2017). Under EU REACH, a standard reference temperature of 12°C (9°C for marine environment) is applied. Since the 32<sup>nd</sup> meeting of the ECHA Member State Committee in 2013, new data generated in biodegradation simulation tests should be carried out at these reference temperatures (ECHA 2017b). Existing half-lives from simulation tests are required to be corrected from their test temperature using the Arrhenius equation and a standard activation energy (ECHA 2017b). This has the impact of increasing half-lives by greater than a factor of 2 (for extrapolation from 20 °C to 12 °C). Obviously, this has a corresponding impact on the stringency of persistence assessments under EU REACH compared with regulatory frameworks that do not apply this temperature correction. The use of the Arrhenius equation to temperature-correct biodegradation half-lives in persistence assessments has been questioned (Boethling, Fenner et al. 2009, Matthies and Beulke 2017, Brown, Camenzuli et al. 2020). These aspects are further discussed in Section 4.1.3.

### **Non-extractable residues**

The formation of non-extractable residues (NER) is a phenomenon that can only be observed in degradation studies 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).

According to REACH, 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. This has in effect increased the stringency of the persistence assessment, when compared with the historical approach where the NER fraction would not be included in the determination of half-lives.

A unified approach has been developed to further divide NER into three types: Type I (physically entrapped parent substance and transformation products), 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).

### **PFAS and substances with extreme persistence**

In recent years there has been significant global awareness and action being taken against per- and polyfluoroalkyl substances (PFAS) (Cousins, Ng et al. 2019, Cousins, DeWitt et al. 2020, EC 2020, Fenton, Ducatman et al. 2021, Panieri, Baralic et al. 2022). These substances are highly resistant to degradation and have been termed 'forever chemicals'. There is a growing body of evidence on their health effects and concern for their continued accumulation in the environment. PFAS have catalysed a renewed interest in the environmental persistence of substances. They have led to the birth of the 'P-sufficient approach' concept, whereby high persistence alone is considered a major cause for concern and justification for regulatory action (Cousins, Ng et al. 2019). Synthetic polymers and the pervasive issue of (micro)plastic pollution has also been discussed in this context (Cousins, Ng et al. 2019, MacLeod, Arp et al. 2021).

However, a distinction should be made between substances that are persistent (P) or very persistent (vP) in the context of a persistence assessment from those that are essentially non-degradable in the environment. These extremely persistent ("eP") substances differ from other P or vP substances with half-lives closer to regulatory cut-offs. The latter substances would still be expected to degrade appreciably in the environment and not accumulate to the extent that eP substances would (EC 1995, Scheringer, Jones et al. 2009). It should be borne in mind that the original P and vP criteria were intended to be used in combination with bioaccumulation (B/vB) and toxicity (T) criteria, and that using these criteria out of context may not be fit for purpose. Arguably, separate criteria for eP should be developed so that these forever chemicals can be distinguished from traditional P/vP substances. However, this comes with some practical challenges, such as the fact that extremely long half-lives are difficult to measure with accuracy in standard simulation tests (EC 2017). It is likely that different approaches would be needed to address this concern and differentiate these substances effectively (Goldenman, Holland et al. 2017).

### **Persistent and mobile substances**

A further new development concerning persistence, again linked to PFAS, is the concern from persistent and mobile substances (EurEau 2019, Hale, Arp et al. 2020, Hale, Neumann et al. 2022). These substances have until recently been less of a focus than the traditional persistent and bioaccumulative substances. However, recently concerns have been raised as to the potential of these substances to contaminate sources of drinking water, and have been argued to pose an equivalent level of concern to PBT/vPvB substances in the context of REACH (Hale, Arp et al. 2020). The EU Chemicals Strategy for Sustainability has committed to developing criteria for PMT/vPvM substances, and adopting this as a new hazard class in both CLP and REACH (EC 2020). At present, the criteria for persistence under the PMT/vPvM hazard class are proposed to align with those of PBT/vPvB.

The introduction of a PMT/vPvM hazard class is expected to bring a distinct chemical space under new regulatory scrutiny, since mobile chemicals are opposite in terms of their hydrophobicity,

compared with the more commonly scrutinised bioaccumulative chemicals. Further, the PMT/vPvM hazard class may be far more successful than PBT/vPvB, in terms of the number of substances falling into its scope, given that degradation transformation products of substances also have to be considered, and most transformation products will be more mobile than their parent compounds. This, and the fact that the exposure-multiplying property of bioaccumulation is not applicable for these substances, may be good reason to reconsider the persistence assessment criteria that are used for PMT/vPvM substances. The introduction of a PMT/vPvM hazard class also further complicates the already very challenging task of performing persistence assessments on complex substances (Hughes, Griffiths et al. 2022b).

The interest in these particular substance types does raise the question of chemical persistence in the groundwater compartment, since this is a major focus of the PMT/vPvM concern. Current persistence testing focuses on degradation in aerobic soil and at the water-sediment interface. It is unclear how well this represents chemical behaviour in the groundwater environment.

### Recent scientific workshops and discussions

In recent years there have been various scientific meetings and workshops focusing on the topic of persistence. In 2008 there was a SETAC Pellston Workshop on 'Science-Based Guidance and Framework for the Evaluation and Identification of PBTs and POPs', leading to several publications (Klečka, Muir et al. 2009). ECETOC also co-hosted workshops in 2007 and 2012 (ECETOC 2007, ECETOC 2013). In 2018, a further workshop was co-hosted by Cefic-LRI and Concawe in Helsinki and discussed findings from a number of recent research projects (Whale, Parsons et al. 2021). Following this workshop, an ECETOC task force was launched titled 'Moving persistence (P) assessments into the 21st Century: Developing a new paradigm to assess degradation potential of chemicals'. This task force produced two papers, one focusing on persistence assessment concepts and methods, and the other on weight of evidence and overall persistence (Davenport, Curtis-Jackson et al. 2021, Redman, Bietz et al. 2021). Most recently, during the SETAC Europe 2022 conference in Copenhagen, a topical discussion session organised by ECETOC on 'Moving persistence assessment into the 21st Century' was held (Ott, Hughes et al. 2022). In this session, a panel of experts from academia, industry and non-governmental organisations (NGO), and an audience of over 100 SETAC delegates via live polling, gave perspectives on a series of questions on persistence assessment, covering concepts, approaches, challenges and research needs. This session gave a broad snapshot of the state of opinion on persistence assessment. In addition there have been several workshops on the specific topics of NER in 2009 and 2021, and PMT in 2011, 2018 and 2021 (ECETOC 2010, UBA 2021, Hale, Neumann et al. 2022).

## 2.4 Key differences between EU REACH and other frameworks

Persistence assessment is incorporated into various regulatory PBT and POP assessment frameworks globally. Table 1 details some key frameworks and their half-life criteria across different environmental compartments. It can be seen that the half-life criteria differ across different legislations. There are other similar legislations in Australia/New Zealand, Japan, China, and Korea (Matthies, Solomon et al. 2016). China recently issued its Order No.12 of the Ministry of Ecology and Environment on 'The Measures for the Environmental Administration Registration of New Chemical Substances', which applies the same persistence criteria as EU REACH (MEE 2020).

Within the EU, the Plant Protection Products (PPP) regulation, Biocidal Products Regulation (BPR), Human Medicinal Products, and Veterinary Medicinal Products regulations share the same half-life criteria as REACH (EC 2006). The EU REACH framework includes comprehensive technical

guidance, including a tiered testing approach and standard guidelines (ECHA 2017b). The EU REACH guidance also forms the basis for assessments carried out under the BPR, and for human and veterinary medicinal products. The PPP regulation however uses its own guidance currently, and this is a source of potential divergence between persistence assessments of plant protection products and that of other chemicals in EU (EC 2009, Rauert, Friesen et al. 2014). An important difference is that for persistence assessment under PPP the half-life is currently normalised to 20°C, whereas under REACH it is normalised to 12°C (EC 2009). This situation may change in the future as the EU Chemicals Strategy aims to align assessment approaches between different legislations (EC 2020). The US EPA Toxic Substances Control Act (TSCA) also employs a tiered approach, and recommends performing simulation tests at a temperature characteristic of the environment that is simulated, or to correct half-lives and kinetic constants to average outdoor temperatures (USEPA 2008). Similar guidance on tiered testing approaches and reference temperatures is lacking for Stockholm Convention and ECCC regulations.

The approach for NER also differs between EU REACH compared with other frameworks, where REACH considers NER a 'hidden hazard', while the PPP regulation considers NER non-bioavailable and hence are not included in P assessment (EC 2009). The US EPA Guidance for Addressing Unextracted Pesticide Residues in Laboratory Studies includes NER in assessment if they are >10%, or if a minor transformation product may be present that is known/suspected to be more toxic than the test compound. If the extraction method is considered adequate, the NER are considered to be strongly sorbed and are not considered bioavailable (USEPA 2014). There is no specific guidance regarding NERs under ECCC or the Stockholm Convention.

Different approaches for testing and assessment, reference temperatures and NER can all influence whether or not a substance will meet the relevant persistence criteria. Furthermore, there is variation in the regulatory consequences of a substance if it meets the criteria (Rauert, Friesen et al. 2014). Under REACH, substances found to be PBT/vPvB have to undergo emission minimisation, and are considered meeting the criteria of substances of very high concern (SVHC). They may be added to the Candidate List for Authorisation, leading to an eventual ban on use (ECHA 2017b). Under the PPP and Biocidal Products regulations, a substance will not be approved and will be included in a list of candidates for substitution if two out of three criteria are met (EC 2009, EC 2012). The Human Medicinal Products regulation stipulates risk mitigation to reduce emissions, while the Veterinary Medicinal Products regulation similarly opts for risk reduction and risk management measures (e.g. post-authorisation monitoring), or no authorisation, or a decision by the Committee for Veterinary Medicinal Products (EC 2001a, EC 2001b). The TSCA will either 'order pending testing/significant new use rule (SNUR)' or 'ban pending testing', according to section 5(e) of TSCA (USEPA 1999). The Stockholm Convention goes down the route of further assessment (risk profile), followed by potential elimination from markets (Annex A), restriction (Annex B), or minimisation of release (Annex C) (UNEP 2001). In Japan a PBT substance is subject to restrictive control (known as Class I Specified Chemical Substances) (Naiki 2010). The recent China MEE Order 12 follows an interesting approach in that registration requirements are not dictated by tonnage as for EU REACH, but by whether the substance has persistence and/or bioaccumulative properties (MEE 2020). This is a relatively unique approach, and raises the importance of the persistence assessment significantly. Substances which are persistent, bioaccumulative and toxic, or 'highly persistent' and 'highly bioaccumulative' are considered highly hazardous substances and carry additional requirements for social and economic benefits analysis.

### 3 Weight of Evidence

Before considering how the use of weight of evidence can be improved in persistence assessments, we will first consider what the concept is, and the guidance currently available on its use. The term 'weight of evidence' is an approach for decision-making, which involves utilising multiple, often disparate, pieces of information. This is carried out by a process of collating, 'weighing' and evaluating evidence in order to form a scientifically plausible conclusion (SETAC 2018). The approach improves robustness by avoiding reliance on individual pieces of information, which on their own may be insufficient to inform a particular question or hypothesis, and is utilised in both ecological and human health assessments (CEPA 1999b).

Definitions of weight of evidence approaches vary in complexity; therefore, it is not entirely a well understood concept used in chemical evaluations and environmental risk assessments. Weight of evidence is conceptually seen as a method for decision-making involving assigning a "weight" to each known line of evidence dependent on the specific relevance and reliability (OECD 2019). This allows for conclusions to be drawn which address the hypothesis proposed (e.g., a substance is persistent). There is no formal adopted definition of the weight of evidence approach however a few definitions are described below.

**(ECHA no date)**

*"Weight of Evidence approach can be generally described as a stepwise process/approach of collecting evidence, assessing, integrating, and weighing them to reach a conclusion on a particular problem formulation with (pre)defined degree of confidence"*

**(WHO 2009)**

*"Weight of Evidence is a process in which all of the evidence considered relevant for a risk assessment is evaluated and weighted"*

**(USEPA 2016)**

*"Weight of Evidence: (1) A process of making inferences from multiple pieces of evidence, adapted from the legal metaphor of the scales of justice. (2) The relative degree of support for a conclusion provided by evidence. The result of weighing the body of evidence."*

**(EFSA 2017)**

*"Weight of evidence assessment is a process in which evidence is integrated to determine the relative support for possible answers to a scientific question."*

**(OECD 2017)**

*"Weight of Evidence refers to a positive expert opinion that considers available evidence from different independent sources and scientific viewpoints on a particular issue, , coming to a considered view of the available, oftentimes conflicting data. It is preferred when every source does not provide sufficient information individually"*

**(SCHEER 2018)**

*"Weight of Evidence: A process of weighted integration of lines of evidence to determine the relative support for hypotheses or answers to a question"*

Furthermore, the weight of evidence approach in the context of PBT/vPvB assessment is described in REACH Annex XIII as:

*“A **weight-of-evidence** determination means that all available information bearing on the identification of a PBT or a vPvB substance is considered together, such as the results of monitoring and modelling, suitable in vitro tests, relevant animal data, information from the application of the category approach (grouping, read-across), (Q)SAR results, human experience such as occupational data and data from accident databases, epidemiological and clinical studies and well documented case reports and observations. The quality and consistency of the data shall be given appropriate weight. The available results regardless of their individual conclusions shall be assembled together in a single weight-of-evidence determination.”*

In this chapter, current practices and methodologies for weight of evidence, as presented in international guidance documents, are discussed. The specific application of the weight of evidence approach to persistence assessments is then discussed.

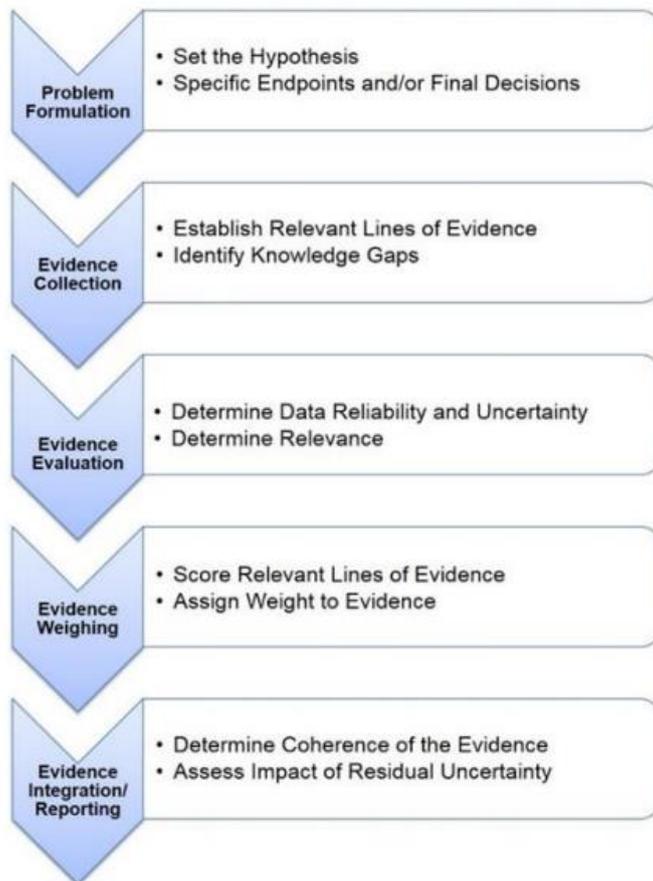
## 3.1 Current guidance on weight of evidence

### OECD (2019)

OECD (2019) recognised that weight of evidence is context dependent, and therefore examined approaches for both human and ecological health, considering universal principles and elements in order to provide a practical guidance document for the formal implementation of weight of evidence in chemical management programs. The document describes a systematic approach to weight of evidence establishing universal guiding principles for “good practice”. The principles are as follows:

- Definition of a hypothesis involving a clear formulation and statement of a problem, as well as possible alternative hypotheses.
- A systematic and comprehensive design documenting the procedure for collating and interpreting evidence.
- The inclusion of a treatment of uncertainty arisen from available data and unknowns/knowledge gaps.
- Consideration of the potential for bias during the systematic approach.
- Transparency across all weight of evidence decisions to allow for understanding, reproducibility, questioning and support by all interested parties.

The document also emphasised the importance of five key elements, detailing necessary steps in formulating a systematic approach to weight of evidence for chemical evaluation (see Figure 2). The first element emphasises the importance of a hypothesis-based approach for chemical evaluation. The formulation and development of hypothesis-based questions during the problem formulation stage is crucial to ensuring correct and meaningful evidence. The scope and goals of the assessment should be defined during the problem formulation stage, as well as an acceptable level of uncertainty and urgency. The level of uncertainty acceptable is typically dependent on the receptor(s) (i.e., human/ecological, single species/multiple species), the context of the decision to be made and the protection goal(s) that are defined within the problem formulation stage.



**Figure 2. Key elements for Weight of Evidence for the assessment of chemicals (from OECD, 2019).**

Secondly, evidence should be collated with lines of evidence and knowledge gaps established. In more recent weight of evidence approaches, assembling evidence into lines of evidence relevant for addressing the hypotheses is common. Lines of evidence are key to ensure transparency within the review and can also allow for the identification of relevant information gaps. Examples of lines of evidence include studies that share a common line of inquiry, i.e. measured endpoints or properties, or evidence, like laboratory data, that come from common sources (Suter and Cormier 2011). To improve transparency, the criteria for line of evidence selection should be documented, as well as the search strategy including key words and sources searched.

The evidence must then be evaluated in order to determine the reliability, uncertainty and relevance of the data followed by assigning a weighting to the evidence. Evidence evaluation can be considered the most crucial step in the weight of evidence assessment, and aims to determine the inherent quality and completeness of the available information. Data quality assessment is discussed further in Section 3.2. Methods for the weighting of evidence can be assigned to two categories: quantitative (numerical) approaches, or qualitative approaches, which are most commonly used. In this step, a weight is assigned to each line of evidence, described either symbolically or numerically, combining influences of relevance and reliability.

Finally, evidence should be integrated and reported. In this step, the coherence of the lines of evidence must be considered, as well as the relationship between the evidence and the weight of evidence conclusion(s). The plausibility and connection between the evidence and the weight of evidence must be coherently examined, for all evidence collated.

A common thread throughout all of the key elements described is the communication and treatment of uncertainty, which can heavily impact the strength of the decision-making as part of the weight of evidence approach. The principles and elements outlined in the document allow for consistent, clear, and transparent decision-making, and enable stakeholders to determine reasons for bias, and determine when bias has become unreasonable.

### **Suter et al. (2020)**

Suter et al. (2020) addressed the benefits of combining the approaches of systematic reviews (SR) and weight of evidence (WoE), based on an understanding of what each practice can offer to environment assessment. The paper recognised that in order to carry out a valuable environmental risk assessment, two broad steps must be taken: the assembly of reasonably complete, relevant, reliable, and unbiased evidence (SR approach) followed by the application of appropriate qualitative and quantitative methods to derive conclusions from the evidence collated (WoE approach). SRs were inspired by (Cochrane 1972) and formalised in 1992 with the aim to provide reliable, consistent, and reproducible summaries of randomised clinical trials of medical treatments. Characteristically SR aims to answer a problem/question through meta-analysis of an assembled data set from a single type of study, whereas WoE addresses a diverse range of evidence in a less methodical manner. Today, SRs have been adapted for environmental applications, including environmental management and environmental risk assessments. The adoption of SRs enabled evidence reviews to limit bias. When organising diverse bodies of evidence, two characteristics should be considered: the type of evidence and the implication of the evidence. The paper recognised that providing an explicit rating system dependent on the identification of the weight of evidence properties is advantageous. However, it was also noted that the creation of a detailed procedure is not always useful in the case of differing questions and inconsistent information. In this case, there is a call for expert judgement as opposed to intricate procedural detail.

Suter et al. (2020) stated that assessors need not choose between SR and WoE approaches when synthesizing evidence, as there is significant overlap in practices. Therefore, an integrated framework of WoE and SR was proposed to defensibly support evidence-based decision making, when classic SR cannot be used, as there is more than one type of study to assess, and meta-analysis is not possible. This framework, similar to the US EPA (2016) weight of evidence approach, has been named 'Methodical Assembly and Weighing of Evidence' (MAWE) and can help derive either qualitative or quantitative conclusions to answer a hypothesis (Figure 3).

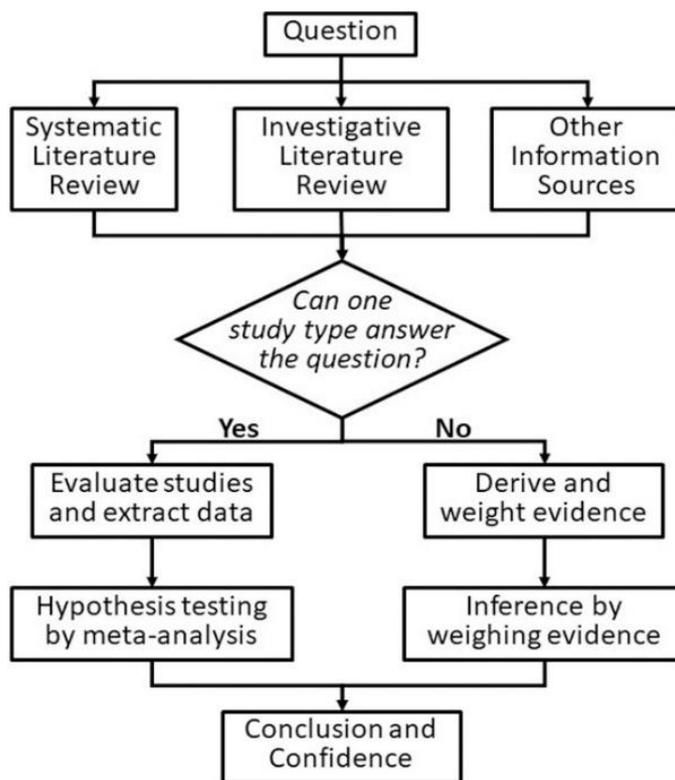


Figure 3: Diagram of MAWE approach (from Suter et al. 2020)

**ECHA (no date)**

A guidance document produced by ECHA recognised the absence of a formal adopted definition of the weight of evidence approach (ECHA no date). The document aimed to harmonise the weight of evidence approach and uncertainty assessment within ECHA processes and improve transparency in regulatory decision making relevant for REACH, CLP and BPR purposes. The document also confers the elements necessary to consider when building a weight of evidence approach, as well as the assessment of uncertainty. The principles described are focused for hazard assessment. However, where the weighing of evidence applies, the principles can also be considered valuable for exposure and risk assessments.

Seven steps were proposed that are considered the backbone of the weight of evidence approach:

1. Problem formulation
2. Collection & Documentation of all information
3. Assessment of quality of individual evidence (reliability, relevance, adequacy)
4. Integration & Weighing of Evidence
5. Application of levels of confidence
6. Uncertainty Analysis
7. Conclusion

Step 4 includes the integration and weighing of evidence using metrics such as consistency and plausibility. However, it is also recognised that weight of evidence also partially occurs at step 3, when assessing the quality of the evidence. The document emphasises that all steps are interconnected, and the weight of evidence approach can be considered iterative.

Step 5 in this approach, the application of levels of confidence, is distinct from other weight of evidence approaches. Confidence levels should be derived considering the outcome of weighing the evidence and assigned to both the lines of evidence and the overall weight of evidence assessment. Confidence levels are usually expressed in a high/medium/low or strong/medium/weak format. In this document, the following criteria has been proposed for the definition of confidence levels, with respect to the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) opinion:

**Strong:** Coherent evidence from human and one or more other lines of evidence (in particular mode/mechanistic studies) in the absence of conflicting evidence from one of the other lines of evidence (no important data gaps)

**Moderate:** good evidence from a primary line of evidence but evidence from other lines of evidence is missing (important data gaps)

**Weak:** weak evidence from the primary lines of evidence (severe data gaps)

**Uncertain:** due to conflicting information from different lines of evidence that cannot be explained in scientific terms

**Weighing of evidence not possible:** No suitable evidence available

### EFSA (2017)

The European Food Safety Authority (EFSA) also developed a guidance document/flexible framework on the use of the weight of evidence approach in scientific assessments for use in all areas under EFSA's remit, as requested by the Scientific Committee (EFSA 2017). The guidance document addresses both qualitative and quantitative approaches to weight of evidence analysis.

Three basic steps were established for the weight of evidence approach:

- (1) Assembling evidence into lines of evidence of similar type
- (2) Weighing the evidence
- (3) Integrating the evidence

These steps may occur at one or more points in an assessment where evidence integration is needed (Figure 4).

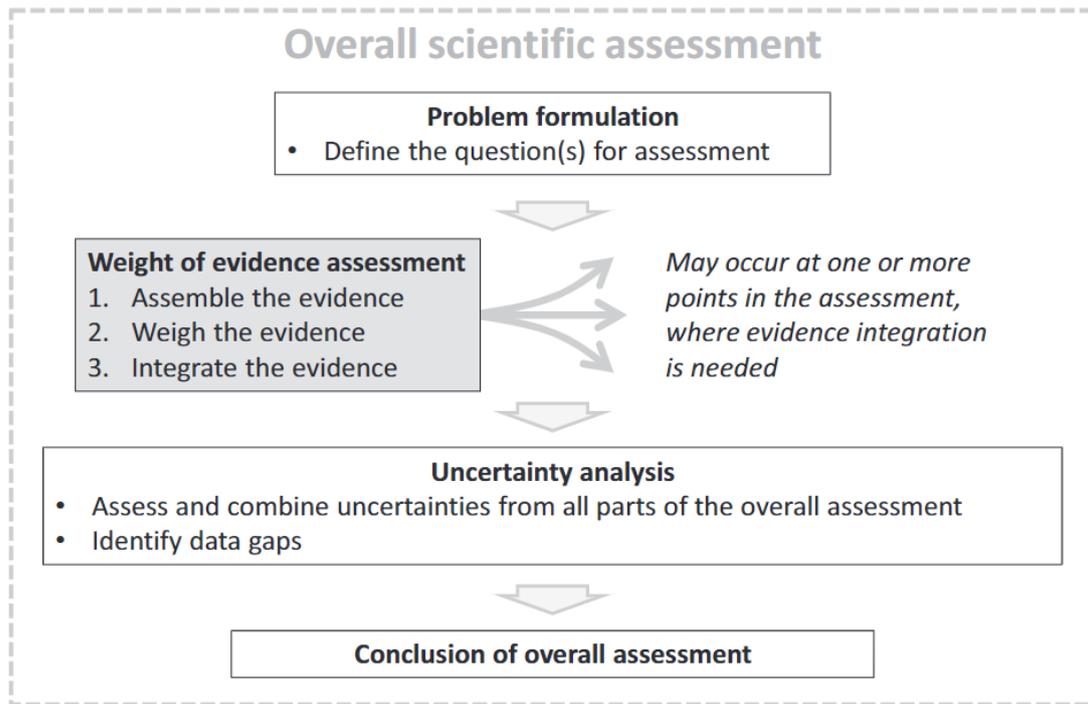


Figure 4: Illustration of the 3-step process of a weight of evidence assessment (from EFSA, 2017).

A conceptual diagram is presented illustrating the three-step approach to weight of evidence and the stages at which assessments of relevance, reliability and consistency are incorporated (Figure 5). Further, various approach classifications for weight of evidence methods, and criteria for assessing their strengths and weaknesses, are discussed. These classifications include ‘best professional judgement’, causal criteria, rating and quantification.

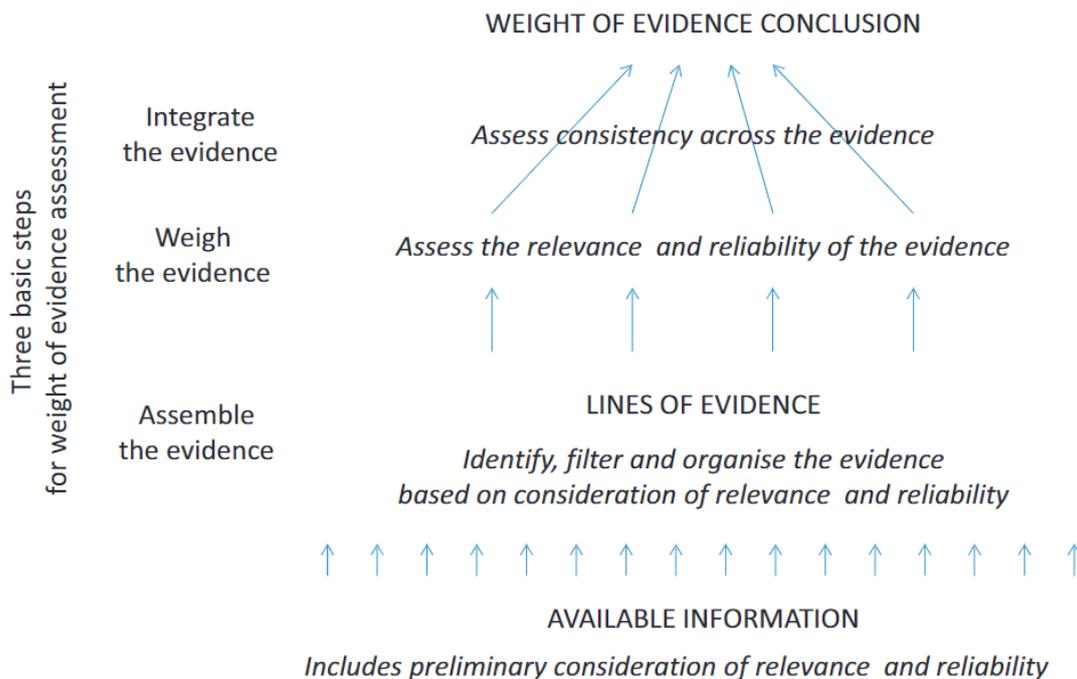


Figure 5: Weight of evidence framework proposed considering relationship of relevance, reliability and consistency to the three basic steps of weight of evidence assessment (from EFSA, 2017).

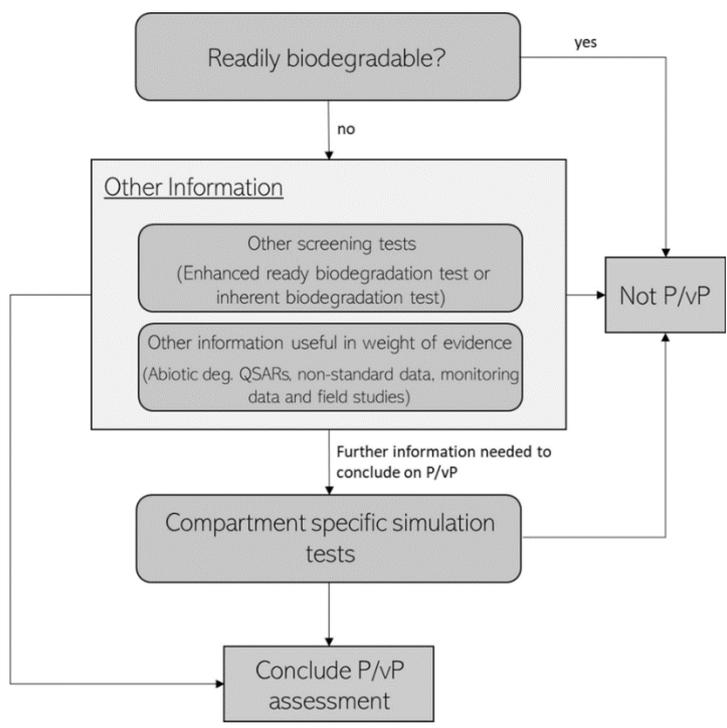
The reporting of a weight of evidence assessment is considered crucial in the EFSA document. Every step of the assessment should be justified, documented in sufficient detail, and clearly state where and how expert judgement has been applied in order to produce a transparent and repeatable review.

## 3.2 Application in persistence assessments

Persistence assessment is a clear example of a process that can benefit from a weight of evidence approach. A range of information types can inform the assessment, and these somehow need to be combined to form a single conclusion. Degradation data are inherently highly variable, and often the datasets are imperfect or incomplete, meaning that a degree of uncertainty needs to be managed. By employing weight of evidence principles, persistence assessments can be made more robust, transparent and consistent.

The framework for assessing persistence under REACH is detailed in Annex XIII of the legal text, with an Integrated Assessment and Testing Strategy (ITS) detailed in ECHA guidance (EC 2011, ECHA 2017b). The assessment considers compartmental degradation half-lives, as well as several other types of relevant information (e.g. screening tests, non-standard experiments, QSARs, field data, etc). The types of information are categorised as 'screening' or 'assessment' information in Annex XIII, with screening information used to indicate whether further testing is necessary to generate assessment information to confirm persistence. Screening information includes biodegradation screening tests (ready, inherent and enhanced ready biodegradability tests), QSARs and 'other information provided that its suitability and reliability can be reasonably demonstrated'. Assessment information includes results from simulation testing on degradation in surface water, in soil or in sediment, and 'other information, such as information from field studies or monitoring studies, provided that its suitability and reliability can be reasonably demonstrated'.

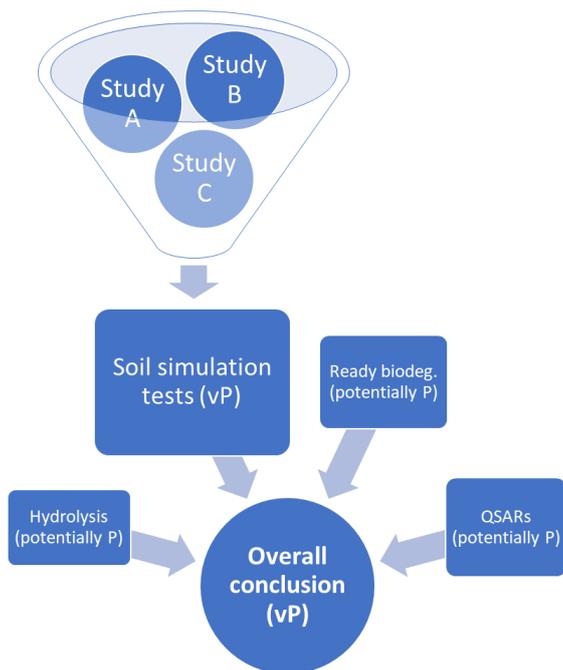
The types of persistence information are further arranged into a tiered workflow in the ITS detailed in guidance (Figure 6). The ITS specifies the order in which testing should be conducted in order to generate information necessary to determine the persistence. If the substance can be concluded as not persistent on the basis of screening information then there is no need to generate further information on persistence.



**Figure 6: Simplified ITS flow diagram for substance persistence assessment according to ECHA guidance (from Hughes et al., 2020).**

As a weight of evidence approach, the persistence assessment should consider all available relevant information together in order to arrive at a single conclusion. However, the further categorisation of different types of information into screening and assessment information, and the tiered approach outlined in the ITS, implies an order of precedence as to how data should be considered in the assessment. This appears somewhat contradictory, since preferring one type of information over others despite contradictory evidence would not be in line with weight of evidence principles. However, this is more familiar for regulatory assessments, where clear conclusions have to be drawn from the results of standard tests. A proposal for applying a weight of evidence approach in persistence assessments is discussed below.

Persistence assessments can be conducted systematically, and in accordance with a weight of evidence approach, by considering the information in each line of evidence (that is each distinct type or category of information) separately, in order to determine what conclusion that particular line of evidence supports. This can take into account the quality, quantity and strength of evidence for that particular line of evidence, and any associated criteria that apply. Then, conclusions for individual lines of evidence can be weighed and combined into a single overall conclusion for the substance, taking into account the prioritisation of different lines of evidence according to the ITS. An illustration of how this approach could be performed is provided in Figure 7. This will also enable an assessment of consistency across lines of evidence, which is an important component of weight-of-evidence approaches described earlier.



**Figure 7. Schematic illustration of a weight of evidence methodology for persistence. Individual studies contribute to conclusions for lines of evidence, which in turn are weighted and combined to an overall conclusion for the substance.**

The above approach could be made consistent with the current approach for persistence assessment, taking into account existing rules such as whether information is considered screening or assessment information, and how to approach multiple or conflicting results for a single line of evidence. Effort would be needed to determine the relative weighting and precedence of individual lines of evidence and a systematic approach for combining this information. Clarification is needed on how certain types of information, such as anaerobic and abiotic data, results from OECD 314B tests, and experiments with single degrading species, can be used in the weight of evidence determination, as currently this remains ambiguous and therefore these data are of limited use in practice (ECHA 2017b). One option would be to incorporate the principles of biodegradability categories (or ‘bins’) as previously proposed (Comber and Holt 2010, Whale, Parsons et al. 2021). These categorisations would operate at the screening level similar to biodegradation screening tests (e.g. ready/inherent biodegradability, fulfilling certain criteria), and would enable other evidence informing the intrinsic degradability of the substance to be utilised in the assessment. The definition of these bins, the data that inform them, and their interpretation in the persistence assessment weight of evidence, would require further discussion. However, this approach would facilitate the consideration of all relevant available information in the assessment, in line with weight of evidence principles.

Most importantly, what is central to an effective weight of evidence approach for persistence assessment is a structured approach to evaluate the quality of individual pieces of evidence, so that these can be meaningfully weighed and combined with other evidence. By using a structured approach to evaluating data quality, and clearly documenting this as part of the assessment, this would facilitate a more structured and objective assessment.

**Evaluating data quality in the context of persistence assessment**

The assessment of data quality is a crucial first step in conducting a robust, transparent and consistent weight of evidence assessment of persistence. Approaches for data quality evaluation in regulatory assessments have been criticised (Ingre-Khans, Ågerstrand et al. 2018). Further, the lack

of utilisation of research from academic literature in regulatory assessments has been highlighted (Ågerstrand, Breitholtz et al. 2011, Tweedale, Lysimachou et al. 2014). This is in part due to a lack of detailed reporting preventing evaluation of data quality. In response to this, the CRED (criteria for reporting and evaluating ecotoxicity data) scheme has been developed for aquatic toxicity studies, which provides a more structured approach to quality evaluation than the Klimisch scheme, currently adopted under REACH (Klimisch, Andreae et al. 1997, Moermond, Kase et al. 2015). The CRED scheme provides a more detailed assessment scheme for evaluating studies and therefore reduces the degree of subjectivity (and thus potential for bias) in the assessment. Substantial progress has also been made in the quality evaluation of bioaccumulation data, built upon significant knowledge gained in recent decades (Armitage, Toose et al. 2021, Arnot, Toose et al. 2022). Similar to persistence, bioaccumulation assessment can be informed by various lines of evidence, and each of these may require separate and dedicated quality assessment schemes. Despite the progress made in data quality evaluation for aquatic toxicity and bioaccumulation assessment, similar progress has been sorely lacking for persistence assessment.

The evaluation of data quality can generally be split into two components: reliability and relevance. Although adequacy is mentioned in ECHA guidance, it appears somewhat poorly defined and a product of reliability and relevance, similar to quality (ECHA no date). In the context of the standard information requirements under REACH, adequacy may indicate whether a piece of information is sufficient to satisfy a particular endpoint requirement. However, this is a separate issue to weight of evidence when applied to persistence assessments. Adequacy is also not considered in CRED, Klimisch and most of the weight of evidence frameworks evaluated. Hence, it is not considered further in these proposals.

Reliability concerns the extent to which the experiment has been designed, executed and reported such that the results can be considered robust and reproducible. This can be informed by whether the experiment has been carried out according to a standard guideline, with quality control procedures in place, such as Good Laboratory Practice (GLP) (OECD 1998). It is also informed by aspects such as the experimental design features (e.g. replication, controls), validation of methods (e.g. analytical method), statistical significance of results and compliance with any associated validity criteria for the experiment.

Relevance concerns the extent to which the information derived can inform the particular question at hand. In the case of persistence assessment, the question is defined by the regulatory endpoints, criteria and relevant conditions. So, for example, whether a substance is persistent, which is defined by its degradation half-life in water, soil, or sediment. The standard OECD guideline tests provide the definitive evidence of this, and thus define the most relevant conditions in the regulatory context. The OECD guideline tests define specific conditions under which degradation is to be assessed (e.g. test system geometry, inoculum sampling and storage conditions etc). These conditions may themselves influence how the substance degrades relative to other potential conditions, or conditions in the field. However, even within these defined conditions there are significant sources of variability which can influence degradation results. These aspects are discussed further in the following chapter.

Reliability and relevance are therefore two distinct and separate categories under which individual studies can be evaluated. These elements can be combined to determine study quality. This provides the basis to move from qualitative, highly subjective evaluations of study quality towards more objective, semi-quantitative and quantitative evaluations. Principles and criteria to determine reliability and relevance of individual (bio)degradation studies still need to be developed. These are likely best informed by the available knowledge contained in standard test guidelines and regulatory guidance, as these inform what are currently considered the most relevant information in the regulatory context. This provides a clear basis with which to evaluate the quality of information derived from standard tests, and this process is largely already implemented. For example, if a study is carried out according

to an OECD guideline, in accordance with GLP, and is observed to have adhered to all the prescribed conditions and validity criteria of that study, then it can generally be considered of high reliability. It can also be considered to be of high relevance, given that the guideline is considered to detail the most relevant conditions. That is not to say that there are not still issues with the suitability and execution of current guidelines, particularly in relation to certain substance types (Ingre-Khans, Ågerstrand et al. 2018, Hughes, Brown et al. 2020, Shrestha, Meisterjahn et al. 2020). However, the greatest challenge comes when dealing with non-standard studies, i.e. those that are not conducted according to standard test guidelines. It is necessary as part of a weight of evidence approach to utilise this information, but it may be unclear how to evaluate their reliability and relevance (Wassenaar and Verbruggen 2021).

In the case of non-standard degradation studies, these can be evaluated in a similar way to standard guideline studies, where their conditions and results resemble those of standard studies. This therefore requires the codification of conditions and requirements of guideline studies so that non-standard studies can be assessed against these requirements. In the case of studies not resembling standard test guidelines, the principles of existing guidelines may still be useful to inform these. In the case of quantitative structure activity relationships (QSARs), existing guidance is available to evidence that that these are reliable (OECD 2004b, ECHA 2008b). However, the question of relevance appears again, and for this there is a need to consider the data on which the models have been trained.

The codification of rules for data quality evaluation has so far not been done for persistence, although, as previously mentioned, encouraging progress has been made for aquatic toxicity and bioaccumulation studies. It was beyond the scope of this project to develop these data quality evaluation criteria, however work is ongoing in a separate project to develop these as part of a software tool to support practitioners in carrying out persistence assessments (Hughes, Griffiths et al. 2022c). It is likely that what is initially proposed will require refinement, since scientific and regulatory consensus would likely be a prerequisite to the implementation of any data quality scoring system. However, once this system has been developed it should provide a much more systematic basis for evaluating the quality of data used in persistence assessments.

The development of a data quality assessment framework for data used in persistence assessment would provide great benefits to the adoption of weight of evidence principles in these assessments, since currently the assessment of data quality is somewhat opaque and subjective. Non-standard studies are commonly afforded little weight or even not considered, since the rules to establish their reliability and relevance are not well defined. However, these studies often provide useful information on the persistence of substances, and should be considered in accordance with weight of evidence principles.

## 4 Sources of variability in degradation data

Degradation of substances in the environment is dependent not only on the properties of these substances, but also the environmental conditions. Degradation reactions are inherently second order in nature, depending not only on the concentration of the substance but on other reactants (e.g. enzymes, free radicals) (Mackay, Hughes et al. 2014). Further, conditions such as temperature and processes such sorption, affecting bioavailability, may vary in the environment (Alexander 1999). Observed rates of degradation are therefore subject to high inherent variability (Boethling, Fenner et al. 2009). These sources of variability can be relevant to both experimental settings as well as the real environment. In order to conduct robust and consistent persistence assessments, often using incomplete and disparate information in a weight-of-evidence approach, there is a need to be mindful of these potential sources of variability, and account for them wherever possible. Admittedly, this can be challenging, particularly where this variability amounts to natural variability within the environment (Schäffer, Fenner et al. 2022). In such cases it is important to remain cognisant of relevant conditions (see Section 3.2) in order to support consistent prioritisation of substances. In this section we will discuss these extrinsic sources of variability in degradation rates of substances. These differ from intrinsic sources of variability related to substance properties, which are covered in the separate 'Guidance for the Persistence Assessment of Difficult Test Substances' document in this series (Hughes, Griffiths et al. 2022a).

### 4.1 Environmental factors

#### 4.1.1 Inoculum

The composition of microbial communities present in environmental settings have been recognised as key influencers in biodegradation kinetics (Kowalczyk, Martin et al. 2015, Ott, Martin et al. 2019, Davenport, Curtis-Jackson et al. 2021). Davenport et al. (2021) described the “quantity and quality” of degrading inocula, with quantity referring to cell concentration or total amount of inoculum, and “quality” referring to the microbial community composition and its activity. This refers to the presence and viability of specific degrader taxa, as well as taxonomic and functional diversity and activity of the inoculum. An increase in concentration of inoculum may not be sufficient without a concomitant increase in diversity (Martin, Snape et al. 2017, Ott, Martin et al. 2020, Davenport, Curtis-Jackson et al. 2021, Wennberg, Meland et al. 2022). A wide variation in both diversity and concentration of bacterial communities is evident across different environmental settings (Mezzanotte, Bertani et al. 2005, Wu, Ning et al. 2019). Different standard biodegradation screening and simulation tests utilise a range of microbial sources, cell concentrations and total numbers of cells (Davenport, Curtis-Jackson et al. 2021). Increased diversity in microbial communities has been shown to influence biotransformation of chemicals in a variety of matrices (Dey and Tribedi 2018, Ott, Martin et al. 2020, Chalifour, Walser et al. 2021, Jayaramaiah, Egidi et al. 2021).

Inocula sampled directly from the field may result in a high variability of bacterial composition dependent on the geographical location, climate, and season from which the sample was taken (Baginska, Haiß et al. 2015). A study by (Wennberg, Meland et al. 2022) found that both sampling depth and season influenced the outcomes of OECD 306 biodegradability in seawater tests. Solutions on how to overcome the issue of inoculum variability in the OECD 306 guideline have been discussed in an ECOTOC workshop (Ott, Martin et al. 2019). Microbial communities also exhibit the ability to adapt and evolve when exposed to a substance, although at present according to REACH pre-

exposure if the inoculum is not permitted for persistence assessment (ECHA 2017a, Poursat, van Spanning et al. 2019).

#### 4.1.2 Matrix characteristics

The degradation of substances can also vary dependent on the properties of the environmental matrix. Persistence assessment under REACH requires the evaluation of degradation in three (or five) distinct compartments: soil, surface waters and sediments, covering both freshwater and marine environments (ECHA 2017b). These compartments are clearly very different from each other, and these contrasting characteristics influence the fate and behaviour of substances differently. The sediment compartment is particularly complex and is discussed separately in Section 4.3.

Previous efforts have been made to extrapolate results of degradation studies from one compartment to another. For example, Boethling (1995) developed scaling factors of 1:1:4 for water:soil:sediment based on a database of degradation half-lives. Similar scaling factor approaches are applied to model degradation in environmental exposure assessments (ECHA 2016). However, the application of such scaling factors in persistence assessments has so far been limited. More recently, efforts have been made to relate degradation in simple batch experiments with activated sludge to more complex soil OECD 307 simulation tests (Fenner, Screpanti et al. 2020). It was found that correction for sorption-related bioavailability differences between the two matrices produced a good correlation of results, showing promise for future prediction and inter-compartment extrapolation of degradation half-lives.

Relevant matrix properties that may influence the persistence of substances include textural composition, pH, cation exchange capacity (CEC), porosity, moisture content, redox conditions, salinity, nutrient supply, organic matter content and the presence of other co-substrates (Alexander 1999, Dechesne, Badawi et al. 2014, Akbari and Ghoshal 2015, Honti and Fenner 2015, Holzmann, Simeoni et al. 2021, Keighley, Ramwell et al. 2021, Sigmund, Arp et al. 2022). Conditions such as redox potential and nutrient availability influence the ability of degraders to utilise substances in their metabolism. Other properties can influence the speciation and bioavailability of substances, affecting their degradation. Factors influencing speciation, sorption and bioavailability are discussed in more detail in Section 4.4 and in Hughes et al. (2022a).

#### 4.1.3 Temperature

Temperature is an important condition affecting degradation of substances. An increase in temperature generally increases reaction rates and aspects such as microbial metabolic activity, which in turn increases observed rates of degradation (Ratkowsky, Olley et al. 1982, Meynet, Davenport et al. 2020). Under REACH, reference temperatures of 12°C for freshwater and 9°C for marine environments have been established at which new degradation data should be generated. These are based on European average temperatures (ECHA 2017a, 2017b). The use of the Arrhenius equation has also been established as a means to extrapolate existing degradation data from test temperature to these reference temperatures (Arrhenius 1889). The Arrhenius equation requires the input of an activation energy ( $E_a$ ), which is rarely known, and a standard  $E_a$  of 65.4 kJ/mol has been defined based on an average for a dataset of pesticides (EFSA 2008). This activation energy appears to represent well the degradation of hydrocarbons in water experiments at varying temperatures (Knudsmark Sjøholm, Birch et al. 2021, Sjøholm, Dechesne et al. 2022). Similar observations were made for micropollutants in sludge systems, although the data suggest some deviation from linearity, and that the Arrhenius relationship may no longer hold outside of a certain temperature range (Meynet, Davenport et al. 2020).

The use of the Arrhenius equation to extrapolate biodegradation data has been questioned due to the unknown influence of microbial adaptation and acclimation to ambient temperatures (Bagi, Pampanin et al. 2013, Lewis and Prince 2018, Brown, Camenzuli et al. 2020, Meynet, Davenport et al. 2020). A retrospective analysis of degradation for hydrocarbons in different systems suggested that the influence of temperature on degradation rates was lower than that predicted by the Arrhenius equation (Brown, Camenzuli et al. 2020). It was suggested that the change in degradation rate in temperature-manipulated experimental systems may be different to that in the natural environment due to the concurrent processes of microbial community adaptation and evolution. It has been suggested that the temperature used in simulation testing should be contingent on the field conditions, in order for the microbial community to remain functional and representative and eliminate variability associated with poorly adapted communities (Davenport, Curtis-Jackson et al. 2021).

#### 4.1.4 Light

Another factor influencing degradation rates of substances is the light conditions. Light can induce the phototransformation of some substances. A substance can be both directly transformed by absorbing the light, or indirectly phototransformed by the formation of reactive species from naturally occurring components, which react with the substance (Fenner, Stamm et al. 2016). The intensity of natural light varies seasonally, latitudinally, and is modulated by increasing depth and turbidity of water bodies (ECHA 2017a). Due to the varying availability of light in different environmental compartments, photodegradation is generally not considered in persistence assessment under REACH (ECHA 2017b).

Guidelines vary regarding lighting conditions (Fenner, Stamm et al. 2016). Most OECD guidelines are carried out in the absence of light. The inclusion of light into such studies may enhance their environmental realism but may reduce their relevance or acceptability for persistence assessment (Davies, Bramke et al. 2013). For example, the degradation half-life of the herbicide pendimethalin was significantly reduced when exposed to simulated sunlight in a scaled up OECD 309 experiment (Hennecke, Hassink et al. 2020). OECD test 309 should preferentially use dark conditions although the option of diffuse light conditions is included. At present the regulatory guidance does not explicitly rule out the inclusion of light in OECD 309 tests, but does require justification for the chosen conditions (along with other conditions) (ECHA 2017b). Hand and Moreland (2013) found that inclusion of diffuse light in OECD 309 was demonstrated to promote the growth of phototrophs, which increased the degradation of the test substance isopyrazam.

#### 4.1.5 Concentration of the substance

Degradation kinetics and degradation mechanisms can vary dependent on the concentration of the compound (Hussain, Arshad et al. 2009, Svendsen, El-taliawy et al. 2020, van Bergen, Rios-Miguel et al. 2021, Alexander 1999). At low concentrations, degradation of substances is assumed to occur co-metabolically. That is, concurrent with the degradation of other primary substrates and not leading to growth of the degrader population (OECD 2004c). In these circumstances, pseudo first order kinetics are typical and can be used to derive degradation half-lives that can be compared to regulatory persistence thresholds. However, when concentrations are higher, first order kinetics are not always followed and can be growth-linked (Davenport, Curtis-Jackson et al. 2021).

The intention of OECD guideline simulation tests is for low test concentrations to be used, so that degradation proceeds by first order kinetics (OECD 2004c, OECD 2006). In standard biodegradation

screening testing, higher test concentrations are used and these typically result in growth-linked degradation kinetics (Alexander 1985, Ahtiainen, Aalto et al. 2003). There is therefore some uncertainty in extrapolating degradation rates with high initial concentrations to environmentally relevant concentrations (Lapertot and Pulgarin 2006, Kowalczyk, Martin et al. 2015). The influence of substrate and degrader concentration on degradation kinetics has been investigated in some detail previously (Alexander 1985, Alexander 1999). Further, at very low concentrations, if a threshold substrate concentration is not surpassed, the activity of bacterial enzymes dedicated to degrading an environmental pollutant may remain low and not evolve (Kolvenbach, Helbling et al. 2014, Kowalczyk, Martin et al. 2015).

## 4.2 Experimental factors

In addition to environmental factors that act as sources of variability in degradation data, there are also factors concerning the conduct of degradation experiments that can similarly affect the results observed. The OECD test guidelines provide some degree of standardisation in degradation experiments that serves to address some of this potential variability. However, even within OECD guideline methods there remain degrees of flexibility which can impact results. Concerning degradation data from non-standard experiments, the potential variation in experimental approaches that can influence results is even greater. Therefore, it is important when evaluating non-standard experiments that consideration is given to the degree to which they follow or resemble conditions that would be applied in the equivalent OECD standard tests. Otherwise, it may be more difficult to compare results between dissimilar degradation experiments in a weight-of-evidence approach. These aspects are discussed in more detail in Section 3.

In degradation tests the test setup can be important. Test setup may include the geometry of test systems, including the size of vessels, the volume of the relevant environmental media and headspace (Ingerslev and Halling-Sørensen 2009, Honti and Fenner 2015, Shrestha, Junker et al. 2016, Birch, Andersen et al. 2017, Seller, Honti et al. 2020). Whether the vessels are open or closed, and how they are aerated and agitated can also be important (Shrestha, Junker et al. 2016, Shrestha, Meisterjahn et al. 2019, Goss, Li et al. 2020, Seller, Honti et al. 2020, Shrestha, Meisterjahn et al. 2020, Shrestha, Hughes et al. 2021). Inoculum pre-treatment, storage and acclimation steps may have an impact on the conditions in the test system, or on the performance of the inoculum (Goodhead, Head et al. 2014, Kowalczyk, Martin et al. 2015, Davenport, Curtis-Jackson et al. 2021, Wennberg, Meland et al. 2022). How the test substance is dosed into the system is also relevant and can affect bioavailability and partitioning of the test substance (Brakstad, Nordtug et al. 2015, Sweetlove, Chenèble et al. 2016, Dorn, Hammel et al. 2021). Particular care should be taken for homogeneous distribution of the substance in the system wherever possible, and avoiding unwanted influence of solvents on the inoculum or system parameters (Hughes, Brown et al. 2020, Shrestha, Meisterjahn et al. 2020). Sampling of the vessels may be relevant, for example in OECD 309 it is possible to sample vessels sacrificially or sequentially, and there is evidence that this may impact the results (Holzmann, Simeoni et al. 2021). Finally, the processing of experimental results to determine degradation endpoints can obviously have a significant bearing on results. Aspects of interpretation, such as kinetic model fitting, inclusion of lag-times, determination and consideration of non-extractable residues (NER), whilst having varying levels of guidance available, still offer significant room for interpretation. Most of these factors have optional degrees of flexibility in existing guidelines. Hence the extent to which standard OECD tests can vary is potentially large, and it is incorrect to assume that each OECD test is equivalent in terms of its experimental conditions. It is necessary to scrutinise the details of tests to understand the specific experimental conditions used and how this may have influenced results.

Finally, the presence of other (co-)substrates in test systems may influence the degradation of test substances. This is generally to be avoided where possible (ECHA 2017a). However, there may be circumstances where this could be necessary/permitted. These aspects are discussed in detail in the separate 'Guidance for the Persistence Assessment of UVCB Substances' (Hughes, Griffiths et al. 2022b).

## 4.3 Sediment compartment

The sediment compartment is worthy of particular attention regarding sources of variability in degradation data. Firstly, the compartment itself is rather poorly defined in persistence assessment. Sediments represent the areas beneath water bodies. These are generally made up of sand, silt, clays and organic matter of a diverse nature (Berlamont, Ockenden et al. 1993). Sediments are in continuous flux owing to the erosion and settling of particulate matter from land and water bodies (Koiter, Owens et al. 2013). They are also known to be a sink for environmental contaminants and have therefore been a focus of environmental quality and risk assessment for decades (Burton 2002, Koiter, Owens et al. 2013). Sediments are generally anaerobic in nature, with a thin aerobic layer near the water-sediment interface, if the overlying water is not anaerobic (Shrestha, Junker et al. 2016). However, bioturbation by benthic invertebrates may serve to increase oxygen penetration into sediments (Krantzberg 1985). Sediments are therefore heterogeneous in nature and stratified by depth. The continuous settling and anaerobic nature of sediments mean that studies of organic contaminants in sediment core samples are common (Hartmann, Quinn et al. 2005, Korosi, Cheng et al. 2015, Thiebault, Alliot et al. 2021). Analysis of contaminants at varying core depths can be compared with settling rates to infer chronological inputs of contaminants.

Despite the heterogeneous nature of sediments, most persistence frameworks do not define the sediment compartment. The fact that sediments act as a record for organic contaminants in their deeper, anaerobic layers suggests that many contaminants are poorly degraded under these conditions. However, it does not appear to be the intention that persistence of substances should be assessed based on their degradation rates in these deeper layers. According to REACH guidance:

*“The “aerobic” OECD TG 308 is a mixture of aerobic and anaerobic sediment. The OECD TG states that the “aerobic test simulates an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient”. By comparison, the anaerobic test “simulates a completely anaerobic water-sediment system”.*

*It is not recommended to judge whether a substance has an environmental half-life exceeding the P and/or vP thresholds using only anaerobic simulation data. Generally it would be expected that an anaerobic half-life would be greater than an aerobic half-life where the main route of degradation is aerobic, i.e. if there is no oxygen, degradation will be hindered. Care should also be taken where the anaerobic data show rapid degradation of a substance. This is because there is generally no immediate discharge of a substance to anaerobic sediment or soil. Instead, the substance will usually need to cross an aerobic zone before reaching the anaerobic zone. This means it is important to understand the rate degradation across that aerobic zone to assess the persistence.”*

It can be inferred from this that the sediment compartment according to REACH is defined by the conditions of an aerobic OECD 308 test (ECHA 2017b). This is further reinforced by the position on the use of sediment core data. It is important to note that the implicit definition of the sediment compartment may differ across other persistence frameworks.

The OECD 308 guideline itself is a less than perfect representation of the sediment compartment. Shrestha et al. (2016) summarised the main criticisms as: 1) the recommended sediment-water ratio does not represent the natural environment and shifts equilibrium mass distribution excessively towards the sediment phase, 2) determination of compartment-specific DegT50 values is challenging and leads to considerable uncertainty, 3) stagnant, stratified design, and 4) does not consider flow velocity and sediment dynamics, not suitable to represent flowing water bodies. Due to the uncertainties in compartment specific DegT50 values in these studies, the degradation in the total system (DegT50<sub>system</sub>) is generally used as the endpoint for persistence assessment (ECHA 2017b).

However, this has been demonstrated to be influenced by the system geometry used, even within guideline boundaries (Honti and Fenner 2015). Other conditions that can be varied within the guideline and may influence results include agitation of the water phase, aeration method, and use of open or closed systems.

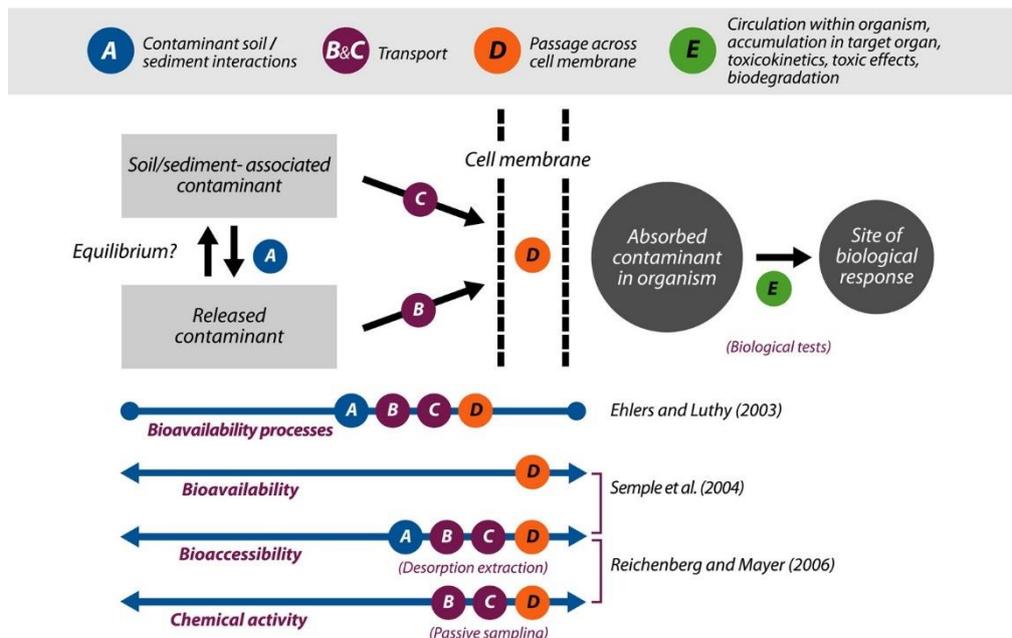
The spatial-temporal behaviour of test compounds has been shown to vary significantly according to substance properties, sediment characteristics, and test system spiking approaches in similar OECD 218 and 219 chironomid toxicity study designs (Dorn, Hammel et al. 2021). This poses significant potential concerns for water-sediment simulation tests, since the spatial-temporal behaviour of test substances is likely to have significant bearing on the rate of degradation observed. The OECD 308 guideline has also shown limitations when testing certain difficult substance types. When testing a slightly volatile, hydrophobic substance in a closed system, biodegradation of the application solvent led to the formation of a biofilm and depletion of oxygen in the water phase, impacting the degradation of the test substance (Shrestha, Meisterjahn et al. 2020). Further work is ongoing to explore improvements to support testing of these substance types according to OECD 308 (Shrestha, Hughes et al. 2021).

Therefore, there are many challenges with the current OECD 308 guideline which affect its robustness, relevance to the natural environment, and applicability to particular substance types. It is important to consider these limitations in undertaking new OECD 308 tests, and in interpreting existing data in persistence assessments. There are promising efforts ongoing to improve the robustness of test methods for persistence assessment in aquatic compartments. Seller et al. (2021) developed a modified biotransformation test system, with a thinner sediment layer. This system showed reduced inter-replicate variability and improved observability of degradation compared with standard OECD 308 and 309 test systems for a wide range of compounds (Seller, Özel Duygan et al. 2021). This study also presented a promising approach for studying inoculum cell densities and cell type diversities using multidimensional flow cytometry data and a machine learning-based cell type assignment pipeline. The data obtained indicated that a sufficient amount of sediment-borne biomass is needed to robustly study the biotransformation of substances in aquatic systems. Developments such as these should be considered for incorporation into regulatory guidance.

## 4.4 Bioavailability

Bioavailability of substances has long been established as playing an important role in their degradation in the environment (Alexander 1999, Alexander 2000, Ehlers and Luthy 2003, Semple, Morriss et al. 2003, Reichenberg and Mayer 2006, Riding, Doick et al. 2013, Naidu, Channey et al. 2015, Ortega-Calvo, Harmsen et al. 2015, Umeh, Duan et al. 2017, Ortega-Calvo, Stibany et al. 2020). Irrespective of whether the structure of a molecule is susceptible to biotransformation, bioavailability is a prerequisite to this process taking place. In this section we attempt to address bioavailability as a source of significant variability in environmental degradation rates, and how this can be accounted for in persistence assessments to arrive at robust and consistent prioritisation of substances.

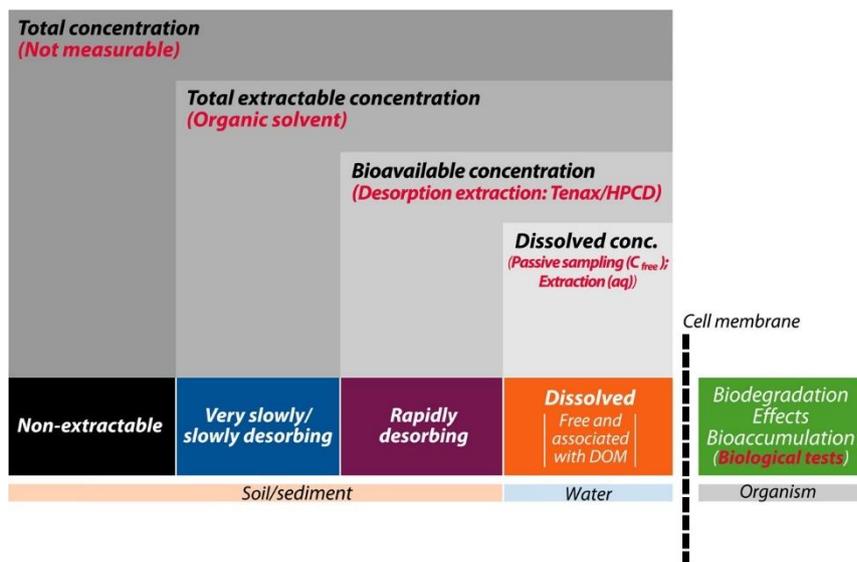
Bioavailability science has advanced significantly over the past 30 years. A SETAC special science symposium held in 2014 concluded that the state of the science was sufficient for bioavailability concepts to be incorporated into chemical regulation to provide for better prospective and retrospective risk assessments (Ortega-Calvo, Harmsen et al. 2015). An overview of the scientific concepts of bioavailability of organic chemicals is presented in Figure 7. This illustrates how different schools of thought have been brought together into a coherent framework describing the different processes involved. Bioavailability can be measured both in terms of chemical activity (potential of the contaminant for direct transport and interaction with the cell membrane) and bioaccessibility (incorporating also the time-dependent phase exchange of the contaminant between the soil/sediment and the water phase). Chemical activity can be measured using equilibrium passive sampling or aqueous extraction. Bioaccessibility is typically measured by more exhaustive desorption extraction techniques, which can be operationalised to recover different fractions of a substance based on their time to desorb from the matrix. As can be seen from Figure 7, substances generally have to desorb from soil/sediment components of environmental matrices before they can cross biological membranes and undergo biodegradation.



**Figure 8. Overview of scientific concepts of the bioavailability of organic chemicals, as explained by Ehlers and Luthy (2003), Semple et al. (2004), and Reichenberg and Mayer (2006). Using the same framework, the figure places different schools of thought that have dissected bioavailability into the different processes that are involved (A to E), the dissimilar end points (bioaccessibility and chemical**

activity), and the different methodologies (desorption extraction, passive sampling and biological tests). Each of these processes, end points and methods has been considered differently in a wide variety of bioavailability scenarios. Depending on the schools and processes investigated, bioavailability can be examined through chemical activity, the potential of the contaminant for direct transport and interaction with the cell membrane (processes B, C, and D), or bioaccessibility measurements, which incorporate the time-dependent phase exchange of the contaminant between the soil/sediment and the water phase (process A). Depending on biological complexity, the passage of the contaminant molecule across the cell membrane (process D) may represent multiple stages within a given organism before the site of biological response is reached (process E). Reprinted with permission from Ortego-Calvo et al. (2015). *Environ. Sci. Technol.* 49, 17, 10255-10264. Copyright 2015 American Chemical Society.

Figure 8 illustrates how the total concentration of a chemical within a soil or sediment matrix may be distributed between different overlapping fractions according to their bioavailability. Within the total concentration there is a total extractable concentration by organic solvents, and a non-extractable fraction. The total extractable fraction constitutes the dissolved concentration (measurable by passive sampling or aqueous extraction), and both the rapidly (measurable by desorptive extraction techniques) and the slowly desorbing (measurable by organic solvent extraction) fractions. It is considered that only the dissolved and rapidly desorbing fractions are bioavailable. It is recognised that sorption of chemicals to soil or sediments generally becomes stronger with time due to a process known as aging (Alexander 2000, Ortego-Calvo, Harmsen et al. 2015, Umeh, Duan et al. 2017).



**Figure 9. Measurement of bioavailability: a simplified scheme for use in regulation.** The color boxes at the left of the cell membrane represent the distribution of pollutant molecules among four classes (nonextractable, very slowly/slowly desorbing, rapidly desorbing and water-dissolved) in soils and sediments. In our scheme, the bioavailable chemical is represented by the rapidly desorbing and dissolved concentrations. The chemical methods able to measure the pollutant present in each specific fraction are given in the gray boxes. The green box to the right of the cell membrane represents the processes that occur within the organism exposed to the pollutant. These biological processes can also serve as the basis for standard methods of bioavailability measurements. Reprinted with permission from Ortego-Calvo et al. (2015). *Environ. Sci. Technol.* 49, 17, 10255-10264. Copyright 2015 American Chemical Society.

The framework presented by Ortego-Calvo et al. (2015) has proposed applications in both prospective and retrospective risk assessment. Bioavailability concepts are of significant interest in retrospective risk assessment (e.g. contaminated land management and remediation), since this can support risk-based decisions and better allocation of scarce resources (Ortego-Calvo, Harmsen et al. 2015, Umeh, Duan et al. 2019, Peijnenburg 2020). Regarding prospective risk assessment,

bioavailability concepts are already considered in the context of toxicity and bioaccumulation assessments. For example, the equilibrium partitioning (EqP) theory facilitates extrapolation of toxicity between the aquatic and soil or sediment compartments (ECHA 2008a). Also, it is well recognised that reduced bioavailability in aqueous exposures can lead to under-estimations of toxicity and bioaccumulation potential (ECHA 2017a). However, in the case of persistence assessments, bioavailability is hardly recognised. The main exception is the modifications permitted for testing poorly soluble substances in biodegradation screening tests. The developing guidance on non-extractable residues (NER) is an example of where bioavailability is not taken into account, since a non-extractable fraction of a substance is also likely to be non-bioavailable (ECHA 2019). At present there is no distinction made between extractable and non-extractable fractions despite the clear difference in the likely risks posed.

#### 4.4.1 Bioavailability in persistence assessments

The issue of bioavailability and its influence of degradation data was discussed in detail in a study by Hughes et al. (2020), which conducted a weight-of-evidence determination of the persistence of phenanthrene. Here, it was discussed that the bioavailability, and hence degradation rate, of phenanthrene was heavily influenced by the dosing method as well as the source material. Degradation in soil of phenanthrene as an *in situ* component of sewage treatment plant sludge was significantly slower than when applied by direct spiking. Similar relationships have also been found for pharmaceuticals and personal care products (Walters, McClellan et al. 2010), and are likely to be applicable in general to organic chemicals with a tendency to sorb (e.g. hydrophobic and cationic substances). Polycyclic aromatic hydrocarbons (PAHs) have the added issue of their source/origin, with pyrogenic PAHs generally being associated with soot, to which they sorb very strongly (Jonker and Koelmans 2002a, Jonker and Koelmans 2002b, Cornelissen and Gustafsson 2004, Jonker, Hawthorne et al. 2005, Rhodes, Carlin et al. 2008, Yu, Duan et al. 2018). This means that pyrogenic PAHs generally have a much lower bioavailability than petrogenic PAHs, and this is supported by evidence from monitoring studies (Burgess, Ahrens et al. 2003, Vane, Harrison et al. 2007, Kim, Vane et al. 2017).

In Hughes et al. (2020) it is argued that it is essential to recognise bioavailability as a source of significant variability in biodegradation data, and to take this into consideration when performing weight of evidence determinations of persistence, in order to arrive at consistent and predictable prioritisation of substances (van Wijk, Chénier et al. 2009, Hughes, Brown et al. 2020). A failure to consider bioavailability risks the substances being prioritised on the basis of conditions under which their degradation has been measured, rather than on their intrinsic properties. Also, if persistence is fundamentally an indicator of exposure potential, bioavailability should be taken into account since bioavailability directly influences exposure (Scheringer 2002, Boethling 2016, McLachlan 2018, Cousins, Ng et al. 2019, Redman, Bietz et al. 2021). If a chemical has been concluded to be persistent under circumstances of low bioavailability, this may in fact be a poor reflection of its exposure potential. Furthermore, in the context of PBT/vPvB assessment, persistence is coupled to bioaccumulation and toxicity, which in combination gives rise to particularly high levels of concern. There is a case therefore that persistence assessment should be based on conditions in which substances are bioavailable and hence also able to bioaccumulate and cause toxicity (Solomon, Matthies et al. 2013).

Given that persistence is currently assessed on the basis of half-lives, it is inevitable that environmental factors will influence the outcome of degradation testing, in addition to intrinsic properties of the substance. Therefore, an assessment of persistence based solely on the intrinsic (bio)degradability properties of substances is somewhat out of reach. However, bioavailability can be

taken into account to some extent by considering the conditions under which degradation has been measured relative to standard test conditions.

#### 4.4.2 How does bioavailability influence degradation?

Based on the principles of bioavailability, described above, substances must be desorbed from matrix components before they can cross biological membranes and be biodegraded. Typically when (bio)degradation of substances is measured, this is expressed on the basis of the observed degradation relative to the total (extractable) concentration present. Bioavailability can therefore influence the observed degradation of substances in three notable ways:

1. Equilibrium partitioning effects
2. Aging effects
3. Free (third) phase effects

These influences are summarised in Table 2 and discussed in greater detail below.

**Table 1. Types of effect on bioavailability that can influence degradation rates of substances.**

Type of effect	Description	Example scenario	Measured by	Environmentally relevant?	Can be addressed?
Equilibrium partitioning	Partitioning of chemical between dissolved phase and other matrix components	Freshly spiked, equilibrated, OECD 307 soil study	Chemical activity	Yes	No
Aging	Gradual sequestration of the substance by the matrix over time	Long-term contaminated site	Chemical activity and bioaccessibility	Yes	Yes (avoid where possible)
Free (third) phase	Substance is present as part of an undissolved third phase	Poorly soluble substance tested at > water solubility	Difficult to measure; by observation or inference	No	Yes (avoid where possible)

##### 4.4.2.1 Equilibrium partitioning effects

Equilibrium partitioning describes the partitioning of a substance between soil or sediment components and interstitial water at equilibrium. This is normally expressed as the adsorption-desorption distribution coefficient ( $K_d$ ), or the organic carbon-water partition coefficient ( $K_{oc}$ ) where this is normalised to the organic carbon content of the sorbent (ECHA 2017a). The partitioning of substances is controlled by many factors, including the composition of the matrix and the properties of the substance, and will be unique to the particular conditions. It might be considered instantaneous and constant, although in reality sorption may increase with time due to aging (see below).

Since desorption of substances is considered a prerequisite for biodegradation, the equilibrium partitioning of substances in environmental matrices plays an important role in determining the proportion of the total concentration present that is available for degradation and thus the observed rate of degradation. The dissolved (mg/L) concentration (or chemical activity) of the substance

controlling degradation of the total (mg/kg) concentration can be measured using equilibrium (passive) sampling, or aqueous extraction (Ortega-Calvo, Harmsen et al. 2015).

Evidence for this influence of partitioning on the observed rate of degradation was provided in a study of the degradation of phthalate esters in natural sediments (Kickham, Otton et al. 2012). Here, it was demonstrated that the rate of degradation of structurally similar substances of varying hydrophobicity was proportional to their octanol-water partition coefficient, indicating that this was largely controlled by sorption to the environmental matrix.

As persistence is assessed based on degradation in natural systems, the equilibrium partitioning of substances is inherent to the environmental sample used and cannot be influenced or mitigated through experimental design. In other words, it is environmentally relevant and a reality of chemical fate in the environment. The variability introduced by this process may be reduced to an extent by the use of consistent or standardised environmental samples for testing, and by adhering to guidelines and regulatory guidance concerning the samples used. For example, for OECD 308 a range of 0.5 – 7.5% organic carbon content is recommended in sediment samples used (OECD 2002b). Also, for OECD 309 testing it is recommended to use a surface water with 10 – 20 mg suspended solids/L (ECHA 2017b). However, other than these limited measures, the influence of equilibrium partitioning on bioavailability and degradation rates of substances is an inevitable product of the process by which persistence is assessed, and therefore must be tolerated. It will affect some substances more than others, particularly strongly sorbing substances, for which the process can become dominant.

#### 4.4.2.2 Aging effects

The term aging describes a well established process of the gradual sequestration of a substance by a soil or sediment matrix over time (Alexander 2000). Over time, substances undergo tortuous diffusion, penetrating pores and migrating to sorption sites deeper within the matrix, from where desorption is increasingly unlikely (Umeh, Duan et al. 2017). The substance may still be extractable with organic solvents, but the strength of sorption will increase and its tendency to desorb (i.e. the “bioaccessibility”) will become reduced.

Aging may affect the observed degradation of substances by affecting both the partitioning of the substance between the sorbent and interstitial water, and the bioaccessibility of the substance. The partitioning will impact the proportion of the substance present that is available for degradation at any one time and hence the rate of degradation relative to the total amount present. The bioaccessibility will reduce the extent to which the substance will desorb from the matrix to replenish the concentration of the substance that has been degraded in the interstitial water. The effect of bioaccessibility may be more visible therefore for more biodegradable chemicals, where the degradation rate is controlled by the rate of desorption. The effects of aging on bioavailability can be measured using equilibrium sampling to determine dissolved concentrations (and hence chemical activity), and desorptive extraction techniques to determine bioaccessibility.

The influence of low bioaccessibility of residues on degradation rates has been observed on various occasions. For example, in a field study conducted on contaminated sediments undergoing landfarming (Harmsen and Rietra 2018). PAHs were observed to degrade rapidly during the first year, followed by a period of slower degradation for the following six years, and a period of very slow degradation up to 25 years. The bioaccessibility of the PAH fractions was measured using Tenax desorptive extraction and the degradation rates of fast, slowly and very slowly desorbing fractions could be determined separately. In a separate study, bioaccessibility of PAHs in contaminated industrial soils was measured using contaminant traps, and compared to biodegradation experiments

(Mayer, Olsen et al. 2011). In both cases a residual amount of the total extractable PAHs remained resistant to removal in the soils, indicating that this was a low bioaccessibility residual fraction that was not amenable to degradation.

The influencing of aging is again an environmentally relevant process. However, it is less comparable to the conditions of standard tests for degradation, where direct spiking is typically used. Degradation of substances under conditions of aging may occur at significantly slower rates than under freshly spiked conditions. These conditions may be considered less relevant than those of standard tests, which are typically used for comparing and prioritising substances. Also, with reduced bioavailability from aging, the exposure and risks from toxicity and bioaccumulation will also be reduced. Therefore a means to account for the influence of aging on bioavailability and degradation rates in a persistence assessment is to factor these aspects into the quality assessment of individual studies, and to score degradation data from freshly spiked experiments more highly. This is justified as these studies will more closely resemble standard test methods, which are considered the most relevant conditions. Also, this will lead to a more consistent assessment of persistence, based on (intrinsic) biodegradation potential between substances.

#### 4.4.2.3 Free (third) phase effects

Free (or third) phase effects result from circumstances where an amount of a substance is present as or in a bulk third phase. Scenarios where a third phase may be present include:

- When a poorly soluble substance is tested at a concentration above its solubility in water, and a proportion if it is present as crystals or droplets.
- When a substance is present as a constituent of a non-aqueous phase liquid (NAPL), such as oil or tar
- Insoluble polymers

In these circumstances, the amount of substance present in the third phase will not be bioavailable for degradation (Yu, Duan et al. 2018). Degradation of this material will be controlled by the partitioning of the substance from the third phase into the surrounding water. Diffusion of the substance within the third phase may also be important, particularly where this is not the pure substance, and the material is semi-solid or viscous. Conversely, it is conceivable that the presence of a third phase may actually increase bioavailability in some cases (e.g. if the substance would otherwise be strongly sorbed to components of the environmental matrix). These aspects are discussed in greater detail in the guidance document from this series on difficult substances (Hughes, Griffiths et al. 2022a).

It is generally difficult to measure whether a substance is present as a free phase within a test system. It may be possible to undertake visual observations, or infer this based on test concentrations and water solubility (e.g. in aquatic studies). However, this becomes more difficult in soils or sediments. Caution should be used when working with viscous materials or at concentrations close to solubility limits. When dosing soil matrices, care should be taken to ensure homogenous distribution throughout the matrix (organic solvents may be useful for this purpose but care should be taken to avoid effects on the microbial inoculum).

For persistence assessments, the influence of free phase material on the bioavailability and degradation of substances can be addressed by avoiding these situations where possible. Persistence assessments should generally consider diffuse, low, environmentally relevant concentrations of substances, whereas the presence of free phase material is more likely a result

experimental artefacts or an unintended release (spill) of the substance (OECD 2006, Brakstad, Nordtug et al. 2015, Birch, Hammershøj et al. 2018, Lewis and Prince 2018). Where it is unavoidable, the effect of the presence of free phase material on observed degradation should be examined carefully. This may be the case for very poorly soluble substances, where it is technically challenging to achieve complete dissolution of the substance. In most cases the bioavailability of the substance will be reduced and the extent to which this has influenced the results should be considered. These considerations should factor in the overall quality score and weighting of the study in the assessment.

Although not strictly a third phase, the influence of the presence of other solid materials (e.g. coke, soot) may have similar effects on the bioavailability of substances. In these cases substances may be adsorbed to the surface of the material, rather than absorbed within the material itself (Alexander 1999). In these situations, desorption from the material may control the bioavailability to the substance, similar to equilibrium partitioning and bioaccessibility of substances within soil or sediment matrices. The application of substances associated with such materials should be avoided wherever possible in degradation studies, as this can significantly impact the bioavailability of the substance and the rate and extent of degradation (Hughes, Brown et al. 2020).

## 4.5 Environmental realism of laboratory tests

The reliance on standard biodegradation test guidelines for the assessment of persistence raises an important question: how well do the results of these tests represent what is happening in the environment? This question is not new (Alexander 1985, Ahtiainen, Aalto et al. 2003). It is particularly pertinent for biodegradation simulation tests, which are intended to test degradation under natural environmental conditions in order to yield realistic degradation half-lives (OECD 2006). It should be recognised from the outset that biodegradation tests in the laboratory can only represent a limited 'snapshot' of the microbial diversity and processes which contribute to degradation in the environment (Mezzanotte, Bertani et al. 2005, Wu, Ning et al. 2019, Davenport, Curtis-Jackson et al. 2021). They also introduce artefacts, which reduce their environmental realism (Kowalczyk, Martin et al. 2015). It is important therefore to approach laboratory biodegradation testing with humility, and not as a panacea from which answers to all questions concerning the fate of chemicals in the environment can be derived.

One common issue that is raised with biodegradation testing is that environmental samples can only ever represent a subsample of the total diversity of microbial communities present in the environment (Davenport, Curtis-Jackson et al. 2021). This implies that within biodegradation test vessels certain key competent degraders of substances may be excluded, and therefore that tests may lead to 'false negative' conclusions (Kowalczyk, Martin et al. 2015, Martin, Snape et al. 2017, Ott, Martin et al. 2019, Ott, Martin et al. 2020). This so-called 'bottle effect' has been studied in various ways. Increasing the concentration of degrader biomass appears to improve results in terms of both consistency and rates of degradation (Martin, Snape et al. 2017, Ott, Martin et al. 2020, Seller, Honti et al. 2020). However, this is not currently permitted in persistence assessments under REACH. The permitted approach of increasing vessel sizes appears to yield inconsistent results (Hammes, Vital et al. 2010, Takekoshi, Takano et al. 2020, Gartiser, Brunswik-Titze et al. 2022).

There will always be a constraint on the microbial diversity that can be represented within laboratory tests. Other relevant environmental processes may also not be well represented by existing test approaches, such as the contribution of biofilms in aquatic systems to microbial degradation (Edwards and Kjellerup 2013). Biofilms carry an important ecosystem function for nutrient cycling and detoxification of the environment, which is thus far excluded from standard persistence assessments in the water compartment. Further, by excluding light in test systems there is evidence that important contributions from phototrophs to degradation in aquatic systems may be excluded (Hand and Moreland 2013, Chalifour, Walser et al. 2021).

Ultimately, the degradation of chemicals in the environment can only be fully represented in the field. However, measuring the actual degradation rates of substances in the environment is notoriously difficult owing to the multitude of potential input, output, mass transfer and removal processes acting in concert (Zou, MacLeod et al. 2013). A limited number of studies have attempted to examine the degradation of chemicals in the field using specific environmental settings where the influence of concomitant processes on the input/removal of substances can be either quantified or excluded (Zou, MacLeod et al. 2013, Zou, Radke et al. 2015a, Zou, Radke et al. 2015b, Álvarez-Ruiz, Hawker et al. 2021). These studies show great promise for improving our understanding of how chemical degradation proceeds in the environment under diffuse conditions. However, the circumstances under which these studies can be carried out are limited (e.g. lake systems with very well characterised inflows and outflows, low-flow estuaries under post-flood conditions), and therefore results are also more specific to these environments. Ultimately such studies should be promoted as means to better improve our understanding of degradation in the field.

The use of benchmarking, whereby the degradation of target chemicals is assessed relatively to the performance of reference chemicals, whose degradation behaviour is more well studied, has also been suggested in this context as a means to improve the reliability and realism of persistence assessments (Zou, Radke et al. 2015a, McLachlan, Zou et al. 2017). This approach would have the benefit of addressing some of the wide variability in degradation rates known to occur in the environment and facilitate a better relative comparison and prioritisation of chemical persistence. The use of benchmarking has already shown value in bioaccumulation assessments, however its use in persistence assessments has been more limited (Chen, L fstrand et al. 2018). One challenge to broader adoption of this approach appears to be concerns about the influence of multiple co-substrates on the degradation of chemicals (ECHA 2017a).

Another effort to improve realism in biodegradability tests has been to explore the influence of test substance spiking on results. Spiking of chemicals into tests leads to a sudden change of conditions, which may affect microbial communities, and generally lacks environmental realism (Kolvenbach, Helbling et al. 2014, Kowalczyk, Martin et al. 2015, Li and McLachlan 2019). Much of the substances present in the environment occur in diffuse, low concentrations. Although it is the aim of persistence assessment to understand the degradation behaviour of substances in these scenarios, our understanding of these processes under these conditions remains relatively poor (Fenner, Elsner et al. 2021). There is a need to better understand these processes, not only for effective persistence assessments, but more widely for effective risk assessment, and to address the challenge of environmental mixtures (Drakvik, Altenburger et al. 2020). OECD 309 experiments have compared the rates of degradation of substances spiked into test vessels with their rates when present as in situ contaminants in the water (Li and McLachlan 2019). The difference in results was striking for some substances, and this raises questions as to the robustness and realism of standard test methods.

Microbial communities also exhibit the ability to adapt and evolve when exposed to a substance (Poursat, van Spanning et al. 2019). For example, (Itrich, McDonough et al. 2015) demonstrated field adaptation of communities to a new consumer product, L-Glutamate-N,N-diacetate (L-GLDA), which exhibited limited biodegradation prior to a 40-50 day acclimation period to allow for growth and establishment of significant degraders. This sudden onset of biodegradation can be attributed to changes in the composition and activity of the microbial community when exposed to a test chemical (Davenport, Curtis-Jackson et al. 2021). Similar examples have been documented elsewhere (Alexander 1999, Poursat, Spanning et al. 2020, Wackett and Robinson 2020). However, it is unclear the extent to which microbial communities will adapt to the presence of a substance when it is present only in very low concentrations (Kolvenbach, Helbling et al. 2014, Poursat, van Spanning et al. 2019). At present, the process of microbial adaptation is not considered within persistence assessments under REACH. Degradation studies should be carried out using inocula that has not been pre-exposed to the test substance (ECHA 2017a). This requirement may carry some practical constraints where substances are subject to widespread dispersive use, for example substances used in consumer products with 'down the drain' applications. In addition, according to REACH guidance, long lag phases observed in biodegradation screening tests are considered as signs of adaptation of the microbial inoculum, and are not considered appropriate for persistence assessments (ECHA 2017a). Whilst microbial adaptation is undoubtedly an environmentally relevant process, there is a need to better understand the mechanisms of this process and the conditions under which it occurs, which may support its greater consideration in persistence assessments (Poursat, van Spanning et al. 2019).

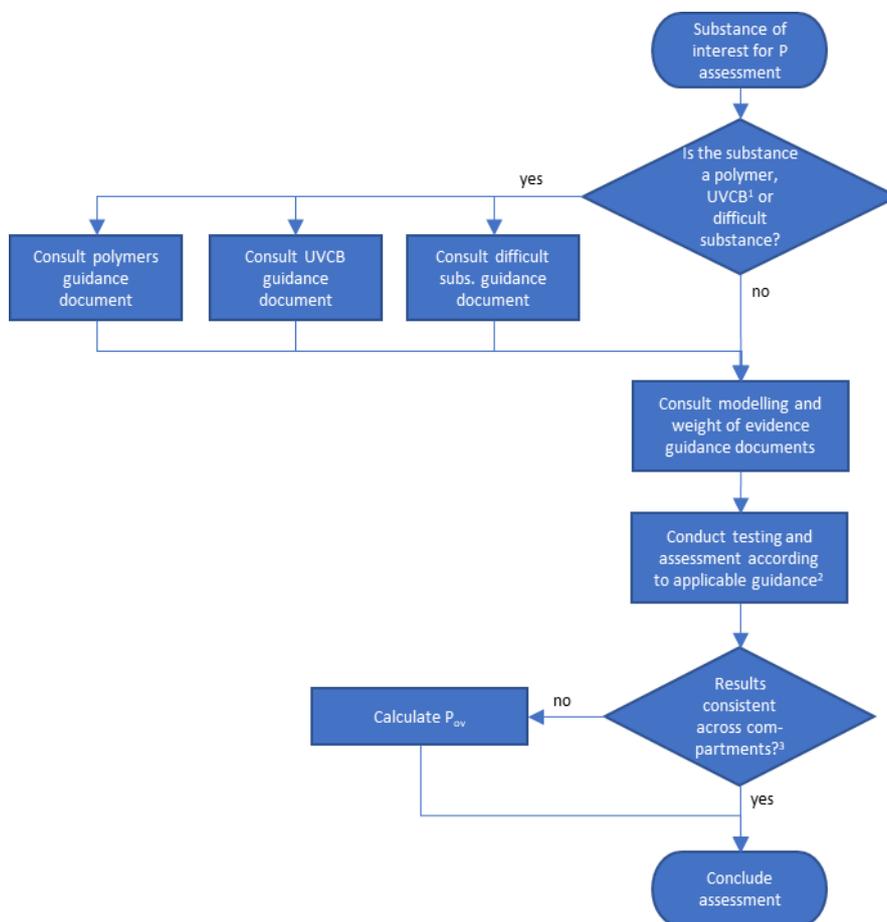
To summarise, the current testing and assessment frameworks for persistence assessment generally rely on standard tests, which can lack environmental realism. There are encouraging developments in the literature which offer the chance to improve our understanding of the fate and degradation of substances when present at low, environmentally relevant concentrations. These are developing

areas, and there may remain some barriers to immediate regulatory adoption. However, the incorporation of developments in this area should be encouraged in order to improve the realism of persistence assessments and ultimately lead to more appropriate risk management decisions.

## 5 Persistence assessment decision tree

A decision tree has been developed to support assessors of persistence to utilise the guidance that has been developed as part of this project. The first step is to understand whether any of the guidance documents for challenging substances are relevant. Following this, the guidance on weight of evidence and multimedia fate modelling should be consulted ahead of carrying out the testing and assessment of persistence. All applicable guidance should be considered in carrying out the testing and assessment of persistence. This includes any available relevant guidance under the applicable regulatory frameworks (e.g. ECHA 2017a and 2017b for REACH).

Following testing and assessment, it should be considered whether the outcome of the persistence assessment is consistent across compartments. If this is not the case, calculation of overall persistence should be considered, as this may provide additional insights to aid the assessment, in accordance with the framework detailed in the modelling guidance. Following these steps the assessment should be concluded.



**Figure 10. Decision tree to support the application of guidance developed during this project in persistence assessments.**

<sup>1</sup> UVCB here should be considered all substances with more than one relevant constituent requiring assessment.

<sup>2</sup> Applicable guidance includes guidance from this project as well as relevant guidance from the framework in question.

<sup>3</sup> If the assessment has been concluded based on biodegradation screening tests these can be considered applicable and consistent for all environmental compartments.

## 6 Conclusions

Persistence assessment is a cornerstone of chemical regulatory assessment and management frameworks, whereby the intrinsic potential of a chemical to remain in the environment after having been released is evaluated. Such assessments are required under numerous regulatory frameworks globally. Persistence is typically assessed alongside bioaccumulation and toxicity as part of PBT/vPvB and POP frameworks. The assessment itself generally considers degradation half-lives of chemicals in different environmental compartments, which are compared to set criteria. Other information may be relevant to the persistence assessment, and a weight of evidence approach is generally required.

Persistence assessments have many challenges to their implementation. Degradation data are often highly variable owing to a range of environmental and experimental factors. Guidance is also needed to support the evaluation of the quality (reliability and relevance) of these data, and in the use of weight of evidence to arrive at conclusions. Further, many substance types pose unique challenges for the application of standard approaches and test methods in persistence assessments. Polymers are a particular class of substances which pose significant challenges under current persistence assessment paradigms and require specific attention. In addition, the opportunities of multimedia fate modelling to support more robust and realistic persistence assessments have so far hardly been implemented.

In this document, the work of the Cefic-LRI ECO52 project has been brought together into a consolidated framework to improve the use of weight of evidence in persistence assessments. The EU REACH persistence assessment is the main focus, although other global frameworks have been considered, and the work of the project is considered to also be relevant to these other frameworks. The other documents from this project are briefly introduced, and these should be read alongside this report.

The concepts, history and recent developments in persistence assessment have been investigated. Persistence is principally a concern due to the potential for increased and poorly reversible exposure to chemicals in the environment. However, other concerns have been raised such as contamination of remote or pristine regions, contamination of important resources, and unforeseeable future or planetary boundary threats. Persistence assessment approaches and criteria have evolved over several decades and differ between legislative frameworks. The EU REACH framework requires a tiered, weight of evidence approach, and the available guidance is particularly detailed. In recent years, there have been several notable developments, and scientific and policy discussion remains active on the role of persistence in global chemicals management and how this should be approached.

Available guidance on weight of evidence has increased in recent years. This guidance has been reviewed and applied in the context of persistence assessments, with particular focus on EU REACH, to provide a framework for applying weight of evidence to improve the consistency, transparency and robustness in these assessments. The need has been identified to develop systematic approaches for evaluating the quality (reliability and relevance) of persistence data, similar to as has been progressed for bioaccumulation and aquatic toxicity. This would facilitate the comparison of experiments carried out under different conditions and the better inclusion of non-standard information in persistence assessments, in line with weight of evidence principles.

Sources of variability in degradation data have also been discussed, as these raise significant challenges to reaching consistent conclusions on persistence. These include both environmental and

experimental factors. In particular, challenges related to the sediment compartment and concerning bioavailability are highlighted. Bioavailability science has advanced significantly in recent years, and an attempt is made to implement these concepts into existing persistence assessment frameworks. The environmental realism of standard persistence assessment test methods, and recent research into this area, is also discussed.

Finally, a decision tree has been developed, which can guide assessors in utilising the guidance that has been developed in the Cefic-LRI ECO52 project. This is intended to direct users to the appropriate guidance for their substance, and to incorporate this guidance in their assessments, along with any applicable guidance from the persistence assessment framework under consideration.

To conclude, the Cefic-LRI ECO52 project has provided a comprehensive review of current persistence assessment frameworks, and developed guidance to support the assessment of challenging substance types and improve the use of weight of evidence. This document provides an overview to the project and other reports; addresses the persistence concepts, history, frameworks and developments; provides a means to improve weight of evidence guidance and account for sources of variability in degradation data; and presents a coherent framework and decision tree for incorporating the guidance developed in the project into current and future persistence assessments. It is hoped that the information provided in this, and other reports from the project, will provide useful support to practitioners in carrying out robust, consistent and transparent persistence assessments, and stimulate further discussion and progress on the development of regulatory policies and guidance.

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.'

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