Hughes *et al. Environ Sci Eur* (2020) 32:148
https://doi.org/10.1186/s12302-020-00427-1

Environmental Sciences Europe

REVIEW
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Can a chemical be both readily biodegradable AND very persistent (vP)? Weight-of-evidence determination demonstrates that phenanthrene is not persistent in the environment

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Abstract

Under the European REACH regulation, chemicals are assessed for persistence as part of weight-of-evidence determinations of persistence, bioaccumulation and toxicity (PBT), as required under Annex XIII and supported by an Integrated Assessment and Testing Strategy (ITS). This study describes the persistence assessment of phenanthrene, a data-rich polycyclic aromatic hydrocarbon (PAH), in accordance with this framework. All available data from screening and simulation tests, for water, soil and sediment compartments, plus other relevant information, have been compiled. These have been evaluated for reliability and relevance, and a weight-of-evidence determination of persistence has been carried out. Aspects relevant to the assessment, such as degradation metabolites, non-extractable residues (NER), test temperature and bioavailability, have also been considered. The resulting assessment considered a wide range of evidence, including 101 experimental data points. Phenanthrene was demonstrated to be readily biodegradable, a first-tier screen for non-persistence in the ITS. Furthermore, weight-of-evidence assessment of data for water, soil and sediment compartments supported a conclusion of "not persistent" (not P). In non-standard soil studies with sludge-amended soils, longer half-lives were observed. This was attributable to pyrogenic sources of and significantly reduced bioavailability of phenanthrene, highlighting the importance of bioavailability as a major source of variability in persistence data. Available simulation test data for the sediment compartment were found to be unreliable due to the anoxic impact of the use of a biodegradable solvent in a closed system, and were inconsistent with the broader weight of evidence. Estimation of photodegradation using AOPWIN and the APEX model demonstrated this to be an important fate process not currently considered in persistence assessments under REACH. The assessment is not in agreement with a recent regulatory decision in which phenanthrene was determined to be very persistent (vP). This assessment provides a case study for persistence assessment using the REACH ITS and highlights the need for improved guidance to improve consistency and predictability of assessments. This is particularly important for complex cases with data-rich chemicals, such as phenanthrene.

Keywords: Phenanthrene, Persistence, REACH, Biodegradation, PBT assessment, SVHC, PAH

Background

Persistence assessment

Characterisation of the risks that anthropogenic chemicals pose to human health and the environment forms

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a fundamental part of chemical management schemes [1]. As one approach to this effort, focusing on intrinsic hazard properties of substances, the identification and management of the so-called persistent, bioaccumulative and toxic (PBT) chemicals has been a subject of international attention and policy action for several decades [2]. The assessment of persistence in the context of PBT assessment is performed by comparing environmental degradation half-lives to set criteria, which are established both based on science and on the aims of policy [2]. The potential of chemicals to exhibit environmental persistence is considered a key indicator of potential risk, associated with increased and poorly reversible exposure [3–7]. More recently, the environmental persistence of chemicals has been gaining increasing attention as an issue of concern in the absence of bioaccumulation potential and/or toxicity [4, 8].

Under the registration, evaluation, authorisation and restriction of chemicals (REACH) regulation of the European Union, the criteria for persistence are specified for various environmental compartments: marine water half-life > 60 days; fresh/estuarine water half-life > 40 days; marine sediment half-life > 180 days; fresh/estuarine water sediment half-life > 120 days and Soil half-life > 120 days. Additionally, under REACH, substances may be identified as very persistent: marine/fresh/estuarine water half-life > 60 days; marine/fresh/estuarine sediment half-life > 180 days and soil > 180 days [9]. Article 14 of REACH stipulates that substances manufactured or imported in quantities > 10 tpa must undergo a chemical safety assessment, which includes a PBT and vPvB (very Persistent, very Bioaccumulative) assessment. In 2011, Regulation 253/2011 (EC) implemented a revision of Annex XIII of REACH, requiring the use of a weight-of-evidence approach to determine whether chemicals have met PBT/vPvB criteria. An Integrated Assessment and Testing Strategy (ITS) for this assessment is outlined in guidance [10]. In the ITS, multiple tiers of testing with increasing complexity are described, ranging from screening tests for ready biodegradability through to higher-tier simulation tests (OECD 307, 308 or 309 guidelines) [10–13].

According to REACH Annex XIII, "A weight-of-evidence determination means that all available information bearing on the identification of a PBT or a vPvB substance is considered together […]. 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". It has been suggested that weight-of-evidence evaluations can be performed either qualitatively or quantitatively [14–16]. All information bearing on the evaluation of the chemical should ultimately be taken into account to draw a conclusion of the assessment, which should assess the coherence of the evidence and remaining uncertainty [15, 17].

Difficulties in persistence assessment

Despite the long-established science of persistence assessment [2], and extensive guidance available under different regulatory frameworks [9, 18, 19], issues of inconsistency and predictability of outcomes in these assessments remain [17, 20, 21]. The compartmental half-life itself is an inherently imperfect metric to assess what is intended to be an intrinsic property of a chemical, as a half-life is implicitly first order in nature, whereas the actual degradation rate depends on both the characteristics of the substance and the environmental conditions the substance finds itself in [3, 5, 22]. Multiple biological and chemical degradation processes, including hydrolysis [17], biodegradation [22], atmospheric oxidation and photolysis [23] and aquatic photodegradation [24–26], contribute to degradation in the environment. Sources of variability in biodegradation rates include, but are not limited to, diversity and quantity of degrading organisms, test substance concentration, presence of oxygen or other electron acceptors, nutrients, organic carbon concentration and bioavailability [17, 27]. Bioavailability in particular, which is defined as the amount of a chemical present in an environmental matrix that is available for uptake by an organism, can be a significant source of variability in environmental degradation measurements, but this process has so far not been addressed in persistence frameworks [21]. This variability raises significant challenges for the consistency of persistence assessments, particularly where substances under assessment have contrasting datasets available, or where physical–chemical properties require the utilisation of divergent testing strategies. Current European Chemicals Agency (ECHA) guidance places heavy emphasis on Organisation of Economic Co-operation and Development (OECD) guideline biodegradation tests. However, even with these standard tests, technical challenges remain that introduce variability and reliability issues, particularly where substance properties require modification to the standard test setups [28, 29–32].

The practice of assessing persistence on a compartment-by-compartment basis, using individual half-lives from biodegradation tests, is also somewhat overly simplistic as it fails to account for other potentially important degradation processes and exchanges of the chemical between compartments in a dynamic environment. This can lead to unnecessarily conservative, or to false-positive persistence assessments [6, 33]. Several have advocated for a multimedia modelling-based approach that also includes consideration of emission patterns as a means of arriving at a better representation of the overall persistence of a substance, and a more risk-based assessment [3, 6, 33–36].

Introduction to case study—phenanthrene

Phenanthrene ($C_{14}H_{10}$, CAS number: 85-01-8) is a polycyclic aromatic hydrocarbon (PAH), considered not classifiable as to its carcinogenicity in animals despite the conduct of cancer bioassays by multiple routes of administration [37–39]. PAHs are a unique class of chemicals characterised by two or more fused aromatic rings. They are found ubiquitously in the environment and have been extensively studied. PAHs have a broad range of both natural and anthropogenic sources: they can be formed as products of incomplete combustion (pyrogenic sources), such as from burning of fuel, forest fires or volcanic eruptions, and are also present as constituents of fossil materials (petrogenic sources), such as crude oil and coal [40, 41]. They are also produced in minor amounts through biogenic and diagenetic processes. Diagnostic ratios are commonly used as a tool to identify and differentiate sources of PAHs in situations of environmental contamination [41, 42]. This study aims to assess the persistence of phenanthrene in the context of the REACH framework. A similar study has recently been completed to assess the bioaccumulation of this substance [43]. Due to the well-studied nature of phenanthrene, a large body of evidence is available to assess its persistence. Phenanthrene was recently the subject of a regulatory evaluation under Annex XV of REACH, in which it was concluded to fulfil the criteria of vPvB and identified as a substance of very high concern (SVHC) [44]. This assessment was originally based on evidence from a previous evaluation of coal tar pitch, but this was later adapted due to evidence received during the public consultation [45, 46]. This study will consider the data contained in that evaluation, along with other available information, to arrive at a weight-of-evidence determination for phenanthrene in line with REACH Annex XIII and in accordance with ECHA guidance [10]. Such a large body of evidence significantly increases the complexity of performing the persistence assessment, since a large amount of non-standard data have to be considered in the weight of evidence in addition to standard guideline tests. Further, for a highly variable parameter, such as degradation rate, it is necessary, where possible, to identify and account for these sources of variability in order to arrive at a consistent and predictable prioritisation. The present study provides a critical test of REACH Annex XIII and the ECHA ITS. It is important that this framework results in consistent and predictable regulatory outcomes in order to support the dual REACH aims of a high level of protection for health and the environment whilst enhancing competitiveness and innovation [9].

Materials and methods

Approach to persistence assessment of phenanthrene

Phenanthrene persistence was assessed in this study using the Integrated Assessment and Testing Strategy (ITS) according to REACH guidance R.11 [10]. Since a range of data types are available, a weight-of-evidence approach has been employed, as prescribed under Annex XIII of REACH. A simplified overview of the ITS scheme is presented in Fig. 1. It begins with the generation and appraisal of Ready Biodegradability test data following OECD guidelines. If the substance is demonstrated to be readily biodegradable, the substance should be concluded "not P". However, if the conclusion of the assessment for ready biodegradability is "not readily biodegradable", the substance is considered "potentially P", by default and the assessment moves to a second tier where enhanced ready and/or inherent biodegradation screening testing is considered. This phase should also consider other data as part of a weight-of-evidence approach to determine persistence. This includes data from standard and non-standard testing, field studies, monitoring data as

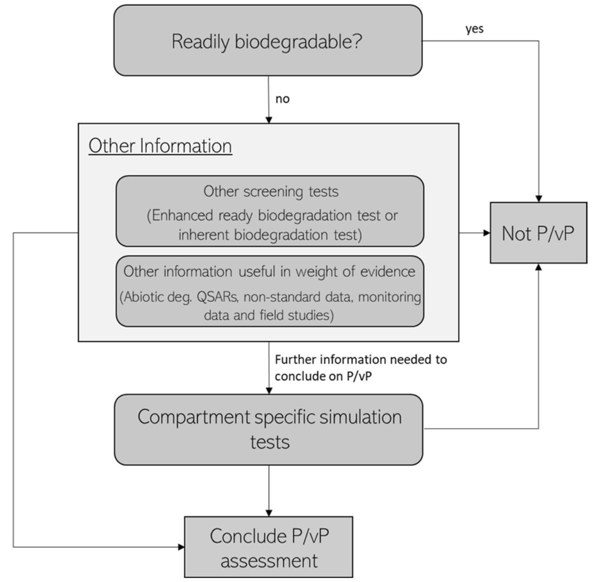

Fig. 1 Simplified ITS flow diagram for substance persistence assessment. Assessment and testing is tiered and progresses until a definitive conclusion on persistence can be drawn. Conclusions on non-persistence can be achieved with ready biodegradation test data. Following this, information from other screening tests in combination with other relevant information can be used in a weight-of-evidence approach to conclude the persistence assessment. In cases where the assessment cannot be concluded based on the available information, definitive compartment-specific simulation tests are undertaken

well as information from applicable quantitative structure–activity relationship (QSAR) models. If, based on the assessment of all available information, it is not possible to reach a conclusion of the persistence assessment, further testing is required in the form of compartment-specific simulation tests. These OECD guideline tests for water (OECD 309), soil (OECD 307) and sediment (OECD 308) are considered the highest tier of information in a persistence assessment and are intended to simulate the degradation of a chemical in the compartment of interest under environmentally relevant conditions. As described in REACH guidance R.11, the aim of these simulation tests is to provide a definitive degradation half-life that can be compared directly to the persistence criteria of Annex XIII of REACH and thus permit a conclusion of the assessment to be reached. It is envisaged that results from testing of one or more compartments can be used to conclude the persistence assessment for other compartments, when supported by proper justifications. In order to reach a conclusion of “not P” for the substance, it is necessary to be able to conclude “not P” in all environmental compartments under consideration.

Whilst the ITS described in R.11 guidance outlines a stepwise approach for generating and appraising information on persistence, such information was available from all tiers of the assessment for phenanthrene. Therefore, all available data has been considered together, in order to apply a weight-of-evidence determination as described in REACH Annex XIII.

Data availability

The data included in this assessment were obtained predominately from the REACH Annex XV dossier for phenanthrene, and the comments provided during the associated public consultation [46, 47]. Additional studies have subsequently been compiled to supplement the persistence assessment of phenanthrene in this review. A systematic search of scientific literature repositories (PubMed, Science Direct, Google Scholar, Deepdyve) was undertaken using relevant keywords, resulting in the identification of approximately 10,000 potentially relevant studies. These were further screened to identify biodegradation data that were relevant to the persistence assessment. An overview of all the sources of information is provided in Additional file 1: Table S1. In summary the dataset is composed of the following:

a. Ready biodegradability information: two ready biodegradability studies, performed according to OECD test guidelines 301C [48, 49]. Five additional 301F ready biodegradation tests were performed in accordance with good laboratory practice (GLP) (Additional file 1: Section S2).
b. Persistence modelling using QSARs: Biodegradability predictions were undertaken using BIOWIN2, BIOWIN3 and BIOWIN6 [50] and half-life was modelled using BioHCwin [51] (Additional file 1: Section S3).
c. Higher-tier simulation test information: data are available for soil and freshwater sediment compartments corresponding to OECD test guidelines 307 and 308, respectively (Additional file 1: Sections S4 and S5).
d. Other information useful for a weight-of-evidence approach:
    i. Activated sludge simulation test according to OECD test guideline 314B [52] (Additional file 1: Section S6).
    ii. Non-standard experimental data from peer-reviewed publications were evaluated according to guidance R.7b and R.11 [10, 53].
    iii. Estimations of overall persistence time and partitioning of phenanthrene between environmental compartments, based on Mackay [54], were performed using the CEMC Level III model (download: https://people.trentu.ca/~mparnis/files/L3410.html). Standard defaults were used as model inputs except half-life values which were based on experimental data presented in this paper.
    iv. Photolysis degradation estimations in air were performed using AOPWIN [55] using standard defaults. Estimates of photodegradation rate in surface water were determined through review of previous literature and also with the APEX model (Aqueous Photochemistry of Environmentally occurring Xenobiotics) [24, 56].

Weight-of-evidence approach

As required under REACH Annex XIII, a weight-of-evidence determination has been applied in the persistence assessment of phenanthrene. All available relevant information bearing on the assessment of each environmental compartment have been considered together. Individual pieces of evidence have been weighed according to their reliability and relevance and have been assembled together in a single weight-of-evidence determination. The assessment has been conducted according to the following principles:

1. Relevance of experimental data has been assessed according to similarity to current standard test guidelines, as such having a higher regulatory impact and

requiring lower extrapolation [15], and applied a corresponding qualitative weight in the assessment. For instance, experiments employing freshly spiked, non-adapted test systems at low, environmentally relevant concentrations, or minimising artefacts from the presence of free-phase material, and incorporating measures to minimise or account for abiotic losses, are afforded greater weight in the assessment. This is consistent with the ECHA ITS, in which simulation testing represents the highest tier in the regulatory assessment of persistence [10].

2. In order to ascertain the coherence of evidence to arrive at an intrinsic determination of persistence [15], and in light of the naturally high variability observed in degradation half-lives [17], data for individual compartments were assessed on the basis of geometric means (geomeans). Where significant outliers were identified, the potential causative experimental or environmental factors were explored. Consistency in evidence between compartments was also a consideration, in line with the ECHA ITS.

Available information on phenanthrene

Ready biodegradation testing

There are a total of seven independent standardised ready biodegradation tests:

i. The first test, published in 1978 by the Japanese Ministry of International Trade and Industry (MITI), resulted in 54% degradation based on biochemical oxygen demand (BOD) measurements after 28 days [48]. The approach used in this test (MITI I) would ultimately become part of the standardised OECD 301 suite (301C) more than a decade later [57]. Additional analysis by gas chromatography (GC) and UV–Vis found degradation levels to be 79% and 72%, respectively, suggesting higher levels of primary degradation of phenanthrene. Limited information is available on this study, so it is not possible to confirm whether other guideline validity criteria were met.

ii. The second study, by Junker et al. [49], also following the OECD 301C (MITI) test guideline, employed ultrasonic homogenisation to increase dispersion of phenanthrene. In this test, 67% of theoretical oxygen demand (ThOD) was achieved in 28 days. Although the 10-day window criterion is not applicable to the MITI method, it was confirmed that phenanthrene did achieve 60% degradation within this window (pers. comm.). Furthermore, the overall validity criteria of this experiment were considered fulfilled.

iii. In 2019, GLP testing under the OECD 301F guideline and using a similar Oxitop© test system as Junker et al. [49] was performed. In this work, a test where phenanthrene was introduced by direct addition was conducted, along with tests employing the following bioavailability improvements, permitted within OECD 301 TG [57] and PBT assessment guidance [10, 53] and described in ISO standard 10634 [58]:

    a. Dispersion using ultrasonication;
    b. Increased solubilisation through initial heating at 40 °C;
    c. Adsorption on an inert support (silica gel) using a volatile solvent;
    d. Introduction of phenanthrene using a volatile solvent followed by emulsion using silicon oil.

Where phenanthrene was not introduced with volatile solvent, the particle size of the phenanthrene crystals was reduced by grinding in a mortar and pestle prior to testing. The degrading inoculum was non-preadapted activated sludge taken from a municipal wastewater treatment plant treating predominantly domestic wastewater. The test using silicon oil was performed after the initial experiments with other bioavailability modifications, using the same experimental conditions and inoculum source. All validity criteria were fulfilled for all tests. Phenanthrene ultimate biodegradation in these tests achieved greater than 60% ThOD (Fig. 2) within 28 days. Therefore, all five tests conducted in this study fulfilled the threshold of oxygen consumption for demonstrating “ready biodegradability”. For the most part, bioavailability improvements provided no significant benefit to the results of phenanthrene biodegradation relative to direct addition, which achieved the highest overall extent of mineralisation (68.3%) (Additional file 1: Figure S1). However, the test using silicon oil did show some potential to increase the rate of biodegradation. It was observed that the extent of mineralisation was 50.3% ThOD during the 10-day window, compared to 44.7% ThOD with direct addition. As these tests were performed with different inocula, a statistical assessment was not performed. However, this result sets apart the use of silicon oil as the only treatment to fulfil the 10-day window criterion for phenanthrene in these experiments (Additional file 1: Section S2).

Six out of seven ready biodegradability tests on phenanthrene report biodegradation $\geq 60\%$ in 28 days. For the purpose of the persistence assessment as part of PBT/vPvB assessment under REACH, it is not necessary to meet the 10-day window in the assessment of ready biodegradability [10]. However, the available

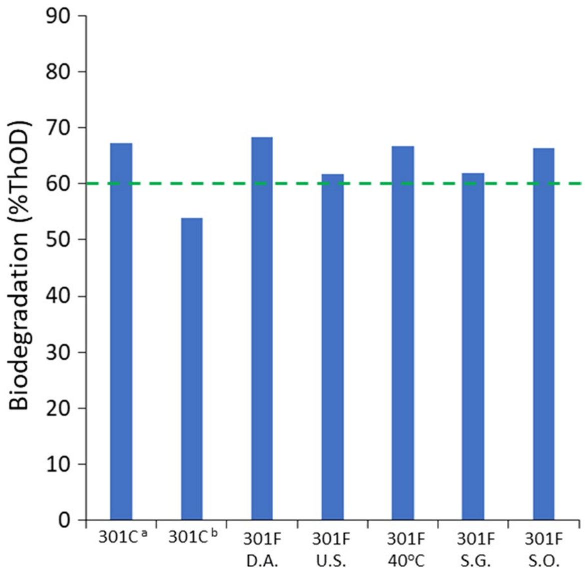

Fig. 2 Summary of phenanthrene ready biodegradability data. Ready biodegradability in OECD 301C tests by [a]Junker et al. [49] and [b]CITI [48]. Summary of 301F test results presented in this study also shown using direct addition (D.A), ultrasonication (U.S.), heating to 40 °C (40 °C), silica gel (S.G.) and silicon oil (S.O). Pass criteria for ready biodegradability indicated by green dashed line

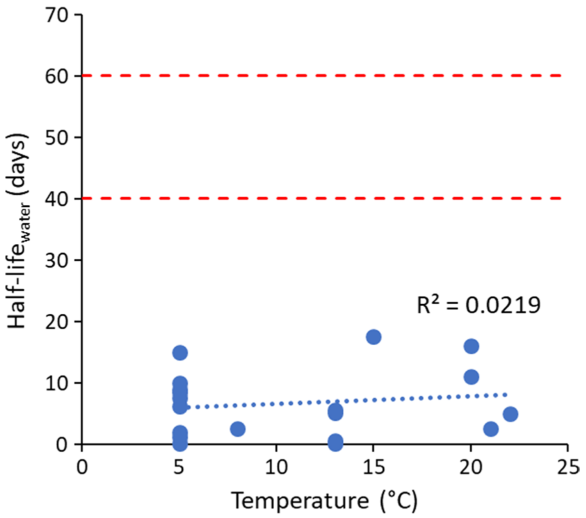

Fig. 3 Phenanthrene biodegradation half-life versus test temperature (°C) for the water compartment ($n=19$). P/vP thresholds for each compartment are shown with the red dotted line

data from 301F testing suggest that phenanthrene also meets this criterion when the appropriate bioavailability improvements are used. The majority of available data therefore support a conclusion of "readily biodegradable" in the context of the persistence assessment as part of PBT/vPvB assessment. Ready biodegradability tests are designed so that positive results are unequivocal, as it is recognised that tests may sometimes fail due to their stringent test conditions, [53, 59, 60]. Therefore, it is generally considered that positive results supersede negative results. Due to the limited information reported in CITI [48], it is not possible to investigate the details of this study. Compared to the results of the other tests, one potential explanation for the lower result in CITI [48] is the difference in inoculum. It is recognised that the inoculum in ready biodegradation tests can have variable performance, and that the inoculum from OECD 301C in particular (continuous culture of inoculum from mixed sources with synthetic sewage) tends to have a lower biodegradation potency compared to other ready biodegradability tests using freshly collected inoculum [49, 59, 61, 62]. A further potential reason for the inconsistency is the poorly water-soluble nature of phenanthrene. Chemicals that have low water solubility are recognised as presenting issues in ready biodegradability testing due to the relatively high test concentrations, and as such a range of approaches to increase the bioavailability of the test compounds are permitted [10, 57, 58].

Overall, the available evidence supports a conclusion of "readily biodegradable". Given the stringent nature of these tests, and the recognition that positive results generally supersede negative results [10, 27, 60], it can be concluded that phenanthrene is readily biodegradable.

Experimental data for the water compartment

No degradation data from water simulation tests are available for phenanthrene, and so the assessment was undertaken using data from non-standard studies. Ten independent studies in both fresh and marine water provided a total of 19 phenanthrene biodegradation half-life measurements ($n=19$, geomean = 3.5 days) (Fig. 3). An extensive study with pristine rural lake water [63] included inocula from seven sampling sites, collected throughout the year and assessed for their ability to biodegrade phenanthrene dosed with the co-solvent acetone. Mean biodegradation was 82% ($n=28$) after 28 days of incubation. Further detailed assessment of a subset of inocula (sites 4 and 7) found that most of the total biodegradation (91–95%) occurred within the first week of incubation, suggesting phenanthrene rapidly biodegrades in water. In other non-standard studies, phenanthrene biodegradation has been measured in both fresh and marine water at low, environmentally relevant, concentrations [64, 65]. Phenanthrene was passively dosed through a loaded polymer as part of a composed mixture. Biodegradation half-lives ranged from 5 to 16 days ($n=3$). Co-substrate effects on hydrocarbon degradation rate resulting from the use of composed hydrocarbon mixtures have been investigated by Hammershøj et al. [66] with hydrocarbons tested in isolation and in 3-, 8- and 16-part mixtures. No significant influence of the number of components on measured degradation rates was found.

Phenanthrene degradation has also been measured in seawater dispersions of oils [61, 68–72]. In only one case was seawater pre-adapted to the test substance [67]. Measured half-lives from oil dispersions ranged from 0.1 to 10 days ($n = 13$). No increase in the phenanthrene biodegradation half-life was observed from pre-adapted seawater. Also, no significant influence of dispersant on the measured degradation rates was observed [68] (Fig. 3). Phenanthrene biodegradation was also observed in a study with B20 biodiesel in freshwater [73] with a measured half-life of 2.6 days.

Primary and ultimate biodegradation of phenanthrene were assessed in an experiment similar to the OECD 306 seawater biodegradability test [74, 75]. Due to deviations from the guideline (temperature, test substance concentration and lack of reference compound controls), the study is included as weight-of-evidence rather than as a screening test. The study was performed at 5 °C using nutrient-amended pristine seawater collected from Trondheimsfjord (Norway) and 2 mg/L phenanthrene was dosed using a hydrophobic adsorbent (Fluortex™). Primary and ultimate half-lives were calculated to be 15.1 and 63.2 days, respectively. However, a lag phase of around 10 days was apparent from the data, and the mineralisation half-life was extrapolated beyond the total test duration (40 days). These results are considered of lower weight in the persistence assessment due to the observations on data treatment and the fact that the test concentration was above the water solubility of phenanthrene.

Rapid aquatic biodegradation rates are supported by a study of the PAH mass balance of the global oceans, indicating that more than 99% of atmospheric inputs of PAHs (1.09 Tg/year) do not settle to the deep ocean [76]. The highest of these imbalances was observed for phenanthrene, and microbial degradation was determined to be the dominant removal process. These types of field data are useful for persistence evaluation as they are based on a mass balance approach. The results demonstrate rapid and extensive degradation of phenanthrene in water, which is consistent with data from laboratory tests described above.

Experimental data for the soil compartment

An experimental system designed for screening chemical biodegradation in soil was developed based on the OECD 301C (MITI) test method [49]. This system, referred to as the "soil screening tool" (SST), replaced the inoculated mineral medium with a sandy loam soil. Using this approach, phenanthrene degradation reached a plateau at 39.8%, based on mineralisation, within 28 days.

Higher-tier simulation tests were performed using the standard OECD 307 aerobic flow-through setup (Additional file 1: Section S4) [32, 46]. In this study, degradation of the ^{14}C-labelled phenanthrene was investigated in four reference soils using acetone as an application solvent. All quality criteria were met, with the very minor exception of a slight over-recovery of radioactivity at one time point in a single soil (111.4% normally limited to 110%). The study can therefore be considered of high reliability and relevance. The DegT50 values ranged from 6.8 to 17.3 days (Additional file 1: Table S6).

In other non-standard studies where phenanthrene degradation has been measured in freshly spiked soils, half-lives are generally in agreement with Shrestha et al. [32] (Additional file 1: Table S1). In total, thirteen such studies were found in the literature, with phenanthrene being dosed to soils using varying approaches, including direct addition or solvent spiking of phenanthrene individually, as a composed PAH mixture, or as a constituent of materials such as crude oil [77–89]. Measured half-lives were between 4.8 and 63 days ($n = 28$; geomean = 22.0 days). A single measured half-life appears to be in exception to this, with 200 days reported in Coover and Sims [77]. This half-life, measured at 10 °C, is significantly longer than the half-lives measured at 20 and 30 °C in the same study, which were both < 60 days. One possible explanation for this is the relatively high amount of application solvent dichloromethane (100 mL/kg soil) that was used in this experiment. Dichloromethane has a measured log Koa of 2.27 and has been reported to reduce enzymatic activity in soil at concentrations as low as 1 mg/kg [90, 91]. It is possible at this lower temperature that some dichloromethane could have remained and affected the microbial activity in the soil. Another potential contributing factor is the relatively high loading of phenanthrene (1000 mg/kg) and total PAH (3718 mg/kg) in this study. In general, it was found that reported half-lives for phenanthrene in soil correlated with the total loading of test material (Fig. 4), with studies that used low, environmentally relevant concentrations reporting lower half-lives, including the OECD 307 study. At high loadings, there is an increased likelihood that phenanthrene will be present as free phase, and therefore having constraints on its bioavailability to degrading organisms, or to be in competition with other substrates. In line with the principle that lower concentrations are more relevant for the persistence assessment, this observed correlation adds further weight to the result from the OECD 307 study.

There is a large disparity between the data described above and those of studies where phenanthrene has

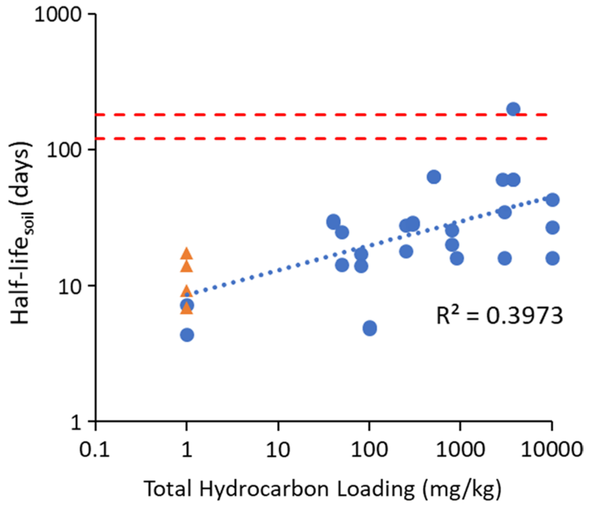

Fig. 4 Phenanthrene biodegradation half-life versus total hydrocarbon loading (mg/kg) for the soil compartment. Orange triangles indicate data from OECD 307 simulation tests (*n* = 4). Blue circles indicate data from non-standard experiments (*n* = 28). Data correspond to experiments with freshly spiked phenanthrene. P/vP thresholds for each compartment are shown with the red dotted line

been introduced to the soil through application of sewage sludge. Data presented by Wild et al. [92] and Wild and Jones [93] suggested phenanthrene half-lives to be in the order 5.7 years for long-term field studies and a range of 83–193 days for corresponding laboratory-based studies. The discrepancies can be interpreted as arising from differences in bioavailability of phenanthrene in these experiments. Bioavailability plays an important role in the microbial degradation of substances and can be a significant source of variability in environmental degradation rates [22, 94–97].

The available data on ready biodegradability and 314B activated sludge simulation testing (see "OECD 314B test data" section and Additional file 1: Section S6) for phenanthrene are indicative of the rapid degradation potential of phenanthrene in wastewater treatment systems. Despite this, concentrations of phenanthrene in sludges appear to be typically in the range of 0.1–10 mg/kg dw [98–101]. This situation is not limited to phenanthrene or PAHs in general, as sewage sludge is a known repository for a wide range of hydrophobic organic contaminants, which preferentially sorb to the organic matter in sludge [98, 99, 102]. Efforts to characterise organic contaminants in sludges have identified hundreds of individual compounds to be present in similar concentrations to phenanthrene, including readily biodegradable substances (e.g. toluene, benzoic acid and alcohol ethoxylates) [103–105].

It is understood that degradation rates of PAHs in sludge are controlled by aqueous concentrations (i.e. freely dissolved or sorbed to dissolved colloidal material), and not by total extractable concentrations [106, 107]. The reported dissolved concentrations of phenanthrene in sludge are in the order of 1–10 ng/L [98, 108], suggesting that bioavailable concentrations are much lower than total extractable concentrations. These very low aqueous concentrations may be very limiting for further degradation [59].

A further explanation for the reduced bioavailability of phenanthrene in sludges and historically contaminated soils and sediments is the increasing prevalence of pyrogenic (soot-associated) material in these matrices [40]. Extensive literature has documented exceptionally strong binding and low bioaccessibility of phenanthrene associated with urban soot and char [97, 109–116]. For example, Jonker et al. [113] estimated that 20 to 116 years would be required to desorb 99% of the bound phenanthrene on various sources of soot, under environmental conditions. Inspection of the available diagnostic ratios (i.e. the phenanthrene/anthracene, fluoranthene/pyrene, and benzanthracene/chrysene ratios) uniformly indicates that the PAHs had predominantly pyrogenic origins [41] in the sewage sludge applied to soils by Wild et al. [92, 93]. Consequently, a significant fraction of soot-associated phenanthrene is expected to be non-bioavailable and have limited potential to biodegrade.

In a study by Harmsen and Rietra [94], the long-term degradation of PAHs in sediment-amended soils was correlated to presence and rates of fast, slowly and very slowly desorbing fractions. Harmsen and Rietra [94] reported that 42–84% of the phenanthrene in PAH-contaminated sediments was rapidly bioaccessible. By contrast, 3 years after these sediments were added to soils, the bioavailable fraction represented only 6–7% of the phenanthrene present in the amended soils, and after 10 years this value was < 3%. Clearly, the bioavailability of chemicals in environmental matrices has a significant bearing on the rates at which they will degrade. Available diagnostic ratios (phenanthrene/anthracene, fluoranthene/pyrene and benzanthracene/chrysene) indicate that the PAHs studied by Harmsen and Rietra [94] had predominantly pyrogenic origins [41], consistent with the interpretation that a significant fraction of the phenanthrene was strongly bound to soot and thus had low bioavailability. This has important implications for phenanthrene and for persistence assessments in general, which are discussed further in "Bioavailability" section.

In addition to the considerations of bioavailability, there are several significant concerns relating to the reliability and relevance of the Wild et al. [92] study. First, the reported half-life of 5.7 years is misleading, because this value results from a poorly fitted exponential model to the data, the field data reported by Wild et al. [92] indicate that their most slowly degrading experimental rural site experienced a 50% decrease in phenanthrene concentration within approximately 2.9 years. Secondly, the initial concentrations in the experiments used to derive half-lives are estimates rather than measured values. Third, phenanthrene concentrations in control plot soils actually increase over the duration of the experiment indicating a continuous influx after the initial application (most likely as a result of aerial deposition) and adding further uncertainty to validity of the degradation calculations. Fourth, in a later publication the authors themselves highlight the inconsistency of this dataset with other field datasets and cite possible artefacts due to the use of archived soils [117]. It should also be noted that the limitations due to the semi-controlled and site-specific nature of field studies are recognised in guidance [53], and as such field studies are not considered to represent high-tier studies, or to be directly comparable with laboratory tests or P/vP criteria. Based on these considerations, and in line with the ECHA ITS, the studies by Wild et al. [92] and Wild and Jones [93] are considered of lower relevance compared with the OECD 307 and other studies where phenanthrene was spiked directly into soil, and are afforded a low weight in the assessment.

Experimental data for the sediment compartment

Phenanthrene was tested in a "water-sediment screening tool" (WSST), based on the OECD 301C (MITI) test method, similar to the "soil screening tool" (SST) described above [49]. In this system, phenanthrene achieved 51.3% mineralisation over 28 days, with a corresponding ultimate degradation half-life of 25.3 days.

Phenanthrene has been tested in a simulation test according to OECD 308 (Additional file 1: Section S5) [31, 46]. The combination of hydrophobicity and volatility makes chemicals, like phenanthrene, particularly difficult to test in this system due to challenges in introducing the chemical into the system and in avoiding evaporative losses that lead to incomplete mass balances and degradation modelling challenges [31]. In pre-tests with the standard flow-through setup, considerable losses (> 40%) of applied radioactivity were observed due to volatilisation over a period of 7 days. Consequently, a static biometer system was designed and used to reduce these losses and ensure mass balances remained within a valid range (90–110%). This did not incorporate gentle stirring of the water surface, contrary to what is recommended in OECD 308. All other parameters were followed as expressed in OECD 308 [12].

Phenanthrene was applied in an acetone–water co-solvent solution, as permitted in the guideline. According to the guideline, the concentration of this organic solvents used should not exceed 1% v/v and should not have adverse effects on the microbial activity of the system. However, the use of acetone for dosing of phenanthrene (0.25% v/v) in this closed setup led to a significant depletion of oxygen in the test system and the formation of a biofilm (evidence of an effect on microbial activity) at the air–water interface (Additional file 1: Figure S6). This led to the onset of anaerobic conditions in the water phase, which was not replenished by headspace oxygen, and consequently a likely reduction in the rate of phenanthrene biodegradation. Although both the use of application solvents and the use of closed systems are permitted in the guideline, their use in combination was likely never envisaged nor validated, and led to significant issues with the performance of this test. The amount of acetone used (approximately 2 g/L) was sufficient to consume all of the available oxygen in the system multiple times over, and exceeds some reported effect concentrations for microorganisms [118, 119].

In order to further assess the extent of oxygen depletion under the conditions employed in the OECD 308 test, a repeat of the experimental setup of parameter samples was conducted employing continuous monitoring of oxygen concentrations in the water phase using optical oxygen sensors (PreSens, Germany). The results indicated a continuous decline of oxygen concentration in the water phase of samples treated with acetone despite the headspace remaining aerobic throughout (Additional file 1: Figure S6). By day 30 the oxygen in the water phase of both sediments was fully depleted. The data provide important evidence as to the depletion of oxygen in this system, which had previously only been judged based on a single measurement of dissolved oxygen at 28 days. This emphasises the importance of taking additional steps to maintain aerobic conditions in the water phase of closed OECD 308 test systems.

Although an anaerobic sediment zone at the bottom of a water-sediment system is unavoidable [30], the water phase and surface layer of sediment of OECD 308 test are typically aerobic. In fact, the OECD 308 TG states that the "aerobic test simulates an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient" and specifies typical dissolved oxygen concentrations of 7 to 10 mg/L in the aqueous phase. Additionally, the regulatory guidance is clear in that the assessment of persistence in the sediment compartment should not be based only on anaerobic data [10]. This is due to the fact that there is generally no immediate discharge to anaerobic sediment or soil, and that for purposes of persistence assessment, it is important to understand the rate of degradation across the aerobic zone [10]. Due to the observations above, there are serious concerns as to the reliability of this OECD 308 test, and it is highly likely that the biodegradation potential of phenanthrene was underestimated. The reported half-lives for the total system were 113 days for both fine- and course-textured sediments (Additional file 1: Table S7).

In addition to the OECD 308 test, there were several non-standard studies found in the literature (Fig. 5). Four of these utilised conditions similar to OECD 308, with test material applied to water-saturated natural sediments, with sediments remaining stagnant throughout the test period. Apitz et al. [120] tested a water-saturated sediment spiked with a mixture of solid PAHs in sand, with no overlying water, resulting in a half-life of 42 days. In a second study, crude oil was mixed directly with sediments and the degradation of phenanthrene measured [121]. Three treatments, one with overlying water and two with a commercial dispersant (Corexit), with and without overlying water, resulted in half-lives of 18.5, 40.3 and 19.4 days, respectively. Two further studies assessed the degradation of phenanthrene in covered mesocosms, with crude oil applied to the surface of sediment cores and a pumping cycle used to simulate natural tides [122, 123]. The degradation half-lives of phenanthrene in these experiments were 9 and 2.5 days. The latter study did not incorporate abiotic controls and is therefore afforded lower weight in the assessment.

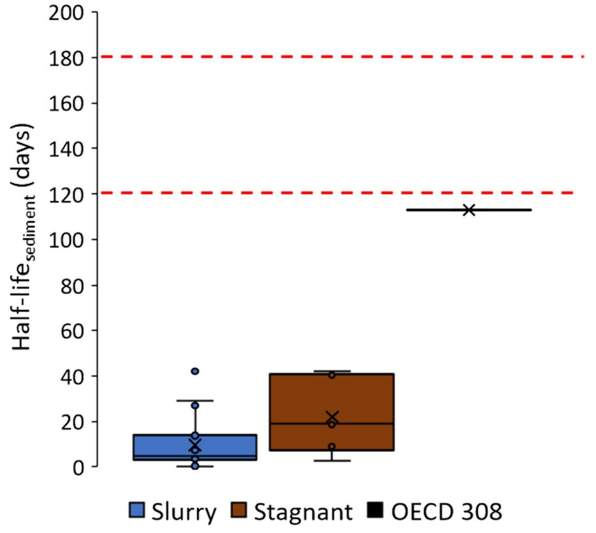

Fig. 5 Box and whisker plots of phenanthrene biodegradation half-life data for the sediment compartment. Data categorised according to slurry systems (*n* = 26), stagnant systems (*n* = 6) and the OECD 308 test (*n* = 2). P/vP thresholds for each compartment are shown with the red dotted line

In addition to the above studies employing stagnant sediment systems, several researchers measured degradation of phenanthrene in water-sediment slurries, where the system is continuously shaken [124–127]. Whilst these conditions are not as comparable to standard OECD 308 methods, it is notable that this is a common approach for assessing chemical degradation in sediments in research settings. Under such conditions, test systems would be more homogeneous and aerobic, and less stratified than the OECD 308 test. This may serve to better harmonise water-sediment biodegradation testing and to address some of the issues raised around robustness and environmental relevance of the OECD 308 test [29, 30, 128]. However, it may also be considered to represent a different scenario than OECD 308, and to produce more favourable conditions for degradation. Phenanthrene degradation half-lives in aerobic water-sediment slurries ranged from 0.3 to 42 days (*n* = 26, geomean = 5.2 days), which were overall faster than in stagnant systems (*n* = 6; geomean = 15.5 days). The available half-lives from non-standard studies are much lower than the results of the OECD 308 test (Fig. 5), indicating the clear potential of phenanthrene to rapidly degrade in this compartment. These studies provide further evidence that the methodological concerns with the OECD 308 study negatively impacted phenanthrene degradation, and as such that the OECD 308 study is unreliable.

Other information useful in a weight-of-evidence approach

In silico predictions of phenanthrene persistence

Phenanthrene was evaluated using the QSAR model BioHCwin (v1.01), which has been developed specifically for the biodegradation half-life prediction of petroleum hydrocarbons [51]. Recent comparisons of BioHCwin predictions to experimental half-lives have found predictions to be conservative [64, 129]. Phenanthrene was assessed to be within the applicability domain of the model (Additional file 1: Section S3), and has a predicted half-life of 15.0 days. Biodegradability of phenanthrene was also evaluated using BIOWIN (v4.10; [50]). In the REACH guidance R.11 [10, 53], screening criteria for persistence are defined for this model. A substance screens as "potentially P or vP" if *either* the result of the BIOWIN 2 model is < 0.5 and the result of the BIOWIN 3 model is < 2.25, *or* if the result of the BIOWIN 6 model is < 0.5 and the result of the BIOWIN 3 model is < 2.25. The model results for phenanthrene were 0.99, 2.22 and 0.19 for BIOWIN 2, 3 and 6, respectively. Therefore, phenanthrene screens as "potentially P or vP" based on the results of BIOWIN models 3 and 6, but not based on BIOWIN 2 and 3. It is notable that in the case of BIOWIN 3 the result (2.22) was very close to the criteria of < 2.25. Overall the results of QSAR predictions for phenanthrene do not provide a clear indication for the persistence assessment.

OECD 314B test data

OECD 314 A-C studies are not currently considered acceptable on their own for the persistence assessment under REACH because they are not considered to be representative of the natural environment, as they represent sewage treatment facilities. Data from these studies, however, can be used as additional lines of evidence for persistence assessment as sewage treatment bacteria do enter surface waters and data from these studies will indicate whether the substance has the potential to degrade [130]. Phenanthrene degradation was assessed in an OECD 314B study (Additional file 1: Section S6). Modifications of the test system were implemented to address abiotic losses and achieve complete mass balance, and all remaining validity criteria were fulfilled. In this test, rapid degradation of phenanthrene was observed and half-life was calculated to be 0.45 days (Additional file 1: Table S7).

Photodegradation in air and water

Photodegradation is an important abiotic degradation mechanism in the environment and is known to occur with PAHs including phenanthrene [131]. AOPWIN predicted a half-life for phenanthrene of 9.9 h in the troposphere [55]. Based on laboratory measurements of aquatic photodegradation rates for phenanthrene, [25, 26, 131–133] and information about reactivity with sunlight-generated natural oxidants [134–138], the APEX model [24, 56, 139] was used to predict a vertically integrated photodegradation half-life in a 3-m depth surface water (the depth conventionally applied for regional-scale multimedia models) having average conditions. The model inputs assumed a median diurnal sunlight intensity (i.e. early spring or early autumn) and partial cloud cover at mid-latitude (45° N), with a typical natural water composition and a moderate dissolved organic carbon concentration (3 mg C L^{-1} DOC). With these inputs, the APEX model indicates that phenanthrene photodegrades in a typical surface water according to a half-life of about 30 days. The modelled photodegradation rate depends primarily on the season, cloud cover and DOC concentrations, and the rate can reasonably vary from 3.5 days (for fair-weather conditions in summer with 1 mg C L^{-1} DOC) to 140 days (for cloudy conditions in winter with 5 mg C L^{-1} DOC). These data demonstrate that photodegradation in the atmosphere and in surface waters are important fate processes for phenanthrene persistence, in addition to biodegradation.

Multimedia fate modelling of overall persistence

Persistence criteria are based on compartment-specific biodegradation half-lives. However, in the environment, advection processes and inter-compartmental mass transfers (e.g. resuspension) also play a role on the residence time of a chemical in each compartment. In recent work by Mackay et al. [36], the recommendation is that both reactive degradation half-life and transport processes should be considered in the persistence assessment of a chemical. Mass balance model calculations were performed using the CEMC Level III model to determine the steady-state distribution of phenanthrene. The total (steady-state) residence time is a metric commonly used to estimate “overall persistence” (P_{ov}) [33]. This model framework was applied with the geometric means of degradation half-life data compiled in the present work. Input values of 3.5, 21.6 and 25.4 days were selected for the water, soil and sediment compartments, respectively. The geomean for sediment included the OECD 308 data but excluded the slurry data as a conservative measure. This resulted in a total residence time for phenanthrene of 13 days, which is considerably lower than the REACH P cut-offs, even for the water compartment. Previously, a P_{ov} of ~ 90 days has been suggested as a threshold for assessing compounds based on overall persistence, as this indicates essentially complete removal from the environment within a one year timeframe [33].

Monitoring data

Monitoring data can provide supporting evidence in a persistence evaluation. However, it is acknowledged in guidance that monitoring data in itself cannot demonstrate persistence because the presence of a substance in the environment is dependent on a range of factors, including emissions and distribution rates, as well as degradation processes [10]. Potential sources, volume trends, uses and releases should be considered when evaluating the suitability of monitoring data [10]. Unlike most industrial chemicals, PAHs in the environment may be attributed both to anthropogenic activities (e.g. coal, biomass and petroleum use, and vehicle exhaust) as well as a range of natural sources (e.g. forest fires and natural seepage from petroleum deposits) [140]. Global atmospheric emissions of 16 PAHs were estimated to be 504 Gg in 2007, with the majority being from residential/commercial biomass burning (60.5%) [141, 142]. Despite these large quantities, greater than 99% of atmospheric inputs to oceans have been found to be degraded near the surface (0–200 m) [76]. Evidence from soil and sediment monitoring studies suggest that environmental occurrence of PAHs is predominantly from pyrogenic sources—both natural and anthropogenic [143–145].

This is in part due to a greater relative input of PAHs from these sources, but is also a result of the contrasting fate and degradation of PAHs from pyrogenic versus petrogenic sources [40]. Pyrogenic PAHs are associated with soot carbon, which renders them resistant to degradation due to severely reduced bioavailability [97, 112]. This is in contrast to petrogenic PAHs, which are able to diffuse out of their carrier materials and into a freely dissolved form which is biologically labile. The variety, diffuse nature and magnitude of emission sources, and their relative impact on fate processes, render monitoring data to be of limited value for use in the persistence assessment of phenanthrene.

Other considerations

Assessment of phenanthrene degradation metabolites

Based on a survey of the Swiss Federal Institute of Aquatic Science and Technology (Eawag) EAWAG-BBD (Eawag Biocatalysis/Biodegradation Database), a database of microbial biocatalytic reactions and biodegradation pathways [146], phenanthrene is degraded by bacteria through one of two different routes (Additional file 1: Figures S9–S10). Fungi are also known to metabolise phenanthrene (Additional file 1: Figure S11). Demonstration of rapid mineralisation of phenanthrene to CO_2 (Fig. 2, Additional file 1: Sections S4, S8) suggests that degradation metabolites are unlikely to accumulate in the environment. This is further supported by BIOWIN2 and BIOWIN3 QSARs that predict rapid biodegradation of all phenanthrene metabolites described in the Eawag survey (Additional file 1: Table S9). These data support the conclusion that metabolites produced from phenanthrene biodegradation are labile and not persistent in the environment.

Temperature correction

Guidance for persistence assessment under REACH was recently updated to require a European average environmental reference temperature [53]. As such there is a requirement for new degradation studies to be carried out at 12 °C, which is considered the mean surface water temperature of Europe. For existing test data, the guidance suggests a temperature correction using the Arrhenius equation should be considered [10]. This recommendation assumes that biodegradation in the environment varies with temperature according to Arrhenius, and is based on an analysis of plant protection product biodegradation in soils [147]. However, there remains a body of evidence that does not support this practice [148–151]. In particular, the Arrhenius law, which is used to describe simple chemical reactions, does not consider the possible influence of temperature adaptation on biodegradation rates in complex biological systems. In a systematic analysis of 993 hydrocarbon biodegradation half-lives, Brown et al. [150] found that the use of the Arrhenius equation in accordance with ECHA guidance over-estimated the influence of temperature on biodegradation half-lives when comparing temperature-adapted inocula. A comparison of surface water biodegradation half-lives for phenanthrene did not yield any correlation with temperature (Fig. 3). As such, temperature correction of data for phenanthrene was considered inappropriate and was not performed, consistent with previous recommendations [17, 130, 152].

Non-extractable residues (NER)

Chemicals that enter the environment have the potential to form non-extractable residues (NER) [153]. The relevance of NER, which are only detectable in radiolabelled studies, to environmental hazard and risk assessment continues to be debated, i.e.: are they a "safe sink" or a "hidden hazard" [154–158]? Current ECHA guidance applies the latter interpretation [10, 159]. Based on ECHA guidance, the NER fraction should by default be considered to be composed entirely of non-degraded substance, and should be included in the calculation of the degradation half-life for the purpose of persistence assessment [10]. This has the potential to greatly impact the conclusions of existing and future persistence assessments, with NER fractions for instance reported to vary widely for pesticides [160]. Three separate types of NER in environmental matrices have been proposed by Schäffer et al. [158]: NER type I (physically trapped in the matrix), Type II (covalently bound to the matrix) and Type III (incorporated into the biomass), with Types II and III to be considered of low or no concern and removed from the assessment. However, the methods proposed for distinguishing these fractions are still undergoing validation [157].

In the soil simulation studies presented in this assessment, rapid primary biodegradation and mineralisation of phenanthrene were concurrent with the formation of NER (Additional file 1: Figure S3). A lack of NER produced in sterile controls suggests that in biotic tests NER formation was unlikely to be derived from physically entrapped substance (i.e. Type I NER). Chemicals that are rapidly mineralised are prone to the formation of biomass (Type III NER) [153, 161]. Phenanthrene, as a chemical that undergoes rapid mineralisation, is therefore likely to form appreciable amounts of Type III NER. Furthermore, contributions of several PAH metabolites to the formation of covalently bound (i.e. Type II) NER have been described previously [162, 163]. Although no additional characterisation was performed to quantify the different types of NER produced during phenanthrene biodegradation, the above evidence indicates that phenanthrene

NERs can be regarded as of low or no concern and not a contributing factor in the calculation of half-lives.

Bioavailability

For persistence assessment it is essential to recognise bioavailability to degraders as a source of significant variability in biodegradation data, and to take this into consideration when performing weight-of-evidence determinations if we are to arrive at a consistent and predictable prioritisation of chemicals [21]. In this context, since most substances will be evaluated on the basis of standard simulation tests, and these data are considered as having the greatest weight in the assessment, it follows that non-standard data derived under similar conditions should also be assigned greater weight in the assessment. In standard simulation tests, the chemical is directly spiked into the test system, and as such is introduced in a readily available state giving microorganisms maximum opportunity for degradation. This contrasts with situations where chemical bioavailability is reduced, e.g. as a result of aging or being entrapped in soot [40, 96, 97]. A failure to consider these factors raises the significant risk of substances being prioritised purely on the basis of differences in conditions under which their degradation has been measured, rather than on their intrinsic properties. This is clearly an undesirable outcome if the goal is to have an effective prioritisation scheme for regulation.

In light of these considerations, it is worthwhile to reconsider the purpose of persistence assessment, which was developed for the specific purpose of addressing concerns posed by substances that were also bioaccumulative and/or toxic [2]. As such, persistence is fundamentally an indicator of exposure potential, with persistent chemicals assumed to have greater potential for increased and poorly reversible exposure [3–7]. However, if a chemical has been concluded to be persistent based on a situation of low bioavailability, this may in fact be a poor reflection of its exposure potential. This is especially the case for readily biodegradable substances, which are assumed to undergo rapid removal from the environment and therefore unlikely to pose a concern for bioaccumulation and long-term toxicity. As well as affecting the biodegradation of chemicals, bioavailability is also known to limit exposure to other ecological receptors [95, 96, 164, 165]. This is further justification to assess persistence on the basis of direct spiking methods, as this ensures that the degradation of the substance is assessed under circumstances in which it also has the potential to bioaccumulate and/or cause toxicity. Ultimately, these measures will lead to more consistent prioritisation of chemical persistence and will prevent the undesirable scenario of rapidly degradable substances being mis-prioritised as persistent.

Synthesis of all data and conclusions on persistence

Ready biodegradability

Phenanthrene has been demonstrated to be readily biodegradable. Readily biodegradable substances are assumed to undergo rapid and ultimate biodegradation under most environmental conditions [10, 60], and a conclusion of "readily biodegradable" carries significant regulatory implications for a substance. From the perspective of the REACH and CLP (Classification, Labelling and Packaging; (EC) No 1272/2008 [166]) regulations, "readily biodegradable" is a criterion which limits the amount of further testing required for a substance (including simulation testing), reduces environmental exposure estimates in risk assessment and may lead to a more favourable environmental hazard classification. According to the ITS for persistence assessment under REACH, the role of the ready biodegradability test as a first tier in the assessment is explicit in that a readily biodegradable substance can be concluded as not fulfilling the criteria of P or vP.

Persistence in water

The measured half-lives for phenanthrene degradation in surface water are consistent across studies, geographical regions and testing approaches. In summary, measured biodegradation rates cover a range of 0.1–16 days, with a geometric mean of 3.5 days (Fig. 3, Table 1). The available measured half-lives for phenanthrene are substantially lower than the cut-offs for persistence of 40 days for freshwater and 60 days for marine water and indicate phenanthrene to be not persistent (not P) in these environmental compartments.

Persistence in soil

The OECD 307 simulation test is intended to provide a definitive degradation half-life that can be compared directly to the persistence criteria as defined in REACH Annex XIII [53]. The results from the reliable OECD 307 study on phenanthrene (DegT50 = 6.8–17.3 days)

Table 1 Summary of available half-live data for phenanthrene

Compartment	Half-life (days)		
	n	Range	Geomean
Water	19	0.1–17.5	3.5
Soil	32	4.4–200	21.6
Sediment	32 (34)	0.3–42 (113)	15.5 (25.4)[a]

Values in parentheses include data from OECD 308 study, which have been demonstrated to be unreliable

[a] Values do not include data from slurry experiments

therefore carry the highest weight in the assessment in soil and are well below the P cut-off. These results are consistent with non-standard studies which followed similar methods to the OECD 307 test (Fig. 4, Table 1). For reasons explained in "Experimental data for the soil compartment" and "Bioavailability" sections, studies where bioavailability of phenanthrene was severely limited, such as when introduced via sewage sludge, have been given a low weight in the assessment. Therefore, based on the weight of evidence, phenanthrene is concluded as not persistent in soil (not P).

Persistence in sediment

The OECD 308 simulation test is intended to provide a definitive degradation half-life that can be compared directly to the persistence criteria of REACH Annex XIII [10]. However, the OECD 308 study on phenanthrene had a number of serious methodological issues. Specifically, the use of a readily biodegradable solvent to apply phenanthrene in a closed system resulted in oxygen depletion in the water phase and effects on the microbial activity. For these reasons, the study is not considered reliable for use in persistence assessment. Several additional non-standard studies are available in the literature that provide degradation half-lives for phenanthrene in aerobic water-sediment systems. All of these studies support a conclusion of "not P" for phenanthrene, with half-lives well below the P cut-offs (Fig. 5, Table 1).

As described in "Materials and methods" section, the ITS for persistence assessment envisages the use of simulation testing from one compartment to potentially draw conclusions on the other compartments [10]. In principle, this means that data from a valid simulation test in soil or water are relevant to the persistence of phenanthrene in the sediment compartment as well. The available half-lives from the OECD 307 test for phenanthrene are around an order of magnitude lower than the P and vP cut-offs for both soil and sediment. Such a short half-life in soil supports the concerns regarding the reliability of the OECD 308 study and provides further evidence that phenanthrene is not P in sediment. Perhaps the most compelling evidence is the fact that phenanthrene is readily biodegradable. As already highlighted, a positive conclusion from stringent ready biodegradability testing is interpreted as evidence that a substance will undergo rapid and ultimate degradation in the environment, and carries several important regulatory implications. This fact, along with the evidence from the soil and water compartments, and the non-standard data for the sediment compartment all collectively point towards a conclusion of not P. In line with the principles of a weight-of-evidence determination, phenanthrene is therefore concluded to be not persistent (not P) in sediments.

Comparison of this study to the phenanthrene SVHC listing

The findings of this study are not in agreement with the regulatory decision surrounding phenanthrene [44], and three key differences stand out as follows:

1. The regulatory assessment by the authorities did not reach a conclusion on the ready biodegradability of phenanthrene [44]. The regulatory assessment concluded "biodegradation of phenanthrene may occur in water". However, ready biodegradability testing is not intended to reflect only degradation in water, but under "most environmental conditions" [53, 60]. Moreover, "readily biodegradable" is a specific condition for waiving simulation testing in the REACH legal text. This demonstrates that REACH, which has been underpinned by the precautionary principle in its construction [9], recognises that readily biodegradable chemicals cannot also be P/vP. Thus, phenanthrene should be concluded "not P".
2. The regulatory assessment by the authorities did not reach a conclusion for the persistency assessment of phenanthrene in soil, despite the highest tier of information being available [44]. Whilst it was acknowledged that rapid biodegradation occurred in the OECD 307 study, a seemingly equivalent weight was applied to biodegradation results from non-standard and field-based studies, where biodegradation was slow. It was acknowledged that field-based studies depend on local conditions, matrix and methodological conditions, and that these confounding factors played a dominant role in the slow biodegradation rate of phenanthrene. These facts pertaining to field studies are recognised in guidance and, as such, field studies are considered "not directly comparable to laboratory tests or P/vP criteria" [10]. It is because of such confounding factors that standardised simulation testing is afforded the greatest weight in the regulatory assessment. Therefore, the data from the valid simulation test should be given the highest weight as the most reliable and relevant information to conclude in the assessment, as envisaged in the ITS and REACH legal text. Thus, phenanthrene should be concluded "not P".
3. Ultimately the vP conclusion for phenanthrene relied solely on the results from one study—the OECD 308 water-sediment simulation test—after applying a temperature correction to extrapolate the result from 113 days (not P) to > 180 days (vP). As discussed above, due to serious methodological issues, the OECD 308 test on phenanthrene is not reliable.

Further, as discussed in "Temperature correction" section, the practice in guidance of temperature correction of degradation half-lives using the Arrhenius equation remains scientifically questionable and uncertain [150]. As has been demonstrated in this study, several relevant studies from the literature have measured the degradation of phenanthrene in water-sediment systems, and all resulted in half-lives well below the P cut-off. These studies are in line with results from other compartments, including the soil OECD 307 study. Moreover, a conclusion of vP in sediments for phenanthrene is in direct contradiction with the result from biodegradability screening and carries important implications for the suitability of the current regulatory framework, and assessments of over 22,000 substances registered under REACH. Ultimately, the conclusion of phenanthrene to be vP based solely on a single piece of information, which is significantly out of step with the broader evidence, is not in line with the principles of a weight-of-evidence determination as defined under Annex XIII of REACH. Thus, phenanthrene should be concluded "not P".

Conclusions

Phenanthrene is a chemical rich in persistence data, and therefore provides for an ideal case study for persistence assessment and a critical test of the Integrated Testing and Assessment Strategy (ITS) applied under REACH. In this work, the validity of the available data was consistently assessed, and integrated to produce a weight-of-evidence determination on the persistence of phenanthrene. Experimental data from all compartments, as well as screening tests, consistently indicate the rapid biodegradation potential of phenanthrene, and support a conclusion of not P. Although some non-standard testing and field studies indicate longer residence times, these data are generally attributable to pyrogenic sources of phenanthrene, or to situations of significantly reduced bioavailability. This highlights the importance of bioavailability as a major source of variability in data compilation for persistence assessment, and the importance of standard testing methods to arrive at consistent prioritisation of chemicals.

This work highlights the importance of applying weight-of-evidence principles to the assessment of persistence. Thoroughly assessing the validity of each line of evidence, assigning the appropriate weight to different lines of evidence, and considering the coherence of the overall dataset in the final determination, is critical. This is particularly important for data-rich chemicals, like phenanthrene.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12302-020-00427-1.

Additional file 1. Supplementary information.

Abbreviations

APEX: Aqueous Photochemistry of Environmentally occurring Xenobiotics; BOD: Biochemical oxygen demand; CLP: Classification, labelling and packaging; DOC: Dissolved organic carbon; EAWAG-BBD: Eawag Biocatalysis/Biodegradation Database; ECHA: European Chemicals Agency; GC: Gas chromatography; GLP: Good laboratory practice; ITS: Integrated Testing and Assessment Strategy; MITI: Ministry of International Trade and Industry; NER: Non-extractable residues; OECD: Organisation of Economic Co-operation and Development; P: Persistent; PAH: Polycyclic aromatic hydrocarbon; PBT: Persistent, bioaccumulative and toxic; Pov: Overall persistence; QSAR: Quantitative structure–activity relationship; REACH: Registration, evaluation, authorisation and restriction of chemicals; SST: Soil screening tool; SVHC: Substance of very high concern; ThOD: Theoretical oxygen demand; vP: Very persistent; vPvB: Very persistent, very bioaccumulative; WSST: Water-sediment screening tool.

Acknowledgements

The authors would like to acknowledge Sarah Bagwell and Megan Griffiths from Ricardo Energy & Environment for their support in the preparation of this manuscript. They are grateful to Thomas Junker of ECT Oekotoxikologie GmbH for providing comments and clarifications regarding OECD 301C data from his 2016 study. They thank Boris Meisterjahn, Dieter Hennecke and Prasit Shrestha of Fraunhofer IME, who performed the simulation testing, and they are indebted to Prasit for his helpful comments and for providing additional control O_2 data relating to his 2019 paper. Finally, they appreciate Stefan Gartiser and Andrea Brunswik-Titze of Hydrotox GmbH for their discussions around phenanthrene biodegradability and who performed the ready biodegradability testing presented in this paper.

Authors' contributions

CBH and DMB are contributed to conception of study, acquisition, analysis and interpretation of data, and drafting of manuscript; LC and ADR are contributed to conception of study, acquisition, analysis and interpretation of data, and substantive review; JSA is involved in acquisition, analysis and interpretation of data on persistence in soil and sediment, photodegradation and bioavailability, and substantive review; DV is involved in acquisition, analysis and interpretation of section on photodegradation, and substantive review; NW and EV are contributed to conception of study and substantive review. All authors read and approved the final manuscript.

Funding

The funding for this research was provided by Concawe. Representatives of Concawe and its members have contributed to the preparation of this manuscript (see "Authors' contributions" section).

Availability of data and materials

All data generated or analysed during this study are included in this published article and its Additional files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare the following financial and non-financial interests which may be considered as potential competing interests: The authors are employed, or have previously been employed, by companies or industry associations involved in the manufacture and marketing of petroleum substances that may contain phenanthrene.

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Received: 28 August 2020 Accepted: 26 October 2020

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