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Persistence, bioaccumulation and toxicity-assessment of petroleum UVCBs: a case study on alkylated three-ring PAHs

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Review

Persistence, bioaccumulation and toxicity-assessment of petroleum UVCBs: A case study on alkylated three-ring PAHs

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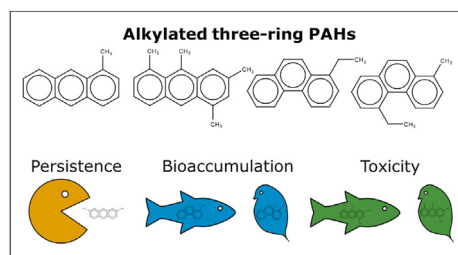
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HIGHLIGHTS

- The hydrocarbon block method is applied in a PBT-assessment of alkylated 3-ring PAHs.
- Trend analyses were conducted as few environmentally relevant data was available.
- Results indicate that alkylated 3-ring PAHs are more P, B and T than parent PAHs.
- As the parents are identified as PBT/vPvB, results could be extrapolated to the group.

GRAPHICAL ABSTRACT



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ABSTRACT

Substances with (very) persistent, (very) bioaccumulative, and/or toxic properties (PBT/vPvB) are of environmental concern and are identified via hazard-based PBT-assessment approaches. The PBT-assessment of well-defined substances is optimized over the past decades, but is under development for substances of unknown or variable composition, complex reaction products or biological materials (UVCBs). Particularly, the large number of constituents and variable composition complicate the PBT-assessment of UVCBs. For petroleum UVCBs, the use of the hydrocarbon block method (HBM) is proposed. Within this method, groups of constituents with similar physicochemical properties and structure are treated as a single entity and are expected to have comparable environmental fate and hazard properties. So far, however, there is a lack of experience with the application of the HBM for PBT-assessment purposes. The aim of this study is to investigate the suitability of the HBM for the PBT-assessment of petroleum UVCBs by evaluating the group of alkylated three-ring polycyclic aromatic hydrocarbons (PAHs). The presented approach is based on experimental data and model predictions and followed the guidelines of the European Chemicals Agency. Because of a lack of relevant experimental data, relative trend analyses were applied. The results indicate that alkylated three-ring PAHs are more persistent, bioaccumulative, and toxic than the parent three-ring PAHs. As the parent three-ring PAHs are currently identified within Europe as PBT/vPvB substances, the alkylated three-ring PAHs could also be considered as PBT/vPvB. Accordingly, this case study provides the prospects for the application of the HBM for the PBT-assessment of UVCBs using trend analysis.

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1. Introduction

Regulatory priority is given to substances that are (very) persistent in the environment, (very) bioaccumulative in organisms, and/or toxic to the environment or to humans (i.e. PBT/vPvB) (ECHA, 2020a). Once emitted, PBT/vPvB substances cannot easily be removed from the environment, and are likely to reach high and potential toxic concentrations in organisms or humans upon continued emission (ECHA, 2017a). Therefore, regulatory agencies try to identify PBT/vPvB substances in order to take relevant regulatory measures.

PBT/vPvB substances are identified following a hazard-based PBT-assessment (ECHA, 2017a). Within this assessment, chemical persistence is evaluated based on environmental half-lives in different environmental compartments. Bioaccumulation is generally assessed based on bioconcentration in aquatic organisms, whereas toxicity is evaluated based on toxic effects to aquatic organisms or specific toxicity to mammalian species including humans. Within the PBT-assessment, these properties are compared to specified criteria to determine whether a substance is PBT/vPvB (ECHA, 2017a).

Within REACH, the European regulation on industrial chemicals (EC/1907/2006), the PBT-assessment principally considers a single constituent assessment. This means that a PBT-assessment needs to be conducted for all constituents within a substance that are present above a concentration of 0.1% (w/w) (ECHA, 2017a). Although this is seemingly clear for well-defined substances, like mono-constituent and (to a lesser extent to) multi-constituent substances, it is more complex for substances of unknown or variable composition, complex reaction products or biological materials (UVCBs). UVCBs contain a large number of constituents, often ranging from hundreds to thousands in number (Quann, 1998), of

which a significant fraction could be unknown, and/or their concentrations could be variable or unpredictable in the composition (ECHA, 2017a). Technically it is not possible to identify, isolate and test all individual constituents (ECHA, 2017a; Salvito et al., 2020), which are often individually present below 0.1% (w/w). However, as the individual constituents are generally very similar in structure to many other constituents, with sum concentrations of structurally similar constituents frequently above 0.1% (w/w), they are considered relevant for the PBT-assessment. This structural complexity complicates the PBT-assessment of UVCBs in comparison to well-defined substances.

Despite the complexity, PBT-assessment approaches for UVCBs are indispensable as approximately 40% of all REACH registered substances are considered UVCBs (Kutsarova et al., 2019). One specific group of UVCBs are petroleum-derived substances. Petroleum substances are considered UVCBs as their composition is highly variable (depending on the source and batch of crude oil, as well as specific production processes) and partially unknown, as it is not possible to identify each individual constituent. Petroleum UVCBs mainly contain hydrocarbons in the form of paraffins (alkanes), naphthenes (cycloalkanes) and/or aromatics, but can also contain other hydrocarbon structures like naphthenic-aromatics (Fig. 1A). Several of these hydrocarbon constituents are of potential PBT/vPvB concern due to their physicochemical properties (ECHA, 2017a). In addition, these hydrocarbons are potentially emitted to a high extent as petroleum-derived substances are used in large quantities. In 2013, 971 million tons of petroleum-derived substances were manufactured or imported into the European Union. The highest fraction is applied as fuel (64%, 618 million ton) and approximately 4% (37.5 million ton) for industrial or widespread uses (i.e. professional and consumer applications). The remaining fraction is registered for intermediate uses, meaning

A	Paraffins		Naphthenics				Aromatics				Others
	Par	iPar	mNap	diNap	triNap	polyNap	mAr	diAr	triAr	polyAr	...
...											
C10											
C11											
C12											
C13											
C14											
C15											
C16											
C17											
C18											
C19											
C20											
...											

B	Anthracene		#	Phenanthrene		#
C14			1			1

P						
C15			3			5

C1						
C16			18			30

C2						
C17			61			115

C3						
C18			225			425

C4						

Fig. 1. A) Hydrocarbon block method (HBM), with chemical classes in columns and number of carbon atoms in rows. The red rectangle highlights the hydrocarbon blocks that represent the three-ring polycyclic aromatic hydrocarbons (PAHs) that are investigated within this study (more details on these blocks are shown in Fig. 1B). Par = normal alkanes or paraffins; iPar = branched alkanes or paraffins; mNap = mono-naphthenics; diNap = di-naphthenics; triNap = tri-naphthenics; polyNap = poly-naphthenics; mAr = mono-aromatics; diAr = di-aromatics; triAr = tri-aromatics; polyAr = poly-aromatics. B) Three-ring PAHs C14-C18, with representative structures of the different blocks and the total number of structures belonging to the block (#). P are the parent substances anthracene and/or phenanthrene, C1 three-ring PAHs have one extra carbon atom, C2 contain two extra carbon atoms, C3 contain three extra carbon atoms and C4 contain four extra carbon atoms. The extra carbon atoms can be present in the form of methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl and tert-butyl chains. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

that they are further refined on site into other product types (32%, 315 million ton) (CONCAWE, 2019). These products generally consider more refined UVCBs and are more likely to have widespread applications. Because of the widespread applications and presence of potential PBT/vPvB-constituents, PBT-assessments of petroleum UVCBs are essential.

The Technical Guidance Document of the European Chemicals Agency (ECHA) on the assessment of PBTs/vPvBs provides information on approaches to assess UVCBs (ECHA, 2017a). This includes the 'known-constituents approach', the 'whole-substance approach' and the 'fraction profiling approach', also known as the hydrocarbon block method (HBM). The latter approach is specifically suited for petroleum substances. The HBM resolves complex petroleum substances into pseudo-constituents ('blocks') that are defined by, and assessed based on, representative hydrocarbon structures exhibiting similar physicochemical properties (CONCAWE, 1996; ECHA, 2017a). Generally, the constituents are grouped based on their chemical class and number of carbon atoms (Fig. 1A). The underlying assumption of the HBM is that all constituents within a block have fairly similar physicochemical properties, and to a certain extent also a fairly similar biodegradability (P), bioaccumulation (B) and aquatic toxicity (T) potential. Accordingly, a block of constituents could be assessed as if it were a single constituent and the PBT properties of the block/representative constituent could be compared to the PBT criteria, similar to mono-constituent substances (ECHA, 2017a). So far, however, there is a lack of experience with the application of the HBM for PBT-assessment purposes of petroleum UVCBs.

This study aims to investigate the suitability of the HBM for the PBT-assessment of petroleum UVCBs by evaluating the group of three-ring polycyclic aromatic hydrocarbons (PAHs). We specifically selected this group of hydrocarbons as there is a relatively large volume of data available in comparison to other hydrocarbon categories. The group of three-ring PAHs includes the parent three-ring PAHs (i.e. non-alkylated anthracene and phenanthrene) and all alkylated derivatives up to four extra carbon atoms (i.e. C14-C18/P-C4; Fig. 1B).

2. Methods

Within this study, a PBT-assessment on the group of three-ring PAHs was conducted following the ECHA Technical Guidance Document (ECHA, 2017a). The PBT-properties of the parent (i.e. non-alkylated) three-ring PAHs have been investigated for decades, and anthracene and phenanthrene are currently identified within Europe as PBT and vPvB, respectively (ECHA, 2020b). Although the PBT/vPvB-status of phenanthrene is under discussion (Hughes et al., 2020; InfoCuria, 2019), the current PBT/vPvB-status of anthracene and phenanthrene were considered as starting point for our assessment. The current PBT-assessment mainly focusses on the PBT-properties of the alkylated three-ring PAHs, which might be equally or more abundant in crude oils than parent three-ring PAHs (Pampanin and Sydnes, 2013). We specifically focus on alkylated three-ring PAHs with up to four extra carbon atoms (in the form of methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl and tert-butyl chains), as those are most frequently encountered (i.e. C14-C18/P-C4) (Stout et al., 2015). In total, this category includes 884 unique constituents (i.e. all hypothetical constituents with up to four extra carbon atoms with the above-mentioned alkyl substituents) which are grouped based on the number of carbon atoms (Fig. 1B). Within this grouping (and throughout the text), P are defined as the parent substances anthracene and/or phenanthrene and contain 14 carbon atoms. The alkylated three-ring PAHs are defined as C1, C2, C3 and C4. The C1 three-ring PAHs have one extra carbon atom compared to the parent three-ring PAHs (with 15

carbon atoms in total), C2 contain two extra carbon atoms (16 carbon atoms in total), C3 contain three extra carbon atoms (17 carbon atoms in total) and C4 contain four extra carbon atoms (18 carbon atoms in total).

Within this PBT-assessment, we analyze the P-, B- and T-properties separately, using experimental data and model predictions. Model predictions were conducted to substantiate experimental data and/or to fill experimental data gaps. Experimental data were gathered via internet searches and references in relevant literature, and were evaluated for quality and relevance (see below). Searches combined substance related keywords (e.g. alkylated three-ring PAHs, alkylated anthracene, alkylated phenanthrene, substituted anthracene, substituted phenanthrene, etc.) with test related keywords (e.g. degradation, bioaccumulation, bioconcentration, toxicity, etc.), and optionally the compartment of interest (e.g. water, aquatic, fish, invertebrates, etc.).

2.1. Persistence

Experimental laboratory biodegradation data of alkylated three-ring PAHs in water, sediment and/or soil compartments were collected. The studies were evaluated based on their relevance for P-assessment purposes according to the ECHA Technical Guidance Document (ECHA, 2017a) and aspects from related OECD Test Guidelines (e.g. OECD TG 307, 308 and 309). Environmental relevance of the reported biodegradation half-life values has been assessed based on the aspects as provided in Table 1. All criteria apply to aquatic biodegradation studies and where relevant also to sediment and soil biodegradation studies. When degradation data were reported in terms of depletion over time (i.e. in percentage), or in case of data fits that do not directly provide a well-defined half-life, we calculated half-lives using GraphPad Prism (v8), where possible, using first-order degradation kinetics. In addition, biodegradation half-lives were normalized to 12 °C by using the Arrhenius equation (ECHA, 2017b), which generally showed to be applicable to convert the environmental half-lives of hydrocarbons (Sj holm et al., 2020). Based on this analysis, studies were classified as either 'relevant' to determine environmental degradation half-life values', 'not-relevant' to determine environmental degradation half-life values' or 'not assignable' in case of missing data/details. Furthermore, photodegradation data on alkylated three-ring PAHs were collected and analyzed. However, as the contribution of photodegradation to the overall degradation can be considered negligible in several environments (ECHA, 2017a, 2017b), the process of photodegradation is not considered in the persistence conclusion (ECHA, 2017a).

In addition to experimental data, we predicted the degradation potential for all 884 constituents of the category by using two models, Biowin3 and BioHCwin (US EPA, 2012). Biowin3 aims to predict the required time for complete ultimate biodegradation in a typical aquatic environment. The results are given as a quantitative value, ranging from 1 to 5, and P-screening criteria have specifically been adopted for this model (i.e. <2.25–2.75 (ECHA, 2017a)). These semi-quantitative ratings have been transformed to half-life values using Equation (1), which is modified from Rorije et al. (2011). BioHCwin, on the other hand, is specifically designed for the prediction of primary biodegradation of hydrocarbons. Although this model does not provide a half-life for a specific compartment, the outcomes are generally interpreted as half-life values in freshwater compartments (CONCAWE, 2016).

$$\text{Water HL} = 5378 \cdot e^{-1.95 \cdot \text{Biowin3}} \quad (1)$$

Table 1

Relevance assessment of experimental biodegradation data for PBT-assessment purposes. Definitions: ↑ = The application/presence of this aspect will result in an increase in the observed degradation. ↓ = The application/presence of this aspect will result in a decrease in the observed degradation. – = No effect on the observed degradation, or no conclusion is drawn based on the application/presence of this aspect. X = This aspect is not considered relevant for determining environmental degradation half-lives (i.e. not representative of environmental conditions).

Aspect	Effect on degradation	Reasoning	Conclusion on study relevance
1 Application of high non-dissolved concentrations	↓	Non-dissolved constituents are not available for degradation.	X
2 Addition of mineral media	↑	Addition of mineral media increases observed degradation due to an increase in microbial growth. Simulation tests are performed in field sampled water, sediment or soil.	X
3 Application of crude oil or a complex mixture of hydrocarbons	↑↓	Application of crude oil influences the solubility of varying constituents, and can influence the composition of the microbial community resulting in an increase or decrease of degradation.	X
4 Addition of a dispersant	↑	Addition of a dispersant increases observed degradation due to an increase in bioavailability, and co-metabolism might occur concurrent with degradation of the dispersant.	X
5 Pre-adapted or irrelevant inoculum	↑↓	A pre-adapted inoculum increases observed degradation. An irrelevant inoculum can result in higher or lower observed degradation (e.g. artificial seawater).	X
6 Observed evaporation	↑	Inclusion of evaporation in degradation calculations will underestimate the degradation half-life.	X
7 Assumption of lag-phase	↑	Lag-phases are a sign of adaptation. If a lag-phase is assumed but does actually not occur, half-lives are underestimated. In case of no adaptation, lag-phase should be part of the half-life. If significant adaptation occurs see aspect 5.	X –
8 Normalization to an internal marker with varying physicochemical properties	↑↓	Normalization to an internal marker may increase or decrease observed biodegradation due to differences in physicochemical properties (e.g. sorption/evaporation).	–
9 Absence of abiotic controls	↑–	Unknown whether there might be partial dissipation.	–

2.2. Bioaccumulation

The evaluation of experimental bioconcentration data on alkylated three-ring PAHs was limited to laboratory tests, focusing on fish or invertebrate species. Both steady state and/or kinetically determined bioconcentration factors (BCF) were extracted or, where possible, derived from raw data using GraphPad Prism. Besides aquatic exposure experiments, also dietary exposure studies were analyzed. Results of dietary exposure studies were transformed to BCFs using the OECD BCF Estimation Tool (OECD, 2019), as within REACH the B-criteria is based on BCF values for aqueous species. From the BCF Estimation Tool we reported the range of estimated BCF values and specifically highlight the prediction of the model by Sijm et al. (1995). The relevance of the data was scored based on criteria suggested and applied in previous studies (Arnot and Gobas, 2006; Parkerton et al., 2008; Wassenaar et al., 2020) and are related to OECD TG 305 and the ECHA Technical Guidance Document (ECHA, 2017a; OECD, 2012). The criteria were applied to both aqueous and dietary exposure experiments, where relevant. The following substance based criteria were used: 1) the test exposure concentration should be measured and should not be nominal; and 2) water exposure concentrations should be below three times the estimated water solubility (as predicted according to Verbruggen et al. (2008)). With respect to the BCF values, the following criteria were applied: 3) reported BCF values should be substance specific (e.g. not on total radiolabeled content); 4) when BCF steady-state values are reported, exposure duration should have been sufficient to reach steady-state conditions; 5) the BCF should be based on whole body content; and 6) lipid content of the exposed species should be reported. In addition, several experimental test conditions should be met: total organic carbon content must be lower than 2 mg/L, pH should be between 6.0 and 8.5 at the start of the experiment, temperature should be within the recommended ranges (e.g. as reported in the OECD TG 305 (OECD, 2012)), and no toxicity should be observed during the accumulation test. Furthermore, experiments in which organisms were simultaneously exposed to chemicals that stimulate the mixed-function oxygenase (MFO) enzyme system, like four- and five-ring PAHs, were considered not relevant. Earlier research indicates that such exposures result in lower BCFs for three-ring PAHs compared to

single substance exposures due to enhanced metabolic activation (Carlson et al., 1979; Wassenaar et al., 2020). Based on this analysis, studies were classified as either ‘relevant’ to determine the bioconcentration potential’, ‘not-relevant’ to determine the bioconcentration potential’ or ‘not assignable’ in case of missing data/details.

In addition to the experimental data, we estimated the bioconcentration potential of this group with the EU-BCFmax and BCFBAF EpiSuite models (Rorije et al., 2011; US EPA, 2012). The EU-BCFmax model only considers passive uptake and excretion of a chemical, and can be calculated from the octanol-water partition coefficient (K_{ow}). Using log K_{ow} predictions of EpiSuite (US EPA, 2012), the EU BCFmax was calculated with Equation (2), as derived from Rorije et al. (2011). The BCFBAF EpiSuite software consists of two BCF models, the BCFBAF Arnot-Gobas model and BCFBAF regression (US EPA, 2012). As opposed to the EU BCFmax model, the BCFBAF Arnot-Gobas model considers multiple factors, including uptake and elimination through gills, fecal egestion, growth and biotransformation, and other factors like bioavailability and gills absorption efficiencies. As input, the model requires log K_{ow} and first-order metabolism rate constant (k_M) predictions from EpiSuite (US EPA, 2012). We predicted the bioconcentration for the upper trophic level, which were normalized to a 5% lipid content. The BCFBAF regression model, on the other hand, is developed based on a regression through a dataset of experimental BCF values for non-ionic substances versus log K_{ow} values from EpiSuite (US EPA, 2012).

$$\text{Log BCFmax} = \frac{34.43}{2.93 \cdot (2\pi)^{0.5}} \cdot e^{-0.5 \cdot \left(\frac{\text{Log } K_{ow} - 6.52}{2.93} \right)^2} \quad (2)$$

2.3. Toxicity

The toxicity assessment solely focused on aquatic toxicity and did not consider effects on human health. Aquatic toxicity data on alkylated three-ring PAHs were gathered and evaluated, focusing on acute, sub-chronic and chronic exposures to organisms from

varying trophic levels. In case of sediment exposure experiments, data were converted to pore water concentrations using equilibrium partitioning in order to provide indicative water toxicity data. To apply the equilibrium partitioning method, we derived organic carbon-water partition coefficients (K_{oc}) according to Verbruggen et al. (2008), and used the fraction of organic carbon as reported in the respective toxicity studies. The reliability of identified studies was scored according to the technical guidance for deriving environmental quality standards (European Commission, 2018). The following criteria were used: 1) exposure concentrations should be measured or stable concentrations could be guaranteed (e.g. Teflon-lined capping and renewal or flow-through exposure protocol); 2) effect concentrations should be below three times the estimated water solubility (as predicted according to Verbruggen et al. (2008)); 3) effects should be exposure concentration related; and 4) effects/mortality under control conditions should not exceed recommended values from relevant guidelines. Based on this analysis, studies were classified as either 'reliable', 'not-reliable' or 'not assignable' in case of missing data/details. For studies that were considered as reliable, NOEC or EC10 values were extracted or derived with GraphPad Prism from original data, where possible. In addition, we made a distinction between studies with and without UV-exposure, as PAHs may exert a phototoxic effect (Roberts et al., 2017). UV-exposure regimes were considered relevant for exposure at the earth's surface and shallow surface water, when the applied intensities were below full-strength sunlight as described in literature (Allred and Giesy, 1985; Boese et al., 1997; McCloskey and Oris, 1991; Oris et al., 1984; Peachey and Crosby, 1996).

In addition, we predicted the ecotoxicity of the group of three-ring PAHs by using the Target Lipid Model (TLM) (McGrath et al., 2018) and an Internal Lipid Residues Model (ILRM) (Verbruggen et al., 2008; Verbruggen, 2012) (see Supplemental Material S1). Both models have been developed to estimate the baseline toxicity caused by hydrocarbons/PAHs, and are based on species sensitivity distributions (SSDs) of internal membrane/target lipid concentrations against fraction of affected species. The TLM is based on acute and sub-chronic toxicity data for 54 species, mainly based on hydrocarbon toxicity (but also includes effects of several other chemicals) and results are extrapolated to chronic effects by using an acute-to-chronic (ACR) ratio of 5.22. The ILRM is based on a SSD of chronic PAH toxicity and total petroleum hydrocarbon (TPH) toxicity values for 58 different aquatic, benthic and terrestrial species. In order to estimate the fraction of species that is affected at a specific exposure concentration, only the log K_{ow} is needed. The TLM is based on log K_{ow} estimates from EpiSuite (US EPA, 2012), and the ILRM is based on log K_{ow} estimates from ClogP Biolum (BioByte, 2006), which are subsequently used to predict membrane water partition coefficients (K_{mw}) (Verbruggen et al., 2008). For all 884 three-ring PAH constituents, we predicted the fraction of affected species at an exposure concentration of 10 $\mu\text{g/L}$ (i.e. T-criteria (ECHA, 2017a)) or at maximum water solubility. For both models, water solubility was estimated based on log K_{ow} from ClogP Biolum estimates according to Verbruggen et al. (2008), which are slightly more conservative for the alkylated three-ring PAHs than the WSKOW EpiSuite estimates, which are normally applied in the TLM. For the TLM, this only results in minor differences in the predicted fraction of affected species for C4 three-ring PAHs (<3% difference). In addition, we applied the median affected fraction per constituent, where the TLM generally uses the lower confidence limit of the affected fraction to calculate the impact (McGrath et al., 2018).

3. Results & discussion

3.1. Persistence

3.1.1. Experimental data

In total, 18 studies were identified that investigated degradation of alkylated three-ring PAHs, of which 16 studies analyzed biodegradation in water and one study in either sediment and soil compartments. In addition, three studies were identified that analyzed photodegradation of alkylated three-ring PAHs. The experimental data generally report individual half-life values or percentages of degradation for the parent three-ring PAHs (i.e. anthracene and/or phenanthrene) and averages for the varying alkylated three-ring PAHs (i.e. C1, C2, C3 and C4).

For all records collected from literature, the identified experimental degradation data were considered not relevant to determine environmentally realistic degradation half-lives, due to the inclusion of one or multiple aspects as described in Table 1 (see Table S1–3 for details). Nevertheless, the information of relative degradability of varying constituents within each study could be relevant for a qualitative assessment of the biodegradation kinetics.

The relative persistence of parent and alkylated three-ring PAHs in the aquatic compartment (for which most data are available) is shown in Fig. 2. Only records with half-life values (Table S1) are included in this figure. In general, persistence seems to increase with the level of alkylation, with half-lives for parents < C1 < C2 < C3 < C4 three-ring PAHs. The observed trend is consistent across the studies and gives an indication of the expected relative behavior of alkylated and parent three-ring PAHs, even though the calculated degradation half-lives may not be relevant at environmentally realistic conditions. In addition, within the studies that report biodegradation in terms of depletion over time (in %) rather than half-lives, the same trend is observed (i.e. parents < C1 < C2 < C3 < C4 three-ring PAHs) (see Table S1). Slight variances between studies and overlap in persistence between adjacent blocks could potentially be explained by the presence of varying types of constituents in the different test materials. It has for instance been observed that varying methylated three-ring PAHs can have slightly different biodegradation kinetics (isomer variation) (Bayona et al., 1986; Huang et al., 2004). The exact composition of the tested material may thus slightly influence the observed degradation.

A similar trend as depicted in Fig. 2 is observed when restricting to those studies where the influence of physicochemical properties on the observed relative biodegradation trend is minimal (Fig. S1). Within this subset, studies were included that corrected or controlled for evaporation and photodegradation (i.e. closed (dark) test systems or corrected with abiotic controls). In addition, within these studies all constituents could be considered dissolved to a relative similar extent (i.e. single substance exposures or addition of a dispersant).

Furthermore, the sediment and soil biodegradation studies show a similar trend as observed in the water degradation studies (i.e. parents < C1 < C2 < C3 < C4 three-ring PAHs; Table S2–3). On the contrary, an opposite trend is observed for photodegradation, indicating faster photodegradation with an increase in alkylation for three-ring PAHs (Table S4). The trends as observed in the biodegradation studies are not considered to be influenced by photodegradation processes, as many studies corrected half-lives for abiotic degradation or were conducted in the dark (Table S1 and Fig. S1).

3.1.2. QSAR data

Besides experimental data, environmental biodegradation was predicted with Biowin3 and BioHCwin for all constituents

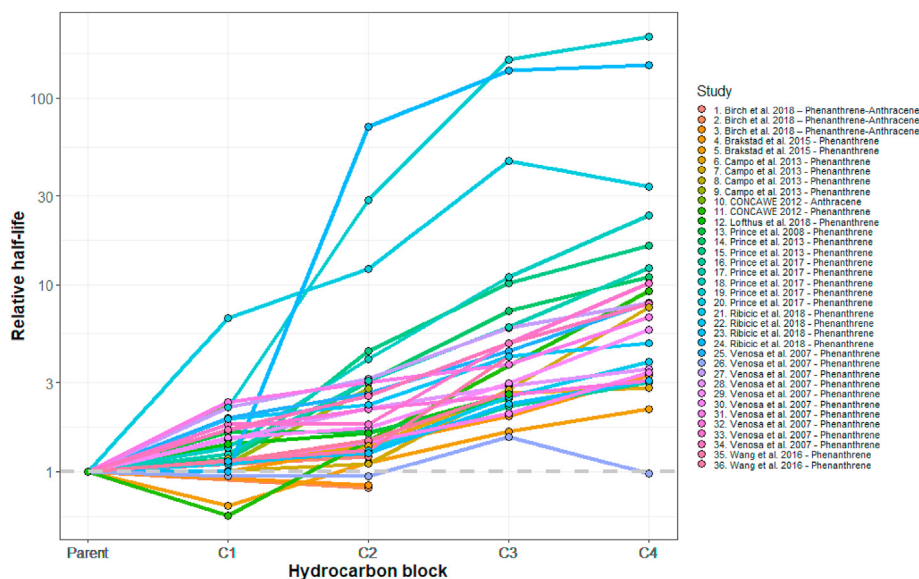


Fig. 2. Trends in relative water biodegradation half-lives across parent and alkylated three-ring PAHs (see Table S1 and S5 for more details on test designs). Half-life values were normalized to the observed half-life for the parent substances and 'larger than values' (i.e. '>') were not included (see Table S5). Parent represents anthracene and/or phenanthrene, C1 are alkylated three-ring PAHs with one extra carbon atom, C2 contain two extra carbon atoms, C3 contain three extra carbon atoms and C4 contain four extra carbon atoms.

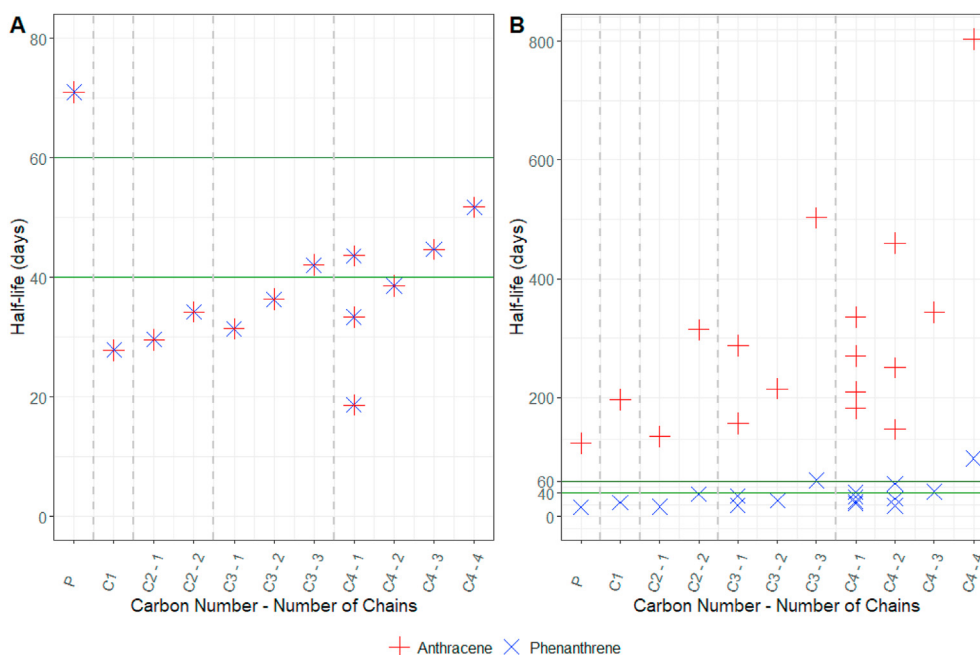


Fig. 3. Predictions of the biodegradation half-lives for the group of three-ring PAHs. A) Predictions by Biowin3. B) Predictions by BioHCwin. P are the parent substances anthracene and/or phenanthrene, C1 are alkylated three-ring PAHs with one extra carbon atom, C2 contain two extra carbon atoms, C3 contain three extra carbon atoms and C4 contain four extra carbon atoms. In addition, the constituents within these blocks are further categorized based on the number of alkyl chains.

belonging to the group of three-ring PAHs.

According to Biowin3 predictions, the parent three-ring PAHs are much more persistent than the alkylated three-ring PAHs (Fig. 3A). However, when alkylated, a slight increase in persistence is observed with an increase in carbon number and/or chains. A higher half-life is particularly predicted for constituents with an increased number of alkyl-chains. Furthermore, it can be observed that there is a substantial spread in the half-life values across the total group of three-ring PAHs, of which several carbon number blocks contain non-persistent and persistent constituents.

In contrast to Biowin3, the BioHCwin model predicts different half-lives between anthracene and phenanthrene and their derivatives (Fig. 3B). In addition, this model predicts that the alkylated three-ring PAHs are more persistent than the parent three-ring PAHs. Similar to Biowin3, the BioHCwin model also predicts a higher half-life with an increase in the number of chains for the alkylated three-ring PAHs. Overall, BioHCwin predicts a wide variation in half-life values within carbon number groups, in which all groups contain non-persistent and very persistent constituents.

3.1.3. Persistence discussion

Based on available experimental data, a clear pattern in biodegradation was observed for parent and alkylated three-ring PAHs in water, sediment, and soil compartments, in which parent three-ring PAHs seem to be less persistent than alkylated forms (parent < C1 < C2 < C3 < C4). Less weight is given to the results of the QSAR analyses, as two fundamental contradictions between the models were observed that also deviate from the experimental data. Therefore, the QSAR predictions should be interpreted with caution (see discussion below).

The pattern as observed in the experimental data might be caused by interference of the alkyl substituents with oxidation enzymes via steric hindrance (Prince and Walters, 2006). Similar conclusions are obtained in other studies. For instance, Leblond et al. (2001) concluded that an increase in the number of methyl substituents and an increase in the size of a substituent, result in a decreased primary biodegradation rate based on data on naphthalene (including varying methyl, dimethyl and ethyl derivatives). In addition, the same pattern was observed for phenanthrene in field studies by Douglas et al. (1996) and Prince et al. (2003). Furthermore, the observed pattern is in accordance with the expected overall pattern of biodegradation of crude oil components as described by Prince and Walters (2006).

In addition, there might be other factors that also contribute to the biodegradability of parent and alkylated three-ring PAHs that were not observed in the current evaluation. For instance, Wammer and Peters (2005) concluded that naphthalene substances with a substituent in an α -position have lower biodegradation rates compared to naphthalenes without substituents or with a substituent in the β -position (see Fig. 4A). Within the study of Wammer and Peters (2005) no strong correlation was observed with the presence and length of alkyl substituents for naphthalenes.

Furthermore, the biodegradation might also be influenced by the presence/absence of specific sites or regions, like bay-regions and k-regions (see Fig. 4B). It has been suggested that these regions can be used by (bacterial) enzymes to break down PAHs and thus potentially influence the biodegradation rate. As can be observed from Fig. 4B, such regions can be formed by the PAH backbone (also called angular PAHs) or by alkyl substituents on specific locations (Abdel-Shafy and Mansour, 2016; Tuarze, 2015). Currently, these regions have not (extensively) been linked to biodegradation differences and are specifically related to

carcinogenic effects of PAHs due to reactivity of the oxidized metabolites (i.e. epoxides) formed in a first step. Specifically, the high molecular weight PAHs with four- and five-rings can be transformed to 'bay-region' diol epoxides, which are highly reactive (Ghosal et al., 2016).

Within the QSAR analyses, two fundamental differences between the models were observed that also deviate from the experimental data. First, the models differently weigh the contribution of alkyl substituents to persistence. In comparison to parent three-ring PAHs, alkylation increases persistence according to BioHCwin (which is in line with experimental data) and decreases persistence according to Biowin3. These differences are not related to differences in the predicted endpoint (i.e. Biowin3 predicts ultimate biodegradation and BioHCwin predicts primary biodegradation), as Biowin4 (which predicts primary degradation) predicts a similar trend to Biowin3 (data not shown). Secondly, both models differently weigh the contribution of the parent backbone to persistence. Biowin3 makes no distinction between the parent backbones, whereas BioHCwin predicts that anthracene is very persistent and phenanthrene is not persistent. This difference is not specifically apparent from experimental data. Based on this evaluation it can be concluded that predictions of the two QSAR models (Biowin3 and BioHCwin) for the group of three-ring PAHs (and probably other alkylated PAHs) do not specifically match the experimental data, and therefore should be interpreted with caution. Apparently, the models are based on too few experimental data points to generate reliable predictions for the group of three-ring PAHs.

Besides the identified trends (as described above), there is also monitoring data available that indicates the presence of alkylated three-ring PAHs in the environment, both in populated and more remote areas. For instance, alkylated three-ring PAHs have been detected in water (González-Gaya et al., 2019; Khairy et al., 2014; Takeuchi et al., 2009), sediment (Foster et al., 2015; Harvey et al., 2014) and soil compartments (Kim et al., 2017; Vane et al., 2014), in which the measured concentrations of alkylated three-ring PAHs are generally comparable to the concentrations of parent three-ring PAHs. Although the sources of PAHs may vary (e.g. petrogenic and/or pyrogenic), these monitoring studies indicate that alkylated three-ring PAHs are present across the world. It should be noted, in this respect, that monitoring information on alkylated three-ring PAHs is not as extensive as monitoring data on parent three-ring PAHs, as most studies only focus on parent PAHs. To monitor the wide variety of alkylated PAHs, generally more specialized analytical techniques are required.

3.1.4. Persistence conclusion

In conclusion, no environmentally relevant half-lives were identified for the alkylated three-ring PAHs following the included validity criteria. However, considering the weight of evidence, independent of the test-setup and shortcomings, a clear trend is observed in experimental data across parent and alkylated three-ring PAHs, indicating an increase in persistence with the level of alkylation (i.e. parent < C1 < C2 < C3 < C4). This trend was consistent in the water, sediment and soil compartments. Therefore, it can be concluded that C1 to C4 anthracene and phenanthrene are more persistent than the parent three-ring PAHs.

3.2. Bioaccumulation

3.2.1. Experimental data

In total, seven fish and three invertebrate bioconcentration studies were identified that report BCFs for alkylated three-ring PAHs (Table S6-7). These studies mainly report BCFs for C1 and

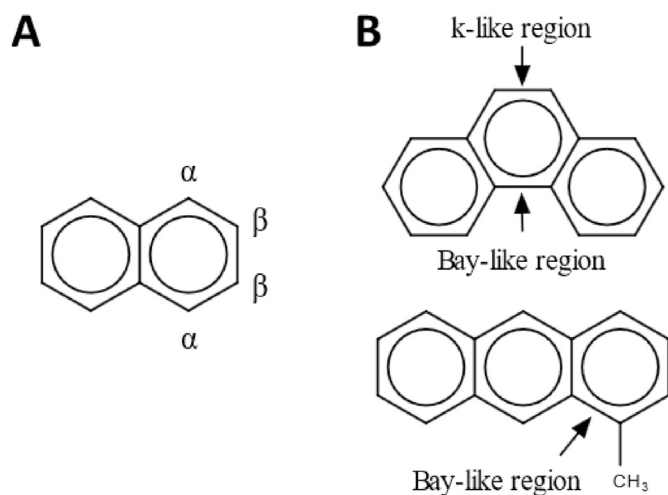


Fig. 4. A) Characterization of substituent positions on the rings of naphthalene according to Wammer and Peters (2005). B) Bay-like regions and k-like regions in three-ring PAHs.

C2 alkylated three-ring PAHs, and only one study reported BCFs for C3 and C4 constituents. Furthermore, within the reported bioconcentration tests, data is generally only available for a few constituents specifically. This is in contrast to persistence tests, where often groups of constituents are analyzed and followed over time (i.e. parents, C1, C2, C3 and/or C4).

Of all available bioconcentration data on alkylated three-ring PAHs, only one relevant BCF for one specific constituent is identified (i.e. BCF of 3896 for 9-methylanthracene, C1 three-ring PAH (Southworth et al., 1978)). All other data were considered not-relevant to determine the bioconcentration potential, mainly due to simultaneous exposure to four and five-ring PAHs (see section 2.2). We therefore restricted our analyses to bioconcentration trends across parents and alkylated three-ring PAHs that were tested within the same study.

The trends that can be observed in the individual fish bioconcentration studies, testing a parent and alkylated three-ring PAH in a mixture test design, are shown in Fig. 5A. These data show a quite consistent BCF trend for phenanthrene, in which the BCF tends to decrease with an increase in alkylation. For anthracene on the other hand, a slight increase in BCF is observed with an increase in alkylation. However, as only one study analyzed the bioconcentration trend for anthracenes, less weight can be given to this observation, especially because for phenanthrene nearly the same trend was observed in this study.

The bioconcentration trends as observed within the three invertebrate studies are visualized in Fig. 5B. These studies show a consistent pattern that deviates from the trend as observed in the fish bioconcentration studies with phenanthrene. Available invertebrate data indicate an increase in BCF with increases in alkylation/hydrophobicity, both for anthracene and phenanthrene.

3.2.2. QSAR data

Besides experimental data, the bioaccumulation potential of the group of alkylated three-ring PAHs was predicted with BCF estimates of the EU-BCFmax and BCFBAF models (i.e. BCFBAF regression and BCFBAF Arnot-Gobas model).

The EU-BCFmax and the BCFBAF regression model predict a similar bioconcentration pattern across parent and alkylated three-ring PAHs (Fig. 6A–B). Both models predict an increase in bioconcentration potential until a certain optimum (mainly related to $\log K_{ow}$), after which a decline in BCF values is predicted. However, the absolute predicted BCF values differ between both models. For instance, the EU-BCFmax model predicts that the parent three-ring PAHs already meet the B-criteria, whereas the BCFBAF regression model suggests that the B-criteria is exceeded at a higher carbon number. In addition, the BCFBAF regression model predicts a different bioconcentration potential for anthracene and phenanthrene derivatives. The predictions by the BCFBAF Arnot-Gobas model, on the other hand, indicate a different pattern in which no optimum can be observed (Fig. 6C). This model suggests that none of the hydrocarbon blocks meet the B-criteria.

3.2.3. Bioaccumulation discussion

Based on available fish bioconcentration data, a trend for phenanthrene was observed that indicates lower BCF values with increased alkylation. The steepness of the decrease, however, cannot be assessed based on the limited amount of data. According to these results, the alkylated three-ring PAHs are potentially more readily metabolized than parent three-ring PAHs. Remarkably, however, the observed bioconcentration trend for alkylated phenanthrenes in fish deviates from the observed biodegradation trends, despite commonality of reactions in microbial and fish transformations (Dimitrov et al., 2019). Potentially, this is related to

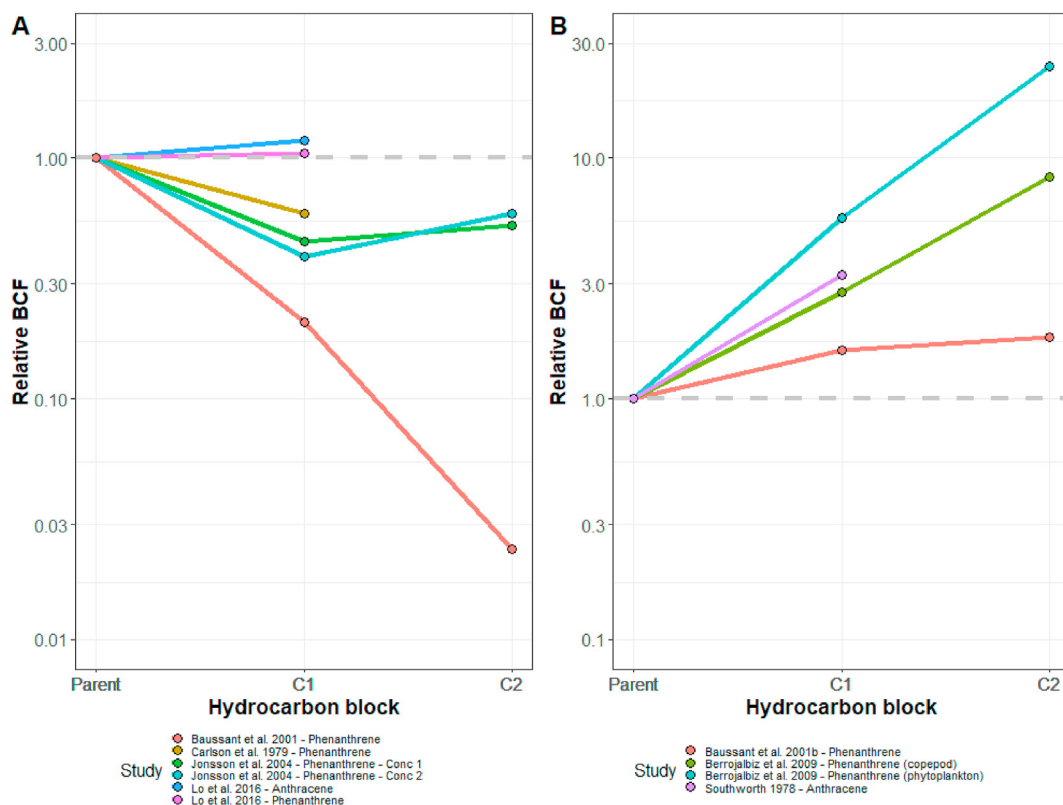


Fig. 5. Trends in relative bioconcentration factors (BCF) across parents and alkylated three-ring PAHs. Where possible, BCFs are based on kinetic data (see Table S6-7). A) Fish data. B) Invertebrate data. Parent represents anthracene and/or phenanthrene, C1 are alkylated three-ring PAHs with one extra carbon atom and C2 contain two extra carbon atoms.

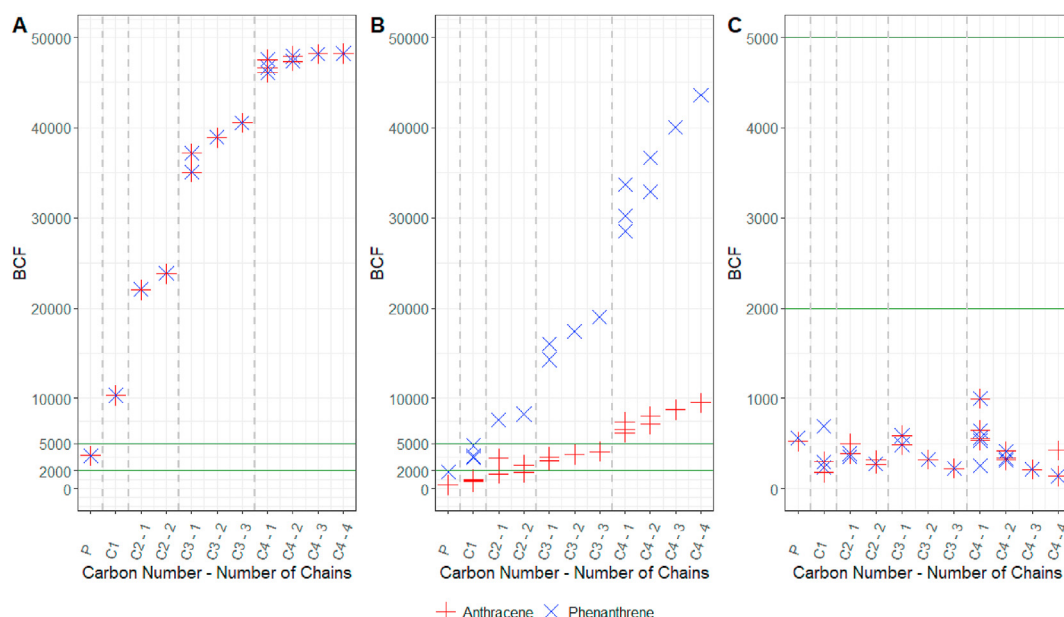


Fig. 6. Predictions of bioconcentration factors (BCF) for the group of three-ring PAHs. A) Predictions by the EU BCFmax model. B) Predictions by the BCFBAF regression model. C) Predictions by the BCFBAF Arnot-Gobas model, upper trophic level, normalized to 5% lipid content. P are the parent substances anthracene and/or phenanthrene, C1 are alkylated three-ring PAHs with one extra carbon atom, C2 contain two extra carbon atoms, C3 contain three extra carbon atoms and C4 contain four extra carbon atoms. In addition, the constituents within these blocks are further categorized based on the number of alkyl chains.

(functional) differences between microbial and fish enzymes, but could also be related to other fate processes that take place in the degradation experiments (e.g. sorption). Moreover, a decrease in bioavailability in the fish bioconcentration experiments seems not a plausible explanation for the observed difference, as an opposite bioconcentration trend is observed for invertebrates.

For anthracene on the other hand, an opposite trend was observed in fish bioconcentration data that indicates a slight increase in BCF with an increase in alkylation. However, as only one study analyzed the trend for anthracene, less weight can be given to this observation, especially because for phenanthrene nearly the same trend was observed in this study. Potentially, differences in fish bioconcentration potential between phenanthrene and anthracene derivatives could be related to factors as discussed in Fig. 4, including the position of the alkyl substituent(s) and the presence of bay- and k-regions.

In general, the fish bioconcentration trends correspond to the trends as predicted by the BCFBAF Arnot-Gobas model. However, the reliability of the absolute values as estimated by this model could be questioned, as for instance the parents are not predicted to be bioaccumulative (see discussion below).

The invertebrate bioconcentration data indicate a clear trend across all available studies. These studies show increases in BCF values with increases in alkylation. The difference between fish and invertebrate bioconcentration trends could be explained by a general lower metabolic capacity in invertebrates (ECHA, 2017a). Such differences have been observed for PAHs (Bleeker and Verbruggen, 2009), and also for several other chemicals, like polychlorobiphenyls (Koenig et al., 2012). In addition, the trend as observed in experimental invertebrate bioconcentration data corresponds to the predictions of the EU-BCFmax model, which also does not take metabolism into account (i.e. increases in bioconcentration with increases in log K_{ow}).

The predictions by the EU-BCFmax model (and BCFBAF regression model) are very different from the predictions by the BCFBAF Arnot-Gobas model. One of the main factors contributing to the observed difference is the metabolism correction. When applying

the BCFBAF metabolism correction to the EU BCFmax model, similar results are obtained as in the BCFBAF Arnot-Gobas model, and vice versa (Fig. S2). Although the application of metabolism correction could be considered relevant, particularly for fish, the reliability of the first-order metabolism rate constant (k_M) predictions for three-ring PAHs by the BCFBAF model could be questioned. The data that are used to develop the k_M BCFBAF model were not primarily set up to determine k_M values and are derived from studies with varying study set-ups and species. Metabolism rate constants are derived from these studies based on BCF and/or total elimination rate constants following several assumptions. The related three-ring PAH data that were used in the development of the k_M BCFBAF model consider average k_M values that are mainly derived from studies in which fish were exposed to multiple substances simultaneously (including four- and five-ring PAHs). These mixtures significantly influence the metabolism within the fish, resulting in faster transformation of three-ring PAHs compared to single substance exposures (Carlson et al., 1979; Wassenaar et al., 2020). When only k_M data were used from studies that applied single substance exposures, the derived half-lives are a factor 3 to 6 higher than current applied values (Table S8). Consequently, the k_M BCFBAF corrections may overpredict the metabolism, resulting in lower predicted BCF values.

In addition to the bioconcentration trends as observed in the laboratory experiments and the predictive models, several studies have measured alkylated three-ring PAHs in organisms/food chains in the field, including in invertebrates, fish and seabirds (Khairy et al., 2014; Nfon et al., 2008; Takeuchi et al., 2009; Waszak et al., 2020). These field studies, however, do not provide a clear bioaccumulation/biomagnification pattern across parent and alkylated three-ring PAHs, as no (consistent) trends can be identified. For instance, the data of Takeuchi et al. (2009) indicates an increase in bioaccumulation with an increase in alkylation, whereas no clear trend can be observed from the data by Khairy et al. (2014) and Nfon et al. (2008) (see Table S9). In addition, the field data cannot directly be compared to the B-criteria, as the criteria is based on bioconcentration potential (ECHA, 2017a). Nevertheless, this data

indicates that alkylated three-ring PAHs are identified in organisms, and can be found at comparable concentrations as parent three-ring PAHs.

3.2.4. Bioaccumulation conclusion

In conclusion, alkylated three-ring PAHs can be considered more bioaccumulative than the parent three-ring PAHs in invertebrates, which seem to follow the trend of increased bioconcentration with increases in log K_{ow} . Therefore, the observed trend for parents, C1 and C2 three-ring PAHs could be extrapolated to C3 and C4 three-ring PAHs based on QSAR predictions. In contrast to invertebrates, the BCF tends to decrease in fish with an increase in alkylation for phenanthrenes. For alkylated anthracenes no conclusions can be drawn for bioconcentration in fish, mainly due to a lack of data.

3.3. Toxicity

3.3.1. Experimental data

In total, 20 toxicity studies with aquatic organisms were identified that specifically report the toxicity of alkylated three-ring PAHs (Table S10). Investigated endpoints include mortality, growth, morphology (including symptoms related to blue sac disease in fish) and hatching. Of the identified studies, ten were considered reliable. These studies are reported in Tables 2 and 3 for alkylated phenanthrenes and anthracenes, respectively. Within these tables, only studies on parent three-ring PAHs toxicity are included when they also analyzed toxicity of alkylated three-ring PAHs. For the parent three-ring PAHs there is much more toxicity data available, for which some critical studies are reported in Table S11.

Based on available data, it can be observed that alkylated anthracenes and alkylated phenanthrenes exert a phototoxic effect at relevant UV-exposure conditions (i.e. applied UV-intensities were

below the intensities of full-strength sunlight). This effect seems to be stronger for alkylated anthracenes compared to alkylated phenanthrenes. Furthermore, it can be observed that some species (like the mysid shrimp, *Americamysis bahia*) seem to be more sensitive to (alkylated) three-ring PAHs compared to others (Tables 2 and 3). Differences in sensitivity between organisms to hydrocarbon and PAH exposure have been shown in SSDs (Supplemental Material S1, McGrath et al. (2018), Verbruggen et al. (2008) and Verbruggen (2012)), and the order of species in these SSDs matches the species sensitivity as observed for three-ring PAHs in this study rather well (Table S12).

In general, only few toxicity data on alkylated three-ring PAHs are available, and only a low number of different constituents have been tested. For the lower carbon number blocks (i.e. parents, C1 and C2), one or more constituents show to be toxic to a sensitive aquatic organism below an exposure concentration of 10 µg/L (i.e. T-criteria (ECHA, 2017a)). For the higher carbon number blocks very few data are available, including only one test with a C3-constituent and four tests with the same C4-constituent (i.e. retene). For all these studies, none of the more sensitive species were used (Table S12) and it is unclear whether UV radiation has been applied within the set-up of these studies.

3.3.2. QSAR data

Ecotoxicity was also predicted with the TLM and ILRM for all constituents that belong to the group of alkylated three-ring PAHs. The predictions of both models are shown in Fig. 7, and express the fraction of species that is potentially affected at an exposure concentration of 10 µg/L or at the maximum water solubility level. These predictions indicate a clear and consistent trend across both models, with higher toxicity being predicted for constituents containing more carbon atoms (i.e. parents < C1 < C2 < C3 < C4 three-ring PAHs). Only slight differences between the TLM and ILRM are

Table 2

Reliable toxicity data on alkylated phenanthrenes. Toxicity data are reported as EC10 or NOEC values for the most sensitive endpoint. When EC10 and/or NOEC values were not reported and could not be derived, reported EC50 values are presented. Bold values are toxicity values below 10 µg/L. More details on the specific tests are included in Table S10 and detailed study references are provided in the supplemental material. 1) The NOEC/EC10 values for phenanthrene and methyl-phenanthrene from Finch 2017 are around the T-threshold upon acute (48h) exposure. For phenanthrene an exposure concentration-response curve fit could be conducted to derive an EC10. 2) Derived with GraphPad Prism from original data. 3) Sediment toxicity test. 4) Only one concentration was tested, at which more than 50% effect was observed.

UV conditions	Carbon number	Substance	Species	Tox (µg/L)	Ref	Remarks
With UV	Parents	Phenanthrene	<i>Americamysis bahia</i>	12.5	EC10	Finch 2017
		Phenanthrene	<i>Rhepoxynius abronius</i>	117.9	EC50	Boese et al. (1998)
	C1	3-methylphenanthrene	<i>Americamysis bahia</i>	8.65	NOEC	Finch 2017
		2-methylphenanthrene	<i>Rhepoxynius abronius</i>	36.5	EC50	Boese et al., (1998)
	C2	3,6-dimethylphenanthrene	<i>Americamysis bahia</i>	3.24	NOEC	Finch 2017
		3,6-dimethylphenanthrene	<i>Rhepoxynius abronius</i>	>60.6	EC50	Boese et al., (1998)
Without UV	Parents	Phenanthrene	<i>Americamysis bahia</i>	<13.3	NOEC	Finch 2017
		Phenanthrene	<i>Oryzias latipes</i>	>200	NOEC	Rhodes 2005
		Phenanthrene	<i>Rhepoxynius abronius</i>	123.5	EC50	Boese et al., (1998)
	C1	3-methylphenanthrene	<i>Americamysis bahia</i>	21.2	EC10	Finch 2017
		2-methylphenanthrene	<i>Rhepoxynius abronius</i>	36.5	EC50	Boese et al., (1998)
	C2	3,6-dimethylphenanthrene	<i>Americamysis bahia</i>	>16.3	NOEC	Finch 2017
		3,6-dimethylphenanthrene	<i>Oryzias latipes</i>	>200	NOEC	Rhodes 2005
		3,6-dimethylphenanthrene	<i>Rhepoxynius abronius</i>	>60.6	EC50	Boese et al., (1998)
	Not-specified	Phenanthrene	<i>Oryzias latipes</i>	42.8	EC10	Turcotte 2011
		Phenanthrene	<i>Oryzias melastigma</i>	84.5	EC10	Mu 2014
		1-methylphenanthrene	<i>Oryzias latipes</i>	1.8	EC10	Fallahtafti 2012
		1-methylphenanthrene	<i>Oryzias latipes</i>	95.3	EC10	Turcotte 2011
		3-methylphenanthrene	<i>Raph. subcapitata</i>	280	EC50	Kang 2016
		1,7-Dimethylphenanthrene	<i>Oryzias latipes</i>	18.7	EC10	Turcotte 2011
Not-specified	C2	2,7-Dimethylphenanthrene	<i>Oryzias latipes</i>	16.1	EC10	Turcotte 2011
		2-Ethylphenanthrene	<i>Oryzias latipes</i>	32	EC10	Turcotte 2011
		3,6-dimethylphenanthrene	<i>Raph. subcapitata</i>	>37	EC50	Kang 2016
	C3	7-Ethyl-1-methylphenanthrene	<i>Oryzias latipes</i>	>36	EC10	Turcotte 2011
	C4	7-Isopropyl-1-methylphenanthrene	<i>Oryzias melastigma</i>	45.2	EC10	Mu 2014
		7-Isopropyl-1-methylphenanthrene	<i>Oncorhynchus mykiss</i>	<9	EC50	Brinkworth 2003
		7-Isopropyl-1-methylphenanthrene	<i>Oryzias latipes</i>	47.5	EC10	Turcotte 2011
		7-Isopropyl-1-methylphenanthrene	<i>Oryzias latipes</i>	50.7	EC50	Lin et al., (2015)

Table 3

Reliable toxicity data on alkylated anthracenes. Toxicity data are reported as EC10 or NOEC values for the most sensitive endpoint. When EC10 and/or NOEC values were not reported and could not be derived, reported EC50 values are presented. Bold values are toxicity values below 10 µg/L. More details on the specific tests are included in Table S10 and detailed study references are provided in the supplemental material. 1) Derived with GraphPad Prism from original data. 2) Sediment toxicity test.

UV conditions	Carbon number	Substance	Species	Tox (µg/L)	Ref	Remarks
With UV	Parents	Anthracene	<i>Americamysis bahia</i>	0.44	EC10	Finch 2017
	C1	2-methylanthracene	<i>Americamysis bahia</i>	0.27	NOEC	Finch 2017
		2-methylanthracene	<i>Rhepoxynius abronius</i>	4.5	EC50	Boese et al., (1998)
		9-methylanthracene	<i>Rhepoxynius abronius</i>	10.9	EC50	Boese et al., (1998)
	C2	2,3-dimethylanthracene	<i>Americamysis bahia</i>	0.055	NOEC	Finch 2017
		9,10-dimethylanthracene	<i>Rhepoxynius abronius</i>	>41.9	EC50	Boese et al., (1998)
	Without UV	Parents	Anthracene	>44.2	NOEC	Finch 2017
		Anthracene	<i>Oryzias latipes</i>	>40	EC10	Turcotte 2008
		2-methylanthracene	<i>Americamysis bahia</i>	>6.37	NOEC	Finch 2017
		1-methylanthracene	<i>Oryzias latipes</i>	25	EC10	Turcotte 2008
	C1	9-methylanthracene	<i>Oryzias latipes</i>	130	EC10	Turcotte 2008
		2-methylanthracene	<i>Rhepoxynius abronius</i>	>36.5	EC50	Boese et al., (1998)
		9-methylanthracene	<i>Rhepoxynius abronius</i>	105.5	EC50	Boese et al., (1998)
		2,3-dimethylanthracene	<i>Americamysis bahia</i>	>0.37	NOEC	Finch 2017
	C2	9,10-dimethylanthracene	<i>Oryzias latipes</i>	<12	EC10	Turcotte 2008
		9,10-dimethylanthracene	<i>Rhepoxynius abronius</i>	>41.9	EC50	Boese et al., (1998)
	Not specified	C1	9-methylanthracene	320	EC50	Kang 2016
		C2	9,10-dimethylanthracene	>7.9	EC50	Kang 2016

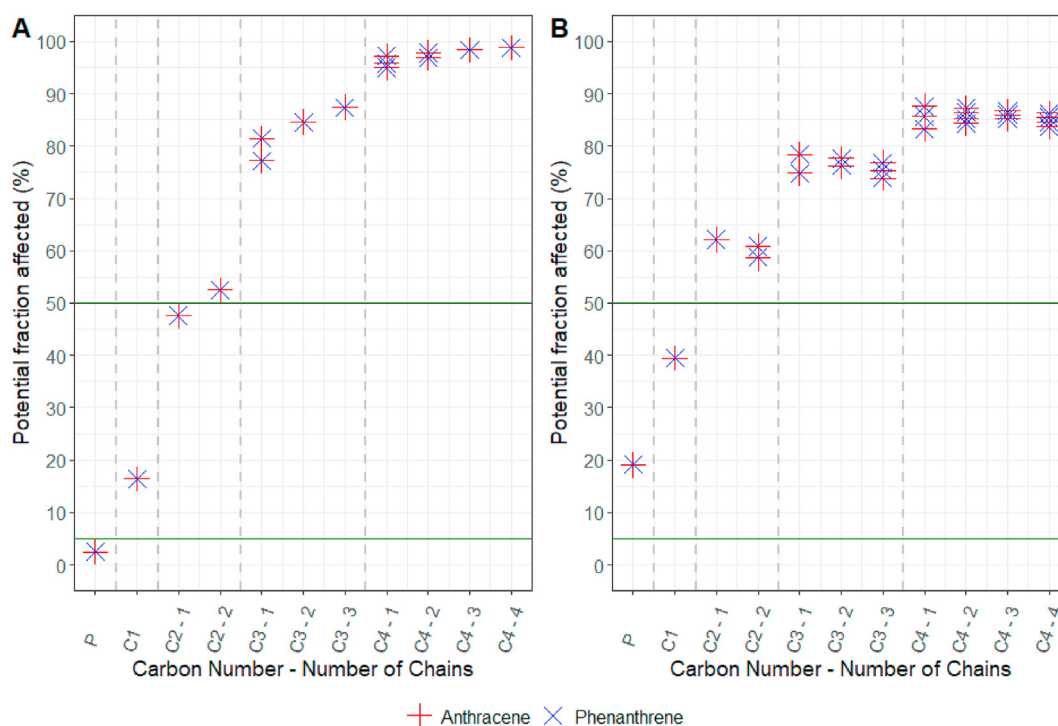


Fig. 7. Predictions of ecotoxicity in terms of fraction of affected species at an exposure concentration of 10 µg/L or at maximum water solubility for the group of three-ring PAHs. A) Predictions by the Target Lipid Model. B) Predictions by the Internal Lipid Residues Model. P are the parent substances anthracene and/or phenanthrene, C1 are alkylated three-ring PAHs with one extra carbon atom, C2 contain two extra carbon atoms, C3 contain three extra carbon atoms and C4 contain four extra carbon atoms. In addition, the constituents within these blocks are further categorized based on the number of alkyl chains.

observed, in which the ILRM predicts a higher fraction of affected species for the lower carbon number blocks (P, C1 and C2) and the TLM for the higher carbon number blocks (C3 and C4).

3.3.3. Toxicity discussion

Several reliable experimental toxicity studies were available, particularly for the lower hydrocarbon blocks (i.e. parents, C1 and C2). Although the identified studies mainly consider short-term or sub-chronic exposures, (photo)toxic effects are observed below the T-threshold of 10 µg/L for these lower carbon number constituents.

According to the available data, alkylated anthracenes seem

slightly more toxic than alkylated phenanthrenes. In particular, alkylated anthracenes seem to have a higher phototoxic potential, which might be related to the molecule's HOMO-LUMO gap (i.e. the energy difference between the highest occupied and the lowest unoccupied molecular orbital). De Lima Ribeiro and Ferreira (2005) predicted that substances with a HOMO-LUMO gap between 6.5 and 7.9 eV have a high phototoxic potential, whereas substances with a HOMO-LUMO gap beyond this range are predicted to have a much lower or no phototoxic potential. The HOMO-LUMO gap of anthracene is within these boundaries (7.279 eV), whereas the HOMO-LUMO gap of phenanthrene is above the upper value

(8.209 eV) (De Lima Ribeiro and Ferreira, 2005). It has been shown that methylation generally reduces the HOMO-LUMO gap of substances. Methyl- and dimethyl-phenanthrene, for instance, have a HOMO-LUMO gap of 8.13 eV and 8.05 eV, respectively (Boese et al., 1998). This may explain the observed phototoxic effects of alkylated phenanthrenes in comparison to the parent three-ring PAH. Furthermore, the difference in the HOMO-LUMO gap between alkylated anthracenes and phenanthrenes may explain the different phototoxic potencies as observed for these constituents.

The predictive models, which are mainly based on non-phototoxic effects, estimate a consistent increase in toxicity with an increase in alkylation. This trend is not particularly confirmed by the experimental data without UV-exposure, in which in general no toxicity below 10 µg/L is observed. However, it should be noted that available experimental data consider acute or sub-chronic exposures (which includes many larger than values: '>'), and no information on chronic effects without UV-exposure is available. Therefore, in the absence of data, it cannot be concluded that the alkylated three-ring PAHs will not exhibit any long-term effects below 10 µg/L without UV-exposure. The available experimental phototoxicity data (with mysid shrimp, *Americamysis bahia*) show a clear trend that is consistent and fits the trend as predicted by the models, indicating an increase in toxicity with hydrophobicity (see Fig. S3). Accordingly, a similar trend is expected for long-term toxicity, for which the models were developed. Therefore, higher (photo)toxic effects are expected for C3 and C4 constituents, compared to the parent, C1 and C2 constituents.

Besides aquatic toxicity, alkylated three-ring PAHs may also exert toxic effect to mammalian species. However, in the current evaluation of alkylated three-ring PAHs we did not consider toxicity to mammalian species, including humans. It should be noted, in this respect, that for the parent three-ring PAHs, anthracene and phenanthrene, there are currently no notified classifications to mammals/humans that would give rise to a T-identification within the PBT-assessment (ECHA, 2021).

3.3.4. Toxicity conclusion

In conclusion, experimental data on C1 and C2 alkylated three-ring PAHs indicate aquatic toxicity below the T-threshold of 10 µg/L, and these alkylated PAHs can therefore be considered as T. For the constituents belonging to the higher hydrocarbon blocks (i.e. C3-C4), far less experimental data are available and none of the more sensitive species have been tested (Table S12). Therefore, no conclusions could be drawn based on experimental data for the C3-C4 constituents. Nevertheless, based on the predictive models, a clear trend of increasing toxicity with an increase in alkylation/hydrophobicity is predicted. Accordingly, C3-C4 constituents are

expected to be more toxic than the lower carbon number constituents.

3.4. PBT conclusion and outlook to other UVCBs

Based on the P-, B- and T-assessment, it can be concluded that the alkylated three-ring PAHs are more, or at least equally persistent, bioaccumulative in invertebrates and toxic to aquatic organisms when compared to the parent three-ring PAHs, anthracene and phenanthrene (Table 4). As anthracene and phenanthrene are currently both considered as PBT and vPvB, respectively (ECHA, 2020b), the alkylated three-ring PAHs could also be considered to have PBT/vPvB properties.

It should be noted, however, that the current PBT/vPvB-status of phenanthrene is under discussion (Hughes et al., 2020; InfoCuria, 2019). If these discussions would result in a removal of the PBT/vPvB-status of phenanthrene, it will be more difficult to derive a conclusion for the whole group of alkylated three-ring PAHs based on current available data. Particularly, as this would mean a diverging PBT/vPvB-starting point for the group of three-ring PAHs. It should be noted, in this respect, that a difference in PBT/vPvB-properties between the two parent three-ring PAHs would, in principle, not be in accordance with the block homogeneity assumptions of the HBM.

Application of the HBM in the PBT-assessment for other hydrocarbon blocks is likely more complicated and challenging compared to the three-ring PAHs, as for many hydrocarbon categories far less experimental data are available and/or parent structures have not been assessed (yet). For instance, difficulties arise when no (unambiguous) PBT-trends could be derived based on available experimental data. Potentially, a part of these experimental data gaps could be filled by extrapolating the results of the current evaluation of three-ring PAHs to other categories. For instance, several studies indicate comparable P-, B- and T-trends for two-ring PAHs (Elmendorf et al., 1994; Jonsson et al., 2004; Leblond et al., 2001; NITE, 2020) and four-ring PAHs (Elmendorf et al., 1994; Lin et al., 2015), and may suggest a generic trend across parent and alkylated PAHs. Nevertheless, a systematic evaluation of available data would be necessary to conclude on such trends for other categories. Furthermore, challenges with the application of the HBM arise when a relative PBT-trend could be derived, but no clear PBT/vPvB-starting point is established or the starting point is below or around the border of the PBT-criteria. In such cases, quantitative data would be necessary in order to conclude the PBT-assessment.

Several methodologies have been proposed that could be used to assess and generate data on UVCBs, including constituent-based approaches and whole substance-based approaches (ECHA, 2017a;

Table 4

Conclusions of the PBT-assessment on the group of alkylated three-ring PAHs. Parent represents anthracene and/or phenanthrene, C1 are alkylated three-ring PAHs with one extra carbon atom, C2 contain two extra carbon atoms, C3 contain three extra carbon atoms and C4 contain four extra carbon atoms.

Carbon number	Persistence	Bioaccumulation	Toxicity ^a
Parent ^b	vP	B/vB	nT/T ^c
C1	More persistent than parent ^d	More bioaccumulative than parent ^e	T ^f
C2	More persistent than parent ^d	More bioaccumulative than parent ^e	T ^f
C3	More persistent than parent ^d	More bioaccumulative than parent ^e	More toxic than parent, C1 and C2 ^g
C4	More persistent than parent ^d	More bioaccumulative than parent ^e	More toxic than parent, C1 and C2 ^g

^a The toxicity assessment solely focused on aquatic toxicity and did not consider effects on human health.

^b Based on the SVHC-dossiers of anthracene, phenanthrene and coal-tar-pitch high temperature. It should be noted that the current PBT/vPvB-status of phenanthrene is under discussion see (Hughes et al., 2020; InfoCuria, 2019) and text.

^c Phenanthrene is currently not identified to be toxic, though data is available showing toxic effects below 10 µg/L (Table S11).

^d Based on a relative persistence trend to parent three-ring PAHs for water, sediment and soil compartments.

^e Based on a relative bioconcentration trend to parent three-ring PAHs for invertebrate data.

^f Based on experimental data indicating toxic effect below 10 µg/L.

^g Based on a relative aquatic toxicity trend to parent and alkylated three-ring PAHs according to modelled data.

Salvito et al., 2020). Ideally, for PBT-assessment purposes of these substances, additional data are generated via a constituent-based approach (like the HBM), as the physicochemical properties differ significantly between the varying constituents of petroleum UVCBs. Although all constituents within a petroleum UVCB could be emitted as a whole, the constituents will fractionate in the environment due to the varying physicochemical properties and persistence, resulting in different distribution, fate and exposure patterns (ECHA, 2017a; Salvito et al., 2020). Therefore, one or more (representative) constituents or groups/blocks of very similar constituents should be tested, and only in cases where all constituents are (structurally) very similar, a whole substance-based approach might be followed. Nevertheless, further scientific discussions and analyses are necessary to improve the understanding of which specific data would be sufficient to derive a PBT-conclusion on a block or UVCB when (additional) quantitative data are necessary.

4. Conclusion

Within this study, we applied the fraction profiling approach or so-called hydrocarbon block method (HBM) for a PBT-assessment of alkylated three-ring PAHs to investigate the suitability of the HBM for the assessment of UVCBs. Evaluation of available data revealed that the absolute degradation half-lives and BCF values from many studies are of insufficient relevance for PBT-assessment purposes. Nevertheless, by using trend analyses on a block of hydrocarbons with a known PBT/vPvB starting point, it was possible to derive a PBT-conclusion for 884 constituents in one assessment. This case study on the alkylated three-ring PAHs gives promising perspectives for other hydrocarbon blocks and possibly for other UVCBs. For these cases, further work is required to evaluate the suitability of the HBM when trend analyses are not possible and/or conclusive.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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