Semipolar Polycyclic Aromatic Compounds: Identification of 15 Priority Substances and the Need for Regulatory Steps Under REACH Regulation

Markus A Schwarz,† Andreas Behnke,‡ Marc Brandt,§ Adolf Eisenträger,‡§ Martin Hassauer,† Fritz Kalberlah,† and Albrecht Seidel‡*

†Forschungs- und Beratungsinstitut Gefahrstoffe, Freiburg, Germany
‡Biochemisches Institut für Umweltcarcinogene, Prof. Dr. Gernot Grimmer Stiftung, Grosshansdorf, Germany
§Umweltbundesamt, Postfach, Dessau-Rosslau, Germany

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ABSTRACT

Semipolar polycyclic aromatic compounds (sPACs) are frequently found in association with homocyclic polycyclic aromatic hydrocarbons (PAHs) in substances of unknown or variable composition, complex reaction products, or biological materials (UVCBs) from coal or crude oil and products derived thereof. However, major information deficiencies exist with regard to their prevalence and their toxicological and ecotoxicological potential, persistency, and bioaccumulation characteristics. Therefore, in this work, the environmental concern and relevance of sPACs was addressed in a general, stepwise approach. First, a large list of sPACs was collected and subsequently refined by assessing their persistence, bioaccumulation, and toxicity (PBT) properties by quantitative structure–activity relationship (QSAR) methods and their relevance by determining their respective frequency of occurrence. In this way, 15 priority sPACs were identified. These 15 priority sPACs were further characterized in detail with respect to their ecotoxicological properties, environmental behavior, carcinogenicity, and genotoxicity attributes. All of these 15 substances were quantified in distillate or product samples. In the next step, some principles for nomination of indicator substances, indicative for the overall content of sPACs, are derived. Data gaps on ecotoxicological endpoints preclude final conclusions, but the respective necessary supplemental tests were identified. Five of the 15 sPACs were tentatively characterized as potential substances of very high concern (SVHC) for the environment. The overall results of this study also clearly show that regulatory risk management of homocyclic PAHs within the European Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) does not address the environmental concern created by sPACs within UVCBs from coal or crude oil. The study proves the need for additional regulatory steps under REACH and suggests indicator substances for their enforcement. *Integr Environ Assess Manag* 2014;9999:XX–XX. © 2014 SETAC

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INTRODUCTION

Many polycyclic aromatic hydrocarbons (PAHs) are regarded as substances with problematic properties for humans and the environment. Therefore, they have been the focus of extensive research in the last 5 decades. Chemical legislation addresses some problematic PAHs, mostly in the form of more or less representative groups, such as the 8 carcinogenic PAHs currently addressed by the European chemical legislation Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; EC no.1907/2006) in Annex XVII (REACH PAH) or the 16 PAHs that were originally proposed by the US Environmental Protection Agency (USEPA) as priority pollutants in the Clean Water Act of 1977. Much less is known about semipolar polycyclic aromatic compounds (sPACs) than about PAHs, and thus they are not yet addressed in chemical legislation. This work establishes the necessary background to conclude on possible regulatory gaps regarding sPAC.

The sPACs are a heterogeneous group with heteroatoms in the aromatic ring systems (S-, N-, O-heterocycles; i.e., thiaarenes, azaarenes, oxaarenes) or substituents including these heteroatoms. Not considered in this work are compounds with fewer than 2 condensed aromatic rings or nonaromatic rings, such as piperazine, diphenylether, or hydroxybiphenyls, with the exception of 2-hydroxybiphenyl, which is a known degradation product of dibenzothiophene (Bressler et al. 1998). Toxicological characterization of sPACs is most often insufficient, in spite of indications of toxic properties comparable to their homocyclic analogs.

No uniform set of compounds of sPACs have been analytically assessed in routine analyses, as is mostly the case for PAHs, even though they are frequently found in association with homocyclic PAH in substances of unknown or variable composition, complex reaction products, or biological materials (UVCBs; see ECHA 2012 for substance definition) from coal or mineral oil and derived products thereof. Quantitative data are often lacking, and much less is known in regard to their relevance for refined products.

One main source for sPACs is coal tar, the condensation product obtained by cooling of the raw gas generated in coal pyrolysis. Creosote is a fraction of the coal tar distillation

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* To whom correspondence may be addressed: markus.schwarz@fobig.de

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process (boiling temperature ~230–350°C) and is often used as a synonym for the anthracene fractions of the coal tar. In creosote, high concentrations of PAH and sPAC are found. The main use of creosote is as a wood-preserving agent. Creosote also has been a component of roofing pitch and is used in blends of coal tar oils as a source of carbon black (Sundström et al. 1986; Betts 1997; WHO 2004).

The other important sources for sPACs are mineral oils, which are separated in a first step by fractionated atmospheric distillation. This process yields the gaseous and lower boiling fractions, including lubricating oil (>360°C), and a heavy hydrogen mixture of higher boiling compounds (long residue). The latter is separated by a further fractionating distillation process under vacuum yielding lubricant base oils, heavy fuels, paraffin waxes, and bitumen (short residue). Process or extender oils (e.g., for tire production) originate from the lubricant base oil fraction of vacuum distillation of crude oil after several refining steps (e.g., solvent extraction). The so-called distillate aromatic extracts (DAE) have a high PAH content (10%–25% dimethylsulfoxide [DMSO]-extractable fraction); fractions with reduced PAH (DMSO-extractable fraction < 3%) content are mild extracted solvate (MES), treated distillate aromatic extract (TDAE, hydrotreated or solvent extracted), treated residual aromatic extract (TRAE), and hydrotreated naphthenic process oils. The latter were not included within the framework of this study. The DMSO-extractable fraction is a surrogate indicator for the carcinogenic potential (originating at least in part from the PAH content) of the oils. Oils with values less than 3% by weight are regarded as safe and avoid being labeled as carcinogenic (Null 1999; Prince 2010). Process and extender oils are used for various purposes as components of tires, thermoplastics, industrial rubber, printing inks, and polyvinyl chlorine (PVC; Nynas Corporation, product information on process and extender oils). The PAH emissions caused by tire abrasion exceeded the amount of PAH generated by exhaust emissions (Null 1999). Because of the high content of carcinogenic PAH in high aromatic extender oils, this burden has been reduced by specifications of car manufacturers and by legislative steps in the European Union: According to entry number 50 of Annex XVII, REACH upper limits for PAH concentrations in extender oils used for production of tires are set. Recently, this entry was extended, with provisions for PAH in consumer products.

This work contributes by filling knowledge gaps with regard to both properties of ecological concern (persistence, bioaccumulation, toxicity according to REACH) as well as prevalence in refined or final products such as carbon black, bitumen, processing oils, or rubber products to conclude a need for additional regulatory steps under REACH explicitly addressing sPACs. To do this, a manageable list of priority sPACs was compiled and evaluated.

METHODS

The methodological approach for the prioritization and evaluation of sPACs that was chosen in this study is summarized in Table 1. The results of the approach are also shown.

Table 1. Outline of the methodological approach for the prioritization and evaluation of sPAC with corresponding results

Step	Task	Result
1	Collecting semipolar polycyclic aromatic compounds (sPAC) with potential environmental concern	Initial list of 443 substances
2	Refining the initial list by QSAR methods to a list containing substances fulfilling the PBT-screening threshold values according to European REACH-regulation (Annex XIII)	List of 154 substances
3	Identifying priority sPAC. The primary indicator of relevance was frequency of occurrence of the substances in matrices (mainly UVCB: literature data). Not considered were compounds formed exclusively because of environmental processes.	List of 15 substances (priority sPAC); see Table 3.
4	Confirming the environmental relevance of the priority sPAC through analytical quantification in extender oils (4 different types) coal tar pitch, carbon black, bitumen as well as 4 typical consumer products (tire and corresponding tube, flip-flop sandals and childrens' rubber boots)	All 15 priority sPAC could be determined in at least some of these matrices, confirming their environmental relevance.
5	Proposal of indicator substances for priority sPAC	Benzo[*b*]naptho[2,1-d]thiophene (2,1-BNT) was identified as the most appropriate candidate within 15 priority sPAC
6	Confirming environmental concern for the priority sPAC through extensive evaluation of existing data	Derivation of provisional PNECs, environmental classifications, and PBT assessment based on available data, including mutagenic and carcinogenic properties. Identification of most relevant data gaps for the environmental assessment.

sPAC = semipolar polycyclic aromatic compounds; QSAR = Quantitative Structure Activity Relationship; PBT = persistence, bioaccumulation and toxicity; REACH = Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals; UVCB = substances of unknown or variable composition, complex reaction products or biological materials; PNEC = predicted no-effect concentration.

Step 1: Collecting sPACs with potential environmental concern

To establish an initial list of identified sPACs, as a first step the earlier work on sPACs by the KORA project (see http://www.natural-attenuation.de/for more information) and LAWA AG (LAWA AG 2010) on priority compounds of creosote-contaminated sites was used. Thus, an initial set of approximately 256 substances (isomers only partly included) by KORA (Blotevogel et al. 2007) was taken as input. Within prioritization for compounds with relevance for partitioning into groundwater at contaminated sites by KORA, those with log octanol–water partition coefficient (K_{OW}) greater than 4.5 were excluded. In contrast, these compounds are of interest in this project.

To make the initial list as comprehensive as possible, an extensive literature search was performed on UVCBs derived from coal or mineral oil and final products (see Supplemental Data for full references). Substances were included when sPAC content was specified.

In addition, REACH registration information on UVCBs and single compounds derived from coal (compound lists from CEFIC Coal Chemicals Sector Group "REACH for Coal Chemicals," http://www.r4cc.org/) as well as dossiers on distillates derived from mineral oil and used for process oils (information from telephone survey, including manufacturers and organizations as well as material safety data sheets for process oils) were evaluated for information on sPACs.

All collected compounds were identified by chemical name, Chemical Abstracts Service numbers, or simplified molecular-input line-entry system code, and their respective origins (analyzed matrix plus reference) were recorded. For final evaluation, compounds were tabulated together with their frequency of occurrence in matrices (respective matrices were assigned to 14 generic matrix types).

Step 2: Refining the initial list by QSAR methods to a list containing substances fulfilling the persistence, bioaccumulation, and toxicity screening threshold values according to European REACH regulation (Annex XIII)

For the prioritization of the identified sPACs relating to properties of persistence, bioaccumulation, and toxicity (PBT), a screening method based on quantitative structure–activity relationship (QSAR) software tools was employed.

The programs of the USEPA QSAR software package for prediction of environmentally relevant properties (EPI Suite version 4.1) were used. This enables a screening based on numerical criteria for PBT or not fulfilling screening criteria for T but being persistent and having a high potential for bioaccumulation (vPvB) assessment given in Annex XIII of the REACH regulation and REACH guidance documents (Table 2). The following programs were applied: KOWWIN (v. 1.68) for estimation of the octanol–water partition coefficient ($\log K_{OW}$), with preference on experimental values from the PhysProp-DB (as far as available); BIOWIN (v. 4.1) modules 2, 3, and 6 for biodegradability estimation; and ECOSAR (v. 1.00) for estimation of acute aquatic toxicity.

For bioaccumulative properties (B), the log K_{OW} was employed as a screening parameter instead of the calculated bioconcentration factor. Literature evaluation of programs predicting bioconcentration factor from log K_{OW} against validated experimental data indicates a high percentage of false negatives; that is, substances with bioaccumulative

Table 2. PBT- and vPvB-criteria according to REACH and applied cutoff values for QSAR-based screening on PBT

Property	Persistence*	Bioaccumulation	Toxicity
Criterion according to REACH Annex XIII	T½ freshwater > 40 d	Bioconcentration factor (BCF) > 2000	Long-term NOEC < 0.01 mg/L OR
	T½ sediment (freshwater) > 120 d		CM cat. 1A or 1B, R cat. 1A, 1B, or 2 OR
	T½ soil > 120 d		STOT RE cat. 1 or 2 according to (EC) No. 1272/2008
Screening-criterion according to REACH guidance	Not readily biodegradable or	Log K_{OW} > 4.5	EC50/LC50 (algae, *Daphnia* or fish)
	Not inherently biodegradable or		< 0.1 mg/L: T presumably fulfilled
	Not degradable by physicochemical processes (e.g., hydrolysis)		< 0.01 mg/L: T definitely fulfilled
QSAR-Screening	BIOWIN 2/3 and 3/6-evaluation according to REACH-Guidance R.11 P1 or P2	Log $K_{OW} \geq 4.0$ Log $K_{OW} \geq 4.5$ for binary combinations BT, PB	E(L)C50 <= 1.0
Property	**Very persistent and very bioaccumulative (vPvB)**		
Criteria according to REACH Annex XIII	T½ water > 60 d T½ sediment > 180 d T½ soil > 180 d		Bioconcentration factor (BCF) > 5000

*Only criteria for freshwater compartment are given in this table.

PBT = persistence, bioaccumulation and toxicity; vPvB = not fulfilling screening criteria for T but being persistent and having a high potential for bioaccumulation; REACH = Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals; BCF = bioconcentration factor; NOEC = no observable effect concentration; CM cat. 1A or 1B = carcinogen or germ cell mutagen category 1A or 1B; R cat. 1A, 1B, or 2 = toxic for reproduction category 1A, 1B, or 2; STOT RE = specific target organ toxicity after repeated exposure category 1 or 2; EC50 = median effectice concentration; LC50 = median lethal concentration; QSAR = Quantitative Structure Activity Relationship; BIOWIN = US EPA EPI Suite BIOWIN modules; BT = bioaccumulation and toxicity; PB = persistence and bioaccumulation.

properties are classified as not bioaccumulative. Because the screening criterion for B according to REACH guidance documents of log K_{OW} greater than 4.5 is very strict (risk of too many false negatives), we used a log K_{OW} of 4.0 or greater as the criterion for the screening on B. This is in accordance with criteria on classification, labeling, and packaging (CLP regulation, EC no. 1272/2008). Moreover, the applicability of this criterion was successfully tested on 10 compounds (PAH and sPAC) with experimental data on bioconcentration, mainly from substances of very high concern (SVHC) support documents for UVCB anthracene oil published by the European Chemicals Agency (ECHA).

For persistence (P), following the REACH guidance documents (ECHA 2008b), a pairwise combination of the results of 3 of the BIOWIN programs of the EPI Suite is proposed to assess persistence, BIOWIN2/3 and BIOWIN6/3. BIOWIN 2 and 6 give probabilities for fast biodegradation, and BIOWIN 3 predicts ultimate biodegradability timeframe. In our assessment, we used the following output: P1 = persistent according to BIOWIN 2/3 results (i.e., $<0.5/\leq 2.25$); P2 = persistent according to BIOWIN 6/3 results (i.e., $<0.5/\leq 2.25$). One combination indicating persistence was sufficient for a positive screen on P. These criteria were tested on 5 compounds (PAH and sPAC), with experimental data on persistence from SVHC support documents for UVCB anthracene oil published by the ECHA.

For toxicity (T), acute toxicity values were estimated by ECOSAR for algae, daphnids, and fish, and the effect value of the most sensitive organism group was compared with the screening value for T. The more specific structure–activity relationships were preferred over the general structure–activity relationships "neutral organics" as far as available. A decisive effect concentration was taken 1) regardless of a lower solubility than the effect concentration indicated by ECOSAR or 2) regardless of the substance being outside the model domain because of a log K_{OW} higher than the limit of the model (e.g., log K_{OW} of 5). In these cases, ECOSAR predicts no acute toxicity at saturation concentration. We proceeded this way for 2 reasons: First, for long-term effects caused by bioaccumulation, a low water solubility may be sufficient for buildup of higher concentrations in the organism over time. Second, acute toxicity prediction is taken as an indication of long-term hazards. Median effective concentration divided by median lethal concentration being 1 mg/L or less was chosen as the criterion for T, based on QSAR screening results. This approach follows the criterion for acute aquatic hazard category 1 and chronic hazard category 1 based on acute data according to the classification, labeling, and packaging (CLP) regulation (EC no. 1272/2008) and not the screening values according to REACH. The reason for this is that, according to the REACH guidance documents, the thresholds for the screening value and the definite T-criterion for chronic ecotoxicity data differ only by a factor of 10 (0.1 mg/L for acute median lethal concentration or median effective concentration compared with a no observable effective concentration <0.01 mg/L). This translates in an acute to chronic ratio of 10. With our approach, we apply a more conservative acute to chronic ratio of 100, which is according to REACH guidance documents R.10 on predicted no-effect concentration (PNEC) derivation.

Considering the inherent uncertainty associated with QSAR predictions, we decided to consider further those compounds that were positively screened for only 2 of the 3 PBT properties. For the combinations PB and BT, we set a stricter selection limit on B, namely, log K_{OW} of 4.5 or greater instead of 4.0.

The QSAR selections of critical sPAC predicted to be PBT, PB, PT, or BT, according to the selection methodology described, were evaluated subsequently with the CATALOGIC QSAR-Models (Jaworska et al. 2002; Dimitrov et al. 2007) to substantiate the predictions of the EPI suite model. CATALOGIC is able to predict the biodegradability of compounds, based on the predicted biological oxygen demand. This QSAR is based on experimental results from tests according to Organisation for Economic Co-operation and Development guidelines 301 C and 301 F.

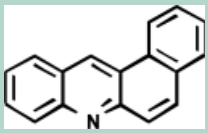
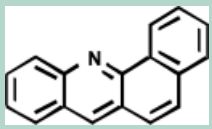
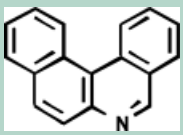
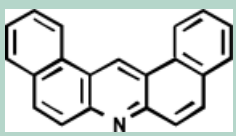
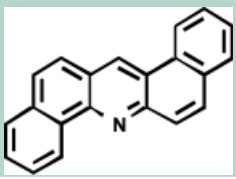
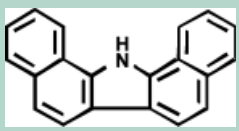
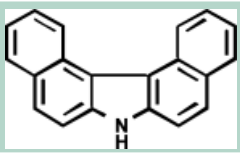
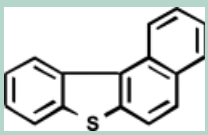
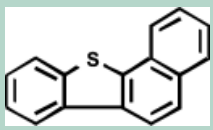
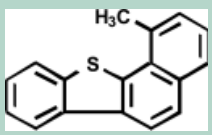
Step 3: Identifying priority sPACs

Because quantitative analytical data were only rarely available, the earlier recorded frequency of occurrence in matrices (as recorded for all 443 sPACs of the original list based on analytical determinations published in the literature) was used as a surrogate indicator. A frequency of 6 or higher was taken as the primary indicator for relevance. The resulting set of compounds was expanded by application of other indicators for relevance, such as the amount of experimental data available in the Organisation for Economic Co-operation and Development QSAR toolbox, listing in Annex XIV support documents under REACH or in national PBT priority lists. The number of compounds was then reduced by disregarding those substances for which information from literature reliably could demonstrate their origin being exclusively attributable to secondary environmental formation processes (e.g., combustion processes, atmospheric formation caused by irradiation and/or oxidation processes). Those latter compounds were considered as not relevant because of the scope of REACH (the focus of this work was on substances in need of regulation by REACH). The resulting substances make up the list of priority sPACs.

Step 4: Confirming the environmental relevance of the priority sPACs through analytical quantification

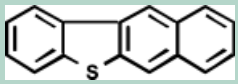
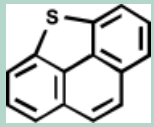
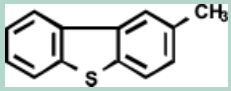
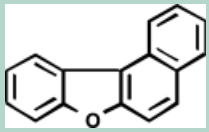
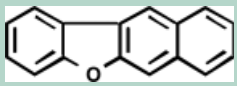
Analytical investigations were performed to confirm relevance as deduced from the literature for important samples in the sense of this project. This was necessary because information on occurrence was generally very limited to nonexistent, for example, for occurrence of sPACs in articles (as defined by European REACH regulation), no data were available at all. Fifteen priority sPACs (Table 3) complemented by 25 PAHs ("Grimmer" PAH, including 16 EPA-PAH and 8 European Union priority PAH indicated in Commission Regulation [EC] 552/2009) were analyzed in consumer products, extender oils, carbon black, bitumen, and coal tar pitch (Table 4). The PAHs were analyzed to enable a comparison between PAH and sPAC concentrations for later conclusions regarding the necessity of regulatory actions. Samples were selected based on literature information, expert knowledge, or elevated PAH concentrations found in earlier investigations. Reference materials were largely from the Biochemisches Institut für Umweltcarcinogene repository; purity was greater than 98% as determined by gas chromatography with flame ionization detector (GC-FID). 1-Methylbenzo[*b*]naphtho[2,1-*d*]thiophene was synthesized in isomeric pure form. Because of the great similarities of the physicochemical properties between PAHs, thiaarenes, and oxaarenes (sPACs containing S and O in their aromatic ring system, respectively) all 3 classes of compounds can be determined together in 1 profile analysis. In principle, the determination of the PAH profile according to the Grimmer

Table 3. Identified 15 priority sPAC, literature data on their occurrence as well as results from analytical determinations performed within this project (detections ≥ LOQ)

CAS-No.	Chemical name	Structure	Frequency of occurrence lit.	Occurrence analytics (step 4 of this project) [mg/kg]
225-11-6	Benz[*a*]acridine		Environment (2); tar (1); tar fraction (2)	Coal tar pitch (1149.1), spare tire (61.9), spare tube (36.9), flip-flop (0.71), bitumen (0.07), rubber boots (0.05), furnace black (0.03), MES (0.03), RAE (0.01)
225-51-4	Benz[*c*]acridine		Environment (2); pitch (2); tar (1); tar fraction (7)	Coal tar pitch (985.0), spare tire (81.0), spare tube (55.9), flip-flop (0.63), bitumen (0.07)
195-29-9	Benzo[*a*] phenanthridine (mixed isomers)		Environment (1); pitch (4); tar (1); tar fraction (6)	Coal tar pitch (22.1), spare tire (1.8)
224-42-0	Dibenz[*a,j*]acridine		Environment (3); pitch (1); tar fraction (2)	Coal tar pitch (310.8), spare tire (10.4), spare tube (4.9)
226-36-8	Dibenz[*a,h*]acridine		Environment (2)	Coal tar pitch (517.7), spare tire (24.7), spare tube (17.4), flip-flop (0.13), furnace black (0.043), bitumen (0.02), rubber boots (0.02), RAE (0.01)
239-64-5	13H-Dibenzo[*a,i*] carbazole		Tar fraction (1)	Coal tar pitch (187.6), spare tire (11.3), spare tube (7.3), flip-flop (0.13), bitumen (0.06), RAE (0.03)
194-59-2	7H-Dibenzo[*c,g*] carbazole (mixed isomers)		Environment (2); pitch (1); tar fraction (2)	Coal tar pitch (73.3), spare tire (9.3), spare tube (6.9)
205-43-6	Benzo[*b*]naphtho[1,2-*d*]thiophene		Environment (1); pitch (2); tar (1); tar fraction (1)	Coal tar pitch (380.2), spare tire (126.6), spare tube (105.9), DAE (1.9), flip-flop (1.3), TDAE (0.21), MES (0.08), RAE (0.07), rubber boots (0.04), bitumen (0.03), furnace black (0.02)
239-35-0	Benzo[*b*]naphtho[2,1-*d*]thiophene		Bitumen (8); carbon black (2); environment (3); pitch (2); tar (2); tar fraction (2)	Coal tar pitch (2141.8), spare tire (615.7), spare tube (519.9), DAE (6.2), flip-flop (6.8), TDAE (1.0), MES (0.41), RAE (0.32), rubber boots (0.12), bitumen (0.07), furnace black (0.09)
4567-41-3	1-Methylbenzo[*b*]-naphtho[2,1-*d*] thiophene (mixed isomers)		Environment (1); pitch (1); tar (2); tar fraction (1); oil fraction hydrotreated (1)	Spare tube (9.7), spare tire (8.3), DAE (2.3), flip-flop (0.98), TDAE (0.42), RAE (0.17), MES (0.11), rubber boots (0.03), bitumen (0.017)

(*Continued*)

Table 3. (*Continued*)

CAS-No.	Chemical name	Structure	Frequency of occurrence lit.	Occurrence analytics (step 4 of this project) [mg/kg]
243-46-9	Benzo[*b*]naphtho[2,3-*d*]thiophene		Environment (2); pitch (2); tar (1); tar fraction (1)	Coal tar pitch (691.0), spare tire (209.4), spare tube (178.6), flip-flop (1.6), DAE (1.0), RAE (0.17), TDAE (0.14), MES (0.04), bitumen (0.04), furnace black (0.04), rubber boots (0.02)
30796-92-0	Phenanthro[4,5-*bcd*]thiophene		Carbon black (3); environment (3); pitch (2); tar (2); tar fraction (1)	Coal tar pitch (426.3), spare tire (153.7), spare tube (141.7), furnace black (7.4), flip-flop (1.1), TDAE (0.10), DAE (0.08), RAE (0.08), rubber boots (0.04), MES (0.03), bitumen (0.02)
20928-02-3	2-Methyldibenzothiophene (mixed isomers)		Environment (3); tar (2); tar fraction (2); oil fraction hydrotreated (1)	Spare tire (36.8), spare tube (36.3), coal tar pitch (36.4), TDAE (2.2), flip-flop (0.82), MES (0.53), RAE (0.36), DAE (0.27), rubber boots (0.06), bitumen (0.02)
205-39-0	Benzo[b]naphtho[1,2-*d*]furan (mixed isomers)		Carbon black (1); environment (3); pitch (2); tar (2); tar fraction (2)	Coal tar pitch (474.9), spare tire (73.4), spare tube (61.0), flip-flop (0.89), DAE (0.37), rubber boots (0.08), TDAE (0.04), MES (0.01), RAE (0.01)
243-42-5	Benzo[b]naphtho[2,3-*d*]furan (mixed isomers)		Environment (2); tar (3); tar fraction (2)	Coal tar pitch (895.6), spare tire (112.2), spare tube (92.0), flip-flop (1.1), DAE (0.14), rubber boots (0.07)

Flip-flop: flip-flop sandals (soft-PVC).
sPAC = semipolar polycyclic aromatic compounds; MES = medium extracted solvate; RAE = residual aromatic extracts; DAE = distillate aromatic extracts; TDAE = treated distilled aromatic extracts.

method is based on a stable isotope dilution methodology (Grimmer et al. 1997). For the determination of azaarenes (sPACs containing N in their aromatic ring system), an advanced version of a previously published gas chromatography mass spectrometry method (Grimmer et al. 1978; Grimmer et al. 1983; Grimmer and Naujack 1985) was used including a deuterated internal standard. An Agilent 6890N gas chromatography mass spectrometry instrument connected to a 5973N MSD was used. Separation of thiaarenes and oxaarenes was performed on an Agilent DB-35MS capillary (30 m × 0.25 mm × 0.25 μm), whereas an Agilent VF-200MS capillary (30 m × 0.2 5 mm × 0.25 μm) was selected for separation of azaarenes after a pulsed splitless injection. Helium was used as carrier gas, and an individually optimized temperature program had to be developed. A 3-point calibration curve was performed, with each individual reference material of sPAC showing a linear range of 2 orders of magnitude for thiaarenes (range, 0.05–2.0 mg/L), oxaarenes (range, 0.05–2.0 mg/L), and azaarenes

Table 4. Samples tested for 15 priority sPAC and 25 PAH

Rubber processing oil	DAE (distillate aromatic extract)	TDAE (treated distillate aromatic extract)	RAE (residual aromatic extract)	MES (mildly extracted solvate)
Source	Germany	Thailand	Germany	Europe
Material	Coal tar pitch	Bitumen	Carbon black (Furnace black)	
Source	Germany	Germany	Germany	
Rubber product	Tire from DIY market	Tube of the tire from DIY market	Flip-flop sandals (soft PVC)	Children's Rubber-boots
Source (all bought in Germany)	Product of Asia	Product of Asia	Origin unclear	Product of China

sPAC = semipolar polycyclic aromatic compounds; PAH = polycyclic aromatic hydrocarbons; MES = medium extracted solvate; RAE = residual aromatic extracts; DAE = distillate aromatic extracts; TDAE = treated distilled aromatic extracts; DIY = do-it-yourself; PVC = polyvinyl chlorine.

(range, 0.1–4.0 mg/L). The limit of quantification (LOQ = 0.01 mg/kg) for the thiaarenes, oxaarenes, and azaarenes was roughly estimated using the signal-to-noise method (10:1).

Step 5: Proposal of indicator substances for priority sPACs

Analytical data analysis was essentially restricted to quantitative results derived within step 4 of this project. The reason for this is that analytical results for different matrices published in the literature were most often lacking quantitative information, and target compounds in these investigations were much too heterogeneous to be comparable. One exception is a coal tar pitch sample with a comparable range of analyzed PAH and sPAC (Domínguez et al. 2004), which was included in the analysis. The following ratios were formed for the different samples analyzed in step 4 to conclude on potential indicator substances: 1) Priority sPACs to total PAH or to 16 USEPA PAH with a) sum of 15 sPACs and b) separately for thiaarenes, azaarenes, and oxaarenes; 2) ratio of benzo[*b*]naphtho[2,1-*d*] thiophene (2,1-BNT) to sum of priority thiaarenes compared with ratio of thiaarene with maximum concentration in respective sample to the sum of priority thiaarenes; 3) ratios of priority thiaarenes, azaarenes, or oxaarenes to sum of all 15 priority sPACs; 4) ratio of 2,1-BNT to sum of priority sPACs.

Step 6: Confirming environmental concern for the priority sPACs through extensive evaluation of existing data

An extensive literature research was performed to identify experimental data on persistence (biodegradation potential), bioaccumulation, ecotoxicity, genotoxicity, and carcinogenicity (see Supplemental Data for full references). Because of their limited or uncertain reliability, in vitro results for ecotoxicity were generally not considered (data from the fish embryo toxicity test were included, however). The confirmation of QSAR results was generally restricted to 1 unequivocal experimental study if available. Further studies were evaluated if results were inconsistent with QSAR data or if inconclusive study results afforded a weight of evidence approach. Data retrieved from all available sources were carefully evaluated regarding their impact on PBT assessment. Predictivity of the applied QSAR screening procedure was tentatively assessed.

For all 15 priority sPACs, the results from experimental ecotoxicity tests, tests on bioconcentration/bioaccumulation, results from tests relating to persistence, as well as data on mutagenicity and/or carcinogenicity were summarized and data gaps provisionally closed by QSAR assessments. Based on this, provisional freshwater PNECs were derived (according to REACH guidance document R.10 [ECHA 2008a]), a provisional PBT assessment was performed (also including data on genotoxicity and carcinogenicity according to REACH Annex XIII and REACH guidance document R.11 [ECHA, 2008b]), and provisional classifications for environmental hazards according to CLP regulations were assigned.

RESULTS

Step 1: Collecting sPACs with potential environmental concern

As a basis for all further research, we sought to attain a list of compounds as comprehensive as possible using different resources as input.

The initial set by the KORA project (Blotevogel et al. 2007) and the work of LAWA AG (LAWA AG 2010) encompasses approximately 256 substances (isomers only partly included). After filtering for sPACs according to the definition of sPAC chosen in this study (see *Introduction*), 159 substances remained as first input to the list of identified substances.

By extensive research of analytical results for different matrices (UVCB, environment) published in the literature, sPACs were identified in 118 matrices, and the compounds found in these investigations were matched with the initial pool of identified sPACs from KORA/LAWA. In this way, 284 further sPACs were identified, and the overall list of identified sPACs amounted to a diverse set of 443 compounds differing in heteroatoms (N, S, O, or combinations thereof), condensation degree, and alkylation degree. All compounds were uniquely characterized by Chemical Abstracts Service number (for 113 compounds no Chemical Abstracts Service available) and simplified molecular-input line-entry system code. Occurrence in UVCBs and environmental samples varies between 0 (e.g., some compounds from KORA/LAWA not identified in analyzed profiles) and 42. The full compound list including QSAR prediction results and frequency of occurrence in generic matrix types is available as a spreadsheet (sheet "InitialListOf443sPAC") in the Supplementary Data.

Step 2: Refining the initial list by QSAR methods to a list containing substances fulfilling the PBT screening threshold values according to European REACH regulation (Annex XIII)

Applying the selection methodology described in the *Methods* section, persistence (P, assessed using BIOWIN, either P or not P) and toxicity (T, assessed using ECOSAR results) were assessed separately. Compounds predicted at the same time to be P and T were in addition provisionally regarded to be B, if KOWWIN-predicted log K_{OW} was 4.0 or greater. If a substance was positively screened for either P or T only, it was provisionally regarded to be also B if predicted log K_{OW} was 4.5 or greater. By this approach, compounds that are ecotoxic and most probably at the same time have a high potential for bioaccumulation are targeted, although they may not be persistent. Conversely, compounds not fulfilling screening criteria for T but being persistent and having a high potential for bioaccumulation (vPvB candidates) may cause harm even at lower toxicity. One hundred fifty-four compounds were identified. Ninety-four were predicted to be PBT, 3 were predicted to be PB, none was predicted to be PT, and 57 were predicted to be BT. The list of the resulting 154 compounds including QSAR prediction results and frequency of occurrence in generic matrix types is available as a spreadsheet (sheet "154-PBT_OR_BT_OR_PB") in the Supplementary Data. "Occurrence in profiles total" includes published results on matrices, in which the respective compound had been looked for but was not detected.

Step 3: Identifying priority sPACs

The overall relevance of a compound is governed by substance properties together with actual occurrence in UVCBs or environmental samples. Accounting for the latter as outlined in the *Methods* section, from the 154 compounds resulting from step 2 all those were selected for which frequency of occurrence was 6 or more. Further compounds out of the 154 were selected based on the other criteria for relevance. Then, based on scientific literature on these compounds, 3 were removed because of their origin being exclusively attributable to secondary environmental formation processes, to finally yield a diverse set of 15 priority sPACs, consisting of azaarenes, thiaarenes, and oxaarenes predicted to be PBT (14 substances)

or BT (1 compound). Structural representations as well as occurrence in generic matrix types (literature data) are given in Table 3, and further details are available as a spreadsheet (sheet "154-PBT_OR_BT_OR_PB") in the Supplementary Data (sort for priority sPACs in the last column).

Step 4: Confirming the environmental relevance of the priority sPACs through analytical quantification

Concentrations for the 15 priority sPACs measured in different matrices are given in Table 3 (last column). Only concentrations equal to or above LOQ were considered. Please note that 25 PAHs were analyzed in parallel to enable comparison between PAH and sPAC concentrations for later conclusions regarding the necessity of regulatory actions.

All 6 priority thiaarenes were detected in the investigated rubber process oils and commodities. With the exception of 1-methylbenzo[*b*]naphtho[2,1-*d*]thiophene (content less than LOQ), high levels of thiaarenes were detected in coal tar, as expected. The 2,1-BNT proved to be the compound of highest or at least second highest concentration in all thiaarene profiles but furnace black, in which phenanthro[4,5-*bcd*]thiophene was determined as the major component in the ppm range, whereas the other investigated thiaarenes were less than LOQ or in the lower ppb range.

The 2 priority oxaarenes benzo[*b*]naphtho[1,2-*d*]furan and benzo[*b*]naphtho[2,3-*d*]furan were found in high concentration (ppm range) in coal tar pitch as expected, but surprisingly also in the commodities. In contrast, the levels of the 2 oxaarenes are below LOQ in bitumen and furnace black. With regard to the extender oils only in DAE, both isomers occurred in the upper ppb range, whereas concentrations were low or below LOQ for the PAH-reduced extender oils TDAE, MES, and Residual Aromatic Extract (RAE).

The 7 priority azaarenes were found in high concentrations (ppm range) in coal tar pitch and a spare tire from a do-it-yourself market as well as in the corresponding tire tube. In all extender oils, bitumen, furnace black, and children's rubber boots, the concentrations of the investigated azaarenes were almost all below LOQ. In flip-flop sandals, 4 of the priority azaarenes could be determined in the higher ppb range.

Step 5: Proposal of indicator substances for priority sPACs

One or a few indicator substances for priority sPACs would ideally enable conclusions from their targeted analysis on the prevalence of the whole group. Prerequisite for an indicator substance approach are relatively constant ratios of the indicator compound to those substances to which extrapolation should be applied across several matrices and samples of the same matrix type, respectively. Only 1 quantitative PAC analysis from the literature was appropriate for comparison with experimental data derived within this project, and this was for coal tar pitch (Domínguez et al. 2004). For PAH as well as priority sPACs, all compounds examined in both samples or studies were indeed detected, and for most substances very similar concentrations were found. This is demonstrated in Figure 1, in which concentrations for all sPACs examined and quantified in both studies are compared. Thus, at least for coal tar, ratios between priority sPACs across samples seem to be comparable.

Further considerations regarding indicator substances had to be restricted to quantitative data on priority sPACs derived within step 4 of this project to ensure comparability (same set of compounds analyzed). Ratios of priority sPACs to total PAH

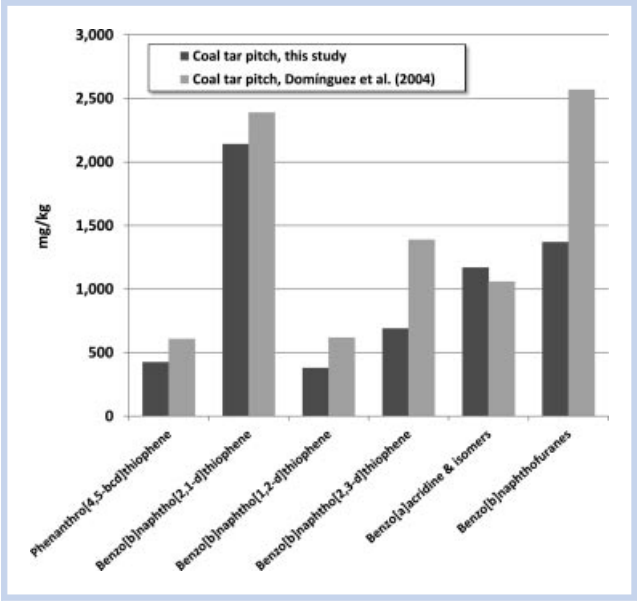


Figure 1. Content of priority sPACs examined both in this study and in Domínguez et al. (2004): Different samples of coal tar pitch.

(data not shown) or priority sPACs to the fraction of 16 EPA-PAH (Figure 2) for all 11 matrices analyzed vary widely over the different product groups, such that PAHs are not suitable for extrapolation to priority sPAC content. Whereas the total concentration of priority sPACs is in most cases considerably lower than the PAH concentration, modern extender oil samples TDAE, RAE, and MES are an exception to this: Their higher relative content of priority sPAC may be explained by the targeted reduction of PAH in these oils. The sPAC obviously are not diminished to the same extent by the production processes applied.

Thiaarenes pronouncedly dominate quantitatively compared with azaarenes and oxaarenes in most samples and are at least equal to or somewhat higher in concentration than azaarenes in coal tar pitch and bitumen. Moreover, occurrence of thiaarenes shows the least variation relative to total sPAC over different matrices, and 2,1-BNT, their 1 predominant representative over all samples, is also present in higher concentration than all azaarenes and oxaarenes analyzed in parallel. For the same reasons, 2,1-BNT had been included in the so-called Grimmer-PAC (Grimmer and Böhnke 1976). Exceptions to this are bitumen and furnace black, which are not further considered here.

For each of the remaining samples (4 household products and 4 processing oils), extrapolation factors were derived from the ratio of 2,1-BNT to total priority sPAC (Table 5). Averaging over these 8 matrices while assuming that content of priority sPAC in the products will be mostly attributable to processing oils used allows calculation of a mean extrapolation factor to estimate the total priority sPAC ($n = 15$) concentration from the concentration of 2,1-BNT. This factor is 3.1 (range, ± 1.1) and remains to be confirmed by further studies.

Step 6: Confirming environmental concern for the priority sPACs through extensive evaluation of existing data

Detailed substance fact sheets were generated for all 15 priority sPACs, and QSAR data were implemented to close experimental data gaps. Table 6 summarizes derived provisional PNECs, PBT assessment, environmental classifications

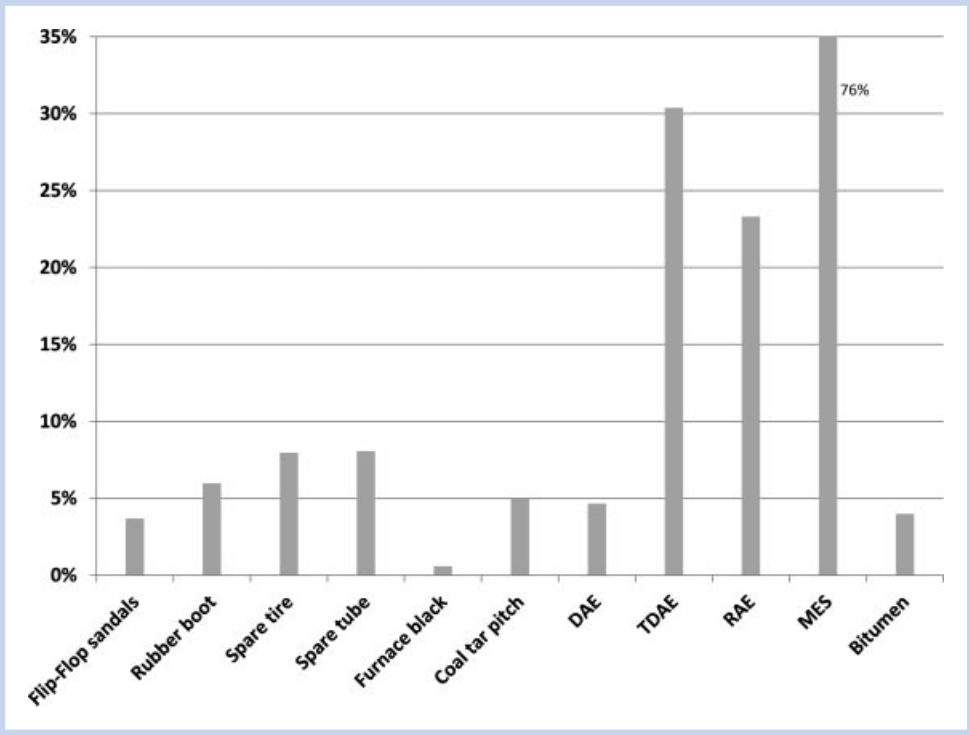


Figure 2. Ratios of the 15 priority sPACs to 16 EPA-PAHs in different analyzed matrices.

according to GHS, data on mutagenic and carcinogenic properties, as well as most important data gaps. Five of the 15 sPACs are tentatively characterized as potential SVHCs for the environment based on the provisional PBT assessment. A more detailed summary of available data is given for dibenz[*a*,*j*] acridine in Table 7 to exemplify the methodology. Details on results and rationale are presented elsewhere (UBA 2012).

DISCUSSION AND CONCLUSIONS

The overall results of this study clearly show that regulatory risk management of homocyclic PAH does not fully address the environmental concern created by sPACs in UVCBs from coal or crude oil (see later discussion); therefore, they must be assessed by themselves. Based on a list of 443 sPACs of potential concern, application of QSAR, literature data on occurrence, and analytical data from this study, we propose 15 substances as potential priority sPACs (see Table 3) because of their occurrence in products and provisional fulfillment of PBT or vPvB properties according to REACH. Although we evaluated a high number of analytical publications for different matrices, we cannot fully rule out that frequency of occurrence could be higher for some substances if the scope of analysis would have been broader for certain matrices in the literature. This implies

Table 5. Benzo[*b*]naphtho[2,1-*d*]thiophene as indicator substance for sPAC: Suggested extrapolation factor and range by extrapolation on total content of priority sPAC in household products and rubber processing oils

Household products	Flip-Flop	Rubber boot	Spare tire	Spare tube	Mean	Range
2,1-BNT/total 15 priority sPAC	42.2%	23.7%	40.1%	40.8%	36.7%	
Deduced extrapolation Factor on total 15 priority sPAC	2.4	4.2	2.5	2.5	2.9	
Processing oils	DAE	TDAE	RAE	MES	Mean	
2,1-BNT/total 15 priority sPAC	50.4%	25.0%	25.7%	33.3%	33.6%	
Deduced extrapolation Factor on total 15 priority sPAC	2.0	4.0	3.9	3.0	3.2	
Mean extrapolation factor on total priority sPAC from all products and processing oils. Range: relation of highest to lowest single extrapolation factor					3.1	±1.1

Flip-flop: flip-flop sandals (soft-PVC).
sPAC = semipolar polycyclic aromatic compounds; MES = medium extracted solvate; RAE = residual aromatic extracts; DAE = distillate aromatic extracts; TDAE = treated distilled aromatic extracts; PVC = polyvinyl chlorine.

Table 6. Data summary for priority sPAC: Provisional PNECaqua, PBT-assessment, and environmental classification based on available experimental data supplemented by QSAR results, mutagenic and carcinogenic properties, as well as most relevant data gaps

Chemical name	PNEC [ng/L]	PBT-assessment	Environmental Class.	Mutagenic and Carcinogenic properties[1)]	Most important data gaps
Benz[*a*]acridine	15	– (P, T, not B)	A 1, M: 10; H400 - C 1; H410	M in vitro: +?	–
				M in vivo: nd	
				C: -?	
Benz[*c*]acridine	6.9	– (P, T, not B)	A 1, M: 100; H400 - C 1; H410	M in vitro: +?	BCF
				M in vivo: nd	
				C: +?	
Benzo[*a*]phenanthridine (mixed isomers)	920	– (P, not T, not B)	A 1, M: 1; H400 - C 1; H410	M in vitro: nd	Any experimental data
				M in vivo: nd	
				C: nd	
Dibenz[*a,j*]acridine	120	PBT	A 1, M: 1; H400 - C 1; H410	M in vitro: +?	Fish, daphnia
				M in vivo: +	
				C: +	
Dibenz[*a,h*]acridine	120	PBT	A 1, M: 1; H400 - C 1; H410	M in vitro: -?	Data on ecotoxicity
				M in vivo: +	
				C: +	
13H-Dibenzo[*a,i*] carbazole	140	vPvB	A 1, M: 1; H400 - C 1; H410	M in vitro: -	Data on ecotoxicity
				M in vivo: +	
				C: +?	
7H-Dibenzo[*c,g*]carbazole (mixed isomers)	140	PBT	A 1, M: 1; H400 - C 1; H410	M in vitro: +	Data on ecotoxicity
				M in vivo: +	
				C: +	
Benzo[*b*]naphtho[1,2-*d*] thiophene	220	– (P, B, not T)	A 1, M: 1; H400 - C 1; H410	M in vitro: -	Fish, algae
				M in vivo: nd	
				C: nd	
Benzo[*b*]naphtho[2,1-*d*] thiophene	220	– (P, B, not T)	A 1, M: 1; H400 - C 1; H410	M in vitro: +?	Fish, algae, (daphnia)
				M in vivo: nd	
				C: +?	
1-Methylbenzo[*b*]-naphtho[2,1-*d*] thiophene (mixed isomers)	73	PBT	A 1, M: 10; H400 - C 1; H410	M in vitro: +	Data on ecotoxicity

Table 6. (*Continued*)

Chemical name	PNEC [ng/L]	PBT-assessment	Environmental Class.	Mutagenic and Carcinogenic properties[1)]	Most important data gaps
				M in vivo: nd	
				C: nd	
Benzo[*b*]naphtho[2,3-*d*] thiophene	190	– (P, B, not T)	A 1, M: 1; H400 - C 1; H410	M in vitro: -	Data on ecotoxicity
				M in vivo: nd	
				C: nd	
Phenanthro[4,5-*bcd*] thiophene	510	– (P, B, not T)	A 1, M: 1; H400 - C 1; H410	M in vitro: -	Data on ecotoxicity, (P)
				M in vivo: nd	
				C: nd	
2-Methyldibenzo-thiophene (mixed isomers)	200	– (B, not P, not T)	A 1, M: 1; H400 - C 1; H410	M in vitro: -	Data on ecotoxicity, (BCF), (P)
				M in vivo: nd	
				C: nd	
Benzo[*b*]naphtho[1,2-*d*] furan (mixed isomers)	420	– (P, B, not T)	A 1, M: 1; H400 - C 1; H410	M in vitro: nd	Data on ecotoxicity, (P)
				M in vivo: nd	
				C: nd	
Benzo[*b*]naphtho[2,3-*d*] furan (mixed isomers)	420	– (P, B, not T)	A 1, M: 1; H400 - C 1; H410	M in vitro: nd	Data on ecotoxicity, (P)
				M in vivo: nd	
				C: nd	

Data gaps: brackets = property/data on organism to be confirmed by reliable exp. data.
[1)]: = negative; +? = questionable positive; −? = questionable negative; + = positive; A = acute; C = chronic; M = M-factor; P = persistent; B = bioaccumulative; T = toxic; nd = no data; sPAC = semipolar polycyclic aromatic compounds; PNEC = predicted no-effect concentration; PBT = persistence, bioaccumulation and toxicity; QSAR = Quantitative Structure Activity Relationship.

the final possible consequence of a slightly different set of priority sPACs.

Concluding from analytical results, 2,1-BNT would be a suitable indicator substance for the 15 priority sPACs in most samples. Overall, the study results prove the need for additional regulatory steps on sPACs.

Selection strategy: Evaluation regarding PBT properties

Retrieved experimental data were used to check for predictivity of the applied QSAR screening procedure. All of the selected 15 priority sPACs were screened as toxic and bioaccumulative and all but 1 compound as persistent. Comparison with experimental data showed that the rate of true positives (sensitivity) is 75% for toxicity, 85% for bioaccumulation potential, and 93% for persistence.

Additionally, the predictions on toxicity of the substances on the list of identified sPACs (443 compounds) were compared with legal classifications available for 9 compounds and another QSAR-based classification by the Danish EPA (2010) that was available for a further 163 of the 443 compounds. Taking the Danish data (employing different QSAR models) together with legal classifications as a reference (with risk phrases R50 or R50/53 as criterion for toxicity), the employed screening approach using ECOSAR would result in 31% false negatives and 6% false positives. Thus, the Danish approach seems to be more conservative compared with ECOSAR-based screening. "Advisory classifications" that existed for 8 of the 15 priority sPACs were fully in line with our QSAR screening results. The results gained from these comparisons support the QSAR-based selection strategy. To test the selection methodology further, a fraction of the substance list from step 2 ($n = 49$) was tested against the CATALOGIC QSAR model. The predictions were generally more conservative: All BIOWIN-based predictions were confirmed, but 21 further substances were predicted to be persistent. The EPISUITE calculations had predicted them to be only BT, or not persistent.

In conclusion, the employed method seems to be useful to identify PBT candidates. Because this evaluation of QSAR data is based on just 14 compounds with existing experimental data for at least 2 properties (1 compound without any data on

Table 7. Data summary for priority sPAC dibenz[a,j]acridine as an example for data collected in step 6

Ecotoxicity	Experimental data on fresh water algae toxicity (EC50 > 0.4 mg/L); QSAR (daphnid): LC50 (48 h) 0.123 mg/L
Bioaccumulation	Experimental log $K_{OW} = 5.63$
Persistence	Half-life >160 d (soil) extrapolated from experimental data
Genotoxicity, carcinogenicity	Reasonably anticipated to be human carcinogen (data, potency):
Tests on mutagenic properties: questionable positive in vitro, positive in vivo. Tests on carcinogenicity were positive.	
Provisional PNEC aquatic	Assessment factor 1000 (acute test data), QSAR derived effect concentrations: $PNEC_{aqua} = 0.12$ μg/L
Provisional SVHC classification	P (experimental data soil) – B (log $K_{OW} > 4.5$) – T (carcinogenic)
Provisional environmental classification	H400, very toxic to aquatic life
H410, very toxic to aquatic life with longlasting effects	
Occurrence	Qualitatively: in tar fractions (2), in pitch (1), environmental (3)
	Experimental (BIU):
	Coal tar pitch: 310.8 mg/kg; Spare tire: 10.4 mg/kg; Spare tube: 4.9 mg/kg; others: below LOQ

sPAC = semipolar polycyclic aromatic compounds; QSAR = Quantitative Structure Activity Relationship; EC50 = median effective concentration; LC50 = median lethal concentration; PNEC = predicted no-effect concentration; SVHC = substances of very high concern; BIU = Biochemisches Institut für Umweltcarcinogene; LOQ = limit of quantification.

persistence, bioaccumulation potential, or toxicity), it may give only a rough impression on usability of the applied QSAR screening methodology.

Analytical results and indicator substance approach

As summarized in Table 3, all of the 15 priority sPACs could be quantified in at least 2 analyzed samples. Twelve priority sPACs were quantified in 5 to 11 of the matrices analyzed. Thus, besides the QSAR-based screening on PBT properties, the screening on relevance with regard to actual occurrence proved to be successful.

Coal tar pitch was included as a kind of positive reference material, because it is known to contain a multitude of heterocyclic compounds in high concentrations. Indeed, all priority sPACs could be quantified in it, with the exception of 1-methyl-benzo[*b*]naphtho[2,1-*d*]thiophene. This fits to the observations that, in contrast to petrogenic PAH, in which often alkyl PAHs are more abundant than the parent compounds, in pyrogenic samples the parent PAHs dominate in relation to alkyl PAH, and alkylation extent is much more limited (Neff et al. 2005).

Whereas modern processing oils (TDAE, (T)RAE, MES) contain a substantially lower level of PAH compared with highly aromatic oils (DAE), a relative enrichment of priority sPACs is observed in these oils, probably because of the treatment methodology aimed to reduce the DMSO extract according to the IP 346 method. This is important for producers, because the mass percent organic compounds contained in the extract correlates with carcinogenicity, and oils containing less than 3% are regarded to keep the limit values set for benzo[*a*]pyrene (1 ppm) and for the sum of 8 priority European Union PAHs (10 ppm) according to REACH Annex XVII and need not be labeled. Besides PAH, also heterocyclic PAC and naphthenes are extracted by DMSO. Nevertheless, priority sPAC content was always lower or at maximum roughly equal to PAH content.

Because of its abundance and dominance over nearly all analyzed profiles, 2,1-BNT was proposed as a possible indicator substance for extrapolation to priority sPAC content. The extrapolation factor of 3.1 (range, ± 1.1) was derived from analysis of only 8 different matrices and therefore has to be considered preliminary. It may be used to estimate total priority sPAC concentration from 2,1-BNT for processing oils and for those consumer products in which these oils are used for production (mainly rubber products). It is not applicable to bitumen, furnace black, and coal tar pitch. No valid extrapolation on the relative composition of the sPAC content from thiaarenes, azaarenes, and oxaarenes is possible from the data because of the high variability of the latter 2.

Limitations of retrieved experimental data on PBT properties and preliminary PBT assessment

The remaining data gaps in regard to the ecotoxicity of priority sPACs are considerable. Not for a single compound could experimental data for 3 trophic levels be retrieved. Thus, a provisional assessment based on data for 1 or 2 trophic levels had to be carried out and the results compared with and complemented by the QSAR screening result. Whereas 1 result below the threshold value is sufficient for a positive classification as "toxic" within the PBT assessment, for a classification of "not toxic" data from at least 3 trophic levels are needed. QSAR proved to be a valuable tool for a first screening approach (see previous sections). However, results are essentially based on a nonspecific (narcotic) type of action (ECOSAR class neutral

organics), and thus predicted effect concentrations may be higher than real ones possibly would be (de Wolf et al. 2005). Therefore, complementation by QSAR data is in this case no substitute for experimental data and especially may underestimate chronic ecotoxicity.

For thiophene derivatives, a comparably high number of biodegradation studies are available. However, these were often performed as a technological approach to reduce the amount of sulfur-containing compounds from raw oil or oil fractions or with respect to enhanced attenuation. Thus, experimental conditions are often far from guideline tests and difficult to evaluate with respect to relevance for the environment. This resulted in a higher uncertainty for several compounds regarding the persistence assessment.

Because experimental data are often missing for certain endpoints, QSAR results based on EPISUITE were used as surrogates. These results are associated with considerable but not quantifiable uncertainty. Also, certain available experimental data points were not sufficiently reliable or were so inconclusive that further studies will be needed to draw definitive conclusions. Therefore, the PNECs, the assessment of PBT properties, and the classifications according to the Globally Harmonised System (GHS) summarized in Table 6 are only preliminary and tentative.

Outlook: Complementation of ecotoxicity database and indications from biomonitoring

As outlined, for many of the priority sPACs no data on ecotoxicity are available (see Table 6). If there are, these do not cover all 3 trophic levels and are restricted to acute tests. To assess the ecotoxicological properties of these substances definitely, further tests are needed. A general drawback for ecotoxicity tests for priority sPACs is that the log K_{OW} is proportional to the potential for bioaccumulation up to a maximum for log K_{OW} of slightly below 6 (Arnot and Gobas 2003; Arnot and Gobas 2006). The log K_{OW} is inversely proportional to water solubility. This implies that for the more lipophilic priority sPACs acute toxicity tests might result in no observable effects because of the low concentration in the test water, and only chronic tests or tests with benthic organisms (because of sediment sorption) would be suited to detect toxic effects. These tests, however, are lengthy and expensive to perform. In this respect, note that the fish embryo toxicity test, for which an International Organization for Standardization guideline (DIN 38415-6) and an Organisation for Economic Co-operation and Development guideline (236, adopted July 2013) are available, may be performed in a modified way to test toxicity of sediments (Hollert et al. 2003; Rocha et al. 2011).

From the literature search, some publications on biomonitoring also were identified. Monitoring data on sPACs in freshwater and marine organisms points to the relevance of alkylated core structures for accumulation in biota. At the same time, only few data in regard to detection in samples and even less in regard to ecotoxicological tests are available for such alkylated sPACs. Therefore, we must also address this knowledge gap.

Implications for regulatory consequences under REACH regulation

The sPACs are a substance group of potential relevance because of their properties and because of environmental exposure, which is not yet explicitly accounted for in the legislation of chemicals. Before this is done, whether the current legislative restrictions on PAH already implicitly cover priority sPACs has to be assessed. This assessment will be done separately for human toxicity and genotoxic and/or carcinogenic effects as well as ecotoxicological effects in environmental media.

Subsumption human toxicology. Substantial doubts exist concerning the implicit coverage of sPACs in the current limit values for PAH in process oils used for tire production and tires themselves under REACH. This holds true especially for the explicit limits on PAH for process oils and tires (poor correlation of sPAC content with PAH content) and the International Organization for Standardization 21461 method applied for tires to conclude indirectly on compliance with PAH limits. This nuclear magnetic resonance (NMR)-based method relies on the ratio of bay protons to total protons (i.e. a relative value) and the structure–activity relationship that bay region PAHs are associated with high genotoxic and/or carcinogenic potency. In the case of sPACs, hetero-atoms in the aromatic ring system may lead to false-negative or false-positive results concerning bay protons because of possible alterations in shielding effects. Further work would be required to demonstrate applicability of the method and the associated cutoff value for sPACs. We have more confidence in the 3% weight limit for the DMSO extract obtained by the IP 346 method used to conclude on compliance with PAH limits, because PAHs as well as sPACs are extracted with DMSO. Considering these uncertainties and possible methodological limitations (International Organization for Standardization 21461 method for tires in regard to sPAC), setting an additional limit value for 1 or more important representative(s) of sPAC should be considered. The suggested indicator compound 2,1-BNT could be suited for this. Further work, however, needs to be done to validate the representativeness of this suggested indicator for the priority sPACs and, even more, to establish a sufficiently conclusive database for toxicological assessment of the compound group to enable designating qualified limit values.

Subsumption ecotoxicology. For assessing the relevance of the 15 priority sPACs and thus the possible regulatory gap with regard to organisms exposed predominantly over the water phase or the sediment, solubility and partitioning behavior of sPACs compared with PAHs are decisive. For sPACs, higher water solubility is to be expected compared with their homocyclic PAH analogs, and relative concentrations in aquatic media are expected to be considerably different from determined concentrations detected in product samples. Overall, however, only few experimental data are available. In addition, the environmental toxicity of the priority sPACs is not yet clear and probably underestimated, because often data on acute toxicity are missing, and no chronic toxicity data are available at all. Nonetheless, relevant environmental exposure to sPACs appears to occur apart from creosote-contaminated sites: Environmental determinations of organic contaminants in several media were recently performed by the Swedish Environmental Research Institute (Brorström-Lundén et al. 2010). Five of the 15 priority sPACs (7H-dibenzo[*c*,*g*]carbazole, benz[*a*]acridine, dibenz[*a*,*h*]acridine, 2,1-BNT, and benzo[*b*]naphthofuran) were included in the work and were indeed detected in environmental media, such as in urban soil and sediment, where concentrations in the μg/kg

(dry weight) range were found, and even higher concentrations are reported for sludge. Some of the compounds also were detected in background areas (areas of assumed low contamination).

The assessment of the gathered data on persistence, bioaccumulation potential, and toxicity leads us to the conclusion that at least 5 of the 15 priority sPACs are potential PBT(4)/vPvB(1) substances. Without supplemental information, no final recommendation of regulatory consequences with regard to the environment can be given at this point. However, no evidence allows assuming that current regulation on PAHs would implicitly protect the environment from potential effects by sPACs; the necessary information should be generated to address the potential very high concern of these substances.

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SUPPLEMENTAL DATA

Table S1: Analytical results for different matrices published in the literature and evaluated for sPAC

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