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Evaluation of the hazardous potential of semipolar polycyclic aromatic hydrocarbons with respect to environment and users and the need to potential regulatory activities

by

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Abstract

The project addresses the identification and (eco-)toxicological characterisation of semipolar polycyclic aromatic compounds (PAC) in coal- and mineral oil-based substances of unknown or variable composition, complex reaction products or biological materials (UVCB) as well as possible regulatory implications under REACH.Available data from related former projects as well as published substance profiles were analysed and relevant compounds subjected to a QSAR (quantitative structure activity relationship) selection process on PBT (persistence, bioaccumulation, toxicity) properties. After comparison of these results with experimental data (including degradation pathways) 15 priority compounds out of initial 443 substances were identified and categorized with respect to substances of very high concern (SVHC) properties according to REACH. On the basis of this selection procedure 5 out of these 15 are possible SVHC candidates. Tests are proposed to validate these results. The analytical part of the project included the quantification of the 15 priority compounds in consumer products and further relevant matrices (11 samples in total), where they were detected in varying concentrations. According to this limited range of examinations benzo[b]naphtho[2,1-d]thiophene may be a suitable indicator substance for the total content of the 15 priority semipolar PAC. Based on the information gained within this project it seems questionable whether the regulation of the concentration limits of polycyclic aromatic hydrocarbons in extender oils and tyres (REACH, Annex XVII, No. 50) sufficiently (indirectly) includes the semipolar PAC. For a decision whether or not an additional regulation for semipolar PAC is required with respect to (eco-)toxicological aspects, the information based on QSAR data has to be corroborated by the results of the proposed tests.

Kurzbeschreibung

Das Projekt befasst sich mit der Identifizierung und (öko-)toxikologischen Charakterisierung von semipolaren polyzyklischen aromatischen Kohlenwasserstoffen (semipolar polycyclic aromatic compounds, PAC) in steinkohle- und mineralöl- basierten UVCB (Substances of unknown or variable composition, complex reaction products or biological materials) sowie möglichen regulatorischen Konsequenzen unter REACH. Bereits veröffentlichte Daten aus Projekten zu diesem Thema sowie publizierte Stoffprofile wurden analysiert und relevante Substanzen einem QSAR- (Quantitative structure activity relationship)-Auswahlverfahren auf PBT- (Persistenz, Bioakkumulierbarkeit, Toxizität)-Eigenschaften unterzogen. Nach Abgleich mit experimentellen Daten (auch zum Abbauverhalten) wurden aus ursprünglich 443 insgesamt erfassten Substanzen 15 prioritäre Verbindungen identifiziert und eine Kategorisierung auf SVHC- (Substances of very high concern)-Eigenschaften nach REACH vorgenommen. 5 Substanzen kommen nach dieser Methodik als mögliche SVHC in Frage. Zur Absicherung wurden Vorschläge für weitere Tests erarbeitet. Der analytische Teil des Projektes umfasste die Bestimmung dieser 15 Substanzen in Verbraucherprodukten und weiteren relevanten Matrices. Alle prioritären Verbindungen wurden dabei in unterschiedlichen Konzentrationen in den insgesamt 11 untersuchten Proben nachgewiesen. Als geeignete Indikatorsubstanz für die Abschätzung des Gesamtgehaltes an den 15 prioritären semipolaren PAC kommt auf Basis der begrenzten Untersuchungen Benzo[b]naphtho[2,1-d]thiophen in Frage. Es ist nach dem Kenntnisstand des Projekts fraglich, ob die gegenwärtige Regulation der Höchstgehalte von polyzyklischen aromatischen Kohlenwasserstoffen in Weichmacherölen und Reifen (REACH Annex XVII, Nr. 50) die semipolaren PAC hinreichend (indirekt) mit erfasst. Ob unter human- und ökotoxikologischen Aspekten die zusätzliche Regulation auf semipolare PAC erforderlich ist, könnten die vorgeschlagenen Untersuchungen zur Erhärtung der Hinweise auf Basis von QSAR-Daten klären.

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List of abbreviations

2,1-BNT	Benzo[b]naphtho[2,1-d]thiophene
BTEX	Benzene, toluene, ethylbenzene, and xylenes
DAE	Distillate Aromatic Extracts, process oil, high aromatics
DNEL	Derived No Effect Level
ECHA	European Chemicals Agency
GC/MS (SIM)	GC/MS with Selected Ion Monitoring
GC/MS	Gas Chromatography coupled with Mass Spectrometry
GFS	Geringfügigkeitsschwellenwert (i.e. de minimis level for ground water contaminants)
GOW	Gesundheitlicher Orientierungswert (i.e. healt based orientation value)
HET	Heterocycles
HET-PAC	Heterocyclic polyaromatic compounds
KORA	“Kontrollierter natürlicher Rückhalt und Abbau von Schadstoffen bei der Sanierung kontaminierter Grundwässer und Böden”, BMBF-Förderschwerpunkt (www.natural-attenuation.de)
MCS	Multi-constituent substance
MCS	Multi-constituent substance: defined by its quantitative composition, in which more than one main constituent is present in a concentration ≥ 10% (w/w) and < 80% (w/w) as a result of the manufacturing process
MES	medium/mildly extracted solvate, process oil, reduced aromatics
PAH	Polycyclic aromatic hydrocarbons
PAK	Polyzyklische Aromatische Kohlenwasserstoffe
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
QSARs	Quantitative structure activity relationships
RCR	Risk Characterization Ratio
RAE	Residual Aromatic Extract, process oil, reduced aromatics
SPE	Solid Phase Extraction
TDAE	Treated Distilled Aromatic Extracts, process oil, reduced aromatics
TEF	Toxicity Equivalency Factor
UVCB	Substance of **u**nknown or **v**ariable composition, **c**omplex reaction products or **b**iological materials
UVCB	Substance of **u**nknown or **v**ariable composition, **c**omplex reaction products or **b**iological materials

1 Summary

Semipolar Polycyclic Aromatic Hydrocarbons (PAH) are a heterogeneous group with heteroatoms in the aromatic ring systems and / or substituents including heteroatoms. Therefore they are named semipolar aromatic compounds hereafter (semipolar PAC). They are frequently found in association with the homocyclic PAH in substances of **U**nknown or **V**ariable composition, **C**omplex reaction products or **B**iological materials (UVCB) from coal or crude oil and derived products thereof. Their toxicological characterization is often insufficient, in spite of the fact that there are indications of toxic properties comparable to their homocyclic analogues.

The aim of this work is to gather and evaluate information about the relevance, occurrence and (eco)toxicological properties of semipolar polycyclic aromatic compounds in UVCB with respect to potential regulatory activities (e.g. restrictions according to Annex XVII, REACH). Fuels are exempt from this project as these are already regulated apart from REACH regulation. Also semipolar PAC contained in tobacco smoke are not a relevant topic in this work.

Evaluation of existing data

In a first step previous work from KORA and LAWA on semipolar PAC was reviewed and summarized. Differences to the current project were elaborated, compounds identified in these projects evaluated for possible availability and relevance of new data, and finally an initial *pool of identified semipolar PAC* of 159 substances was created based on this earlier work.

Evaluation of substance profiles

To broaden this *pool of identified semipolar PAC* and determine their relevance in regard to occurrence in relevant UVCB-matrices, compound profiles were retrieved from the literature. Identified matrices were described and characterized in regard to their PAC content. The identified profiles did not include end-products, but intermediates or by-products of the coal and crude-oil refinery processes, e.g. coal tar, fractions of coal tar distillation or oil distillates, as well as environmental samples. They were heterogeneous in respect to the range of examined substances and qualitative/quantitative determination. In total, semipolar PAC were identified in 118 profiles and the compounds found in these profiles were matched with the initial KORA/LAWA based *pool of identified semipolar PAC*. The resulting cumulative list of compounds (*pool of identified semipolar PAC*) encompasses 443 semipolar PAC.

Characterisation with respect to possible PBT properties using QSAR

To enable fast prioritization of compounds, a QSAR based screening methodology on PBT properties (persistency, bioaccumulation and toxicity) was developed and cut-off criteria successfully “calibrated” using semipolar PAC from REACH Annex XIV support documents with experimental data on PBT properties available. Concurrently, the *pool of identified semipolar PAC* (443 compounds) was subjected to this QSAR based selection. This resulted in an *extended QSAR selection of critical PAC* containing 154 compounds of concern in relation to PBT criteria (94 for all three criteria together, and in addition 57 for the combination BT and 3 for the combination PB; 0 for TP; see Figure S-1).

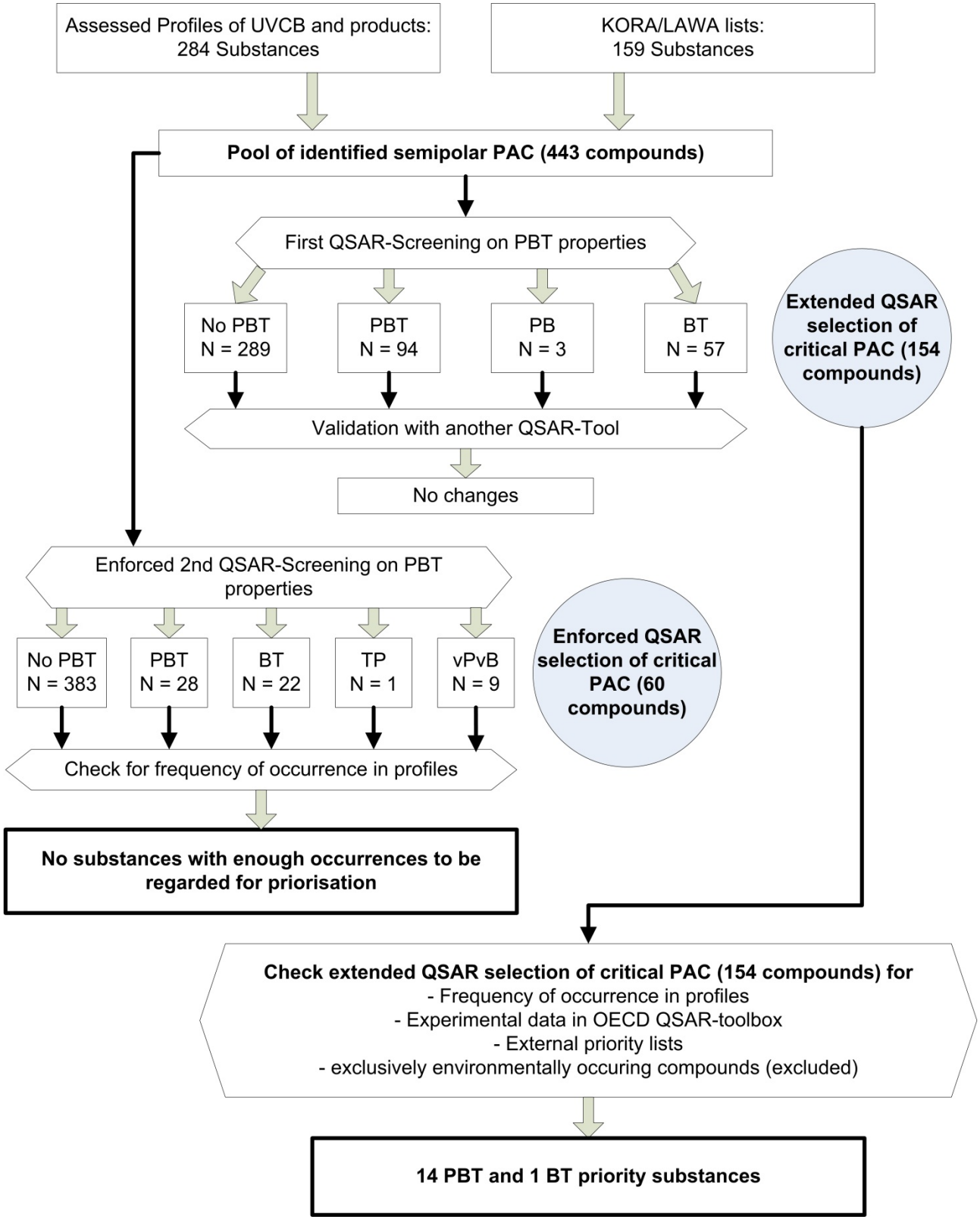


Figure S-1: Selection process of PBT relevant semipolar PAC under consideration of their relevance of occurrence in analysed sample profiles (for details, see section 5.2.1 to 9)

This list was reduced by enforcing the screening criteria, resulting in the *enforced QSAR selection of critical PAC* containing 60 compounds with predicted PBT-properties (28 PBT, 22 BT, 1 TP and 9 vPvB candidates;

see Figure S-1). However, these compounds revealed to occur only rarely in the analysed profiles (usually in 1 or 2 profiles, with a maximum of 6). Therefore the *extended QSAR selection of critical PAC* (154 compounds) was screened for compounds which are listed in at least 6 profiles resulting in a list of 11 *priority compounds*.

To increase the probability of capturing the most important semipolar PAC in the list of priority compounds, additional indicators for relevance were used. In particular, Annex XIV-support documents under REACH and REACH registration dossiers were checked and the prevalence of experimental data on ecotoxicity and environmental fate as well as occurrence on certain PBT-priority lists of other countries rated as relevance criteria. Thus, additional substances were added to the group of *priority semipolar PAC* while some were removed due to aspects of relevance in products yielding a final list *of priority semipolar PAC* of 15 substances (see Figure S-1).

Supporting information from published literature and further sources

In an exhaustive literature research experimental data on PBT properties were retrieved for these 15 compounds to verify the PBT-classifications based on the applied QSAR-selection strategy by real data. Mainly due to considerable data gaps not anyone substance of the priority list could be definitely classified as PBT according to REACH criteria. However, 6 compounds fulfilled at least two of the three PBT-criteria according to REACH. For only 1 compound (benz[a]acridine) experimental data are sufficient to conclude that these are not bioaccumulative. Only one compound most probably does not fulfil T-criteria according to REACH and additionally is clearly not persistent (2-methyldibenzothiophene and isomers).

Comparison and reconciliation of QSAR results with experimental data

Retrieved experimental data on ecotoxicity and environmental fate were used additionally to evaluate the QSAR-based selection-methodology. QSAR-results on T, B and P proved to be appropriate in 75%, 85% and 93%, respectively. Therefore, the applied method is applicable as screening-tool for determination of PBT-candidate compounds. Additional verification by other QSAR-models essentially corroborated this.

Information on occurrence in biota (bio-monitoring)

Identified data from bio-monitoring for the 15 priority semipolar PAC are analyzed. Available data are very limited and therefore not representative. Further, quantitative conclusions are hampered by mostly missing data on environmental concentrations. Totally, from the 15 priority semipolar PAC three thiaarenes, one oxaarene and one azaarene were detected/quantified in crayfish, fish and mussels. The results of several publications on dibenzothiophenes of different alkylation degree point to higher enrichment and persistence in organisms compared to the non-alkylated core structure. This could be due to higher lipophilicity and/or slower metabolism caused by the alkyl residue(s) and beyond the 15 priority semipolar PAC might indicate a generally special relevance of alkylated core structures in terms of PBT properties. However, for these compounds generally no experimental data are available.

Degradation pathways

Aerobic degradation pathways for the 15 priority semipolar PAC are reviewed. Literature information is essentially limited to the thiophene derived heterocyclic PAC. Known degradation pathways are described and conclusions drawn on possibly hardly degradable intermediates resulting from these pathways.

Categorisation in respect to SVHC properties

In respect to the SVHC-definition according to REACH including germ cell mutagenicity and carcinogenicity category 1A and 1B in respect to fulfilment of the T-criterion, a literature research was conducted for the 15 priority semipolar PAC in respect to these properties. For each substance available data were described and summarized.

Comprehensive substance profiles were established now containing also data on genotoxicity and carcinogenicity. Data gaps were closed by “read across” and QSAR-results, such that provisional aquatic PNEC could be derived, a provisional classification in regard to environmental hazards could be conducted and a provisional SVHC-assessment could be performed. Concluding from that, the following five substances were provisionally characterized as SVHC (in part exclusively based on QSAR-derived data):

- Dibenz(a,j)acridine (PBT)
- Dibenz(a,h)acridine (PBT)
- 7H-Dibenzo(c,g)carbazole (PBT)
- 1-Methylbenzo(b)-naphtho(2,1-d)thiophene (PBT)
- 13H-Dibenzo(a,i)carbazole (vPvB)

Supplemental considerations in respect to PBT properties

To check for possibly relevant structural representatives of semipolar PAC not covered by the 15 priority compounds, the extended QSAR selection of critical PAC (154 compounds) was revisited: A plausibility check was undertaken to determine if there were indications that important classes of compounds had been missed by our selection strategy leading to the 15 priority semipolar PAC. To this end, chemical expertise and knowledge on formation and occurrence as well as biological activities of PAC (mainly in regard to genotoxicity) was applied. In conclusion, there are no clear indications that important compounds / compound groups have been missed. Rather, with the current knowledge on occurrence and substance properties the 15 semipolar PAC seem to include or represent the most important compounds / groups of compounds. However, some uncertainty remains as no systematic analysis could be performed within the scope of the project and conclusions are often based on theoretical considerations.

Moreover, structure-activity relationships in regard to PBT-properties were analyzed. In conclusion, general rules derived from QSAR-results could often be confirmed by experimental data as long as narcotic mechanisms of toxic action are involved. Specific or reactive toxicity (e.g. photoenhanced toxicity) is generally not predicted by QSAR, can however play an important role for certain semipolar PAC leading to unexpected results. Also disabling of specific degradation pathways by e.g. sterical hindrance by alkyl substituents is not sufficiently mirrored by QSAR-predictions. General tendencies however seem to be very well represented by the applied QSAR models.

Suggestions for ecotoxicity testing

As for the 15 priority semipolar PAC experimental data on ecotoxicity are often missing or not sufficient for assessment, ecotoxicity tests are recommended. Compounds are ranked in regard to the need for ecotoxicity tests. For the four substances of highest rank, critical parameters for ecotoxicity tests are explained and conclusions drawn with regard to test design and analytical monitoring.

Analytical quantification in household products and further relevant matrices

In the analytics part of the project conducted at the Biochemical Institute for Environmental Carcinogens Prof. Dr. Gernot Grimmer-Foundation (BIU) consumer products (4), processing oils (4), coal tar bitch, bitumen and furnace black were analysed for the 15 *priority semipolar PAC*. In parallel, 25 different PAHs ("Grimmer PAH" and 16 EPA-PAH) were quantified in the different matrices. An unavailable reference compound (1-methylbenzo[*b*]naphtho[2,1-*d*]thiophene) could be synthesized in isomer pure form. An important aspect of this work was the the question of interdependency of the prevalence of semipolar PAC and (rather nonpolar) PAH and, in addition, if quatitative date on occurrence in relevant matrices would confirm the selection of 15 priority semipolar PAC which was in part based on (very limited) data on occurrence from the literature.

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

The two priority oxaarenes benzo[*b*]naphtho[1,2-*d*]furan (1,2-BNF) and benzo[*b*]naphtho[2,3-*d*]furan (2,3-BNF) 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 two oxaarenes are below LOQ in bitumen and furnace black and in the extender oils only in DAE both isomers occurred in the upper ppb range whereas concentrations were low or below LOQ for PAH-reduced TDAE, MES and RAE.

The 7 priority azaarenes and also the examined 10-azabenzo[*a*]pyrene were found in high concentrations (ppm range) in coal tar pitch and a spare tyre from the do-it-yourself market as well as in the corresponding tyre 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 four of the priority azaarenes could be determined in the higher ppb range.

Considerations on possible indicator substances

One objective of the project was to analyze available analytical data for (a) possible indicator substance(s) which could be used to extrapolate on the content of semipolar PAC. Our work was essentially restricted to experimental data on occurrence of the 15 priority semipolar PAC in consumer products, processing oils, coal tar bitch, bitumen and carbon black gained from the analytical data collected within this project (BIU). That was so because often analytical profiles from the literature were qualitative only and target compounds in these profiles were much too heterogeneous to be comparable. In conclusion, the concentration of all determined PAH or the 16 EPA-PAH in particular are not suitable to extrapolate to the total content of the 15 priority semipolar PAC. Based on these analyses, the so-called Grimmer-PAC benzo[b]naphtho[2,1-d]thiophene (2,1-BNT) is suggested as indicator compound to extrapolate on total priority semipolar PAC content. This compound is, however, not suitable as indicator compound for matrices furnace black and bitumen. For processing oils and for those consumer products where these oils are used for production (mainly rubber products), an extrapolation factor of 3.1 (± 1.1, i.e. the relation of highest to lowest single extrapolation factor of only 8 analyzed matrices) on the concentration of 2,1-BNT [mg/kg] is derived. It is emphasized that this extrapolation factor is based and restricted to the 15 priority semipolar PACs identified and analyzed within this project. From qualitative analysis of literature profiles it was shown that 2,1-BNT could be used

also as a qualitative indicator substance in regard to high probability of simultaneous presence of other priority semipolar PAC where this indicator substance had been detected.

Relevance of semipolar PAC in respect to regulatory requirements under REACH

A further central point of the current project was to analyse, if semipolar PAC would be a group of compounds with possible special relevance currently not accounted for in chemicals' legislation and the concurrent need to impose legislative steps for their regulation; or rather, current legislative restrictions on PAH would implicitly cover semipolar PAC. This had to be separately assessed for human toxicity and genotoxic/cancerogenic effects on the one hand and ecotoxicological effects in environmental media on the other.

Subsumption human toxicology: There are substantial doubts concerning the implicit coverage of semipolar PAC in the set limit values for PAH in process oils used for tyre production and tyres itself under REACH. This holds true especially for the explicit limits on PAH for process oils and tyres and the ISO 21461 method applied for tyres to conclude indirectly on compliance with PAH limits. There is considerable more confidence in the 3% weight limit for the DMSO extract obtained by the IP346 method used to conclude on compliance with PAH limits, as in principle semipolar PAC are implicitly assessed. Considering these uncertainties and possible limitations of the currently applied ISO 21461 method for tyres in regard to semipolar PAC, we recommend setting in addition to cut-off values for PAH also a regulatory limit value for one or more important representative(s) of semipolar PAC. The suggested indicator compound benzo[b]naphtho[2,1-d]thiophene could be well suited for this. The content of semipolar PAC in tyres is obviously dominated by the process oil because the substance profile is similar in both. Further work however needs to be done to validate the representativeness of this suggested indicator for the priority semipolar PAC and, even more, to establish a sufficiently conclusive database for toxicological assessment of the compound group to enable one to designate qualified limit values.

Subsumption ecotoxicology: For assessing the relevance of the 15 priority semipolar PAC and thus the possible regulatory gap in regard to organisms exposed predominantly over the water phase (or the sediment) solubility and partitioning behaviour of semipolar PAC compared to PAH are decisive. For semipolar PAC higher water solubility is to be expected compared to their homocyclic PAH-analogues and relative concentrations in aquatic media are expected to be considerably different from determined concentrations in matrices. Experimental data however are mostly not available. In addition, their relevance in regard to environmental toxicity is still not clear and may be underestimated, as often data are lacking and generally no chronic toxicity data are available. Without this supplemental database, no recommendation of regulatory consequences can be given in regard to ecotoxicity of semipolar PAC.

Combination effects

A final, briefly discussed section is mixture toxicity as in the analytics part of the project as a rule, several and up to all 15 priority semipolar PAC could be determined in parallel within a matrix. Identified literature data on mixture toxicity of semipolar PAC were summarized. As a default, it is suggested to assume concentration additivity.

2 Zusammenfassung

Semipolare Polyzyklische Aromatische Kohlenwasserstoffe (PAK) stellen eine heterogene Gruppe von Substanzen dar, bei denen Heteroatome entweder im aromatischen Ringsystem oder auch an Substituenten lokalisiert sind. Sie werden im Folgenden auf Basis des englischen Begriffs „Semipolar Polycyclic Aromatic Compounds (PAC)“ als semipolare PAC bezeichnet. Diese chemischen Verbindungen treten häufig vergesellschaftet mit homozyklischen PAK in steinkohle- bzw. mineralölbasierten Stoffgemischen (Substance of **U**nknown or **V**ariable composition, **C**omplex reaction products or **B**iological materials, UVCB) und darauf basierenden Produkten (Gemischen/ Erzeugnissen) auf. Sie sind im Vergleich zu den homozyklischen Verbindungen toxikologisch oft nur ungenügend charakterisiert, obwohl Hinweise auf vergleichbare oder sogar höhere Toxizität dieser semipolaren Analoga vorliegen.

Ziel der vorliegenden Arbeit war es, Informationen zur Relevanz in Bezug auf Vorkommen und (öko)toxikologische Eigenschaften dieser Substanzgruppe auszuwerten. Diese Arbeiten erfolgten vor dem Hintergrund möglicher regulatorischer Aktivitäten unter der EU-REACH-Gesetzgebung (z.B. Beschränkung über Annex XVII, REACH). Ausgenommen waren in diesem Projekt Kraftstoffe (da anderweitig reguliert) sowie semipolare PAC in Zigarettenrauch.

Auswertung vorliegender Ausarbeitungen

In einem ersten Schritt wurden vorausgehende Arbeiten zum Thema analysiert und kurz zusammengefasst (KORA, LAWA). Methodische Unterschiede zum vorliegenden Projekt wurden herausgearbeitet, in diesen Projekten identifizierte Substanzen hinsichtlich ihrer Relevanz für diese Arbeit bewertet und für die wichtigsten dort identifizierten Verbindungen neuere Literatur hinsichtlich möglicher PBT-Eigenschaften (**P**ersistenz, **B**ioakkumulation, **T**oxizität) geprüft. Schließlich wurde – basierend auf den in diesen früheren Arbeiten identifizierten Stoffen – ein erster *Pool identifizierter semipolarer PAC* von 159 Substanzen gebildet.

Auswertung von Stoffprofilen

Um diesen *Pool identifizierter semipolarer PAC* zu verbreitern und deren Relevanz hinsichtlich Vorkommen in relevanten UVCB-Matrices zu untersuchen, wurden aus der Literatur entsprechende Stoffprofile ermittelt und in Hinsicht auf ihren Gehalt an semipolaren PAC charakterisiert. Es konnten keine auswertbaren Profile für (aktuelle) Endprodukte gefunden werden, vielmehr handelte es sich um Zwischenstufen/Nebenprodukte des Produktionsprozesses, z.B. Steinkohlenteer, Fraktionen der Kohleteerdestillation oder Rohöldestillate sowie Umweltproben. Hierbei zeigte sich in den ausgewerteten Stoffprofilen eine große Heterogenität im Hinblick auf den jeweiligen Untersuchungsumfang und die quantitative Erfassung. Der erste, auf KORA/LAWA basierende *Pool identifizierter semipolarer PAC* wurde um neue Substanzen aus insgesamt 118 Profilen von UVCB auf Kohle- und Erdölbasis sowie daraus hergestellten Produkten ergänzt, so dass ein erweiterter *Pool identifizierter semipolarer PAC* von insgesamt 443 Substanzen resultierte.

Charakterisierung hinsichtlich möglicher PBT-Eigenschaften mittels QSAR

Um eine schnelle Priorisierung in Hinsicht auf mögliche PBT-Eigenschaften zu ermöglichen, wurde eine auf QSAR basierende Selektionsmethodik erarbeitet und an semipolaren PAC aus den sogenannten REACH Annex XIV Support Dokumenten, zu denen dort experimentelle Daten zu PBT-Eigenschaften gesammelt sind, erfolgreich kalibriert und validiert. Im Folgenden wurde der *Pool identifizierter semipolarer PAC* von insgesamt 443 Substanzen diesem QSAR-basierten Auswahlverfahren unterzogen. Die resultierende *erweiterte QSAR-Auswahl kritischer semipolarer PAC* umfasst 154 semipolare PAC mit aus Struktur-

Aktivitätsbeziehungen abgeschätzten PBT-Eigenschaften (94 für alle 3 Kriterien, zusätzlich 60 für entweder P/B, P/T oder B/T; siehe Figure S-1 der Englischen Zusammenfassung).

Durch ein stringenteres QSAR-Auswahlverfahren konnte eine in der Stoffzahl deutlich reduzierte *verschärfte QSAR-Auswahl kritischer semipolarer PAC* mit 60 Stoffen (28 PBT-, 22 BT-, 1 TP- und 9 vPvB-Kandidaten) generiert werden (siehe Figure S-1 in der englischen Zusammenfassung). Allerdings wurden diese 60 semipolaren PAC in den 118 Stoffprofilen nur vereinzelt nachgewiesen (meistens 1-2, maximal 6 Nennungen). Aus diesem Grund wurde mit der *erweiterten QSAR-Auswahl kritischer semipolarer PAC* (154 Verbindungen) weitergearbeitet und als weiteres Kriterium die Vorkommenshäufigkeit in Stoffprofilen (≥ 6) berücksichtigt (Vorkommensrelevanz). Auf diese Weise konnten 11 prioritäre semipolare PAC ermittelt werden.

Ergänzende Informationen aus publizierten Daten und anderen Quellen

Um weitestgehend ausschließen zu können, wichtige semipolare PAC bei der Erstellung der Liste prioritärer Verbindungen zu übersehen, wurden weitere Indikatoren für die Relevanz hinzugezogen. Insbesondere wurden Hintergrunddokumente für die Ermittlung von zulassungspflichtigen Stoffen nach REACH Anhang-XIV sowie REACH-Registrierungsdossiers geprüft und das Vorhandensein experimenteller Daten zu Ökotoxizität und Umweltverhalten sowie die Listung von Stoffen auf PBT-Listen anderer Länder als Kriterien für Relevanz herangezogen. Unter Hinzunahme von auf diese Weise ermittelten Stoffen und unter Berücksichtigung der Relevanz für Produkte resultierten insgesamt 15 prioritäre semipolare PAC (siehe Figure S-1 der englischen Zusammenfassung).

Abgleich der QSAR-Ergebnisse mit experimentellen Daten

Im Weiteren erfolgte eine umfassende Literaturrecherche zu Ökotoxizität und Umweltverhalten der 15 Substanzen, um die auf den QSAR-basierten Selektionskriterien beruhenden Vorhersagen zu den PBT-Eigenschaften anhand experimenteller Daten zu überprüfen. Vor allem aufgrund der großen Datenlücken im Hinblick auf experimentelle Untersuchungen konnte keine der 15 Verbindungen aufgrund dieser Daten eindeutig als PBT-Stoff nach REACH bestimmt werden. Sechs dieser Verbindungen erfüllen aber mindestens 2 der drei PBT-Kriterien nach REACH, nur ein Stoff (Benz[a]acridin) konnte als sicher nicht bioakkumulativ bestimmt werden und lediglich 1 Stoff erfüllt sehr wahrscheinlich nicht die Kriterien für T nach REACH und ist sicher nicht persistent (2-Methyldibenzothiophen und Isomere). Die experimentellen Daten zur Ökotoxizität, Bioakkumulation und Persistenz wurden weiter dazu genutzt, die für den Selektionsprozess verwendete QSAR-Methodik zu überprüfen. Die QSAR-Ergebnisse zu T, B und P erwiesen sich in 75%, 85% bzw. 93% als zutreffend. Die QSAR-basierte Selektionsmethodik wird demnach als geeignet für die Ermittlung von PBT-Kandidaten eingeschätzt. Zusätzliche Überprüfung mittels anderer QSAR-Modelle bestätigte dies im Wesentlichen.

Informationen zum Vorkommen in Biota (Biomonitoring)

Identifizierte Literatur zum Biomonitoring der 15 prioritären semipolaren PAC wurde analysiert. Die Datenlage ist sehr begrenzt und daher nicht repräsentativ, auch sind quantitative Aussagen schwierig, da die Umweltkonzentrationen überwiegend nicht bestimmt sind. Von den 15 prioritären Verbindungen wurden insgesamt 3 Thiaarene, 1 Oxaaren und 1 Azaaren in Krebsen, Fischen und Muscheln nachgewiesen. Die Ergebnisse mehrerer Publikationen weisen anhand unterschiedlich hoch alkylierter Dibenzothiophene darauf hin, dass die Alkylierung zu einer relativ zur unsubstituierten Kernstruktur höheren Anreicherung und Persistenz führt. Dies könnte auf höhere Lipophilie und/oder verlangsamten Metabolismus zurückzuführen sein und deutet

über die 15 prioritären Verbindungen hinaus auf eine bezüglich PBT-Eigenschaften möglicherweise generell höhere Relevanz alkylierter semipolarer PAC hin. Allerdings sind für diese Verbindungen zumeist keine experimentellen Daten verfügbar.

Abbaupfade

Weiter wurde Literatur zu möglichen aeroben Abbauwegen der 15 prioritären Verbindungen gesichtet. Im Wesentlichen sind die Arbeiten beschränkt auf Thiophen-Abkömmlinge (Thiaarene). Bekannte Abbauwege werden beschrieben und mögliche schwer abbaubare Intermediate beschrieben.

Kategorisierung hinsichtlich SVHC-Eigenschaften

Im Hinblick auf die SVHC-Definition nach REACH, wonach sich die Toxizität auch über keimzellmutagene oder karzinogene Eigenschaften der Kategorien 1A oder 1B bemisst, wurde für die 15 prioritären Substanzen eine Literatur-Recherche hinsichtlich dieser Eigenschaften durchgeführt und die Datenlage für die einzelnen Stoffe zusammenfassend beschrieben.

In knappen Stoffprofilen, die auch Gentoxizität und Karzinogenität umfassen und bei deren Erstellung Datenlücken durch QSAR-Ergebnisse und „Read Across“ gefüllt wurden, erfolgte die Ableitung vorläufiger aquatischer PNECs, eine vorläufige Umwelteinstufung gemäß CLP-Verordnung (1272/2008/EC) sowie eine vorläufige SVHC-Beurteilung. Hierbei konnten folgende fünf Substanzen vorläufig (teilweise auch ausschließlich basierend auf QSAR-Daten) als mögliche SVHC charakterisiert werden:

- Dibenz(a,j)acridin (PBT)
- Dibenz(a,h)acridin (PBT)
- 7H-Dibenzo(c,g)carbazol (PBT)
- 1-Methylbenzo(b)-naphtho(2,1-d)thiophen (PBT)
- 13H-Dibenzo(a,i)carbazol (vPvB)

Ergänzende Betrachtungen zu PBT-Eigenschaften

Um zu überprüfen, ob möglicherweise relevante Strukturvertreter übersehen wurden, die nicht durch die 15 prioritären semipolaren PAC repräsentiert sind, wurde die *erweiterte QSAR-Auswahl kritischer semipolarer PAC* (154 Substanzen) erneut überprüft. Zu diesem Zweck wurden fachliche Erfahrungen aus langjähriger Analysepraxis zur Bildung von Substanzen, zum Auftreten und zur biologischen Aktivität (hauptsächlich Gentoxizität) einbezogen und in Bezug auf die einzelnen Substanzgruppen diskutiert. Zusammenfassend kann festgestellt werden, dass diese Plausibilitätsprüfung keine konkreten Hinweise erbrachte, dass wichtige Substanzen oder Substanzgruppen übersehen worden wären. Vielmehr scheinen im Hinblick auf unser gegenwärtiges Wissen zu Vorkommensrelevanz und Stoffeigenschaften die identifizierten 15 prioritären Stoffe tatsächlich auch die wichtigsten Strukturvertreter zu umfassen. Es verbleiben Unsicherheiten, da im Rahmen dieses Projektes keine weitergehende systematische Analyse möglich war und da die Schlussfolgerungen oft auf theoretischen Überlegungen basieren.

Darüber hinaus wurden Gesetzmäßigkeiten im Hinblick auf Bioakkumulationspotential, Persistenz und Toxizität analysiert. Zusammenfassend kann festgestellt werden, dass aus QSAR-Ergebnissen abgeleitete Gesetzmäßigkeiten solange oftmals experimentell bestätigt wurden, als narkotische Mechanismen für die beobachteten Effekte verantwortlich waren. Spezifische oder reaktive Toxizität (z.B. Photoaktivierung) wird

durch die verwendeten QSAR nicht erfasst, kann aber für bestimmte semipolare PAC eine wichtige Rolle spielen und zu unerwarteten Ergebnissen führen. Auch Abbauwege, bei denen sterische Effekte von Alkylsubstituenten Abbauwege blockieren können, werden mit QSAR nicht erfasst. Allgemeine Tendenzen aber werden durch die verwendeten QSAR-Modelle recht gut widergespiegelt.

Testempfehlungen

Da für die 15 ermittelten prioritären semipolaren PAC oftmals experimentelle Daten zur Ökotoxizität fehlen oder für eine Bewertung ungenügend sind, werden Tests empfohlen und anhand verschiedener Relevanzkriterien eine Rangfolge für zu testende Substanzen erstellt. Für die vier ranghöchsten Substanzen werden für Ökotoxizitätstests wichtige Parameter diskutiert und Schlussfolgerungen zu Testdesign und analytischem Monitoring gezogen.

Analytitische Quantifizierung in Verbraucherprodukten und weiteren relevanten Matrices

Im Analytikteil des Projekts - durchgeführt durch das Biochemische Institut für Umweltkanzerogene, Prof. Dr. Gernot Grimmer-Stiftung (BIU) – wurden Verbraucherprodukte (4), Weichmacheröle (4), Steinkohlenteerpech, Bitumen und Furnace-Ruß auf die 15 prioritären semipolaren PAC untersucht. Parallel dazu wurden 25 verschiedene PAK („Grimmer-PAK“ und 16 EPA-PAK) in den unterschiedlichen Matrices quantifiziert. Eine nicht verfügbare Referenzsubstanz (1-Methylbenzo[*b*]naphtho[2,1-*d*]thiophen) wurde im Rahmen des Projekts isomerenrein synthetisiert. Dahinter stand die Frage, ob sich die Auswahl der prioritären semipolaren PAC, der ja unter anderem die Vorkommenshäufigkeit in Matrices zugrunde lag, bestätigen würde. Daten der Literatur waren sehr begrenzt und Informationen zu Erzeugnissen lagen generell nicht vor. Weiterhin sollte geprüft werden, inwieweit das Vorkommen semipolarer PAC an das Vorkommen von (eher unpolaren) PAK gekoppelt ist.

Alle 6 prioritären Thiaarene wurden in den untersuchten Matrices nachgewiesen. Mit Ausnahme des 1-Methylbenzo[*b*]naphtho[2,1-*d*]thiophens (Gehalt kleiner Bestimmungsgrenze, BG) wurden erwartungsgemäß hohe Thiaarengehalte in Steinkohlenteerpech nachgewiesen. Benzo[*b*]naphtho[2,1-*d*]thiophen 2,1-BNT erweist sich in allen Thiaaren-Profilen als die Komponente mit der höchsten oder zumindest zweithöchsten Konzentration. Eine Besonderheit ergibt sich für Furnace-Ruß, wo Phenanthro[4,5-*bcd*]thiophen als Hauptkomponente im ppm-Bereich gefunden wurde, während die übrigen untersuchten Thiaarene nur im ppb-Bereich vorhanden waren oder unterhalb der Bestimmungsgrenze lagen.

Die beiden prioritären Oxaarene Benzo[*b*]naphtho[1,2-*d*]furan und Benzo[*b*]naphtho[2,3-*d*]furan wurden erwartungsgemäß in hoher Konzentration (ppm-Bereich) im Steinkohlenteerpech nachgewiesen, aber überraschenderweise auch in den Bedarfsgegenständen. Demgegenüber liegen die Gehalte der beiden Oxaarene im Bitumen und Furnace-Ruß unterhalb der Bestimmungsgrenze, bei den Weichmacherölen kommen nur im distillate aromatic extract (DAE) beide Isomere im oberen ppb-Bereich vor, wohingegen sie in den PAK-abgereicherten Ölen treated distillate aromatic extract (TDAE), residual aromatic extract (RAE) und mildly extracted solvate MES nur noch im niedrigen ppb-Bereich oder nicht mehr quantifizierbar waren.

Die 7 prioritären Azaarene sowie das ebenfalls untersuchte 10-Azabenzo[*a*]pyren sind in hoher Konzentration (ppm-Bereich) im Steinkohlenteerpech und im Baumarktreifen sowie dessen Gummischlauch zu finden. In allen Weichmacherölen, Bitumen, Furnace-Ruß sowie in den Gummistiefeln liegen die Konzentrationen der untersuchten Azaarene fast ausschließlich unterhalb der BG. In den Badelatschen ließen sich 4 der prioritären Azaarene im höheren ppb-Bereich bestimmen.

Überlegungen zu möglichen Indikatorsubstanzen

Ein wichtiges Projektziel war, aus den zugänglichen analytischen Daten (eine) Indikatorsubstanz(en) vorzuschlagen, aus deren Konzentration auf die Gesamtmenge semipolarer PAC geschlossen werden könnte. Unsere Arbeit beschränkte sich im Wesentlichen auf die im Projekt gewonnenen analytischen Daten zu 15 prioritären semipolaren PAC in Erzeugnissen, Weichmacherölen, Furnace Ruß, Kohlenteerpech und Bitumen (BIU), da analytische Daten aus der Literatur oft nur qualitativ und in ihren Zielverbindungen zu heterogen waren, um Aussagen zu ermöglichen. Generell ließ sich erkennen, dass aus dem Gesamtgehalt der bestimmten PAK (und auch aus dem Gehalt an 16 EPA-PAK) nicht auf die Gesamtkonzentration der 15 prioritären semipolaren PAC geschlossen werden kann. Wir schlagen auf Basis dieser stichprobenartigen Analysen das sogenannte Grimmer-PAK Benzo[b]naphtho[2,1-d]thiophen (2,1-BNT) als Indikatorsubstanz vor, um auf den Gesamtgehalt der 15 prioritären semipolaren PAC zu extrapolieren. Ungeeignet als Indikatorsubstanz ist 2,1-BNT für die Matrices Furnace Black und Bitumen. Auf Basis der untersuchten Weichmacheröle und Erzeugnisse, zu deren Produktion Weichmacheröle zum Einsatz kommen (vor allem Gummiprodukte), wurde ein Extrapolationsfaktor von 3,1 (±1,1 als Verhältnis des höchsten zum niedrigsten Einzelextrapolationsfaktor bei nur 8 analysierten Matrices) abgeleitet. Die Anwendung dieses Extrapolationsfaktors ist mit Unsicherheiten verbunden. Wir betonen ausdrücklich, dass dem Extrapolationsfaktor nur die 15 prioritären semipolaren PAC zugrunde liegen und sich die Extrapolation auch auf den Gesamtgehalt dieser 15 Stoffe beschränkt. Aus der qualitativen Analyse von Literaturdaten konnten wir zeigen, dass sich 2,1-BNT auch als Zeigersubstanz eignet, die auf das gleichzeitige Vorhandensein anderer prioritärer semipolarer PAC hinweist.

Relevanz der semipolaren PAC im Hinblick auf regulatorische Notwendigkeiten unter REACH

Ein weiterer zentraler Punkt des Projekts war die Frage, in wie weit es sich bei den semipolaren PAC um eine Gruppe von Verbindungen mit möglicherweise besonderer Relevanz handelte, die bislang in der Chemikaliengesetzgebung keine Berücksichtigung findet. Die Notwendigkeit regulatorischer Schritte sollte diskutiert werden. Dabei spielt eine Rolle, ob im Rahmen der regulatorischen Beschränkung von PAK die Gruppe der semipolaren PAC implizit miterfasst sind. Dies musste getrennt betrachtet werden im Hinblick auf Humantoxizitiät (speziell gentoxische und kanzerogene Wirkung) einerseits sowie mögliche ökotoxische Effekte in Umweltmedien andererseits.

Humantoxikologische Einordnung: Es bestehen große Unsicherheiten, ob in den geltenden Grenzwerten bei der unter REACH gültigen Beschränkung von PAK in Prozessölen für die Reifenproduktion sowie für Reifen selbst implizit semipolare PAC miterfasst werden. Dies betrifft insbesondere die genannten Grenzen für PAK in Prozessölen und Reifen sowie die für Reifen zulässige ISO 21461 Methode, aus deren Ergebnis derzeit indirekt auf die Einhaltung bzw. Verletzung der genannten Grenzen geschlossen wird.
Dies verhält sich anders in Bezug auf die 3% (Gewicht) Grenze für den DMSO-Extrakt aus Prozessölen nach IP346-Methode, aus deren Einhaltung auf die Konformität mit den expliziten PAK-Konzentrationsgrenzen geschlossen werden darf. Da hier prinzipiell semipolare PAC mit erfasst werden, ist sehr wahrscheinlich von einer ausreichenden Berücksichtigung dieser Gruppe auszugehen.
In Anbetracht dieser Unsicherheiten und den möglichen Einschränkungen der gegenwärtig für Reifen angewendeten ISO 21461-Methode im Hinblick auf semipolare PAC schlagen wir vor, zusätzlich zu den bestehenden Grenzen für PAK die Einführung zusätzlicher Grenzwerte für Vertreter der semipolaren PAC zu prüfen. Eine geeignete Verbindung könnte die vorgeschlagene Indikatorsubstanz Benzo[b]naphtho[2,1-d]thiophen sein. Es bedarf jedoch weiterer Absicherung der Repräsentativität dieser Verbindung für die

Gruppe der prioritären semipolaren PAC und ergänzender toxikologischer Daten zu der Verbindungsgruppe als Voraussetzung für die Benennung qualifizierter Grenzwerte.

Ökotoxikologische Einordnung: Um die Relevanz der 15 prioritären semipolaren PAC im Hinblick auf eine mögliche Regelungslücke zu beurteilen, sind nicht allein die Konzentrationen und Konzentrationsverhältnisse in den Matrices ausschlaggebend, sondern vielmehr die Konzentrationen und Konzentrationsverhältnisse, die sich in der Wasserphase (oder dem Sediment) einstellen. Dafür sind eine Kenntnis der Wasserlöslichkeit und des Verteilungsverhaltens im Vergleich zu unpolaren PAK entscheidend. Für semipolare PAC ist mit höherer Wasserlöslichkeit als für (unpolare) PAK zu rechnen und im Vergleich zu den für die Matrices ermittelten Konzentrationen sind deutliche Unterschiede in den Konzentrationsverhältnissen für die Wasserphase zu erwarten. Experimentelle Daten fehlen jedoch ganz überwiegend. Weiterhin ist die ökotoxikologische Relevanz semipolarer PAC überwiegend unklar und könnte unterschätzt werden, da chronische Daten zur Ökotoxizität fehlen. Um diese Wissenslücken zu schließen, sind daher weitere Arbeiten zu physikochemischen Eigenschaften und zur Ökotoxizität erforderlich. Ohne diese ergänzende Datenbasis kann keine Empfehlung für regulatorische Konsequenzen hinsichtlich der Ökotoxizität semipolarer PAC gegeben werden.

Kombinationswirkungen

Ein letzter, kurz diskutierter Aspekt ist die Gemischetoxizität, da sich im Analytikprojekt das gleichzeitige Vorhandensein mehrerer bis maximal aller 15 prioritären Verbindungen als Regelfall erwies. Identifizierte Literatur zur Gemischetoxizität semipolarer PAC wurde zusammengefasst. Es wird vorgeschlagen, als Defaultansatz von einer Konzentrationsadditivität auszugehen.

3 Previous work on semipolar PAC

3.1 The KORA[1] project

3.1.1 Objective and Organization of the Project

The KORA1 project (full English title "Retention and Degradation Processes to Reduce Contaminants in Groundwater and Soil") aims at establishing background knowledge about processes governing natural attenuation and retention of contaminants as a basis for evaluation of contaminated sites and prospects for development. The focus of KORA thematic network 2 was on tar oil contaminated sites. As parameters for evaluation of soil and groundwater of these sites up to now PAH, BTEX and phenols were used. However, as tar oils consist of 5-13 % of NSO heterocycles (cyclic compounds containing nitrogen, sulphur or oxygen within their cyclic system) with higher polarity compared to their carbocyclic analogues it is that these compounds make up to 40% of the water soluble tar oil fraction and thus are of special importance for monitoring of soil and especially groundwater (Blotevogel, et al., 2008). The German Federal Soil Protection and Contaminated Sites Ordinance (Bundes-Bodenschutz- und Altlastenverordnung, BBodSchV) requires the monitoring of NSO-heterocyclic hydrocarbons however without specifying the extent of such analysis. Therefore, in KORA thematic network 2a project an assessment procedure for the identification of NSO-heterocyclic priority substances was created.

3.1.2 Applied selection scheme and resulting priority list

As a starting point, KORA thematic network 2a established a list of predominantly semipolar compounds found at contaminated sites in soil or water or reported to be part of creosote. As the majority of research focussed on compounds in groundwater, most of these substances are heterocycles of low molecular weight. Of these more than 250 compounds only NSO-heterocycles and only compounds with log Kow $\leq$ 4.5 were further evaluated (160 compounds), as compounds critical for ground water contamination were targeted (Blotevogel, et al., 2007). For details on selection criteria and the evaluation scheme applied by KORA see Table 1. Most important are the following aspects:

- The higher the water solubility, the higher the priority of the compound (higher score)
- The lower the organic carbon partition coefficient, the higher the priority of the compound (higher score, as higher immobilization rate to groundwater). As a low K_{oc} implicates a low K_{ow}, implicitly compounds with low log K_{ow} are given higher priority
- Cancerogenicity, mutagenicity and genotoxicity is given higher priority over ecotoxicity parameters
- Within ecotoxicity parameters, bacteria are included as relevant for microbial degradation; however toxicity to small crustaceans (e.g. Daphnia magna) is not included.

In addition to parameters on intrinsic substance properties prevalence of each compound at creosote contaminated sites was evaluated by a similar evaluation scheme and weighted approximately equally compared to

[1] „KORA – Kontrollierter natürlicher Rückhalt und Abbau von Schadstoffen bei der Sanierung kontaminierter Grundwässer und Böden", Retention and Degradation Processes to Reduce Contaminants in Groundwater and Soil, http://www.natural-attenuation.de/content.php?lang=en

intrinsic substance properties as described above and in Table 1. Points are summed over all criteria and final scores used to rank the substances according to priority.

Table 1: Parameters and criteria for substance evaluation according to KORA (Blotevogel, et al., 2008, modified)

Parameters	Dim.	Weighting	Number of points				
			5	4	3	2	1
Physico-chemical parameters							
Odour threshold value	µg/m³	1	< 1	1 - < 10	10 - < 100	100 - < 1000	≥ 1000
Solubility in water	mg/L	2	≥ 10000	1000 - < 10000	100 - < 1000	10 - < 100	< 10
Henry-Coefficient	--	1	≥ 410^{-2}	4×10^{-3} - < 4×10^{-2}	4×10^{-4} - < 4×10^{-3}	4×10^{-6} - < 4×10^{-4}	< 4×10^{-6}
Partition coefficient K_{OC}	--	2	< 10	10 - < 100	100 - < 1000	1000 - < 10000	≥ 10000
Microbial degradation							
Degradation in 5 relevant redox zones	--	1 – 3 for each zone[(2)]	Persistence proven	--	Degradation not examined	Degradation as well as peristence observed	Degradation proven
Human toxicity parameters							
Cancerogenicity	--	3	Evidence positive		No or. insufficient data		Evidence negative
Mutagenicity	--	3	Evidence positive (even if only in one test system)	Negative as well as positive evidence in the same test system	No data		
Genotoxicity[(1)]	--	3					
Ecotoxicological parameters: Acute toxicity							
Fish	mg/L	1	< 1	1 - <10	10 - < 100	100 - < 1000	≥ 1000
Green algae							
Bacteria							

(1) Prerequisite: All mutagenic substances are genotoxic at the same time. Number of points for genotoxicity therefore is never lower than that for mutagenicity.

(2) Aerobic 2; NO_3^--red. 1; Fe(III)-red. 3; SO_4^{2-}red. 3; methanogenic 1.

This prioritization procedure finally resulted in a priority list of 19 heterocyclic compounds (for isomers treated together) plus 2-hydroxy-biphenyl (biphenyl-2-ol) as a degradation product of dibenzothiophene which itself, however, is not included in the list. Names and CAS-numbers of these 20 priority substances (with three isomers of methyldibenzofuran: 22) are summarized in Table 2.

Table 2: The 20 priority compounds of KORA (Blotevogel, et al., 2008)

Substance name	CAS-number	Substance name	CAS-number
9(10H)-Acridinone	578-95-0	3-Methylbenzothiophene	1455-18-1
Benzo(b)thiophene	95-15-8	1-Methyldibenzofuran	7320-50-5
Benzofuran	271-89-6	2-Methyldibenzofuran	7320-51-6
Biphenyl-2-ol	90-43-7	4-Methyldibenzofuran	7320-53-8
9H-Carbazole	86-74-8	1-Methylisoquinoline	1721-93-3
Dibenzofuran	132-64-9	2-Methylquinoline	91-63-4
2,3-Dimethylbenzofuran	3782-00-1	4-Methylquinolin-2-one	607-66-9
2,4-Dimethylquinoline	1198-37-4	Phenanthridinon	1015-89-0
Isoquinoline	119-65-3	Quinoline	91-22-5
1(2H)-Isoquinolinone	491-30-5	2(1H)-Quinolinone	59-31-4
2-Methylbenzofuran	4265-25-2	Xanthen-9-one	90-47-1

3.2 Derivation of a "de minimis level" (GFS-values) for semipolar PHA by LAWA AG[2] - Methodological aspects and results

To derive GFS values (Geringfügigkeitsschwellenwerte, i.e. *de minimis* level) for ground water contaminating NSO-heterocyclic compounds, LAWA AG used earlier work as input in its selection scheme for relevant compounds. Building on the data from KORA (20 priority substances plus 31 isomers) and taking further substances into account according to work of the Altlastenforum (Kern, et al., 2008), a new list of 71 compounds (including isomers) resulted, presumably including all NSO-heterocyclic compounds relevant for contaminated sites (LAWA AG, 2010).

As criteria with relevance for GFS derivation are differing from selection criteria applied by KORA, modified criteria were applied for selection of the most relevant compounds from the evaluated substances list of KORA (160 compounds) for GFS-derivation. These criteria are limited on substance properties rather than prevalence in groundwater. Most important modifications in criteria and selection scheme are summarized as follows:

- Weighting of persistency reduced, thus equivalence to other criteria, e.g. physicochemical parameters;
- Mutagenicity an genotoxicity evaluated together: only the higher KORA-score of these properties is included in the evaluation;
- Inclusion of toxicity towards daphnids and special weighting with factor 2 compared to the other organism groups (factor 1), as Crustaceans belong to the most important ground water organisms;
- Deviating from KORA methodology: Points of the four criteria blocks are not summed up but rather multiplied. This leads to an increased weight for compounds with scores in several or all blocks of criteria.

[2] LAWA AG, Bund/Länder-Arbeitsgemeinschaft Wasser, federal and state-government consortium water

For details of the GFS evaluation scheme, we refer to Table 3. During this process additional data on ecotoxicity were gathered broadening the data basis in this respect also for the 20 priority compounds from KORA.

Table 3: Parameters and criteria for substance evaluation according to KORA (Blotevogel, et al., 2008, modified) and adapted to prioritisation criteria for GFS-derivation (LAWA AG, 2010)

Parameters	Dim.	Weighting	Number of points				
			5	4	3	2	1
Physico-chemical parameters							
Odour threshold value	µg/m³	0	< 1	1 - < 10	10 - < 100	100 - < 1000	≥ 1000
Solubility in water	mg/L	2	≥ 10000	1000 - < 10000	100 - < 1000	10 - < 100	< 10
Henry-Coefficient	--	1	> 410^{-2}	4×10^{-3} - < 4×10^{-2}	4×10^{-4} - < 4×10^{-3}	4×10^{-6} - < 4×10^{-4}	> 4×10^{-6}
Partition coefficient K_{OC}	--	2	< 10	10 - < 100	100 - < 1000	1.000 - < 10000	≥ 10000
Microbial degradation							
Degradation in 5 relevant redox zones(1)	--	0.1 – 0.3 for each zone	Persistence proven	--	Degradation not examined	Degradation as well as peristence observed	Degradation proven
Human toxicity parameters							
Cancerogenicity	--	3	Evidence positive		No or. insufficient data		Evidence negative
Genotoxicity: only the higher score of mutagenicity and genotoxicity of KORA taken	--	3	Evidence positive (even if only in one test system)		Negative as well as positive evidence in the same test system	No data	
Ecotoxicological parameters: Acute toxicity							
Fish	mg/L	1	< 1	1 - <10	10 - < 100	100 - < 1000	≥ 1000
Green algae		1					
Bacteria		1					
Daphnia		2					

(1) Aerobic 0.2; NO_3^--red. 0.1; Fe(III)-red. 0.3; SO_4^{2-}red. 0.3; methanogenic 0.1: sum for block = 1;

Applying this evaluation scheme, the 71 priority substances (including isomers) from KORA (Blotevogel, et al., 2007) and Altlastenforum (Kern, et al., 2008) were distributed over the whole range of scores. However, the subcommittee of LAWA AG concludes that because of their high prevalence at contaminated sites or in ground water plumes with these compounds the most relevant NSO-heterocycles for analysis of contaminated sites are recorded. Additionally, by reevaluating the 160 compounds of KORA 18 compounds (including isomers) in the highest score range were selected as additional substances with most probably high detrimental effect at creosote contaminated sites. Because of their ubiquitous prevalence furthermore benzotriazole and methylbenzotriazole (2 isomers), which are used in products, were regarded as relevant, leading finally to 92 compounds (including isomers) of relevance for GFS derivation. These compounds are listed in

Table 58 of Annex VI. Toxicity or ecotoxicity data sufficient for risk assessment were only available for 24 substances (see Table 4) of these 92 priority compounds. Where human toxicity data were insufficient for risk assessment, GOW[3] values (Dieter, 2003; UBA, 2003) were derived. Where GFS-derivation could be based on ecotoxicity data only, the PNEC must not be higher by more than a factor of three compared to the GOW value; otherwise, no GFS value was derived.

Table 4: 24 Compounds of the list of 92 priority compounds with sufficient toxicity or ecotoxicity data allowing for risk assessment – GFS values where the data basis allowed for derivation (LAWA AG, 2010, modified)

No..	Name in German (CAS Nr.)	GFS [µg/L]	Human toxicity[4] [µg/L]	Ecotoxicity (PNEC) [µg/L]
1	Acridin (260-94-6)	**0.08**	(GOW: 0.1 – 0.3)	0.08
2	Benzo(b)thiophen (95-15-8)	**0.3**	(GOW: 0.1)	0.3
3	Benzofuran (271-89-6)	**1.8**	2.7	1.8
4	Benzotriazol (95-14-7) und Methylbenzoltriazole (29385-43-1)	**40**	- -	40 75
5	Carbazol (86-74-8)	**0.2**	10	0.2
6	Chinolin (91-22-5)	**0.01**	0.002	0.4
7	Cumarin (91-64-5)	**4.7**	4.7	8
8	Dibenzofuran (132-64-9)	**0.4**	(GOW: 0.3)	0.4
9	Dibenzothiophen (132-65-0)	--	(GOW: 0.1)	0.6
10	2,6-Dimethylchinolin (877-43-0)	--	(GOW: 0.1 – 0.3)	1.9
11	Furan (110-00-9)	**0.35**	0.35	--
12	2-Hydroxybiphenyl (90-43-7)	**0.7**	100	0.7
13	Indol (120-72-9)	--	(GOW: 0.1)	0.9
14	Isochinolin (119-65-3)	--	(GOW: 0.1 – 0.3)	4
15	Methylchinoline (91-63-4, 612-58-8, 491-35-0, 7661-55-4, 91-62-3, 612-60-2, 611-32- 5) Nur 6-Methylchinolin (91-62-3):	--	(GOW: 0.1)	2.2
16	Piperazin (110-85-0)	--	(14)	1250
17	Pyridin (110-86-1)	**0.5**	3.5	1.1
18	Pyrrol (109-97-7)	--	-	-
19	Thiophen (110-02-1)	--	(GOW: 0.3)	13
20	Xanthen (92-83-1)	--	(GOW: 0.1)	0.4
21	2,3-Dimethylbenzofuran (3782-00-1)	**0.3**	(GOW: 0.1)	0.3
22	2-Methylbenzofuran (4265-25-2)	--	(GOW: 0.1)	3.2
23a	3-Methylbenzothiophen (1455-18-1)	--	(GOW: 0.1)	2.6
23b	5-Methylbenzothiophen (14325-14-1)	--	(GOW: 0.1)	14
24	Benzo(b)thiophen-1,1-dioxid (825-44-5)	--	(GOW: 0.1)	14

[3] GOW: Gesundheitlicher Orientierungswert, i.e. health based orientation value

[4] "Trinkwasserwerte" (limit values for drinking water) based on cancerogenic and non-cancerogenic effects

4 Definitions, Outline and Conceptual Work

4.1 Diverging aspects of the current project compared to KORA and GFS-derivation in respect to substance definition and substance properties

Semipolar PAC in the sense of the project are defined as follows:

- Polynuclear (at least 2) heterocyclic aromatic compounds (heteroatoms S, N, O) or
- Polynuclear (at least 2) aromatic compounds with heteroatoms (S, N, O) as substituents or part of substituents

Within this project, heterocyclic polyaromatic compounds (HET-PAC) are of special relevance.

The following compounds are not part of the current project:

- Non-aromatic heterocycles, e.g. piperazine
- Monocyclic aromatic compounds, e.g. pyridine, diphenylether, hydroxybiphenyl
- Alkylated PAH without heteroatoms
- Halogenated polycyclic aromatic compounds

Thus, 19 of the 20 priority compounds of KORA are also within the scope of the current project. As 2-hydroxy biphenyl (biphenyl-2-ol) is itself not amongst the polycyclic (≥ 2) aromatic compounds, it is not directly of relevance for the project. As it is, however, a degradation product of dibenzothiophene it may be of some relevance if the parent compound would be selected as important NSO-heterocyclic compound further on.

Regarding substance properties, in KORA and the work of LAWA AG selection was on compounds with high water solubility and low partition coefficient organic carbon (K_{oc}) to account for a low retention potential of compounds in soil linked with a high susceptibility for leaching into ground water. As semipolar PAC with PBT properties are the scope of the current project, selection criteria regarding these properties will be more sophisticated. A certain degree of water solubility will be necessary to enter the food chain and therefore a prerequisite for bioaccumulation. On the other hand, bioaccumulating properties are increasing with increasing log K_{ow} (limited to log K_{ow}-values up to 6) and therefore decreasing polarity and increasing K_{oc}.

Furthermore, human toxicity properties will not be part of the primary selection criteria of the current project. Ecotoxicity therefore is decisive for the T-criterion of the PBT characteristics. Table 10 of section 6.1 summarizes the PBT-criteria according to REACH Annex XIII. Furthermore, screening criteria for PBT-properties as outlined in the REACH guidance documents for substances with insufficient data to assess the REACH criteria are given in this table.

Table 5: Modification of scores according to the KORA system leaving behind human toxicity criteria: Three compounds (in bold letters) would not be part of the list of the 20 highest scored substances anymore.

20 Priority compounds KORA	Total score	Score human toxicity	% Human toxicity of total score	Score ecotoxicity	% Ecotoxicity of total score	Score without human toxicity
Methylquinoline, 2-	144	36	25.0%	8	22.2%	108
Benzofuran	137	45	32.8%	11	24.4%	92
Methyldibenzofuran	135	27	20.0%	13	48.1%	108

20 Priority compounds KORA	Total score	Score human toxicity	% Human toxicity of total score	Score ecotoxicity	% Ecotoxicity of total score	Score without human toxicity
Phenanthridinone, 6(5H)-	134	39	29.1%	7	17.9%	95
Dimethylbenzofuran	131	24	18.3%	13	54.2%	107
Methylbenzofuran	127	15	11.8%	12	80.0%	112
Methylbenzothiophene, 3-	126	27	21.4%	13	48.1%	99
Dibenzofuran	124	39	31.5%	13	33.3%	91
Methyl-2(1H)-quinolinone, 4-	124	33	26.6%	8	24.2%	85
Hydroxybiphenyl, 2-	122	45	36.9%	10	22.2%	77
Acridinone	121	39	32.2%	10	25.6%	97
2,4-Dimethylquinoline	121	27	22.3%	11	40.7%	94
Benzo(b)thiophene	121	24	19.8%	14	58.3%	82
Methylisoquinoline, 1-	120	27	22.5%	9	33.3%	93
Xanthenone	117	21	17.9%	11	52.4%	96
Isoquinoline	113	33	29.2%	11	33.3%	80
Carbazol	109	39	35.8%	14	35.9%	**70**
Quinolinone, 2(1H)-	107	39	36.4%	8	20.5%	**68**
Quinoline	101	45	44.6%	11	24.4%	**56**
Isoquinolinone, 1(2H)-	100	21	21.0%	7	33.3%	79

Using the scoring system of KORA, leaving behind human toxicity criteria modifies the total score such, that three compounds of the original priority list would have been not selected anymore (Table 5, in bold letters). Instead, 1-methyl-2(1H)-quinolinone, 8-hydroxy-2-methylquinoline and 4-hydroxy-quinoline would be part of the priority list of 20 compounds. For this evaluation inclusion of isomers in the “new list” differing only by the position of the methyl group at the carbon ring atoms was avoided, even if the “new scoring” would have resulted in inclusion of those compounds.

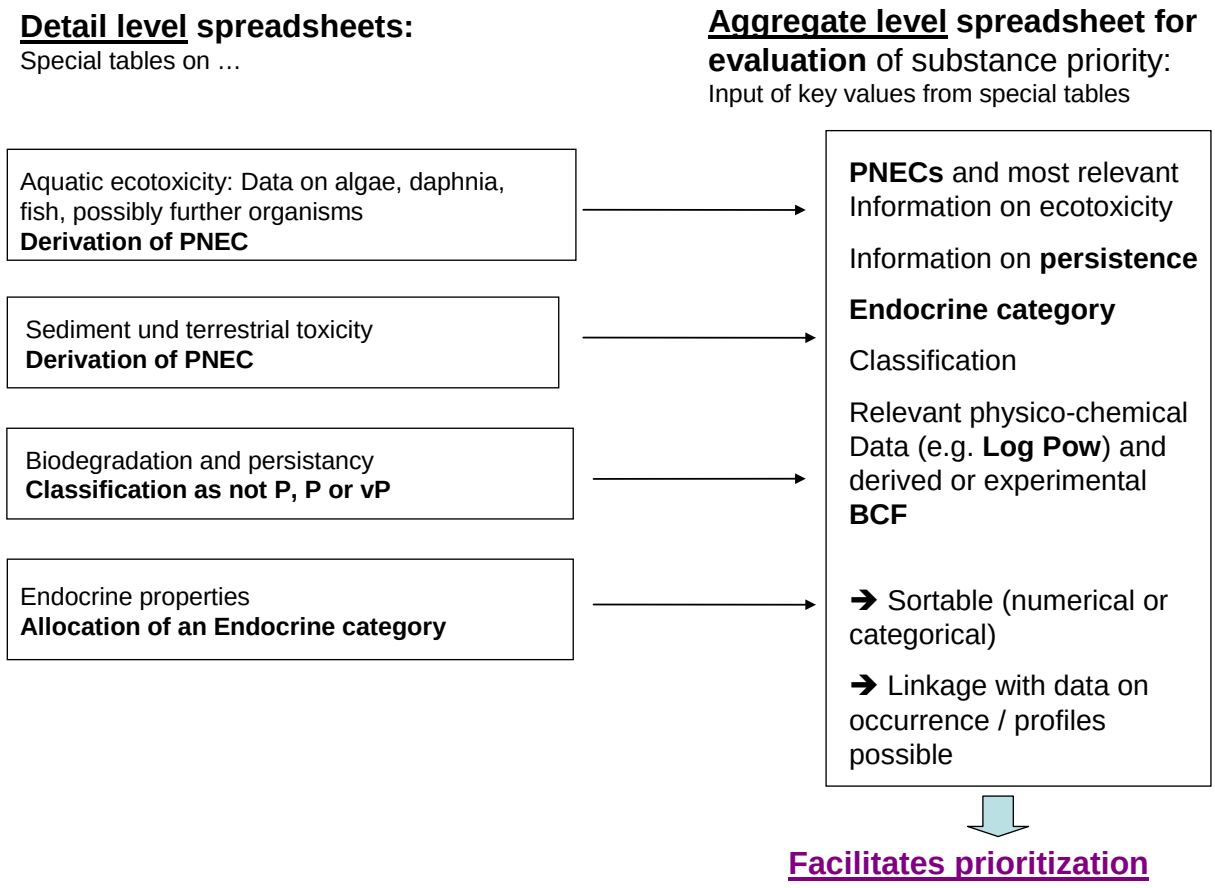
4.2 New concept for data structure facilitating prioritization of compounds

Compounds of the KORA priority list could be potentially relevant also for the current project. As however prioritization criteria are differing in KORA, LAWA and the current project as outlined above, data on these compounds have to be re-evaluated from a different point of view. To enable this, data on these compounds from KORA and LAWA were consolidated to include all available information and restructured to enable easy prioritization according to physicochemical and ecotoxic properties, persistency criteria and the ambition to preserve a clear connection to the original toxicity data. Especially the additional ecotoxicity data gathered during GFS derivation had to be included in the property data collection from KORA. The data structure outlined below is developed using the 20 priority compounds of KORA. Assessments with this level of specification will only be performed for other compounds starting from an elevated priority level.

The concept for data structure with respect to substance properties is summarized in the scheme of Figure 1. In the aggregate level spread sheet only decisive, comparable data (numeric or categorical) are included together with only the most important underlying information. Because of their numeric or categorical nature, sorting according to different combinations of properties is facilitated which enables prioritization of com-

pounds according to flexible criteria. For ecotoxicity for example, derived PNECs (preferably from experimental data) are included together with information on the number of trophic levels covered by experimental data, the assessment factor for PNEC derivation as well as the decisive endpoint.

Figure 1: Hierarchical concept for structuring data on substance properties facilitating prioritization without loss of information



The same or similar structure is intended for other data like sediment or soil toxicity (as far as available), persistence (as a result of evaluation of data on biodegradation or physicochemical transformation) and endocrine properties (see Figure 1).

4.3 Activities on substance identifiers and physicochemical properties of the KORA priority compounds

All data on substance identifiers and physicochemical properties were up to now directly included within the aggregate level spread sheet (see Table 6).

Additionally to CAS-number and chemical name the smiles code for each of the 22 substances (including isomers for methyl-dibenzofuran) was retrieved[5], as this identifier is a prerequisite for further work with QSAR-software.

Classification according to EU regulation number 1272/2008 (CLP, Annex VI) was checked for each substance and the result recorded in the classification-column of the table (nc for not classified).

Physicochemical property data as assembled by KORA were checked and values as well as literature references for dissociation constant, water solubility, log K_{ow} and K_{oc} were adopted for the aggregate level spread sheet. If several values of different sources were available, only one value was recorded in the table:

- experimental values were preferred over calculated values
- if values were in close proximity, one value was chosen with preference for data from the PhysProp Database[6], if available
- if values were different from each other and no obvious reasons existed to prefer one value over the other(s), the arithmetic mean was calculated and the original values or the range of the original values recorded in the literature column together with their references
- calculated values by KORA with EPIWIN version of 2000 which seemed inappropriate were recalculated with version 4.1 of EPIWIN.

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[5] Retrieved via ChemID (http://chem.sis.nlm.nih.gov/chemidplus/)

[6] http://www.syrres.com/what-we-do/databaseforms.aspx?id=386

Table 6: Extract with examples from the evaluation spreadsheet for 22 priority compounds of KORA (Blotevogel, et al., 2008) covering substance identifiers and physicochemical properties. In the literature column, other values or the range (if several values of diverging size available) are given in round brackets together with identification of calculated values. Classification and SMILES code as determined by FoBiG, [26] recalculated by FoBiG using EPI Suite 4.1 (US-EPA).

Chemical name	CAS-NR	SMILES-CODE	Classification according to CLP	Diss.-const. (PKa-value)	Literature / alternative values / comments	Water sol. [mg/L] at 25°C	Literature	log Kow	Literature	Koc (pH=7) [L/kg]	Literature
9(10H)-Acridinone	578-95-0	c12c(c(c3ccccc3[nH]1)=O)cccc2	nc			16.10	[26]	2.96	[28]	34400.00	[2]
Benzo(b)thiophene	95-15-8	c12c(scc1)cccc2	nc			130.00	[1], [3]	3.12	[1], [3]	996.20	[2]
Benzofuran	271-89-6	c12c(occ1)cccc2	nc			451	[3](224) - [1](678)	2.67	[1], [3]	996.20	[2]
Biphenyl-2-ol	90-43-7	c1(c2c(cccc2)O)ccccc1	Eye Irrit. 2; H319 - STOT SE 3; H335 - Skin Irrit. 2; H315 - Aquatic Acute 1; H400	9.97	[1](9,97); [30](9,55)	700.00	[11], [30], [31]	3.09	[1], [30]	10330.00	[2]
9H-Carbazole	86-74-8	c12c3c(cccc3)[nH]c1cccc2	nc	-3.00	[1]	1.80	[1]	3.72	[1] A[3]	11290.00	[2]
Dibenzofuran	132-64-9	c12c3c(cccc3)oc1cccc2	nc			3.10	[1]	4.12	[3]	11290.00	[2]
2,3-Dimethylbenzofuran	3782-00-1	c1ccc2c(C)c(C)oc2c1	nc			62	[3]	3.63	[26]	2271.00	[26]
2,4-Dimethylquinoline	1198-37-4	c12c(nc(C)cc1C)cccc2	nc			1800.00	[2]	3.24	[2]	4920.00	[2]
Isoquinoline	119-65-3	c12c(cccc1)cncc2	nc	5.40	at 20°C [53]	5000.00	[11]	2.08	[54]	63.80	[26] calculated from log

Semipolar polycyclic aromatic hydrocarbons

Chemical name	CAS-NR	SMILES-CODE	Classification according to CLP	Diss.-const. (PKa-value)	Literature / alternative values / comments	Water sol. [mg/L] at 25°C	Literature	log Kow	Literature	Koc (pH=7) [L/kg]	Literature
											Kow
1(2H)-Isoquinolinone	491-30-5	c12c(c([nH]cc1)=O)cccc2	nc	-1.2 / 13.6	[29a] calculated	476.00	[1]	1.42	[2]	144.00	[2]
2-Methylbenzofuran	4265-25-2	c12c(oc(c1)C)cccc2	nc			130.00	[3]	3.22	[2]	1623.00	[2]

Table 7: Extract from the aquatic toxicity data spreadsheet with examples from the 22 priority substances by KORA: *Data section UBA* plus *concluding section* (FoBiG), which is transferred as such into the evaluation spreadsheet. Data from the concluding section may refer to data sections KORA and FoBiG, which are not part of this extract. Literature references to the cited data are given in columns "L".

	Aquatic toxicity data according to UBA - qualified minimum Data on												**Concluding section (identically in evaluation table)**				
	Fish			**Daphnia**			**Algae**			**Microorg. / other**			**PNEC**		**Exp. Data**		
CAS-NR, name	**mg/L**	**End-point, time**	**L / Comment**	**mg/L**	**End-point, time**	**L / Comment**	**mg/L**	**End-point, time**	**L / Comment**	**mg/L**	**Organism, time, end-point**	**L**	**µg/L**	**Decisive data**	**TL**	**AF**	**Comment FoBiG**
1015-89-0 Phenanthridinon	--			--			--			--			0.7	EC50 (Green algae, 96h, calc.), ECOSAR class Amides;	0	1000	ECOSAR-calculation (KORA) for aquatic toxicity obscure recalculated (FoBiG)
119-65-3 Isoquinoline	14.0	LC50 (Poecilia reticulata, 96h)	[46]	4.1	EC50 (Daphnia magna, 48h)	[61]	8.8	EC50 (Scenedesmus acuminatus, 96h)	[58]	125.0	EC50 (Tetrahymena pyrif., replic., 60 h)	[62]	4.0	EC50 (Daphnia magna, 48h)	3	1000	PNEC as derived by UBA
1198-37-4 2,4-Dimethylquinoline	--			2.4	EC50 (Daphnia magna, 16 d, reprod.)	[63]	--			--			--	Yet unclear, only exp. Data on Daphnia magna	1	--	
132-64-9 Dibenzofuran	0.4	LC50 (Danio rerio fish egg, 48h)	[65]	0.6	EC50 (Daphnia magna, 24h)	[65]	1.5	EC50 (Skeletonema costatum, 96h)	Photosynthesis inhibition; [49]	--			0.4	LC50 (Danio rerio fish egg, 48h)	3	1000	PNEC as derived by UBA

Semipolar polycyclic aromatic hydrocarbons

	Aquatic toxicity data according to UBA - qualified minimum Data on												Concluding section (identically in evaluation table)				
	Fish			Daphnia			Algae			Microorg. / other			PNEC		Exp. Data		
CAS-NR, name	mg/L	End-point, time	L / Comment	mg/L	End-point, time	L / Comment	mg/L	End-point, time	L / Comment	mg/L	Organism, time, end-point	L	µg/L	Decisive data	TL	AF	Comment FoBiG
1455-18-1 3-Methyl-benzothio-phene	--			2.6	LC50 (Daphnia magna, 48h)	[66]	--			--			2.6	LC50 (Daphnia magna, 48h)	1	1000	PNEC as derived by UBA, however bacteria more sensitive (Vibrio fisheri)
1721-93-3 1-Methyl-isoquinoline	--			--			--			--			17.4	EC50 (Green algae, 96h, calc.)	0	1000	ECOSAR-Data as calculated by KORA
271-89-6 Benzofuran	1.8	LC50 (Danio rerio fish egg, 48h)	[65]	2.1	EC50 (Daphnia magna, 24h)	[65]	--			152.0	EC50 (Tetrahymena pyriformis, 40h)	[67]	1.8	LC50 (Danio rerio fish egg, 48h)	2		PNEC as derived by UBA

TL: Trophic level; AF = assessment factor; L = literature reference; Exp. = experimental;

4.4 Activities on ecotoxicological properties of the KORA priority compounds

An additional spread sheet within the same file contains available aquatic toxicity data used for derivation of PNECs finally linked to the evaluation spread sheet. In separate column groups identically organized data from KORA, LAWA and new, supplemented or recalculated data by FoBiG are reported. If several data were available for one of the categories Fish, Daphnia, Algae and Microorganisms / other organisms qualified minima, as judged by FoBiG, were selected and reported. As additional, non-reported data are either available from the original KORA or LAWA-data or – in case of data by FoBiG – separately collected, re-evaluation of available data is always possible. On the right side final concluding columns report on calculated PNECs, decisive data for PNEC-derivation, the number of trophic levels with experimental data, the applied assessment factor for PNEC-derivation as well as a comment field. Only this last concluding column-section is transferred to the evaluation spread sheet for prioritization of compounds. For an extract of the table on aquatic toxicity covering the data section from LAWA and the concluding column section transferred to the evaluation spread sheet we refer to Table 7.

4.5 Database searches for relevant (new) data on ecotoxicity and bioaccumulation for the 22 priority compounds from KORA

The ECOTOX-database of US-EPA (http://cfpub.epa.gov/ecotox/index.html) was checked for new data on aquatic toxicity (data added since 2009) and data on toxicity to terrestrial organisms. New aquatic data were only available on toxicity of dibenzofuran to Daphnia magna, and these data had been already reported by KORA. However, data on terrestrial toxicity for 7 of the 22 compounds existed. These were on mammals (rat and mouse) but also on garden snail Helix aspera or on plants like lettuce and cucumber.

Besides this, the following data bases have been checked for (new) data:

- INERIS-data base, which includes data on exotoxicity (http://www.ineris.fr/substances/fr/) – no results
- TERATOX-database, including data on toxicity to Tetrahymena pyriformis (http://www.vet.utk.edu/TETRATOX/index.php): no results
- ERED-database (environmental residue-effects data base, organ/body concentration – effect relation, http://el.erdc.usace.army.mil/ered/): data to 4 of the 22 compounds with effect / no observed effect concentrations in fish
- EDKB-Library (endocrine disrupters knowledge base[7]): data for 2-hydroxybiphenyl (reporter gene assay & receptor binding assay) and quinoline (reporter gene assay)

Data on terrestrial organisms could be decisive especially in cases where persistency and bioaccumulation criteria are fulfilled but at the same time aquatic toxicity is low or moderate or experimental data are missing. Data contained in the ERED-database are taken from the literature where biological effects (e.g. reduced survival, growth, etc.) and tissue contaminant concentrations were simultaneously measured in the same organism. If available, these data could confirm the detrimental effect of a compound with bioaccumulative properties. The EDKB-Library contains data on compounds tested for endocrine effects and their relative po-

7 http://www.fda.gov/ScienceResearch/BioinformaticsTools/EndocrineDisruptorKnowledgebase/ucm135074.htm

tency compared to other tested compounds including known endocrine effectors like estradiol or ethinyl estradiol. Positive effects in in-vitro-systems may therefore be ranked for their relevance.

Other databases checked and judged to be of no use for the current project are

- The ToxRes-Database (http://cfpub.epa.gov/ecotox/index.html): Resource for use in the systematic investigation of hypotheses related to effect/residue relationships. Probably too specialized, none of the 22 priority compounds of KORA and none of the 160 compounds of the initial *pool of identified semipolar PAC* (see section 5.1) included.
- Fathead Minnow data set (http://cfpub.epa.gov/ecotox/index.html): Detailed toxicity data on Pimephales promelas for QSAR-development. Data contained in ECOTOX-database.
- BSAF (Biota-Sediment Accumulation Factor) dataset (http://cfpub.epa.gov/ecotox/index.html): Substantially only data on PCB, dioxins and several PAH as well as some pesticides – no relevance for NSO-heterocycles.
- California Wildlife Biology, Exposure Factor, and Toxicity Database (Cal/Ecotox, http://oehha.ca.gov/cal_ecotox/default.htm): No relevance for NSO heteroyclic compounds.
- GSBL (Gemeinsamer Stoffdatenpool Bund/Länder, http://www.gsbl.de): Publicly accessible part includes only data on substance and physico-chemical parameters, no data on toxicity.
- OECD QSAR-Toolbox (http://www.oecd.org/document/54/0,3746,en_2649_37465_42923638_1_1_1_37465,00.html), experimental data set

5 Compilation of a *Pool of identified semipolar PAC*

5.1 Semipolar PAC determined from the KORA pool and supplemented by substances from LAWA

The extensive work by KORA and LAWA on priority compounds of creosote contaminated sites most probably is at least partially of relevance for the current project. Apart from compounds resulting from transformation or degradation processes, these compounds are presumably also present in significant amount in creosote itself. As unlike in KORA and LAWA also substances with higher log K_{ow}-values than 4.5 are of interest, the very initial set of substances by KORA (approximately 256, isomers only partly included) was chosen as a first step.

As a second step, substances were removed which didn't match the structural criteria as defined in section 4.1. The following substances were removed:

- NSO-heteroycles without aromatic ring (e.g. piperazine)
- NSO-heterocycles with only one aromatic ring
- Aromatic compounds without NSO hetero atoms (only alkyl substituents)
- Diphenylethers
- Hydroxybiphenyls, except 2-hydroxybiphenyl (one of the 20 priority compounds of KORA), as this compound is a degradation product of dibenzothiophene and thus may be of some relevance if the parent compound would be selected as important NSO-heterocyclic compound further on.
- PAH.

10 substances not exactly meeting the prerequisite of more than one aromatic ring and 2-hydroxybiphenyl were – after consultation with UBA – not removed[8]. Thus 149 substances resulted from the KORA project. From these compounds 45 substances had been not further evaluated (essentially no data) within the KORA project (most probably because of log Kow >4.5).

As a third step, for compounds in the list with no or a wrong CAS number assigned the correct number was identified using ChemIDPlus[9] and ESIS[10]. For substances with a wrong structure assigned, the correct structure was identified using the same sources. For isomers with their CAS-numbers put together in one single field of the table, isomers were separated such that specific CAS-numbers were assigned in separate lines of the table. For some compounds with numerous isomers structure and CAS-number of the one or several isomers with the most experimental data were selected (in one line, each).

As such, a list was created with one line of the table per compound or isomer containing one discrete searchable CAS-number per compound / isomer. Because most isomers were now identified separately, the total number of compounds of the list increased to 157 including the 10 compounds8 not exactly meeting substance definition of semipolar PAC as layed down in this project.

[8] 4 indanole isomers (1-, 2-, 4- and 5-indanole), 1-indanone, methylindanone, 4-phenylpyridine, 1,2,3,4-tetrahydroquinoline, 2,3-dihydrobenzothiophene, 2-hydroxybiphenyl, 2,3-dihydroindole

[9] http://chem.sis.nlm.nih.gov/chemidplus/

[10] http://ecb.jrc.ec.europa.eu/esis/

In the fourth step, the list of 92 compounds of LAWA was edited to gain a searchable list of CAS-numbers (i.e. one number for each compound, including isomers). With this list 65 compounds of LAWA were determined which were also part of the list of 158 compounds created thus far from the KORA list. From the substances not included 25 were not meeting the structural criteria as defined in section 4.1. Two substances (including isomers: three), however, meeting the criteria are not part of the list created from KORA. These were benzotriazole (CAS 95-14-7) and the isomeric mixture tolyltriazole (CAS 29385-43-1) consisting of 4- and 5-methylbenzotriazole. These substances are used in products (varnishing, lubricants, cleaning agents and further applications) and found as ubiquitous contaminants in the environment (LAWA AG, 2010). Therefore, these compounds were included in the list to give a final number of initially identified semipolar PAC of 159 compounds based on KORA (Blotevogel, et al., 2007) and LAWA AG (see Table 55: *Pool of identified semipolar PAC* (443 compounds) in Annex II: Column 2 gives information on the origin of compounds).

5.2 Search Strategy for Profiles Containing Semipolar PAC

5.2.1 Data bases

A first *pool of identified semipolar PAC* emanated from the KORA and LAWA lists. To extend this list further, a comprehensive search was performed to identify potentially relevant semipolar PAC in coal tar and crude oil derived products in different data bases:

- PubMed and toxline literature data bases
- HSDB
- U.S. EPA ECOTOX database
- Cal/Ecotox Database (Californian EPA)
- ETOX (UBA)
- ENVICHEM (eChem-Portal)
- Environmental Residue-Effects Database (U.S. Army Corps of Engineers/U.S. Environmental Protection Agency)
- AGRICOLA Database (U.S. Department of Agriculture)
- CHEMPENDIUM databases (Canadian Centre for Occupational Health and Safety)
- BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit - Articles of daily use
- ECHA – registered substances: list of substance-specific UVCB
- submission data from the consortium „REACH for Coal Chemicals” (R4CC)
- google/google scholar
- Benzotriazole-project, UBA

A further search should identify potentially relevant semipolar PAC in basic materials derived from coal and crude oil (which are used for production of commercial products) as well as in merchandised products originating from these basic materials. For this purpose a comprehensive telephone/e-mail survey was performed under manufacturers from the different product groups suspected to contain relevant amounts of PAH (and therefore possibly also semipolar PAC). The list of inquired companies and organizations is attached as An-

nex 5. Subsequently, safety data sheets (SDS) were asked from companies selling the corresponding products to identify components with expected occurrence of semipolar PAC.

5.2.2 Principle search terms

The first search step included the search terms:

PAH or PAK or PAC

combined with *"semi(-)polar" or "(polycyclic or polyzyklisch) and hetero"*

and the list of products: *"profil or bitumen or asphalt (without bitumen) or creosot or pitch or tar or teer or tires or tyres or reifen or extender oil or process oil or plasticiz(s)er oil or softener or weichmacheröl or anthracene oil or anthracenöl or carbon black or wood preserve or holzschutz"*

Next steps were searches for:

PAH or PAK or PAC

combined with *"(polycyclic or polyzyklisch) and NSO (without hetero)"*

NSO-HET (without PAH or PAK or PAC)

"Polyaromatic heterocycles" or"Polycyclic Aromatic Sulfur Heterocycles" or "Polycyclic Aromatic Nitrogen Heterocycles" or thiaarene or azaarene or oxaarene

combined with the list of products (see above)

and *"analysis or analyses or analytical or separation or sample or sampling or sampler or determin or or composition or component or identification or constituent or characterization or characterized or compound"*

Chance findings emerging from links to other sources were also included in the data analysis.

5.2.3 Supplemental search terms

The list of search terms was expanded by the corresponding available CAS numbers, but no additional relevant hits were identified.

A search for Material Safety Data sheets was performed as follows:

SDS or MSDS or sicherheitsdatenblatt

combined with the CAS numbers for the registered UVCB "*65996-79-4" or "65996-93-2" or "68187-58-6" or "84989-04-8" or "84989-06-0" or "85117-10-8" or "85536-20-5" or "90640-80-5" or "90640-84-9" or "90640-86-1" or "90989-38-1" or "90989-41-6" or "92062-36-7" or "101316-62-5" or "121575-60-8"*

A separate search in google was performed for terms as e.g. anthracene oil, tar pitch, marine coating, process/extender oils, carbon black/oils to identify product names and manufacturers thereof.

5.2.4 Additional sources of information

Registered Substances of Possible Relevance for Semipolar PAC

Substances in the highest tonnage range as well as substances classified as CMR category 1A or 1B (above 1 t/a) as well as substances very toxic to aquatic organisms classified as chronic category 1 above 100 t/a registered until November 30, 2010 are now publicly available. The "List of Registered Phase-in Substances" is ready for download at the ECHA web-site[11] in excel-format.

To target substances of possible relevance in respect to semipolar heterocyclic polyaromatic compounds (hetero-PAC), the following two approaches were adopted:

Firstly, from the UVCB compound list of the CEFIC Coal Chemicals Sector Group (CCSG, "REACH for Coal Chemicals", "R4CC"[12]), all CAS-numbers for UVCB-substances were extracted from the pdf-document provided at their web-site and compared to the "List of Registered Phase-in Substances". As coal tar and distillates and residues thereof are rich in HET-PAC, for a fist approximation these UVCB were regarded as possibly relevant sources. 38 Substances were positively identified. As substances registered as intermediates are most probably of low relevance, only substances with full registration were selected, resulting in 15 possibly relevant UVCB from coal distillates (see Table 8). Additionally, brown coal tar was included, being fully registered besides the R4CC-UVCBs. Coal tar creosote (8001-58-9), which is contained in the R4CC-list, is however not (yet) within the registered UVCBs. Until now no detailed verification of the actual relevance of these UVCBs for HET-PAC was carried out. As naphthas are the rather low boiling distillate fractions polyaromatics are probably of lower concentration and low molecular weight, as indicated in the comment field of Table 8.

[11] http://apps.echa.europa.eu/registered/registered-sub.aspx#search

[12] http://www.r4cc.org/

Table 8: UVCBs predominantly from R4CC (CCSG), already registered as of 28 Feb. 2011

CAS RN	Substance Name	Full registration	Coal chemicals sector group-UVCB?	Comment
65996-79-4	Solvent naphtha (coal)	Yes	R4CC UVCB	Polyaromatics probably low
65996-93-2	**Pitch, coal tar, high-temp.**	**Yes**	**R4CC UVCB**	
68187-58-6	**Pitch, petroleum, arom.**	**Yes**	**R4CC UVCB**	
84989-04-8	**Tar acids, methylphenol fraction**	**Yes**	**R4CC UVCB**	
84989-06-0	**Tar acids, xylenol fraction**	**Yes**	**R4CC UVCB**	
85117-10-8	Naphtha, thermal cracked, residues, naphthalene cut	Yes	R4CC UVCB	Polyaromatics probably low
85536-20-5	Solvent naphtha (coal), xylene-styrene cut	Yes	R4CC UVCB	Polyaromatics probably low
90640-80-5	**Anthracene oil**	**Yes**	**R4CC UVCB**	
90640-84-9	**Creosote oil, acenaphthene fraction**	**Yes**	**R4CC UVCB**	
90640-86-1	**Distillates (coal tar), heavy oils**	**Yes**	**R4CC UVCB**	
90989-38-1	**Aromatic hydrocarbons, C8**	**Yes**	**R4CC UVCB**	
90989-41-6	Aromatic hydrocarbons, C6-10, C8-rich	Yes	R4CC UVCB	Polyaromatics probably low
92062-36-7	**Aromatic hydrocarbons, C9-12, benzene distn.**	**Yes**	**R4CC UVCB**	
101316-62-5	**Extract residues (coal), light oil alk., acid ext., indene fraction**	**Yes**	**R4CC UVCB**	
101316-84-1	**Tar, brown-coal, low-temp.**	**Yes**	**No**	
121575-60-8	**Pitch, coal tar, high-temp., heat-treated**	**Yes**	**R4CC UVCB**	

In a second step, single HET-PAC substances listed by R4CC CCSG independent from a full registration were targeted as REACH-dossiers might be a source for toxicity data and data on applications. Additionally, MSDS (material safety data sheets) of a range of process oils from different companies used mainly in the rubber industry (extender oils or plasticizer oils) but also for other polymers (e.g. PVC) and printing inks were analyzed for contained petrol oil distillates (CAS-No.). Where CAS-numbers were not provided (Flexon 683), from description of distillate type a possible UVCB was assigned and the registration number given in 4th column of Table 9. Moreover, Carbon black, Carbon black oil (64741-59-9) and Asphalt was within the already registered UVCB with possible HET-PAC-relevance.

Table 9: Single compounds from R4CC (CCSG) as well as distillates (UVCBs) used for process oils and some other potentially relevant UVCBs, already registered as of 28 Feb. 2011

CAS RN	Substance Name	Full registration	Single compounds R4CC or UVCB used for process oils
86-74-8	**carbazole**	**-**	**R4CC-(Coal chemical sector group): Single compounds**
91-22-5	**quinoline**	**Yes**	**R4CC-(Coal chemical sector group): Single compounds**
119-65-3	**isoquinoline**	**-**	**R4CC-(Coal chemical sector group): Single compounds**
1333-86-4	**Carbon black**	**Yes**	**--**
64741-59-9	**Distillates (petroleum), light catalytic cracked – synonym Carbon black oil**	**Yes**	**--**
(64741-88-4 ?)	**Distillates (petroleum), solvent-refined heavy paraffinic**	**Yes**	**Process Oil (probably: Exxon Mobile Flexon 683, non-labeled, Registration No.: 01-2119488706-23-0016; 01-2119488706-23)**
64741-96-4	Distillates (petroleum), solvent-refined heavy naphthenic	Yes	Process Oil (Nyflex 220, non-labeled)
64742-04-7	Extracts (petroleum), heavy paraffinic distillate solvent	Yes	EXAROL 25, 41 SRD, TOTAL Plasticisers, Aromatics; Process Oil (Rubber, SUNOCO Sundex 790)
64742-05-8	Extracts (petroleum), light paraffinic distillate solvent	Yes	EXAROL 11, TOTAL Plasticisers, Aromatics
64742-10-5	Extracts (petroleum), residual oil solvent	Yes	Process Oil (Rubber, SUNOCO Sundex 790)
64742-46-7	Distillates (petroleum), hydrotreated middle	Yes	Process Oil (Nyflex 8120, non-labeled)
64742-52-5	Distillates (petroleum), hydrotreated heavy naphthenic	Yes	Process Oil (all non-labeled: Nytex 840, 832, 820, 810, 550; Nyflex 210B, 820, 223, 222B)
64742-53-6	Distillates (petroleum), hydrotreated light naphthenic	Yes	Process Oil (all non-labeled: Nyflex 800; Nyprint 841, 862, 863; Nytex 5130, 801)
64742-54-7	Distillates (petroleum), hydrotreated heavy paraffinic	Yes	Process Oil (Shell Catenex T 145 S, non-labeled)
64742-65-0	**Distillates (petroleum), solvent-dewaxed heavy paraffinic**	**Yes**	**PLAXOLENE MS, TOTAL Plasticisers, MES, non-labeled; Process Oil (Shell Catenex SNR; SUNOCO Sundex 790)**
64742-93-4	**Asphalt, oxidized**	**Yes**	**Process Oil (all non-labeled: Nyprint 841, 862, 863)**
72623-85-9	Lubricating oils (petroleum), C20-50, hydrotreated neutral oil-based, high-viscosity	Yes	PLAXENE 6110, TOTAL Plasticisers, Paraffinics, non-labeled
72623-86-0	Lubricating oils (petroleum), C15-30, hydrotreated neutral oil-based	Yes	PLAXENE 185, TOTAL Plasticisers, Paraffinics, non-labeled
72623-87-1	Lubricating oils (petroleum), C20-50, hydrotreated neutral oil-based	Yes	PLAXENE 1100, TOTAL Plasticisers, Paraffinics, non-labeled
8052-42-4	**Asphalt**	**Yes**	**--**
91995-70-9	**Extracts (petroleum), deasphalted vacuum residue solvent**	**Yes**	**PLAXOLENE 50, TOTAL Plasticisers, RAE, non-labeled**

Non-labeled process oils have an IP 346-extract (DMSO) of less than 3%, as carcinogenicity correlates with the mass% organic compounds contained in the extract. As besides PAH also HET-PAC and naphthenes are extracted by DMSO, most probably the HET-PAC content of these oils will be rather low. However, as up to

now no actual evidence for this assumption was provided by industry upon our requests, it would be interesting to evaluate the REACH dossiers in this respect. Especially regarding PBT properties, the threshold level for assessment of constituents is 0.1% (w/w) according to REACH guidance part R.11. As such, PBT-assessments for these UVCBs might be informative.

This list was forwarded to German Federal Environmental Agency (UBA) in order to gain information on details of registration dossiers via official channels. UVCB in grey-shaded fields are of special relevance for the occurrence of unidentified semipolar PAC.

Results from Evaluation of Registration Dossiers by UBA

German Federal Environmental Agency (UBA) evaluated 22 registration dossiers for UVCBs and single substances (grey shaded substances from Table 8 and Table 9) for information on semipolar PAC. As far as the lead registrant´s dossier could be determined this was chosen for evaluation. The sole semipolar PAC mentioned at all were carbazole, quinoline, isoquinoline and acridine. However, these documents contained no genuine information on PBT properties for any of these substances.

Concluding from these fruitless efforts, screening of further registration dossiers for information on PBT properties of semipolar PAC was considered to be of no use.

6 Screening Strategy on PBT-Properites

6.1 Outline of the Screening Strategy for PBT-Properties based on QSARS – Extended QSAR selection

To enable a fast prioritization of identified semipolar PAC relating to properties of persistence, bioaccumulation and toxicity, a screening method based on QSAR software tools is developed.

We decided to use programs of the US EPA QSAR software package for prediction of environmentally relevant properties ("EPI Suite" version 4.1) instead of US EPAs "PBT Profiler". This enables a screening based on EC REACH criteria (see Table 10), whereas the PBT Profiler is based on the US PBT-criteria differing from those of the EC. The proposed criteria (see below) are evaluated as a first step on eight compounds of the UVCB Anthracene Oil, for which experimental data are provided in the SVHC (substances of very high concern) support documents available from ECHA. As a second step 20 random compounds are selected from the initial *pool of identified semipolar PAC* and feasibility of screening with the proposed criteria for bioaccumulation (B), persistency (P) and toxicity (T) is demonstrated.

For B-criteria, we decided to use log K_{ow} as a screening parameter instead of the calculated bioconcentration factor (BCF). Literature evaluation of programs predicting BCF from log K_{ow} against validated experimental data indicates a high percentage of misses, i.e. substances with bioaccumulative properties are classified as not bioaccumulative. As at the same time the screening criterion for bioaccumulation according to REACH guidance documents of log K_{ow} 4.5 is demonstrated to be very strict (too many false negatives, i.e. misses), we used log K_{ow} of 4.0 as cut off criterion for the screening on B-properties. This is in accordance with criteria on classification, labelling and packaging (CLP regulation, EC no. 1272/2008).

Table 10: Summary of PBT-criteria according to REACH Annex XIII and screening criteria according to REACH guidance documents

Property	Criterion according to REACH Annex XIII	Screening-criterion according to guidance
Persistency	$T_{1/2}$ fresh water > 40 days $T_{1/2}$ sediment (fresh water) > 120 days $T_{1/2}$ soil > 120 days	Not readily *biodegradable* and / or Not *inherently biodegradable* and /or Not degradable by physicochemical processes (e.g. hydrolysis)
Bioaccumulation	Bio-concentration factor (BCF) > 2000	Log Kow > 4.5
Toxicity	Long term NOEC < 0.01 mg/L *OR* CM cat. 1 or 2, R cat. 1, 2, or 3 *OR* T, R48, oder Xn, R48 according to 67/548/EWG	EC50 / LC50 (alga, daphnia or fish) < 0.1 mg/L: T *presumably fulfilled* < 0.01 mg/L: T *definitely fulfilled*

Criteria for very persistent (vP) and very bioaccumulative (vB) substances according to REACH annex XIII: **vP**: $T_{1/2}$ (water) > 60 days, $T_{1/2}$ (sediment/soil) > 180 days; **vB**: BCF > 5000

For P-criteria, following REACH guidance documents a pairwise combination of the results of three of the BIOWIN-programs of EPI Suite is proposed to assess persistency, i.e. BIOWIN2/3 (<0.5/≤2.25) and BIOWIN6/3 (<0.5/≤2.25). One combination indicating persistence is sufficient for a positive screen on P.

For T-criteria we decided to use acute toxicity results calculated by ECOSAR-software of EPI Suite for algae, daphnids and fish and to compare the effect value of the most sensitive organism group with the cut-off screening-value for T. The screening value for T according to REACH guidance documents (0.1 mg/L for acute L(E)C_{50}) differs only by a factor of 10 from the T-criterion of Annex XIII of REACH (0.01 mg/L long

term NOEC), i.e. by an acute to chronic ratio (ACR) of 10. We propose to apply an ACR of 100 according to REACH guidance documents R.10 on PNEC derivation. Deriving the screening criterion on T from the T-criterion of Annex XIII REACH (0.01 mg/L long term NOEC) by applying an ACR of 100 a cut-off value of 1 mg/L for acute L(E)C_{50} values results which we will directly compare with the ECOSAR-result for the most sensitive organism group. This screening criterion is in accordance with the criterion for acute aquatic hazard category 1 of CLP regulation (EC no. 1272/2008).

Concluding from the results of the application of the above outlined screening criteria on 20 random compounds we decided to consider also those compounds further, which only fulfil two of the three criteria, considering some uncertainty with the prediction by QSAR. For the combinations BT and BP we set a stricter selection cut-off on B, namely log K_{ow} 4.5 instead of 4.

6.2 Extended QSAR Screening Approach: Evaluation and Fitting on Experimental Data for compounds of UVCB anthracene oil from SVHC support documents

6.2.1 Screening for bioaccumulative properties

As a first step, log K_{ow} values and bioconcentration factors (BCF) were determined by the EPI suite software in batch mode and compared to experimental data from the SVHC support documents.

Log K_{ow} is estimated by KOWWIN software from chemical structure. Whenever experimental values are available in the EXPKOW.DB database, these are preferred. BCF values are estimated from log K_{ow} values, with preference on experimental values on log K_{ow} if available from the EXPKOW.DB database. Resulting values from EPI suite (BCFBAF) compared to experimental results from UVCB support documents are summarized in Table 11. Additionally, two compounds from Table 15 (20 compounds chosen by random from the initial *pool of identified semipolar PAC* of up until then 160 compounds to demonstrate feasibility of final screening criteria for PBT-properties) are included and denoted with an asterisk, as for these compounds experimental values for BCF from the OECD QSAR-Toolbox[13] (OECD, 2010) were available for comparison with calculated values. This data source was used also for acenaphthene and carbazole, as for these compounds no data on BCF were available from the SVHC support documents.

As summarized in Table 11, upon includision of the QSAR-Toolbox experimental values, experimental data on compounds of UVCB anthracene oil are available for all the 8 compounds and additionally for the two compounds from the pool of randomly chosen semipolar PAC of Table 15 below. Determined values show high variability, with mean measured values for BCF however always above 2000, the criterion for B according to REACH, with the exception of acenaphthene and carbazole as well as 3-hydroxy-2-naphthalenecarboxylic acid (not part of the UVCB anthracene oil), where measured BCFs are clearly below 2000. For 4 compounds from anthracene oil and additionally dibenzothiophene upper reported BCF values are exceeding even 5000, the criterion for very bioaccumulative (vB) substances according to REACH Annex XIII: Therefore, conclusion from the SVHC support documents is for three compounds (phenanthrene, fluoranthene and pyrene) very bioaccumulative (vB) and for two compounds (fluorene and anthracene) bioaccumulative (B). Dibenzofuran was self-classified by the authors based on the only reported experimental value of the support document, which was evaluated as reliable with restrictions (reliability category 2). Anthracene was evaluated in a separate document. Following the B-assessment procedure applied for the other compounds with experimental data where upper reported values were applied for vB-assessement, also an-

[13] http://www.oecd.org/document/54/0,3746,en_2649_34377_42923638_1_1_1_1,00.html

thracene would be judged as vB. Additionally dibenzothiophene (not part of the UVCB) would be classified according to REACH criteria as at least bioaccumulative.

Table 11: Compounds of the UVCB Anthracene oil – results on log K_{ow} and BCF from EPI suite software compared to results from CAESAR BCF-tool and experimental results and conclusion on bioaccumulation according to SVHC support documents (ECHA)

Chemical name	CAS-No.	Measured Log K_{ow} (EXPKOW. DB)	BCFBAF estimated BCF	CAESAR estimated BCF	Experimental (SRC-DB)	SVHC support documents BCF measured: range (mean)	Conclusion in SVHC support documents
Phenanthrene	85-01-8	4.46	1865	1215	**2512**	700-6760 (2092)	**vB**
Fluoranthene	206-44-0	**5.16**	1179	1483	**3631**	3388-5920 (4476)	**vB**
9H-Fluorene	86-73-7	4.18	266.1	1064	525	1050-3500 (2275)	B
Pyrene	129-00-0	**4.88**	770.6	1498	1514	2700-35000 (12694)	**vB**
Acenaphthene	83-32-9	3.92	179.2	808	759	(387-1000)**	--
9H-Carbazole	86-74-8	3.72	132.3	76	170	(200-500)**	--
Dibenzofuran	132-64-9	4.12	242.9	87	1514	3430	B[s]
Anthracene	120-12-7	4.45	401	1210	1820	900-10400 (4300)	B
2-Naphthalenecarboxylic acid, 3-hydroxy-*	92-70-6	3.05	3.162	13	4	(1.4-6.7)**	--
Dibenzothiophene*	132-65-0	4.38	360.5	584	1130	(1440-6610)**	B[s]

(*) Not part of Anthracene-UVCB: The only compounds of Table 15 with experimental data from the OECD QSAR toolbox

(**) Experimental values from the OECD QSAR-Toolbox

(s) Selfclassification according to REACH - no classification in SVHC support document for this compound

Calculated BCF-values by BCFBAF (EPI Suite) are below 2000 for all compounds and only for phenanthrene and fluoranthene beween 1000 and 2000 (orange / light grey shading in Table 11). Thus, applying criteria of REACH Annex XIII on calculated BCF values, none of these compounds would have been judged to be bioaccumulative.

Surprisingly, experimental BCF-values from the SRC-database retrieved by BCFBAF and used for building the QSAR-model are pronouncedly higher than the calculated ones. Following these values, at least phenanthrene and fluoranthene would be judged as B, being vB according to data from the SVHC support documents. The reason for the discrepancy between modelled and experimental values of SRC-DB might be the assumption of metabolic biotransformation and the inclusion of modelled biotransformation rates as default in the BCF calculation by BCFBAF. As biotransformation is an important parameter determining bioconcentration and bioaccumulation, in respect to the uncertainty associated with modelled biotransformation rates and the importance to avoid a large number of false negatives in the first screening run potential biotransformation should be carefully judged in a later evaluation step on bioaccumulative properties.

As these results are highly unsatisfactory, more recent QSAR software funded by the European Commission taking account of the REACH requirements was applied on these 10 compounds to compare the results with BCFBAF calculated as well as the experimental values. The CAESAR web tool is freely available on the web and a SMILES-based batch procedure is implemented[14] CAESAR models have been assessed according

[14] http://www.caesar-project.eu/index.php

to the OECD principles for the validation of QSAR. A description of the algorithm and a publication on the model validation for BCF in fish are available (Lombardo, et al., 2010; Zhao, et al., 2008). Applying this model BCF values are derived which still would lead to a classification of not B for all of the 10 compounds. However, at least for five of the 10 compounds BCF values above 1000 are resulting for compounds rated as B or vB according to the SVHC support documents. Thus, CEASAR results seem to be slightly better than results by BCFBAF, but still not convincing regarding the chemicals in question.

Applying the screening criterion of log K_{ow} > 4.5, pyrene and fluoranthene (both vB according to experimental data) would be evaluated as bioaccumulative (red / dark grey shading in Table 11), still underestimating bioaccumulative properties of phenanthrene, fluorene, dibenzofuran, anthracene and dibenzothiophene. Applying the criterion of log $K_{ow} \geq 4.0$ according to CLP-regulation (EC number 1272/2008) for a bioaccumulation potential (a criterion for classification in one of the chronic hazard categories), a good agreement with the experimental results is accomplished: All compounds classified according to experimental values from the SVHC support documents or experimental values from the OECD QSAR-Toolbox as B or vB are correctly assigned as bioaccumulative (see Table 11, orange / light grey shading for ≥ 4.0 red / dark grey shading for ≥ 4.5) concluding from their log K_{ow}.

A review by Arnot and Gobas (2006) on BCF and BAF (bioaccumulation factor) assessment for organic chemcials is further corroborating this log K_{ow}-based evaluation proposal as reasonable for screening for B-properties without taking the risk of a large number of false negatives which would be lost in the first selection run. Based on 5317 BCF and 1656 BAF-data on 842 organic chemicals in 219 aquatic species a validated data set is created by applying 6 confidence scoring criteria. 45% of BCF-values proved to be subject of at least one major source of uncertainty, leading generally to an underestimation of actual BCF values. The resulting validated experimental data set of BCF-values was compared to calculated values from different models. According to the real data, BCF-values above 2000 and 5000 were found already for (some) compounds with log K_{ow}-values ≥ 3.5 and ≥ 3.7, respectively. For the BCFWIN-model of EPI Suite (now BCFBAF), however, BCF-values above 2000 and 5000 were only found for compounds with much higher log K_{ow}-values, namely ≥ 5.2 and ≥ 5.7, respectively. This means, that the probability of false negatives (misses) according to these validated experimental data for substances with BCF of ≥ 5000 was 70.6% for BCFWIN model.

Thus, for screening of substances on a potential for bioaccumulation with sufficient sensitivity to avoid a high number of false negatives, taking log K_{ow} values (with preference on experimentally determined values, as far as available) with a cut off criterion of log $K_{ow} \geq 4$ according to CLP-regulation seems to be most appropriate for bioaccumulation screening. An even lower log K_{ow} cut off is expected to result in a number of false positives too high as to permit a close examination and expert judgement on bioaccumulation properties as is envisaged as a later step.

To yield reasonable results, a prerequisite for this approach is confidence in the calculated log K_{ow}-values. The KOWWIN-program as part of the EPI Suite software package provides the user with validation information. A validation with 10946 compounds not included in the training set resulted in r^2 (correlation coefficient) of 0.94 and a standard deviation of 0.479. The probability for an estimation error ≤ 0.5 log units and 1.0 log units is reported to be 75.6% and 97.7%, respectively. Several publication compare KOWWIN-results with results of other software for log K_{ow}-prediction and real measured values (e.g. Benfenati, et al., 2003; Lazewska, et al., 2007; Pyka, et al., 2006; Zhang, et al., 2010). KOWWIN proved to be one of the best software tools tested for log K_{ow} prediction and mostly good correlations of KOWWIN-results with experimental values are reported. Zhang et al. (2010) compared the results of different partitioning property estimation methods including KOWWIN and SPARC, the latter based on fundamental calculated molecular proper-

ties. As in conclusion SPARC performed not significantly better than KOWWIN[15] we prefer the latter as batch processing is possible with KOWWIN only.

To verify prediction accuracy further, compounds from Table 11 and Table 15 with experimental log K_{ow}-values available are listed in Table 12. Their calculated and experimental log K_{ow}-values are compared to the experimental ones and difference in log units are given. For four from 17 compounds the difference in log units was larger than 0.25 but in any case lower than 0.5 log units. Thus, the prediction accuracy is acceptable to screen for bioaccumulative properties of semipolar compounds.

Table 12: Measued and estimated log K_{ow}-values by KOWWIN program of EPI Suite. Differences in log-units higher than 0.25 are shaded orange / light grey.

Chemical name	CAS number	Estimated Log K_{ow}	Measured Log K_{ow} (EXPKOW.DB)	Δ log-Units
Phenanthrene	85-01-8	4.35	4.46	0.11
Fluoranthene	206-44-0	4.93	5.16	0.23
9H-Fluorene	86-73-7	4.02	4.18	0.16
Pyrene	129-00-0	4.93	4.88	0.05
Acenaphthene	83-32-9	4.15	3.92	0.23
9H-Carbazole	86-74-8	3.23	3.72	0.49
Dibenzofuran	132-64-9	3.71	4.12	0.41
Anthracene	120-12-7	4.35	4.45	0.1
2-Naphthalenecarboxylic acid, 3-hydroxy-	92-70-6	3.42	3.05	0.37
Dibenzothiophene	132-65-0	4.17	4.38	0.21
Phenanthrothiophen, 4,3-b	195-68-6	5.34	5.35	0.01
Benzo(b)naphtho(1,2-d)thiophene	205-43-6	5.34	5.19	0.15
Benzo[b]naphtho[2,3-d]furan	243-42-5	5.23	5.05	0.18
9H-Fluoren-9-one	486-25-9	3.55	3.58	0.03
Quinoline, 8-methyl-	611-32-5	2.69	2.6	0.09
8-Quinolinol, 2-methyl-	826-81-3	2.21	2.33	0.12
Dibenzofuran	132-64-9	3.71	4.12	0.41

6.2.2 Screening for persistency

Most relevant for persistency assessment in general is the potential for biodegradation, while especially for volatile substances also atmospheric degradation processes e.g. by UV-irradiation and hydroxyl radical triggered degradation processes are of importance. For certain substances hydrolysis in water might also be of relevance. This latter process is however a form of primary degradation in most circumstances, leading to transformation products which in turn might be of detrimental effect. As these physicochemical degradation processes are relevant in certain cases only, within the first screening for PBT-properties the persistency screening by QSAR is restricted on the potential for ultimate biodegradation.

For the persistency screening by QSAR, BIOWIN-programs within the EPI Suite software package are used. BIOWIN 1-7 are all based on molecular fragment constants that were developed using multiple linear or non-linear regression analyses, depending on the model. From 295 compounds 36 molecular fragments were

[15] For the aquatic bioaccumulation potential (BAP_{aq} determined from criterion log $K_{ow} \geq 5$) in table 1 the number of false positives is reported as 15 and 16 and the number of false negatives as 4 and 5 for EPI suite and SPARK, respectively.

derived which affect biodegradability. The 37th variable is molecular weight. BIOWIN 1 and 2 predict aerobic fast biodegradation (ready biodegradability), are based on the same data set and represent the linear and non-linear mode, respectively. A biodegradation probability greater than 0.5 is considered as fast biodegradable, a biodegradation probability less than 0.5 is considered as not fast biodegradable. These results give however no information on the potential for complete, ultimate biodegradability in a wider time frame. This is covered by BIOWIN 3 results. Based on a training set of 200 compounds, experts rated the time frame for complete ultimate biodegradation on a scale of 1 to 5: 5 - hours; 4 - days; 3 - weeks; 2 - months; 1 – longer. Based on these ratings, the program output is scaled, e.g. > 1.75 - 2.25 corresponding to "months", < 1.75 "recalcitrant". BIOWIN 5 and 6 also address ready biodegradability, the training set of compounds however is based on the MITI test on ready biodegradability (OECD 301C). 884 chemicals (385 readily biodegradable, 499 not readily biodegradable) were used to derive the fragment probability values used for the QSAR model. Biowin 5 is the linear, Biowin 6 the nonlinear model. As with Biowin 1 and 2, a biodegradation probability greater than 0.5 is considered as readily biodegradable, a biodegradation probability less than 0.5 is considered as not readily biodegradable.

BIOWIN 2, 3 and 6-results are assessed in combination according to ECHA[16]-guidance on Information requirements and Chemical Safety Assessment, part R11. According to this guidance, for combinations of BIOWIN 2 and 3 (< 0.5 and < 2.2, respectively) or BIOWIN 6 and 3 (< 0.5 and < 2.2, respectively) indicating persistence, compounds are judged as persistent according to REACH for screening purposes. This method has been already applied for prioritization of low production volume substances under REACH regarding PBT-criteria by the Environment Agency UK (Brooke and Burns, 2009). QSAR-results for compounds of anthracene oil evaluated that way are compared to experimental data on persistence as well as the conclusion on persistence (as far as available) from the SVHC support documents in Table 13. However, as the REACH guidance is slightly imprecise in giving the limit for BIOWIN 3-results as < 2.2 ("months or longer"), the cut off-value as given in the BIOWIN 3 documentation for two decimal places (≤ 2.25) for the time range "months or longer" is taken.

The screening performed according to the evaluation procedure as suggested by REACH guidance R.11 is consistent with the experimental data for fluoranthene, pyrene, anthracene and phenanthrene (P1 = persistent according to BIOWIN 2- and 3-results; P2 = persistent according to Biowin 6- and 3-results). For carbazole, only one experimental result is reported in the SVHC support document. According to this, the half-life in soil is larger than 184 days. Therefore, also carbazole fulfils the persistence criterion (> 120 d). However, BIOWIN 3 doesn't recognize the poor biodegradability of carbazole (score 2.8 corresponding to weeks). Nevertheless, the screening procedure with BIOWIN 3 cut off of ≤ 2.25 in combination with BIOWIN 2 or 6-results according to REACH guidance R.11 seems to give reasonable results and will be applied for the QSAR screening for P.

Table 13: Compounds of the UVCB Anthracene oil – results on persistence based on EPI suite software and evaluated according to REACH guidance R.11 compared to experimental results and conclusion on persistence according to SVHC support documents (ECHA)

		Persistency assessment according to Guidance R11 with persistence according to	SVHC support documents

[16] European Chemicals Agency, http://echa.europa.eu/web/guest/home

Chemical name	CAS-No.	BIOWIN 2/3 : <0.5/≤2.25 = P1 Biowin 6/3 : <0.5/≤2.25 = P2	Half life soil in days (d), years (a) for field studies	Persistency conclusion SVHC support documents
Phenanthrene	85-01-8	P2	83 d – 5.7 a	vP
Fluoranthene	206-44-0	**P1P2**	173 d – 7.8 a	vP
9H-Fluorene	86-73-7	not P	--	--
Pyrene	129-00-0	**P1P2**	131 d – 8.5 a	vP
Acenaphthene	83-32-9	not P	--	--
9H-Carbazole	86-74-8	not P	>184 d	--
Dibenzofuran	132-64-9	not P	--	--
Anthracene	120-12-7	P2	210 d – 7.9 a	vP

Data on persistence as given by KORA results for several NSO-heterocyclic compounds are in most cases of no use for the current project. Predominantly primary biodegradation was assessed (personal communication with Dr. Thomas Held, Arcadis GmbH) on the one hand and biodegradation was assessed on the other hand at contaminated sites where over the years specialized microbial communities could establish themselves. In some cases even enhanced natural attenuation was applied (KORA, 2005; Reineke and Hollender, 2007). Thus, for example carbazole and anthracene are rated as aerobic biodegradable.

6.2.3 Screening for toxicity

QSAR screening for ecotoxicity is performed with the ECOSAR program of US-EPAs EPI suite.

Taking 31 compounds with experimental data, Blotevogel et al. (2007) compared ECOSAR results for algae and fish acute toxicity to experimental values. For these compounds 25 experimental LC_{50}-values were available for acute fish toxicity. ECOSAR results deviated in both directions with an underestimation of toxicity for 60% of the data; however deviations were low: for 84% below a factor of 5, for 96% below a factor of 10. The maximum deviation observed was by a factor of 11 (overestimation of toxicity).

Algal toxicity data (EC_{50}-values) were available for 19 of these compounds. Also for algae deviation of ECOSAR results was in both directions but underestimation of toxicity was for 74% of the compounds observed. However deviations were low also for algae. For 74% of the 19 compounds with experimental values for algae deviations were below a factor of 5, for 89% below a factor of 10. The maximum deviation observed was by a factor of 28 (underestimation of toxicity).

Blotevogel et al. concluded that ECOSAR is applicable for a screening on ecotoxicity of NSO-heterocycles.

For screening of semipolar PAC from the ecotoxicity results of ECOSAR the lowest acute toxicity value from the representatives of three trophic levels (algae, daphnia and fish) is taken, which can be compared to the screening criterion for T according to REACH guidance of < 0.1 mg/L for the acute EC_{50}. The decisive effect concentration was taken irrespectively of a lower solubility than the effect concentration indicated by QSAR results of EPI suite, as for long term effects caused by bioaccumulation a low water solubility may be sufficient for build-up of higher concentrations in the organism over time.

As a first test for this procedure, for compounds of UVCB anthracene oil the resulting conclusions based on QSAR (T or not T) is confronted with experimental data and the conclusion on T from the SVHC support documents from ECHA and – where no data were available from these documents – from KORA (Blotevogel, et al., 2007), LAWA AG (2010) or the ETOX-database[17] with conclusion on T according to the

[17] http://webetox.uba.de/webETOX/index.do

criteria of REACH Annex XIII or the REACH guidance document R.11 (see Table 10). The results are summarized in Table 14. According to the experimental data available, only three from these eight compounds are not T, namely fluorene, acenaphthene and dibenzofuran. However, according to the ECOSAR results, not any of these compounds would have been judged as T applying the screening criterion of REACH guidance R.11 (< 0.1 mg/L). Therefore, the combination of ECOSAR results and the screening criterion according to REACH underestimates the ecotoxicity for 5 from 8 compounds.

According to CLP-regulation (EC number 1272/2008) compounds are classified as hazardous to the aquatic environment chronic category 1 if EC/LC50-values for fish, algae / plants or crustaceans are below or equal to 1 mg/L and the compound is at the same time not rapidly degradable or log K_{ow} is ≥ 4.

For PNEC fresh water derivation REACH guidance R.10 foresees an assessment factor of 1000 on the lowest $L(E)C_{50}$ if only acute data for the three trophic levels are available to account for the uncertainty in respect to chronic toxicity having only acute data (acute to chronic ratio, ACR: factor 100) and the uncertainty relating to extrapolation from laboratory test results for single representative species to field conditions (10). The employed acute to chronic ratio for the screening criterion on T according to REACH guidance (< 0.1 mg/L) compared with the criterion of REACH Annex XIII (long term NOEC < 0.01) is only 10. As Ahlers et al. (2006) point out, the median ACR determined from validated experimental data regarding new and existing chemicals was 10.5 for fish, 7.0 for daphnids and 5.4 for algae. However, as individual ACRs vary considerably they conclude that even the assessment factor of 100 currently employed is not protective for all chemicals and trophic levels. A similar conclusion is drawn by Raimondo et al. (2007): From 456 ACRs for aquatic invertebrates and fish employing a diverse set of chemicals a median value for ACR of 8.3 was determined, however with a 16,000-fold range in values (1.1-18,550) and still a 32-fold range in 10th and 90th percentile values (2.5-79.5).

To corroborate this further, a closer look on additional ecotoxicity data to compounds phenanthrene, fluoranthene, pyrene, carbazole and anthracene classified as T by SVHC support documents or self-classified as probably T from experimental data from KORA, LAWA AG or ETOX-DB was taken. Experimental ecotoxicity data available via OECD QSAR-Toolbox[18] (OECD, 2010) and CHRIP Japan[19] for these compounds were assessed.

Taking acute experimental data it was examined to what extent ECOSAR is underestimating the actual acute toxicity of these compounds. UV-activation, which reportedly lead to a marked activation of some of these compounds (reported e.g. in SVHC support documents) was deliberately not taken into account, as this is not foreseen in standard test according to OECD guidelines on aquatic toxicity. As a result ECOSAR underestimates the toxicity towards small crustaceans for phenanthrene between a factor of 0.84 and 4.6 (depending on the study), for fluoranthene by a factor of 12, for pyrene by a factor of 7 and for anthracene between a factor of 1.3 and 26 depending on the study. Algal toxicity is underestimated for carbazole by a factor between <10 and 28 (without taking into account the values reported by ETOX stemming from one and the same publication, as these are strikingly low compared to other publications). Thus, ECOSAR indeed underestimates acute aquatic toxicity of these compounds (for all of them the class “neutral organics”, meaning baseline toxicity, had been applied) by approximately factor 10 (minimum 0.86, maximum 28). However,

[18] http://www.oecd.org/document/54/0,3343,en_2649_34379_42923638_1_1_1_1,00.html

[19] http://www.safe.nite.go.jp/english/db.html

taking into account the observed variability of experimental data itself we conclude that for our screening approach QSARs applied using ECOSAR are well applicable.

For an assessment of the adequacy of the acute to chronic ratio of 10 applied for the screening criterion according to REACH guidance R.11 we examined which of the five compounds would have been classified as T by acute ecotoxicity data alone (standard tests without UV activation) by application of the screening criterion of 0.1 mg/L. This would be not the case for phenanthrene whereas for anthracene it depends on the study on Daphnia taken into account (EC_{50}-values between 0.025 to 0.75 mg/L). Available acute data for fluoranthene and pyrene are at least in part below the screening criterion of 0.1 mg/L. For carbazole chronic data for daphnids and fish are missing and data on algal toxicity are equivocal, thus a reliable assessment of the T-criterion is not possible. Besides this, acute to chronic ratio could be determined for phenanthrene, fluoranthene, pyrene and anthracene, as acute and chronic data for the same trophic level were available. The ratio was each time above 10 and three times near (80) or even above 100. From these results we conclude that the acute to chronic ratio of 10 implicitly applied for the REACH screening criterion on T of 0.1 mg/L is too low.

In conclusion,

- Considering the underestimation of acute toxicity by ECOSAR,
- The insufficient acute to chronic ratio of 10 applied for the screening-criterion on T according to REACH guidance R.11 and
- The special property of photo activation by UV of some polyaromatic compounds not accounted for by ECOSAR and standard aquatic toxicity tests,

we consider EC50-values $\leq$ 1 mg/L as sufficiently low for a screening assessment as T by QSAR. Evaluating the ECOSAR data by this screening-criterion for T, a much better agreement with the experimental data is accomplished. As column 5 of Table 14 illustrates, all compounds are correctly assigned except carbazole, which is still judged as not T according to QSAR-Data. As mentioned above, decisive chronic data for T-assessment of carbazole are missing and the preliminary self-assessment as T may be not justified.

It seems therefore most promising to use the lowest acute toxicity value for fish, daphnia and algae from ECOSAR together with the criterion for environmental hazard class 1 according to CLP-regulation (EC number 1272/2008) ($\leq$ 1mg/L, i.e. an ACR of 100 on the NOEC specified by REACH Annex XIII) to screen for T-properties.

Table 14: Compounds of the UVCB Anthracene oil – results on ecotoxicity based on ECOSAR software and evaluated according to the proposed screening criterion for T (L(E)C50-values ≤ 1 mg/L) compared to experimental results and conclusion on persistence according to SVHC support documents (ECHA), LAWA AG, KORA and ETOX database (UBA);

		ECOSAR: lowest effect value algae, daphnids (EC50), fish (LC50)			Experimental data: SVHC support documents - data by KORA or LAWA AG or ETOX db (UBA), if annotated	
Chemical name	**CAS-No.**	**[mg/L]**	**Organism, effect value [Ecosar-class]***	**QSAR conclusion on T**	**Species, effect values [mg/L]: not all reported values are listed here**	**Conclusion on T**
Phenanthrene	85-01-8	0.95	Daphnid, 48h [NO]	T	Daphnia magna, EC50 (48h): 0.207 (ETOX); Oncorhynchus mykiss, embryo-larval toxicity, 23d LC50: 0.04 (ETOX)	T[s]
Fluoranthene	206-44-0	0.36	Daphnid, 48h [NO]	T	Pseudopleuronectes americanus (winter flounder), 96h LC50: 0.0001 (UV-light exp. to > 0.188 (fluorescent light exp.); Pimephales promelas, NOEC (11w, 32d): <0.0062 – 0.0014; Pseudokirchneriella subcapitata, 72h EC10 (growth): 0.0086	T
9H-Fluorene	86-73-7	1.64	Daphnid, 48h [NO]	Not T	Daphnia magna, EC50 (48h): 0,212 (ETOX)	Not T[s]
Pyrene	129-00-0	0.36	Daphnid, 48h [NO]	T	Mulinia lateralis (Dwarf Surf Clam), 96h LC50: 0.00168 (UV-irradiation); Ceriodaphnia dubia, EC10 (reproduction, 7d): 0.0021; Pseudokirchneriella subcapitata, 72h EC10 (growth): 0.0012	T
Acenaphthene	83-32-9	1.18	Daphnid, 48h [NO]	Not T	Daphnia magna, EC50 (48h): 3.45 (ETOX); Salmo trutta, LC50 (96h): 0.58 (ETOX)	Not T[s]
9H-Carbazole	86-74-8	5.83	Green Algae EC50 [NO]	Not T	Scenedesmus subspicatus, 96h EC50 (growth): 0.21 (LAWA AG); Pseudokirchneriella subcapitata, 72h EC50 (growth): 0.015 (ETOX); Scenedesmus acutus, 72h EC50 (growht): 0.005 (ETOX)	T[s]
Dibenzofuran	132-64-9	2.92	Green Algae EC50 [NO]	Not T	Danio rerio, fish egg 48h LC50: 0.40 (KORA / LAWA)	Not T[s]
Anthracene	120-12-7	0.95	Daphnid, 48h [NO]	T	Selenastrum capricornutum, EC50 (22h, UV-light): 0.004; Chlorelle protothecoides, EC50 (24h with or w/o UV-light): 2.53; Daphnia magna, NOEC (21d): 0.0022-0.0068;	T

(*)Abbreviations for ECOSAR classes: NO = neutral organics;

(s) Self classification according to REACH and REACH guidance R.11 - no classification in SVHC support document for this compound

6.2.4 Application of the proposed QSAR screening criteria for PBT properties on 20 randomly selected compounds from the initial pool of NSO-heterocyclic compounds from KORA and LAWA AG

For an exemplary application of the PBT-screening criteria by QSAR as developed in sections 6.2.1 to 6.2.3, 20 compounds were chosen randomly from the initial *pool of identified semipolar PAC* of 160 compounds using random numbers. As by this procedure one of the chosen compounds was dibenzofuran, which already was assessed as one of the compounds of the UVCB anthracene oil, the next compound in the random order, 2,3-dihydro-indole, was chosen instead. For ECOSAR data on aquatic toxicity, the more specific structure activity relationships (SAR) were preferred over the general SAR "neutral organics" as far as available. Values were taken even if they were outside the model domain because of a log K_{ow} higher than the limit of the

model, namely log K_{ow} of 5. For this case ECOSAR predicts no acute toxicity at saturation concentration. As this affects acute models only for toxicity on fish and daphnia (in the current selection of compounds relevant only for daphnia) while the current screening is for chronic toxicity, it is justified to take a precautious approach. Poor water solubility combined with persistence and a potential for bioaccumulation after prolonged exposure might result in body concentrations high enough to cause harm.

For compounds selected by random for this screening together with the screening results see Table 15. If criteria for P, B or T are fulfilled, cells are coloured red. From the 20 compounds only three compounds match all three criteria. These are phenanthro(4,3-b)thiophene, benzo(b)naphtho(1,2-d)thiophene and 7,10-dimethylbenz(c)acridine. These are the only compounds predicted to be persistent. As such, from this first screening list no candidates for vPvB-substances (very persistent and very bioaccumulative) could be found. Moreover, all compounds with a log $K_{ow} > 4.5$ (the REACH screening criterion) are predicted to be T at the same time according to criteria of the CLP-directive (≤ 1 mg/L). Two compounds are predicted to be T without fulfilment of criteria for P or B (3-methylindole and 6-phenanthridone). Dibenzothiophene is predicted to be B according to the criterion of the CLP-directive (log $K_{ow} \geq 4$), without fulfilling further criteria.

Concluding from these results, it is proposed to consider also those compounds further, which only fulfil two of the three criteria, considering some uncertainty with the prediction by QSAR. While substances not considered to be P are at least in their majority probably either biodegradable or at least only of moderate persistence, under these circumstances a higher threshold for B, namely the criterion of log $K_{ow} > 4.5$ according to REACH guidance document R.11 is proposed. By this approach compounds which are ecotoxic and most probably at the same time have a high potential for bioaccumulation are targeted. These compounds could cause harm already at half-lives shorter than the limits for persistence according to REACH. This procedure would also consider benzo[b]naphtho[2,3-d]furan, 1-methyldibenzofuran, 4-methyldibenzothiophene and 2-methyldibenzothiophene as possibly relevant semipolar PAC. Similarly, compounds fulfilling only screening criteria for B and P are most probably not exceptionally toxic and the higher threshold for B of log $K_{ow} > 4.5$ is justified. Applying these criteria, candidates for vPvB-properties are selected.

Thus, finally 13 from 20 compounds (65%) would be dismissed as not relevant for a further evaluation of available data on persistency, bioaccumulation and ecotoxicity. 7 compounds (35%) would be considered further on.

Table 15: 20 randomly chosen compounds from the initial list of identified semipolar PAC from KORA and LAWA AG and results of the PBT screening according to sections 6.2.1 - 6.2.3.

Chemical name	CAS	Persistency assessment	Measured Log K_{ow} where available	ECOSAR effect concentration [mg/L] [Ecosar-class]*	Organism, effect value	QSAR conclusion on T ($\leq$ 1 mg/L)
1H-Indole, 3-methyl-	83-34-1	not P	2.6	0.42 [PP]	Fish LC50 (96h)	T
Benzo f quinoline, 3-methyl-	85-06-3	not P	3.87	2.53 [NO]	Daphnid LC50 (48h)	Not T
2-Naphthalenecarboxylic acid, 3-hydroxy-	92-70-6	not P	3.05	15.42 [PA]	Daphnid LC50 (48h)	Not T
1H-Indole, 2,3-dihydro-	496-15-1	not P	2.06	22.60	Green algae, EC50	Not T
Dibenzothiophene	132-65-0	not P	4.38	1.37 [NO]	Daphnid LC50 (48h)	Not T
Phenanthrothiophen, 4,3-b	195-68-6	P1P2	5.35	0.19	Daphnid LC50 (48h)	T
Benzo(b)naphtho(1,2-d)thiophene	205-43-6	P1P2	5.19	0.19 [NO]	Daphnid LC50 (48h)	T
Benzo[b]naphtho[2,3-d]furan	243-42-5	not P	5.05	0.22 [NO]	Daphnid LC50 (48h)	T
9H-Fluoren-9-one	486-25-9	not P	3.58	3.96 [NO]	Green algae, EC50	Not T
Quinoline, 8-methyl-	611-32-5	not P	2.6	10.91 [NO]	Green algae, EC50	Not T
8-Quinolinol, 2-methyl-	826-81-3	not P	2.33	6.33 [P]	Daphnid LC50 (48h)	Not T
6(5H)-PHENANTHRIDINONE	1015-89-0	not P	1.3	0.67 [A]	Green algae, EC50	T
7,10-Dimethyl benz(c)acridine	2381-40-0	P1P2	5.59	0.13 [NO]	Daphnid LC50 (48h)	T
9H-CARBAZOLE, 2-METHYL-	3652-91-3	not P	3.84	2.49 [NO]	Daphnid LC50 (48h)	Not T
1-Methyldibenzofuran	7320-50-5	not P	4.6	0.60 [NO]	Daphnid LC50 (48h)	T
4-METHYLDIBENZOTHIOPHENE	7372-88-5	not P	4.71	0.53 [NO]	Daphnid LC50 (48h)	T

Chemical name	CAS	Persistency assessment	Measured Log K_{ow} where available	ECOSAR effect concentration [mg/L] [Ecosar-class]*	Organism, effect value	QSAR conclusion on T ($\leq$ 1 mg/L)
Acridinol, 4-	18123-20-1	not P	2.84	3.42 [P]	Daphnid LC50 (48h)	Not T
Methylbenzofuran, 5-	18441-43-5	not P	3.09	5.68 [NO]	Green algae, EC50	Not T
2-METHYLDIBENZOTHIOPHENE	20928-02-3	not P	**4.71**	0.53 [NO]	Daphnid LC50 (48h)	**T**
2-HYDROQUINOLINE	70254-42-1	not P	2.41	4.43 [P]	Daphnid LC50 (48h)	Not T

(*)Abbreviations for ECOSAR classes: NO = neutral organics; PP = Pyrazoles/Pyrroles; PA = Phenols-acid; P = Phenols; A = Amides;

6.3 Enforced QSAR-selection criteria on PBT properties

By application of the QSAR screening strategy outlined in section 6.2 on the *pool of identified semipolar PAC* of 443 compounds (see Annex II), the *extended QSAR selection of critical PAC* of 154 compounds resulted (see section 8.3). To narrow down further the group of substances with potential PBT, BT and vPvB properties (94, 57 and 3, in total 154), selection criteria were further enhanced to screen for the most potent semipolar PAC.

PBT-properties criteria according to REACH guidance R.11 were applied, i.e. log Kow >4.5 for B, L(E)C_{50} <0.1 mg/L and the persistency assessment according to R.11 by pairwise evaluation of BIOWIN 2/3 and 3/6-results (i.e. P-assessment unchanged compared to first screening criteria set).

For enforcement of criteria for potential vPvB-compounds, REACH and REACH guidance specifies no extra screening criteria. According to REACH annex XIII, compounds with half-lives > 60 days in water are regarded as very persistent (vP). US EPAs PBT profiler uses nominal (verbal) output of BIOWIN 3-results to extrapolate on half-lives of compounds in water. As nominal output of BIOWIN 3 is by nature discontinuous, resulting half-lives are graded (15, 37.5, 60, 180 days). The PBT-profiler methodology was applied by MS Excel routines on data from the 443 compounds from the *pool of identified semipolar PAC*. To select for compounds with vPvB-potential, in the first place no special criteria were applied (i.e. log Kow >4.5 and persistency resulting from pairwise evaluation of BIOWIN 2/3 and 3/6-results). To further enforce screening, the persistency criterion of REACH annex XIII for water (>60d) was applied on the output created by PBT profiler methodology. As explained above, the nature of these data implies the factual selection criterion of 180 d (no in-between 60 and 180 days data existing).

These enforced criteria on PBT / BT and vPvB applied on QSAR results were used to further prioritize the *extended QSAR selection of critical PAC* (154 compounds) from the first QSAR screening approach.

6.4 Comparison with other QSAR-models

The *extended QSAR selection of critical PAC*, i.e. 154 compounds which have been predicted to be PBT, BT or PB according to the selection methodology described in section 6.2 above, were evaluated subsequently with the CATALOGIC QSAR-Models (Dimitrov, et al., 2007; Jaworska, et al., 2002) to corroborate the predictions of the EPI suite model. This model run was kindly performed by Ms Böhnhardt, UBA. CATALOG-

IC is able to predict the biodegradability of compounds, based on the predicted Biological Oxygen Demand (BOD). This QSAR is based on experimental results from tests according to OECD guidelines 301 C and 301 F. An interim analysis with a preliminary list of 248 heteroaromatic compounds identified in profiles revealed a markedly higher percentage of substances within the structural domain of model type 301 C compared to model type 301 F. This indicated a broader data basis for the 301 C type model. Therefore the output of this model was preferred for the further analysis.

7 Occurrence of Semipolar PAC in UVCB

The presence of semipolar PAC in some coal tar and crude oil fractions is known for a long time. Due to the possible occurrence in corresponding marketed UVCB and products derived from these materials, an analysis of the content of semipolar PAC in relevant base materials and products derived from the relevant coal tar and crude oil fractions will be presented in the following chapter.

7.1 Technical process to generate coal tar- and mineral oil – UVCB containing semipolar PAC

7.1.1 Coal tar

The coal carbonisation or pyrolysis is performed by heating the material in low-temperature (up to about 400 °C) and high-temperature processes (up to about 1100 °C) by using various technical equipments. Coal tar is the condensation product obtained by cooling of the raw gas generated in coal pyrolysis. The crude coal tar is pretreated to reduce the water content and the concentration of the corrosive ammonium chloride. A typical high-temperature coal tar fractionation distillation (coke oven tar process) yields the following fractions: benzene fraction or light oil (<180 °C), carbolic oil (180-205 °C), naphthalene oil (200-230 °C), methylnaphthalene oil/wash oil/(light) creosote (230-290 °C), light anthracene oil/(creosote) (260-310 °C), heavy anthracene oil/heavy oil/heavy creosote (> 310 °C) and medium soft coal tar pitch as non-volatile residue. The majority of PAH are contained in the fractions naphthalene oil to tar pitch. The higher fractions (wash oil and higher boiling oils) are often blended to obtain miscellaneous creosote oils. Another technical process is the continuous vertical retort with lower distillation temperature tar, yielding the related fractions light oil (90-170 °C), carbolic oil (180-240 °C), light creosote oil (230-300 °C), heavy creosote oil (275-360 °C), residual oil (300-395 °C) and pitch residue. Content and profiles of PAH vary in dependence of the technical process (Betts, 1997; Collin and Höke, 2005; Fiedler, et al., 1997b; IARC, 1985; Kern, et al., 2008; McNeil, 1966).

Several other coal treating processes (e.g. Solvent Refined Coal process (SRC) and modified procedures) are reported. The SRC II process yields fuel oils (coal liquids) rich in Amino-PAH, which originate from hydrogen treatment of heterocyclic PAH (Nishioka, et al., 1985a; Nishioka, et al., 1985b; Nishioka, et al., 1986b). Due to the main intended use as fuels, these coal liquids and the contained semipolar PAC are not viewed in detail (except compounds originating from other sources).

7.1.2 Crude oil derived fractions

The crude oil is separated in a first step by fractionated distillation up to 400 °C at normal pressure. This process yields the fractions gaseous propane/butane (< 25 °C), gasoline (naphtha, 25-200 °C), petroleum (kerosene, 180-250 °C9), gas oil (Diesel, 180-360 °C), lubricating oil (> 360 °C), and a heavy hydrogen mixture of higher boiling compounds (long residue). The latter is separated by a low pressure fractionated distillation (10-100 mm Hg, 1,3- 13 kPa) at 300-400 °C which separates lubricant base oils, heavy fuels, paraffin waxes and bitumen (short residue). All the fractions are further refined (e.g. solvent-extracted, de-waxed and/or hydrocracked). The majority of PAH is contained in kerosene and higher boiling fractions. Crude oil contains predominant sulfur heterocyclic PAH as heterocyclic polyaromatic compounds (Kern, et al., 2008; PHTG, 2003; Prince, 2010; Read and Whiteoak, 2003; Romanow-Garcia and Hoffman, 2007). A synonym for bitumen is petroleum pitch, sometimes the term asphalt is also used (Betts, 1997).

7.2 Semipolar PAC in anthracene oil and creosote and products derived from coal tar

7.2.1 Definition, associated products containing these UVCB

Creosote is a fraction of the coal tar distillation process with a boiling temperature in the range of about 230-350 °C, and creosote is often used as synonym for the anthracene fractions. About 20-30% of the crude coal tar is separated in the tar distillation as anthracene oil fraction (boiling point range 260-400°C), mostly separated in the technical process as light and heavy anthracene oil (boiling points 260-310 °C and > 310 °C, respectively). Dependent on the technical process, there are different origins of creosote with varying nomenclature and chemical composition: LFU (1997) define creosote as the wash oil fraction (also named methyl naphthalene oil) of high-temperature coal tar with a boiling point of 230-290 °C. Betts (1997) named fractions of the continuous vertical retorts (low-temperature distillation, LFU, 1997) of 230-300°C boiling point as light creosote and 275-360 °C as heavy creosote. LFU (1997) named these two fractions as heavy oil and wax oil. This heterogeneity is also reflected in several CAS-numbers for different creosotes, e.g. 8001-58-9 for creosote/wash oil or 90640-84-9 for creosote oil/acenaphtene fraction and also in the individual composition of different creosote samples (see Appendix III). However, marketed creosote oils are often blends of wash oil, anthracene oils and higher boiling coal tar fractions (Betts, 1997). Anthracene oils serve further as a source for isolation of crude anthracene, and are components other aromatic oils (e.g. carbon black oils) and also pitch-oil blends (Betts, 1997; Collin and Höke, 2005).

The main use of creosote is as wood preserving agent, but some minor uses as herbicide, fungicide, disinfectant and insecticide are reported (< 2% in total). Creosote has also been a component of roofing pitch and is used in blends of coal tar oils as source of carbon black (Betts, 1997; Sundström, et al., 1986; WHO, 2004b). Creosote may also be a component of coatings resistant to crude oil and seawater (result of telephone survey).

7.2.2 Comments on search strategy

In addition to the general search strategy (see section 3) a separate search in Google was performed for the terms anthracene oil, creosote and marine coatings to identify product names and manufacturers thereof. Wood creosote was excluded from the search, because it is completely different from the coal tar-derived creosote: it is a mixture of mainly phenols and used as antiseptic and expectorant (Sundström, et al., 1986).

7.2.3 Generated profiles

About 25 profiles could be identified of different creosotes and creosote-contaminated groundwater, soil and sediment. Further 7 profiles are available for the related anthracene oils (see Annex II). No profiles were available for creosote-based products such as marine coatings or other high-resistant coatings. The list of semipolar PAC identified in profiles (together with the relevant substances from the KORA/LAWA list) is attached in Annex III. Based on the evaluated data, no additional semipolar PAC compared to the initial list (KORA, LAWA) could be identified in creosote profiles. However, 11 additional compounds were present in creosote-contaminated ground-water in comparison to the initial *pool of identified semipolar PAC* from the KORA and LAWA lists. These substances are mostly alkylated or oxidized derivatives of core structures known to be present in creosote, so they are possible environmental metabolites.

No relevant information could be gathered by the telephone survey, under manufacturers from different product groups suspected to contain semipolar PAC, because no analysis of these substance group was performed, or confidentiality was claimed, or no replies were obtained in supplemental e-mail requests.

The examination of Material Safety Data Sheets (MSDS) clearly confirmed the use of creosote in wood preserving and as component of resistant coatings, but no relevant information on semipolar PAC could be gathered from these MSDS.

In general the most frequently occurring semipolar PAC compounds in creosote are (illustrated by one example from IARC, 2004, in the order of percental occurrence, but varying between different samples):

Azaarenes: Benzoquinoline, Carbazole, Dibenzocarbazoles, Benzocarbazoles, Indole, Quinoline, Acridine, Methylcarbazoles, Isoquinoline and Methylbenzoquinoline

Thioarenes: Dibenzothiophene and Benzothiophene

Oxaarenes: Dibenzofuran and Benzofuran

7.2.4 Discussion

A relative broad database exists for the composition of various creosotes, including data on the mobility of their components into water, soil and sediment. However, the quantitative determination is in most cases restricted to the more predominant compounds. The simultaneous analysis of PAH and semipolar PAC has not been performed in all identified profiles, and in addition the composition of the individual components varies between different creosotes. So the comparison data base for the ratio of PAH/semipolar PAC seems to be less substantiated. As the profiles mostly differ in the range of examined semipolar PAC, the identification of a representative indicator substance might become problematic.

There is often the drawback within the screened profiles that components are listed as mixtures of isomers or structurally related semipolar PAC (e.g. C2-alkyl quinolones/isoquinolines). This is to all appearance due to the restriction in the separation capacity of the used chromatographic procedures, where compounds of very similar structure co-elute in a single peak. Therefore the identification of individual semipolar PAC is not possible in these cases. This problem is addressed in detail in chapter 9.4.

The occurrence of cyano-substituted PAH can only be detected by sophisticated analysis procedures, and therefore has only been performed by few authors, despite the fact that some of these compounds reveal mutagenic responses (Later, 1985; Later, et al., 1984).

7.3 Semipolar PAC in coal tar pitch and derived products

7.3.1 Definition, associated products containing this UVCB

Coal tar pitch is the non-volatile residue of the coal tar distillation, which amounts to about 50-55% of the high temperature coal tar distillation and to about 25% of the low-temperature process. Dependent on the distillation process soft or hard pitches remain after the tar distillation. Most of the pitch is processed to electrode pitch, roofing tar, protective paints and coatings. Mixtures of tar pitch with various tar oils (such as middle, heavy or anthracene oils) as well as bitumen serve as road tars in North America (Betts, 1997; Collin and Höke, 2005).

7.3.2 Comments on search strategy

In addition to the general search strategy (see section 3) a separate search in Google was performed for the term tar pitch to identify product names and manufacturers thereof.

The telephone survey and the MSDS evaluation revealed that carbon electrodes containing electrode pitch are used only in certain industrial processes (aluminum electrolysis), and other graphite electrodes were indeed manufactured by using electrode pitch, but subsequently subjected to ultra-high temperature processes,

were all carbon material is converted to graphite and no PAH or semipolar PAC were remaining in the electrode material.

7.3.3 Generated profiles

Profiles of pitch bitumen were not considered, as they were not regarded as representative for either tar or bitumen.

About 10 profiles could be identified for tar pitch and about 25 profiles for coal tar or tar contaminations of groundwater and soil (see Annex II). The list of semipolar PAC identified in profiles (together with the relevant substances from the KORA/LAWA list) is attached in Annex III. Based on the evaluated data, no additional semipolar PAC could be identified in tar pitch profiles. However, about 15 additional compounds were present in tar, tar-contaminated ground-water or soil in comparison to the initial *pool of identified semipolar PAC* from the KORA and LAWA lists. These substances are partially alkylated or oxidized derivatives of core structures known to be present in pitch or tar, and therefore possible environmental metabolites.

No relevant information could be gathered by the telephone survey, because no analysis of semipolar PAC was performed, or confidentiality was claimed, or no replies were obtained in supplemental e-mail requests. One manufacturer of clay pigeons mentioned the use of “natural resins” as binding material with a PAH-content in total of < 0.5 mg/kg.

The examination of MSDS yielded no relevant information on semipolar PAC.

The predominant semipolar PAC in coal tar pitch are in general (according to Burchill et al., (1983d), Domínguez et al., (2004) and (ECHA, 2009)):

Azaarenes: Carbazole, Benzocarbazoles, methylated Carbazoles and Benzocarbazoles, Acridine, Azapyrenes

Thioarenes: Dibenzothiophene and higher-aromatic dibenzothiophene derivatives

Oxaarenes: Dibenzofuran and higher-aromatic dibenzofuran derivatives (including alkylated forms thereof)

A more detailed analysis of the potential priority semipolar PAC will be performed later in this project.

7.3.4 Discussion

A relative small database exists for the composition of various tar pitches, more information is available for coal tar, including data on the mobility of their components into water, soil and sediment. The more general restrictions of the data base are already discussed in chapter 7.2.4.

7.4 Semipolar PAC in bitumen matrices

7.4.1 Definition, associated products containing this UVCB

Most of the marketed bitumen originates from the non-volatile residue of the fractionated distillation of crude oil. It is also a component of asphaltenes, naturally occurring in form of asphalt lakes as well as in asphalt rock, which both have been emerged by evaporation of lower-boiling fractions of crude oil.

The oldest known use is as waterproofing for e.g. drinking water tanks. The predominant part of the produced bitumen is to date used for road construction and roofing, but numerous other purposes of use are known (e.g. in materials for damp proofing, sealing and sound reduction). In Northern America “asphalt” is a synonym for bitumen, in Europe the term asphalt means mixtures of bitumen as a binder of aggregate particles and additives (Read and Whiteoak, 2003; Rühl, 2006; WHO, 2004a).

7.4.2 Comments on search strategy

In addition to the general search strategy (see section 3) no further modification was performed, except that the main oil companies were generally included in the telephone survey.

7.4.3 Generated profiles

17 profiles for bitumen could be identified and additionally a set of 5 fractions of fume from oxidized (heat-treated) asphalt (see Annex II). The list of semipolar PAC identified in profiles (together with the relevant substances from the KORA/LAWA list) is attached in Annex III.

Based on the evaluated data, no additional semipolar PAC could be identified in bitumen profiles in comparison to the initial *pool of identified semipolar PAC* from the KORA and LAWA lists.

No relevant information could be gathered by the telephone survey. Some of the inquired companies analyze their bitumen for quality control, but only with respect to regulatory requirements, i.e. for homocyclic PAH like benzo[a]pyrene and not for semipolar PAC. Others claimed confidentiality or no replies were obtained in supplemental e-mail requests.

The examination of MSDS yielded no relevant information on semipolar PAC.

In general the most frequently occurring semipolar PAC compounds in bitumen are S-heterocycles:

Benzo[b]naphtho[2, 1 -d]thiophene, Dibenzothiophene, Benzothiophene

Azaarenes have not been detected or were below the limit of detection in bitumen samples (Knecht, et al., 1999; Kriech, et al., 2002).

A more detailed analysis of the potential priority semipolar PAC will be performed later in this project.

7.4.4 Discussion

Profiles of pitch bitumen were not considered, as they were not regarded as representative for either tar or bitumen.

The examination range of the bitumen profiles is generally more restricted than those for e.g. creosote or tar pitch and mainly focused on homocyclic PAH. Therefore there is only a restricted data base for the assessment of the contents of semipolar PAC. However, the analysis of the examined heterocyclic PAC revealed only low contents for azaarenes and relevant amounts only for thiaarenes (up to about 15 mg/kg for individual compounds (Knecht, et al., 1999; Kriech, et al., 2002)).

The profiles of asphalt fumes are based on oxidized, heat-treated asphalt and therefore the proportion of oxidized semipolar compounds was higher compared to bitumen profiles. But this was not regarded to be representative for bitumen used for products. Accordingly these profiles were not included in the analysis. However, oxidized asphalt itself was identified as component of (not labeled) process oils and is therefore relevant for the occurrence of semipolar PAC.

7.5 Semipolar PAC in extender oils and process oils

7.5.1 Definition, associated products containing this UVCB

Process or extender oils (e.g. for tyre 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% DMSO-extractable fraction), fractions with reduced PAH (DMSO-extractable fraction < 3%) content are Medium Extracted Solvate (MES), Treated Distilled Aro-

matic Extracts (TDAE, hydrotreated or solvent extracted), Treated Residual Aromatic Extracts (TRAE), and hydrotreated naphthenic process oils. The DMSO-extractable fraction stands for the carcinogenic potency (originating at least in part from the PAH content) of the oils and values < 3% are regarded as safe and avoid their labeling as carcinogenic (Null, 1999; Prince, 2010).

Process and extender oils are used in various purposes as components of e.g. tyres, thermoplastics, industrial rubber, printing inks and PVC (Nynas Corporation, Product information on process and extender oils).

The PAH emissions due to tyre abrasion exceeded the amount PAH generated by exhaust emissions (Null, 1999).

Due to the high content of carcinogenic PAH in high aromatic extender oils, this burden has been reduced by specifications of car manufacturers and lately by regulatory regulations in the EU: According to entry number 50 of Annex XVII, REACH the following is stated:

"*From 1 January 2010, extender oils shall not be placed on the market, or used for the production of tyres or parts of tyres if they contain:*

— more than 1 mg/kg (0,0001 % by weight) BaP, or,

— more than 10 mg/kg (0,001 % by weight) of the sum of all listed PAHs.

These limits shall be regarded as kept, if the polycyclic aromatics extract is less than 3 % by weight as measured by the Institute of Petroleum standard IP346" (DMSO-extract) (EC, 2009a)

7.5.2 Comments on search strategy

In addition to the general search strategy (see section 3) a separate search in Google was performed for the terms process/extender/plasticizer oils to identify product names and manufacturers thereof.

7.5.3 Generated profiles

No more recent profiles (> 1980) could be identified from extender, process or related oils, which would be expected to reflect the actual state of the art in composition.

No relevant information could be gathered by the telephone survey, because no analysis of semipolar PAC was performed, or confidentiality was claimed, or no replies were obtained in supplemental e-mail requests.

The examination of MSDS yielded no relevant information on semipolar PAC.

7.5.4 Discussion

No information on the up-to-date composition of the process and extender oils could be gathered by our searches. From a publically available information of a manufacturer (Nynas Naphthenics magazine 2-1995, http://www2.nynas.com/naph/start/article.cfm?Art_ID=772&Sec_ID=55) it can be concluded that the content of PAH has been generally largely reduced in the recent years and the sum of PAH in typical products is in the range of <10 ppm (compared to a high aromatic extract with 850 ppm). In these aromatic extracts the content of heterocyclic PAC was higher than the homocyclic PAH. During the hydrotreatment of the base high aromatic oils the percentage of aromatic compounds in marketed oils is reduced in general, and heterocyclic PAH are destroyed to a greater amount as the homocyclic analogues. Therefore it can be expected that the "modern" process and extender oils contain only very little amounts of semipolar PAC.

This is further corroborated by Null (1999) who compared DAE to more recent process oils in regard to PAH content (see Table 16). PAH content in TDAE (treated distillate aromatic extracts), NAP (naphthenic process

oils) and two types of MES (medium/mildly extracted solvate) are reduced between a factor of 220 (TDAE and MES 2) and 74 (MES 1). Interestingly, with benzo(b)naphtho(2,1-d)thiophene (shaded in grey in Table 16) also a HET-PAC with relevance for the current project is included in this investigation. In fact this HET-PAC is also considerably reduced, however to somewhat lesser extent compared to PAH (between a factor of 150 for NAP and 38 for TDAE).

Table 16: PAH and benzo(b)naphtha(2,1-d)thiophene (shaded in grey) in DAE and process oils with DMSO-extract < 3%m (IP 346 method; from Null, 1999, modified)

Individual PAH [mg/kg]	DAE	TDAE	NAP	MES1	MES 2
Fluoranthene	1	<0.1	0.1	<0.1	0.1
Pyrene	7	0.1	0.3	<0.1	0.3
Benzo[a]fluorene	26	0.1	0.4	<0.1	0.2
Benzo[b+c]fluorene	15	0.1	0.1	<0.1	<0.1
Benzo[b]naphtho[2,1-d]thiophene	15	0.4	0.1	<0.1	0.3
Benzo[g]fluoranthene,-[h],-[i]-	6	<0.1	<0.1	<0.1	0.1
Benzo[a]anthracene	6	<0.1	0.1	<0.1	0.1
Chrysene + Triphenylene	48	0.2	0.4	0.2	<0.1
1+2+3+4+5+6-Methyl-chrysenes	73	0.4	1.7	0.9	0.3
Benzo[b+j+k]fluoranthen	64	0.1	0.3	<0.1	<0.1
Benzo[e]pyrene	45	0.1	0.4	0.5	0.1
Benzo[a]pyrene	12	<0.1	0.2	0.2	0.1
Perylene	49	<0.1	0.4	0.3	<0.1
Dibenz[a,j]anthracene	1	<0.1	<0.1	<0.1	<0.1
Indeno[1,2,3-c,d]pyrene	8	<0.1	<0.1	<0.1	<0.1
Dibenz[a,h]anthracene,-[a,c]-	8	<0.1	<0.1	0.5	<0.1
Benzo[b]chrysene		0.1	0.1	<0.1	0.1
Dibenzo[g,h,i]perylene	49	<0.1	0.2	0.8	<0.1
Anthanthrene	6	<0.1	<0.1	<0.1	<0.1
Coronene	4	<0.1	0.1	2.8	<0.1
Total sum of PAH	443	2	5	6	2

7.6 Semipolar PAC in carbon black/ carbon black preparations

7.6.1 Definition, associated products containing this UVCB

Carbon black consists mainly of elemental amorphous carbon that is manufactured by pyrolysis and incomplete combustion of various hydrocarbon sources. In contrast to soot (product of incomplete combustion of all carbon-containing materials), carbon black possesses a higher surface/volume ratio than soot. Several sources can be used to produce carbon black, and the production process is eponymous for the different carbon blacks produced. About 95% of carbon black originates from burning of oils, the oil-furnace process. Heavy fuel oils and high-boiling fractions of coal tar are sources for carbon black. Carbon black contains solvent-extractable by-products, mainly homocyclic and heterocyclic PAH as well as nitro-PAH. Modifications of the manufacture processes resulted in a largely reduced content of nitro-PAH since the eighties of the last century. Considerable variations are observed in dependence of type and grade of carbon black and also between different batches (IARC, 2010; Wang, M.-J., et al., 2007).

Carbon black is used predominantly for the reinforcement of rubber (about 70% of the total produced amount for tyres, 20% for other rubber products) and as black pigment for inks, paints, paper and plastic materials (about 10% of total production). Several types of furnace-oil carbon black with a wide range of desired properties are available for different purposes (IARC, 2010; Wang, M.-J., et al., 2007).

7.6.2 Comments on search strategy

In addition to the general search strategy (see section 3) a separate search in Google was performed for the term carbon black to identify product names and manufacturers thereof.

7.6.3 Generated profiles

Seven profiles could be identified for carbon black, but all of older origin (1990 and older; see Annex II). The list of semipolar PAC identified in profiles (together with the relevant substances from the KORA/LAWA list) is attached in Annex III.

Most commonly identified heterocyclic PAH are:

Dibenzothiophene, Benzo[def]dibenzothiophene, Phenanthro[4,5-bcd]thiophene and Triphenyleno[4,5-bcd]thiophene.

The predominant Nitro-PAH are mostly nitronaphthalenes, -anthracenes, fluuorenone, -pyrenes, di- and trinitropyrenes. However, the content of nitro-PAH has been reduced since about 1980 by modified manufacturing processes (IARC, 2010) and no actual profiles could be evaluated.

A more detailed analysis of the potential priority semipolar PAC will be performed later in this project.

One working group examined a further group of substituted PAH, the cyano-PAH, and found 9 two- to five ring systems with cyano groups. No quantitative analysis was performed, but the GC-MS chromatogram indicates highest concentrations for cyanonaphthalenes and –acenaphthylenes (Later, et al., 1984).

7.6.4 Discussion

There are uncertainties about the composition of more recent carbon black formulations. Though it is reported that the nitro-PAH content has been largely reduced by technical modifications since about 30 years, the recently published IARC monograph on carbon black (IARC, 2010) lists the above mentioned nitro-PAH as present in carbon blacks. But this information is again based on studies published at least 20 years ago.

Due to the desired low amounts of sulphur compounds in carbon black oils (IARC, 2010), it can be expected that sulphur-containing heteroaromatics (S-HET) are present in only very low concentrations in carbon black. Seven S-HET compounds have been identified qualitatively in older carbon black samples (Lee and Hites, 1976).

A comparison of the content of homocyclic PAH in older and more recent samples shows a marked reduction of the PAH content: analysis of formulations of the 1980ies revealed benzo[a]pyrene contents of up to 5 mg/kg, whereas data from 2005 report a concentration of <0.001 mg/kg (IARC, 2010). With an extrapolation to semipolar PAC it can be assumed that this compound class is also present in lower concentration than in the 1980ies.

Therefore the entire knowledge on semipolar PAC is only useful under qualitative aspects.

7.7 Semipolar PAC in carbon black oil

7.7.1 Definition, associated products containing this UVCB

About 95% of carbon black originates from burning of oils, the oil-furnace process. Heavy fuel oils (catalytically cracked oils, steamcracked oils) and high-boiling fractions of coal tar (e.g. anthracene and creosote fraction) are typical sources for carbon black. In contrast to other purposes of use, a high percentage of PAH is desired in these carbon black oils due to the resulting high carbon content, the sulphur content of these oils should be low (IARC, 2010; Wang, M.-J., et al., 2007).

7.7.2 Comments on search strategy

In addition to the general search strategy (see section 3) a separate search in Google was performed for the terms carbon black/furnace oils to identify product names and manufacturers thereof.

7.7.3 Generated profiles

No profiles could be identified for carbon black oils.

The list of registered UVCB contains e.g. under the CAS no. 64741-59-9 the UVCB distillates (petroleum), light catalytic cracked (synonym Carbon black oil, see chapter 5.2.4). However, this registration dossier did not contain information on the detailed composition of this carbon black oil manufactured under state of the art conditions.

7.7.4 Discussion

There is no detailed information on the composition of carbon black oils. The intended use as a rich carbon source implicates a high content of PAH (as it is documented in MSDS for carbon black oils). However, it is the objective of the manufacturing process of carbon black to yield an almost complete pyrolysis of these compounds, which is reflected in the largely reduced content of PAH in the product carbon black. Due to the desired low amounts of sulphur compounds, it can be expected that sulphur-containing heteroaromatics are present in only very low concentrations in carbon black.

7.8 Semipolar PAC in other matrices

Several profiles could be identified in environmental matrices (contaminated groundwater, soil and sediment as well as urban dust) and ambient air (see Annex II). These profiles may serve as indicators

- for the relevance of the occurrence of semipolar PAC in the environment
- for analysis of metabolic processes when compared to the origin of contamination

E.g. the nitro-PAH identified in air profiles are typical for oxidation reactions of PAH with nitrogen oxides under the influence of UV radiation, but not relevant for the most UVCB described in the preceding chapters. They may serve, however, as a comparison basis for nitro-PAH detected in carbon black.

On the other hand, metabolites of semipolar PAC in environmental media could be of interest in the context of industrial sites and waste disposal sites, and selected compounds with a particular high predicted PBT concern should not be neglected.

8 Iterative Process: Identification of Semipolar PAC with Relevant PBT-Properties

8.1 Starting list

Semipolar PAC have been identified in 118 profiles and the compounds found in these profiles were matched with the initial *pool of identified semipolar PAC* from KORA/LAWA. The final *pool of identified semipolar PAC* (443 compounds) screened by QSAR for PBT-properties is attached to this document (Annex II: Substance list). The list consists of 159 semipolar PAC from KORA/LAWA and 284 substances derived from evaluated profiles (see Annex I: Profile list), giving 443 compounds in total.

8.1.1 Strategy for selection of semipolar PAC from profiles

If no CAS could be identified for a specific compound a SMILES code was created manually from chemical name or structural representation from profile report by using MarvinSketch implemented in ChemIDplus. Using structure search within ChemIDplus in some cases CAS numbers could finally be retrieved. For substances without CAS, SMILES codes were used for identification throughout different profiles. As smiles codes are redundant, SMILES codes were generated by MarvinSketch and in the Kekulé-form only.

If substance name did not allow for identification (only very few cases), this compound was excluded from the further selection process.

8.1.2 PBT screening methodology

For toxicity screening, the lowest acute L(E)C_{50}-value as reported by ECOSAR for the three trophic levels *green algae*, *daphnids* and *fish* was taken. The bioaccumulation screening was based on log K_{ow}-values as calculated by KOWWIN with preference on experimental values from the PhysProp-DB (as far as available); and for the persistence screening BIOWIN2, 3, and 6-results were evaluated basically according to REACH guidance R.11 (see section 6.2 for further details).

8.2 Amendment of SMILES Structural Representations for Thiophene- and Furan-derived semipolar PAC

In this chapter need for amendment of some SMILES structural codes is explained and conclusion on requirements for corrections on SMILES codes and concomitant QSAR-screening results are drawn. Faulty smiles, as outlined in the following paragraphs, were discovered ex post after the selection processes leading to the 15 priority semipolar PAC were already completed and literature research started (see chapters below regarding these steps). Before application of corrections as described below, 16 priority compounds had been determined. Rectification of SMILES then resulted in the exclusion of one compound leading to the final number of 15 and other minor changes as outlined below. The chapter on literature research therefore still includes the later dismissed 16th compound. Apart from that, in all the later sections following this chapter results and numbers represent the status obtained after rectification of SMILES codes.

As will be demonstrated in section 14.7, Het-PAC isomers in relative position of the aromatic ring systems are commonly indistinguishable with regard to QSAR-results affecting PBT-properties. While analysing the list of priority compounds gained from activities summarized under sections 8.3 through 9.3 it was striking that persistency predictions for benzo(b)naphtho(2,3-d)thiophene und benzo(b)naphtho(2,1-d)thiophene were divergent (P for the latter, not P for the first) while experimental data on biodegradation for both compounds from the same publication (Lundstedt, et al., 2003) were identical. A closer look at the SMILES codes of both compounds within the MarvinSketch tool of ChemIDplus revealed missing aromaticity for the thiophene ring of benzo(b)naphtho(2,3-d)thiophene. As outlined above, SMILES codes used for compounds of

the final *pool of identified semipolar PAC* were retrieved from the following sources (order denoting preference of source):

1) Use of SMILES-database in EPI-Suite as far as entry available (batch process)
2) ChemIDplus as far as SMILES available
3) Self-generated SMILES by use of MarvinSketch from name or structural formula in publications

SMILES codes for both compounds were from source 1) implying that only part of the SMILES codes for thiophene compounds may be faulty. As such, BIOWIN calculation using CAS-number as input delivers the wrong SMILES code for benzo(b)naphtho(2,3-d)thiophene (c1cc2c3cc4ccccc4cc3Sc2cc1) with missing aromaticity in the thiophene moiety and the BIOWIN3-result "weeks to months", whereas direct input of the correct SMILES taken from ChemIDplus (c4ccc2c(c4)sc1cc3ccccc3cc21) gives the BIOWIN3-result "months". The structural output of EPIWIN is misleading (Figure 2) as the sulphur in the aromatic thiophene ring (left hand side) has a hydrogen atom associated which is factual not the case. As such, the "wrong" structure depicted on the right hand side seems to be rather the correct one.

Figure 2: EPI Suite structural outputs using SMILES input (correct SMILES; left side image) and input of CAS number (use of EPI Suite SMILES database to retrieve SMILES, missing aromaticity in thiophene ring, right side image)

Analysis of all 16 priority compounds (15 after SMILES correction, see above) for aromaticity in heterocyclic rings detected further faulty SMILES codes. In total the following 4 compounds with thiophene or furan ring systems were affected (not affected: six-membered rings like pyridine) with consequences for the PBT-screening results:

- Benzo(b)naphtho(2,3-d)thiophene (CAS: 243-46-9): BT, now PBT
- Phenanthro(4,5-bcd)thiophene (CAS: 30796-92-0): BT, now PBT
- 1-Methyldibenzofuran (CAS: 7320-50-5): BT, now B
- Benzo(b)naphtho(2,3-d)furan (CAS: 243-42-5): BT, now PBT

SMILES from all 4 compounds were from the EPI Suite data base. Regarding concomitant changes in QSAR outcome, aromaticity generally increased persistence. For furans, aromaticity decreased toxicity (reduced reactivity). Thus, 1-methyldibenzofuran was dismissed from the list of priority semipolar PAC, as only one of three criteria (B, former BT) was actually fulfilled, reducing the total number of priority compounds from 16 to 15.

Moreover, further compounds of the *pool of identified semipolar PAC* could have been affected, potentially having led to misses in selection of priority compounds. However, as priority compounds were selected in regard to both, possible PBT-properties *and* occurrence in profiles, the complete *pool of identified semipolar PAC* (443compounds) was filtered for compounds with a minimum of six occurrences in profiles (see chapters below for further explanations on methodology). The resulting compounds included only 4 compounds with furan rings and 2 compounds having thiophene rings. These were checked and the SMILES codes found to be correct. Smiles codes of the above mentioned four compounds were corrected in the *pool of identified semipolar PAC,* including the altered QSAR-results. The following alterations are the consequence thereof:

- The number of PBT compounds of the *extended QSAR selection of critical PAC* (154 compounds) increases by 3 compounds from 91 to 94
- The number of BT compounds of the *extended QSAR selection of critical PAC* decreases by 4 compounds from 61 to 57
- No changes in the number of PB compounds (3)

Because it was not possible to validate all SMILES codes for compounds containing furan or thiophene moieties these numbers most probably are still not fully correct however give a good assumption on substance properties of semipolar PAC. Possibly undiscovered faulty SMILES however cannot have any influence on selection of priority semipolar PAC (see below).

8.3 Results of (initial) Extended QSAR Screening

The extended QSAR screening for PBT-properties (see section 6.2 for details) resulted in the *extended QSAR selection of critical PAC* with a total of 154 compounds.

Compounds fulfilling cut-off criteria (see section 6.2 for details) for P (P1, P2 or P1P2), B (log $K_{ow} \geq 4.0$) and T ($L(E)C_{50} \leq 1.0$ mg/L) are shown in Table 17 together with their descriptors and calculated values for $L(E)C_{50}$, log K_{ow} as well as the results from the persistency screening. According to these results, 94 substances fulfil all three criteria and therefore are regarded as PBT according to the QSAR-screening methodology.

Table 17: 94 compounds predicted to fulfil all PBT-criteria according to QSAR results (US-EPA EPI Suite version 4.1[20])

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
KORA / LAWA	225-11-6	Benz(a)acridine	c4ccc3nc2ccc1ccccc1c2cc3c4	0.9200	Daphnid	4.48	P1P2
KORA / LAWA	225-51-4	Benz(c)acridine	c4ccc3nc1c(ccc2ccccc12)cc3c4	0.9200	Daphnid	4.48	P1P2
KORA / LAWA	205-43-6	Benzo(b)naphtho (1,2-d)thiophene	c1ccc3c(c1)ccc4sc2ccccc2c34	0.1920	Daphnid	5.19	P1P2
KORA / LAWA	239-35-0	Benzo(b)naphtho (2,1-d)thiophene	c1ccc3c(c1)ccc4c2ccccc2sc34	0.1920	Daphnid	5.19	P1P2
KORA / LAWA	243-42-5	Benzo(b)naphtho (2,3-d)furan	c1ccc2c(c1)oc1cc3ccccc3cc21	0.4160	Daphnid	4.89	P1P2
KORA / LAWA	243-46-9	Benzo(b)naphtho(2,3-d)thiophene	c1ccc2c(c1)sc1cc3ccccc3cc21	0.1920	Daphnid	5.34	P1P2
KORA / LAWA	224-42-0	Dibenz(a,j)acridine	c1ccc4c(c1)ccc5nc3ccc2ccccc2c3cc45	0.1230	Daphnid	5.63	P1P2

[20] http://cfpub.epa.gov/crem/knowledge_base/crem_report.cfm?deid=74897

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
KORA / LAWA	215-62-3	Dibenz(a,c)acridine	n5c2c(cc3c5c1c(c4c3cccc4)cccc1)ccc2	0.1230	Daphnid	5.66	P1P2
KORA / LAWA	226-36-8	Dibenz(a,h)acridine	c1ccc4c(c1)ccc5nc2c(ccc3ccccc23)cc45	0.1230	Daphnid	5.63	P1P2
KORA / LAWA	224-53-3	Dibenz(c,h)acridine	c1ccc2c3nc4c5ccccc5ccc4cc3ccc2c1	0.1230	Daphnid	5.63	P1P2
KORA / LAWA	194-59-2	Dibenzocarbazole (7H-Dibenzo(c,g)carbazole)	c12c3c4c(ccc3[nH]c2ccc2c1cccc2)cccc4	0.1390	Daphnid	6.4	P1P2
KORA / LAWA	2381-40-0	Dimethylbenz(c)acridine	Cc4ccc3c(C)c2ccc1ccccc1c2nc3c4	0.1320	Daphnid	5.59	P1P2
KORA / LAWA	84258-62-8	Methylbenzo(b)-naphtho(1,2-d)thiophene	CC1=CC=CC2=C1C1=C(SC3=C1C=CC=C3)C=C2	0.0730	Fish	5.89	P1P2
KORA / LAWA	4567-41-3	Methylbenzo(b)-naphtho(2,1-d)thiophene	CC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	0.0730	Fish	5.89	P1P2
KORA / LAWA	36821-08-6	Methylbenzo(b)-naphtho(2,3-d)thiophene	CC1=CC=CC2=C1SC1=C2C=C2C=CC=CC2=C1	0.0730	Fish	5.89	P1P2
KORA / LAWA	195-52-8	Phenanthrothiophene, 3,4-b	c12c(ccs1)c1c3ccccc3ccc1cc2	0.1920	Daphnid	5.35	P1P2
KORA / LAWA	195-68-6	Phenanthrothiophene, 4,3-b	c12c(ccs1)ccc1ccc3ccccc3c21	0.1920	Daphnid	5.35	P1P2
KORA / LAWA	224-10-2	Phenanthrothiophene, 3,2-b	c12c(ccs1)cc1ccc3ccccc3c1c2	0.1920	Daphnid	5.34	P1P2
Profiles	195-29-9	Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline = Benzo(a)phenanthridine, 195-29-9)	c12c3c(cccc3)cnc2ccc2c1cccc2	0.9200	Daphnid	4.49	P1P2
Profiles	205-39-0	2,3-Benzodiphenylene oxide (3 CAS, Benzo(b)naphtho(1,2-d)furan, 205-39-0;	c1ccc2c(c1)oc1ccc3ccccc3c21	0.4160	Daphnid	4.89	P1P2
Profiles	21064-50-6	Mixture of Methylbenzocarbazoles (6-Methyl-3,4-benzocarbazole = 7H-Benzo(c)carbazole, 10-methyl- ,21064-50-6)	c12c3c(ccc4c3cccc4)[nH]c1ccc(c2)C	0.3910	Daphnid	4.95	P1P2
Profiles	3074-00-8	Benzo(cd)pyren-6-one	O=C1C2=C3C(C=CC4=C3C3=C1C=CC=C3C=C4)=CC=C2	0.2190	Daphnid	5.31	P1P2

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
Profiles	5315-79-7	1-Pyrenol	Oc4ccc2c3c4ccc1cccc(c13)cc2	0.4650	Daphnid	4.45	P1P2
Profiles	5522-43-0	1-Nitropyrene	O=N(=O)c(c(c(c(c(c1)ccc2)c2cc3)c3c4)c1)c4	0.6130	Daphnid	5.06	P1P2
Profiles	68967-09-9	Mixture of Pyrene carboxaldehydes	O=Cc1cc2ccc3ccc4ccc(c1)c2c34	0.2760	Daphnid	4.65	P1P2
Profiles	76895-43-7	Pyrene-3,4-dicarboxylic acid anhydride = (3H,5H-Pyreno(1,10-cd)pyran-3,5-dione)	c1cc2c3c4c(C(OC(c4cc2)=O)=O)cc2cccc1c32	0.4160	Daphnid	5.01	P1P2
Profiles	78020-40-3	Fluorantheneamine	NC1=CC2=CC=CC3=C2C(=C1)C1=CC=CC=C31	0.8160	Daphnid	4.02	P1P2
Profiles		Phenanthro(4,5-bcd)thiophene, -ethyl; (Phenanthro(4,5-bcd)thiophene, C2-substituted, different isomers)	CCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	0.0830	Daphnid	5.79	P1P2
Profiles		Phenanthro(4,5-bcd)thiophene, -propyl; (Phenanthro(4,5-bcd)thiophene, C3-substituted, different isomers)	CCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	0.0330	Fish	6.28	P1P2
Profiles		Phenanthro(4,5-bcd)thiophene, -butyl; (Phenanthro(4,5-bcd)thiophene, C4-substituted, different isomers)	CCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	0.0130	Fish	6.77	P1P2
Profiles		Phenanthro(4,5-bcd)thiophene, -pentyl; (Phenanthro(4,5-bcd)thiophene, C5-substituted, different isomers)	CCCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	0.0050	Fish	7.26	P1P2
Profiles		Phenanthro(4,5-bcd)thiophene, -Hexyl; (Phenanthro(4,5-bcd)thiophene, C6-substituted, different isomers)	CCCCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	0.0019	Fish	7.76	P1P2

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
Profiles		Benzo(b)-naphtho(2,1-d)thiophene, -ethyl; (benzo(b)-naphtho(2,1-d)thiophene, C2-substituted, different isomers)	CCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	0.0280	Fish	6.38	P1P2
Profiles		Benzo(b)-naphtho(2,1-d)thiophene, -propyl; (benzo(b)-naphtho(2,1-d)thiophene, C3-substituted, different isomers)	CCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	0.0110	Fish	6.87	P1P2
Profiles		Benzo(b)-naphtho(2,1-d)thiophene, -butyl; (benzo(b)-naphtho(2,1-d)thiophene, C4-substituted, different isomers)	CCCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	0.0040	Fish	7.36	P1P2
Profiles		Benzo(b)-naphtho(2,1-d)thiophene, -pentyl; (benzo(b)-naphtho(2,1-d)thiophene, C5-substituted)	CCCCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	0.0017	Fish	7.85	P1P2
Profiles		Chryseno(4,5-bcd)thiophene, C1-substituted	CC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	0.0190	Fish	6.6	P1P2
Profiles		Chryseno(4,5-bcd)thiophene, C2-substituted, different isomers	CCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	0.0070	Fish	7.09	P1P2
Profiles		Chryseno(4,5-bcd)thiophene, C3-substituted, different isomers	CCCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	0.0030	Fish	7.58	P1P2
Profiles		Chryseno(4,5-bcd)thiophene, C4-substituted, different isomers	CCCCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	0.0011	Fish	8.08	P1P2
Profiles	72072-20-9	Benzophenanthrothiophene (Benzo(2,3)phenanthro(4,5-bcd)thiophene)	C1=CC=C2C(=C1)C=C3C=CC4=C5C3=C2SC5=CC=C4	0.0690	Fish	5.93	P1P2
Profiles	102859-52-9	Nitrocyclopenta(cd)pyrene (4-Nitrocyclopenta(cd)pyrene, 102859-52-9)	C1=CC2=C3C(=C1)C=C4C(=CC5=C4C3=C(C=C2)C=C5)[N+](=O)[O-]	0.5210	Daphnid	4.89	P1P2

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
Profiles	104313-09-9	Dibenzo(b,def)carbazole	N1C2=C3C4=C1C=C1C=CC=CC1=C4C=CC3=CC=C2	0.3780	Daphnid	4.99	P1P2
Profiles	12041-95-1	Benzacridine (12041-95-1) and/or Azachrysene	C1=CC=C2C(=C1)C=CC3=NC4=CC=CC=C4C=C32	0.9200	Daphnid	4.48	P1P2
Profiles	189-90-2	Azabenzopyrenes (2-Azabenzo(b)pyrene, 189-90-2)	c4c5ccccc5c2c3c4ccc1cncc(c13)cc2	0.3370	Daphnid	5.08	P1P2
Profiles	218-08-6	Azachrysenes (Naphtho(2,1-f)quinoline = 1-Azachrysene, 218-08-6)	c4cc2c(c3c4c1c(cc3)nccc1)cccc2	0.9200	Daphnid	4.49	P1P2
Profiles	219-25-0	Phenanthro(2,1-b)thiophene	c1ccc2ccc3c4C=CSc4ccc3c2c1	0.1920	Daphnid	5.35	P1P2
Profiles	224-07-7	Phenanthro(2,3-b)thiophene	c12c(ccs1)cc1c3ccccc3ccc1c2	0.1920	Daphnid	5.34	P1P2
Profiles	227-86-1	Anthra(1,2-b)thiophene	C1=CC=C2C=C3C(=CC2=C1)C=CC4=C3SC=C4	0.1920	Daphnid	5.34	P1P2
Profiles	236-01-1	Phenanthro(9,10-b)thiophene	c32c(ccs3)c4ccccc4c1ccccc21	0.1920	Daphnid	5.34	P1P2
Profiles	239-64-5	13H-Dibenzo(a,i)carbazole	c12c(c3ccc4c(c3[nH]2)cccc4)ccc2c1cccc2	0.1390	Daphnid	6.4	P1P2
Profiles	24496-65-9	Dibenzophenanthridine or isomer (Dibenzo(i,lmn)phenanthridine = 12-Azabenzo(a)pyrene)	c12c3c4ncc1c1c(cccc1)cc2ccc3ccc4	0.3370	Daphnid	5.08	P1P2
Profiles	30796-92-0	Phenanthro(4,5-bcd)thiophene	c1cc2sc3cccc4ccc(c1)c2c34	0.6070	Fish	4.95	P1P2
Profiles	3634-16-0	Methylbenzoacridine (5-Methylbenz(a)acridine, 3634-16-0)	CC1=CC2=NC3=CC=CC=C3C=C2C4=CC=CC=C14	0.3490	Daphnid	5.04	P1P2
Profiles	386-77-6	Aza-benzofluoranthenes (Indeno(1,2,3-kl)acridine, 386-77-6 or azabenzopyrenes	c1cccc2nc3cccc4c3c(c12)c1ccccc41	0.3370	Daphnid	5.08	P1P2
Profiles	4107-64-6	Cyanopyrenes or -fluoranthenes (1-Cyanopyrene, 4107-64-6)	C1=CC2=C3C(=C1)C=CC4=C(C=CC(=C43)C=C2)C#N	0.9370	Daphnid	4.48	P1P2
Profiles	58426-99-6	Phenanthro(1,2-b)thiophene	C1=CC=C2C(=C1)C=CC3=C2C=CC4=C3SC=C4	0.1920	Daphnid	5.35	P1P2

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
Profiles	63041-90-7	6-Nitrobenzo(a)pyrene	O=N(=O)c2c1ccccc1c3ccc4cccc5ccc2c3c45	0.0810	Daphnid	5.93	P1P2
Profiles	65777-07-3	Dibenzacridines	C1=CC=C2C(=C1)C=C3C4=CC=CC=C4C5=CC=CC=C5C3=N2	0.1230	Daphnid	5.66	P1P2
Profiles	68518-20-7	Dinaphthofurans	c1ccc2cc3c(cc2c1)oc1cc2ccccc2cc31	0.0550	Fish	6.07	P1P2
Profiles	72076-98-3	Benzo(def)naphthobenzothiophene (= Chryseno(4,5-bcd)thiophene)	c15c3c4ccc5c2c(cc1sc3ccc4)cccc2	0.0690	Fish	5.93	P1P2
Profiles	75321-19-6	1,3,6-Trinitropyrene	O=N(=O)c4cc(c2c3c4ccc1c3c(c(cc1)N(=O)=O)cc2)N(=O)=O	0.2870	Fish	4.39	P1P2
Profiles	75321-20-9	1,3-Dinitropyrene	O=N(=O)c4c2c3c(c(c4)N(=O)=O)ccc1c3c(ccc1)cc2	0.1900	Fish	4.57	P1P2
Profiles	218-19-9	Naphthoquinoline (Naphtho(1,2-h)quinoline 218-19-9)	C1=CC2=C(C=C1)C1=C(C=C2)C2=C(C=CC=N2)C=C1	0.9200	Daphnid	4.49	P1P2
Profiles	84-56-0	Azabenz(a)anthracenes (Naphtho(2,3-h)quinoline, 84-56-0)	c3c4ccccc4cc2c3c1c(cccn1)cc2	0.9200	Daphnid	4.49	P1P2
Profiles	892-21-7	3-Nitrofluoranthene	O=N(=O)c2ccc3c1ccccc1c4cccc2c34	0.6130	Daphnid	4.75	P1P2
Profiles		Azabenzofluoranthenes (SMILES for 2-Aza-isomer)	C1=CC2=C3C(=C1)C1=CN=CC4=C1C3=C(C=C2)C=C4	0.9120	Daphnid	4.49	P1P2
Profiles		Dibenzoisoquinolines (SMILES for Dibenzo(c,f)isoquinoline)	C1=CC2=NC=C3C=CC4=CC=CC=C4C3=C2C=C1	0.9200	Daphnid	4.49	P1P2
Profiles		4H-Naphtho(1,2,3,4-def)carbazole or isomer	N1C2=CC=CC3=C4C=CC=CC4=C4C=CC=C1C4=C23	0.3380	Daphnid	5.06	P1P2
Profiles		Dibenzophenanthridine or isomer (Dibenzophenanthridine)	C1=CC2=C(C=C1)C1=C(C=C2)N=CC2=CC3=CC=CC=C3C=C12	0.1230	Daphnid	5.67	P1P2

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
Profiles		3-Azaphenanthro(4,3-b)thiophene	S1C=C[N+]2=C1C1=C(C=CC3=C1C=CC=C3)C=C2	0.3370	Daphnid	5.05	P1P2
Profiles		Dimethylbenzo(def)carbazole = Dimethylphenanthro(bcd)pyrrole (SMILES for 1,7-dimethylphenanthro(bcd)pyrrole)	CC1=C2C=CC3=C(C)C=CC4=C3C2=C(N4)C=C1	0.4010	Daphnid	4.91	P1P2
Profiles		Methylphenanthro(4,5-bcd)thiophenes (SMILES for 1-Methyl isomer)	CC1=C2C=CC3=CC=CC4=C3C2=C(S4)C=C1	0.1960	Daphnid	5.3	P1P2
Profiles		Methylhydroxypyrenes or -fluoranthenes (SMILES for 3-Methylpyren-1-ol)	CC1=CC(O)=C2C=CC3=CC=CC4=CC=C1C2=C34	0.2200	Fish	5	P1P2
Profiles		Methyldibenzoquinolines (SMILES for Methyldibenzo(c,f)quinoline)	CC1=CC=C2C=CC3=NC=C4C=CC=CC4=C3C2=C1	0.3490	Daphnid	5.04	P1P2
Profiles		Dimethyl- or ethyl-azafluoranthenes/pyrenes or azabenzofluorenes (SMILES for 1,6-Dimethyl-4-azapyrene)	CC1=CC=C2N=CC3=C(C)C=CC4=CC=C1C2=C34	0.3580	Daphnid	5	P1P2
Profiles		Trimethyl- or methylethylbenzo(def)carbazoles or dimethylindenoindoles (Trimethylbenzo(def)carbazole = Trimethylphenanthro(bcd)pyrrole (SMILES for 1,7,8--trimethylphenanthro(bcd)pyrrole)	CC1=CC2=C(C)C=CC3=C2C2=C(N3)C=CC(C)=C12	0.1520	Daphnid	5.46	P1P2
Profiles		10-Methyl-1-aza-pyrene	CC1=CC2=C3C(C=CC4=CC=NC1=C34)=CC=C2	0.9410	Daphnid	4.45	P1P2
Profiles		Methylbenzophenanthro(4,5-bcd)thiophene (SMILES for 2-Methyl isomer)	CC1=CC2=C3C(SC4=C5C=CC=CC5=CC(C=C2)=C34)=C1	0.0190	Fish	6.6	P1P2
Profiles		Methylphenanthro(4,5-bcd)thiophene (SMILES for 10-Methyl-isomer)	CC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	0.1960	Daphnid	5.3	P1P2

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
Profiles		5-Methyl-1aza-fluoranthene	CC1=CC2=CC=NC3=C2C(=C1)C1=CC=CC=C31	0.9410	Daphnid	4.45	P1P2
Profiles		Methylbenzophenanthridine or isomer (SMILES for 10-methylbenzo(a)phenanthridine)	CC1=CC=C2C(C=CC3=C2C2=C(C=CC=C2)C=N3)=C1	0.3490	Daphnid	5.04	P1P2
Profiles		Cyanofluoranthene or -pyrene or azabenzo(ghi)fluoranthene (SMILES for Fluoranthene-1-carbonitrile)	N#CC1=C2C3=CC=CC=C3C3=C2C(C=C1)=CC=C3	0.9370	Daphnid	4.48	P1P2
Profiles		Cyanopentapyrenes or -benzo(ghi)fluoranthenes (SMILES for Benzo(ghi)fluoranthene carbonitrile)	N#CC1=CC2=C3C(=C1)C1=CC=CC4=C1C3=C(C=C4)C=C2	0.3430	Daphnid	5.07	P1P2
Profiles		Chrysenenitriles (SMILES for Chrysene-6-carbonitrile)	N#CC1=CC2=C3C=CC=CC3=CC=C2C2=CC=CC=C12	0.3460	Daphnid	5.07	P1P2
Profiles		Naphthoindoles (SMILES for 1H-Naphtho(1,2-g)indole	N1C=CC2=C1C1=C(C=C2)C2=C(C=CC=C2)C=C1	0.0690	Fish	4.41	P1P2
Profiles		Benzophenanthro(bcd)pyrrole	N1C2=C3C4=C1C1=C(C=CC=C1)C=C4C=CC3=CC=C2	0.3780	Daphnid	4.99	P1P2
Profiles		Dibenzo(c,g)phenanthro(12,9-bcd)thiophene = Benzo(6,7)perylo(1,12-bcd)thiophene (only defined by structure,isomers exisiting)	S1C2=C3C4=C1C=CC1=C4C4=C(C=C1)C=CC1=C4C3=C(C=C2)C=C1	0.0080	Fish	7.11	P1P2
Profiles		Phenanthro(7,6,5,4b,4a,4:12,12a,12b,1,2,3)peryleno(6,7-bcd)thiophene	S1C2=C3C4=C1C=CC1=C4C4=C5C(C=CC(C=C2)=C35)=CC2=C4C3=C(C=CC=C13)C=C2	0.0003	Fish	8.87	P1P2
Profiles		Tribenzo(2,1,4,5,6,7)chryseno(10,11,bcd)thiophene	S1C2=C3C4=C1C1=C5C(C=CC6=C5C4=C(C=C6)C4=C3C(C=C2)=CC=C4)=CC=C1	0.0008	Fish	8.28	P1P2

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assessment[6]
Profiles		Benzo(9,10)pyreno(3,4,5,6-jklma)dibenzothiophene	S1C2=CC=C3C=CC4=C5C=CC=CC5=C5C=CC6=C7C(=C1C=C6)C2=C3C4=C57	0.0008	Fish	8.28	P1P2
Profiles		Benzo(1,2)pyreno(3,4,5,6-jklma)dibenzothiophene	S1C2=CC=C3C4=CC=CC=C4C4=CC=C5C=CC6=C7C(=C1C=C6)C2=C3C4=C57	0.0008	Fish	8.28	P1P2
Profiles		Coroneno(2,3,4-bcd)benzothiophene	S1C2=CC=CC3=C2C2=C4C5=C(C=CC6=C5C5=C(C=C6)C=CC6=C5C4=C3C=C6)C=C12	0.0002	Fish	8.99	P1P2
Profiles	68558-73-6	Triphenyleno(4,5-bcd)thiophene	S1C2=CC=CC3=C4C=CC=CC4=C4C=CC=C1C4=C23	0.0540	Fish	6.05	P1P2

(1) For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets

(2) SMILES codes including substituents; for mixtures or groups of isomers detected together (e.g. C2-substituted) they are however not specific for each isomer regarding the position of substituents on the ring system, the position of a ring-heteroatom within the ring system or the relative position of aromatic rings within the ring system

(3) Fish: always 96h-value (not 14d, as for some classes available)

(4) In case of several applicable classes, the one with the most conservative effect value was chosen

(5) Measured log K_{ow}-values as far as available from PhysProp-Database within EPI-Suite

(6) Persistency assessment according to Guidance R11 (cut-off for Biowin3 $\leq$ 2.25 (months), Biowin2 and Biowin6 <0.5

As outlined in section 6.2.4, besides the substances screened positively as PBT also compounds fulfilling only 2 of the three criteria, namely PB, BT or PT should be critically assessed further concerning their prevalence in products (profiles) and experimental data (as far as available).

The *pool of identified semipolar PAC* of 443 compounds contained no substances exclusively fulfilling criteria for PT without fulfilling the third criterion at the same time. However, 57 substances fulfil the criteria for BT (see Table 18) and 3 substances for PB (see Table 19). As outlined in section 6.2.4, for both of these binary combinations the cut-off value for B was raised from log $K_{ow} \geq 4.0$ to ≥ 4.5. For less toxic substances a higher bioaccumulation may be acceptable and the same holds true for non-persistent substances as a higher bioaccumulation potential is deemed to be necessary to cause harm compared to persistent compounds. The persistence criterion and the T-criterion were left unchanged ($L(E)C_{50} \leq 1.0$ mg/L for T).

Table 18: 57 compounds fulfilling screening-criteria for B and T. For this binary combination cut-off-criteria for B were raised to log Kow ≥ 4.5

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assess-ment[6]
KORA / LAWA	16587-52-3	Methyldibenzothiophene, 3-	Cc1cc2c(c3c(s2)cccc3)cc1	0.53	Daphnid	4.71	not P
KORA / LAWA	7372-88-5	Methyldibenzothiophene, 4-	Cc3cccc2c3sc1c2cccc1	0.53	Daphnid	4.71	not P
KORA / LAWA	31317-07-4	Methyldibenzothiophene, 1-	Cc3cccc2c3c1ccccc1s2	0.53	Daphnid	4.71	not P
KORA / LAWA	20928-02-3	Methyldibenzothiophene, 2- (SMILES for 4-methyl-is.)	Cc3cccc2c3sc1c2cccc1	0.53	Daphnid	4.71	not P
KORA / LAWA	239-30-5	Benzo[b]naphtho-[2,1-d]furan	O1C2=C(C=CC=C2)C2=C1C1=C(C=CC=C1)C=C2	0.42	Daphnid	4.89	not P
KORA / LAWA	1136-77-2	Dimethyldibenzofurans (DMDBF)	c12c3cccc(C)c3Oc1c(C)ccc2	0.23	Daphnid	5.14	not P
KORA / LAWA	1207-12-1	Dimethyldibenzothio-phenes (DMDBT)	Cc1cccc2c1c3c(s2)ccc(c3)C	0.20	Daphnid	5.26	not P
KORA / LAWA	7320-51-6	2-Methyldibenzofuran	c12c3ccc(C)cc3Oc1cccc2	0.60	Daphnid	4.6	not P
KORA / LAWA	7320-52-7	3-Methyldibenzofuran	c12c3cc(C)ccc3Oc1cccc2	0.60	Daphnid	4.6	not P
KORA / LAWA	7320-53-8	4-Methyldibenzofuran	c(ccc1c2cc3)cc1Oc2c(c3)C	0.60	Daphnid	4.6	not P
KORA / LAWA	16587-33-0	Tetrahydrodibenzo(b)-thiophene	S(c(ccc1)c2c1)C(CCC3)=C2C3	0.32	Daphnid	4.95	not P
Profiles	79313-23-8	Propyldibenzothiophene; (Dibenzothiophene, C3-substituted, different isomers)	c12c3c(cccc3)sc1cccc2CCC	0.10	Daphnid	5.69	not P
Profiles	79313-25-0	Butyldibenzothiophene; (Dibenzothiophene, C4-substituted, different isomers)	c12sc3ccccc3c2ccc1C(CCC)	0.04	Fish	6.19	not P
Profiles	82-05-3	Benz(de)anthracene-7-one	O=C(c(c(c(c1c(ccc2)cc3)c3)ccc4)c4)c12	0.60	Daphnid	4.81	not P
Profiles	87688-44-6	2-(9H-Xanthene-9-yl)-1H-indene-1,3(2H)-dione	O=C1C(C(=O)C2=C1C=CC=C2)C1=CC=CC2=C1[O]=C1C=CC=CC1=C2	0.66	Daphnid	4.95	not P

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assess-ment[6]
Profiles		Dibenzothiophene, -butylmethyl; (Diben-zothiophene, C5-substituted, different isomers)	CCCCC1=CC=CC2=C1C1=C(S2)C=C(C)C=C1	0.01	Fish	6.73	not P
Profiles		(1)Benzothieno(3,2-b)(1)benzothiophene, C3-substituted, different isomers	CCCC1=CC2=C(C=C1)C1=C(S2)C2=CC=CC=C2S1	0.01	Fish	6.82	not P
Profiles		(1)Benzothieno(3,2-b)(1)benzothiophene, C4-substituted	CCCCC1=CC2=C(C=C1)C1=C(S2)C2=CC=CC=C2S1	0.01	Fish	7.31	not P
Profiles		Naphthobenzothiophane, C3-substituted	CCCC1=CC2=C(C=C1)C1C=CC3=C(C=CC=C3)C1S2	0.02	Fish	6.49	not P
Profiles		Dinaphthothiophene, C2-substituted	CCC1=C2SC3=C(C=CC4=C3C=CC=C4)C2=CC2=C1C=CC=C2	0.00	Fish	7.68	not P
Profiles	1090-13-7	Naphthacene-5,12-dione	O=C(c(c(C(=O)c1cccc2)cc(c3ccc4)c4)c3)c12	0.99	Daphnid	4.52	not P
Profiles	128-66-5	Dibenzo(b,def)chrysene-7,14-quinone	O=C(c(c(c(c1c(c(c(c(C2=O)ccc3)c3)cc4)c2c5)c5)ccc6)c6)c14	0.04	Fish	6.28	not P
Profiles	14461-85-9	C4-Naphtholes (1,6-Diethyl-2-naphthol, 14461-85-9)	CCC1=CC2=C(C=C1)C(=C(C=C2)O)CC	0.28	Fish	4.77	not P
Profiles	18028-56-3	C3-Carbazoles or benzo-indoles (9H-Carbazole, 1,4,6-trimethyl-RN: 18028-56-3	CC1=CC2=C(C=C1)NC3=C(C=CC(=C23)C)C	0.37	Daphnid	4.93	not P
Profiles	194-65-0	Dinaphtho(2,1-b:1',2'-d)thiophene	S(c(ccc1cc2)c-3c1cc2)c(ccc4cc5)c3c4cc5	0.02	Fish	6.64	not P
Profiles	200-23-7	Benzo(kl)xanthene	O(c(c(c(c1c(ccc2)cc3)c3)ccc4)c4)c12	0.22	Daphnid	5.23	not P
Profiles	29062-95-1	C2-Dibenzofurans (Di-methyldibenzofurans, 29062-95-1)	CC1=C(C2=C(C=C1)OC3=CC=CC=C32)C	0.23	Daphnid	5.14	not P
Profiles	3067-13-8	Benzo(a)pyrene-1,6-quinone	O=C1C=CC2=C3C1=CC=C1C4=C(C=CC=C4)C(=O)C(C=C2)=C31	0.59	Daphnid	4.94	not P

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assess-ment[6]
Profiles	3067-14-9	Benzo(a)pyrene-3,6-quinone	O=C1C=CC2=CC=C3C4=C(C=CC=C4)C(=O)C4=C3C2=C1C=C4	0.59	Daphnid	4.94	not P
Profiles	3074-03-1	11H-Benzo(b)fluoren-11-one	O=C1C2=C(C=CC=C2)C2=C1C=C1C=CC=CC1=C2	0.60	Daphnid	4.73	not P
Profiles	31473-75-3	Perylo(1,12-bcd)thiophene (4 isomers)	S1C2=CC=C3C=CC=C4C5=CC=CC6=CC=C1C(C2=C34)=C56	0.03	Fish	6.37	not P
Profiles	6051-98-5	7H-Benzo(b)fluoren-7-one	O=C1C2=C(C=CC=C2)C2=C1C=CC1=C2C=CC=C1	0.60	Daphnid	4.73	not P
Profiles	70021-48-6	C3-Dibenzothiophenes (Trimethyldibenzothiophenes)	Cc3ccc(c2c3sc1c2cc(cc1)C)C	0.08	Daphnid	5.81	not P
Profiles	76723-60-9	Benzofluorenones (Benzofluorenone, 76723-60-9; 11H-Benzo(b)fluoren-11-one, 3074-03-1)	C1(c4c3c(ccc4c2ccccc12)cccc3)=O	0.60	Daphnid	4.73	not P
Profiles	79313-22-7	5-Ethyldibenzothiophene (CAS for unspecified isomer)	c12c(c3c(s1)cccc3)c(ccc2)CC	0.23	Daphnid	5.2	not P
Profiles	80440-44-4	Dibenzanthracenone (7H-Dibenz(de,j)anthracene-7-one 80440-44-4)	O=C1C2=C3C(C=CC=C3C3=C1C1=C(C=CC=C1)C=C3)=CC=C2	0.08	Fish	5.9	not P
Profiles	81503-62-0	2,4,6-Trimethylbenzo(h)quinoline	n1c(cc(c2cc(c3c(c12)cccc3)C)C)C	0.37	Daphnid	4.96	not P
Profiles	84540-55-6	Methylnaphthobenzofurans (Methylbenzo(b)naphtho(2,3-d)furan)	CC1=C2C(OC3=C2C=CC=C3)=CC2=C1C=CC=C2	0.08	Daphnid	5.77	not P
Profiles	89816-99-9	C2-Dibenzothiophenes (4-Ethyldibenzothiophene 89816-99-9)	c12c(c3c(s1)cccc3)cccc2CC	0.23	Daphnid	5.2	not P
Profiles		Dibenzonaphthofuran or isomers (SMILES for Anthra(2,1-b)benzo(d)furan)	O1C2=CC=CC=C2C2=C1C=CC1=C2C=C2C=CC=CC2=C1	0.03	Fish	6.4	not P

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assess-ment[6]
Profiles		Dimethyl- or ethylhy-droxypyrenes or -fluoranthenes (SMILES for 2,3-Dimethylpyren-1-ol)	CC1=C(C)C2=CC=C3C=CC=C4C=CC(=C1O)C2=C34	0.44	Daphnid	5.21	not P
Profiles		C3-Hydroxyanthracenes or -phenanthrenes (SMILES for 1,9,10-Trimethylanthracene-2-ol)	CC1=C2C=CC=CC2=C(C)C2=C(C)C(O)=CC=C12	0.10	Fish	5.51	not P
Profiles		Dimethyl- or ethylydroxyanthracenes or -phenanthrenes (SMILES for 9,10-Methylanthracene-2-ol)	CC1=C2C=CC=CC2=C(C)C2=C1C=CC(O)=C2	0.23	Fish	4.96	not P
Profiles		C5-Acenaphthenols (3,4,5,6,8-Pentamethylacenaph-thene-1-ol)	CC1=CC(C)=C2C(O)CC3=C(C)C(C)=C(C)C1=C23	0.01	Daphnid	5.35	not P
Profiles		C4-Acenaphthenols (SMILES for 3,5,6,8-Tetramethylacenaph-thene-1-ol)	CC1=CC(C)=C2CC(O)C3=C(C)C=C(C)C1=C23	0.04	Daphnid	4.8	not P
Profiles		Methylhydroxy-phenylnaphthalenes (SMILES for 5-Methyl-2(naphthalen-2-yl)-phenol)	CC1=CC=C(C(O)=C1)C1=CC=C2C=CC=CC2=C1	0.22	Fish	5	not P
Profiles		Dimethylbenzonaphtho-furan (SMILES for 9,11-dimethylben-zo(b)naphtho(2,3-d)furan)	CC1=CC2=C(C=C1)C=C1OC3=C(C=CC=C3)C1=C2C	0.03	Fish	6.32	not P
Profiles		Methylnaphthobenzothi-ophenes (SMILES for 5-Methylnaphtho(1,2-b)thiophene)	CC1=CC2=C(SC=C2)C2=C1C=CC=C2	0.53	Daphnid	4.71	not P
Profiles		Methylazadihydropyrene or C2-azacyclopen-ta(def)phenanthrene (SMILES for 7-Methyl-5-aza-2,3dimethylpyrene)	CC1=CC2=C3C(=C1)N=CC1=C3C(C=C2)=CCC1	0.84	Daphnid	4.52	not P
Profiles		Dimethyldiben-zonaphthofurans or iso-mers (SMILES for 3,7-Dimethylanthra(2,1-b)benzo(d)furan)	CC1=CC=C2C(OC3=C2C2=C(C=C4C=CC=CC4=C2)C(C)=C3)=C1	0.00	Fish	7.5	not P

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assess-ment[6]
Profiles		Azabenzonaphthothio-phene isomers, C1-substituted	CC1=CC=CC2=C1C=C1C(SC3=C1C=CC=C3)=N2	0.40	Daphnid	4.99	not P
Profiles		C5-Naphtholes (SMILES for 4-Methyl-1,6-diethyl-2-naphthol)	CCC1=CC2=C(C)C=C(O)C(CC)=C2C=C1	0.12	Fish	5.31	not P
Profiles		C3-Fluorenols (SMILES for 7-Ethyl-9-methylfluoren-2-ol)	CCC1=CC2=C(C=C1)C1=C(C=C(O)C=C1)C2C	0.34	Fish	4.71	not P
Profiles		C7-Naphtholes (SMILES for 8-Methyl-1,4,6-triethyl-2-naphthol)	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C(C)=C1	0.02	Fish	6.35	not P
Profiles		C8-Naphtholes (SMILES for 1,4,6,8-Tetraethyl-2-naphthol)	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C(CC)=C1	0.01	Fish	6.84	not P
Profiles		C6-Naphtholes (SMILES for 1,4,6-Triethyl-2-naphthol)	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C=C1	0.06	Fish	5.8	not P
Profiles		Only defined by structure (isomers existing, SMILES for Cyclopen-ta(kl)phenanthro(4,5-bcd)thiophene)	S1C2=CC=C3C=CC4=CC5=CC=CC1=C5C2=C34	0.11	Daphnid	5.65	not P

For annotations (1) to (6) please see annotations to Table 17

Table 19: 3 compounds fulfilling screening-criteria for B and P. For this binary combination cut-off-criteria for B were raised to log $K_{ow} \geq 4.5$

Source	CAS	Name[1]	SMILES[2]	ECOSAR L(E)C50 [mg/L][4]	Organism[3]	Log K_{ow}[5]	P-Assess-ment[6]
Profiles	19694-02-1	Pyrene carboxylic acid (1-Pyrenecarboxylic ac-id, 19694-02-1)	OC(=O)C1=C2C=CC3=CC=CC4=CC=C(C=C1)C2=C34	5.42	Daphnid	4.81	P1P2
Profiles	42397-64-8	1,6-Dinitropyrene	O=N(=O)c1ccc2ccc3c(ccc4ccc1c2c34)N(=O)=O	1.02	Daphnid	4.57	P1P2
Profiles	42397-65-9	1,8-Dinitropyrene	O=N(=O)c1ccc2ccc3ccc(N(=O)=O)c4ccc1c2c34	1.02	Daphnid	4.57	P1P2

For annotations (1) to (6) please see annotations to Table 17

8.4 Enforced QSAR-selection criteria on PBT properties

To narrow down further the group of substances with potential PBT, BT and vPvB properties, selection criteria were further enhanced to screen for the most potent semipolar PAC (see section 6.3).

For PBT-properties criteria according to REACH guidance R.11 were applied, i.e. log Kow >4.5 for B, $L(E)C_{50}$ <0.1 mg/L and the persistency assessment according to R.11 by pairwise evaluation of BIOWIN 2/3 and 3/6-results (i.e. P-assessment unchanged compared to first screening criteria set).

Applying these criteria to select for PBT-substances on the *pool of identified semipolar PAC*, 28 out of 443 compounds with a potential to fulfil PBT-criteria are selected, compared to 94 resulting with the extended QSAR selection criteria on B and T applied in the first place (30%).

Using these same criteria to select for compounds with predicted BT-properties, 22 compounds are judged to fulfil both properties according to the enforced QSAR selection criteria compared to 57 according to the extended QSAR selection (i.e. 39%).

After enforcement of criteria for potential vPvB-compounds, 9 semipolar PAC with a potential for fulfilling vPvB-criteria are selected (with an associated log Kow ≥4.57).

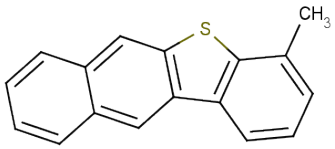
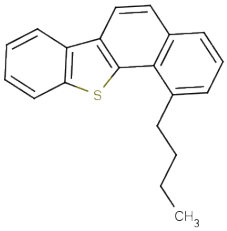
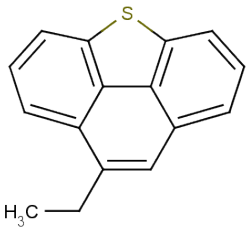
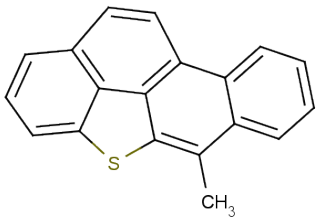
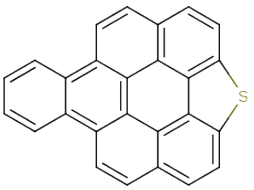
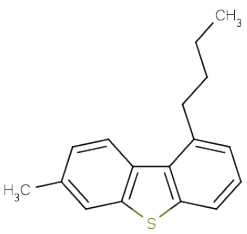
One compound results predicted to be TP according to enforced QSAR selection criteria.

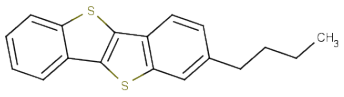
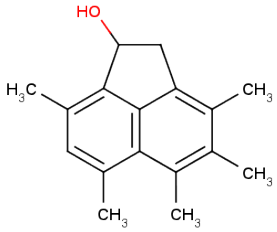
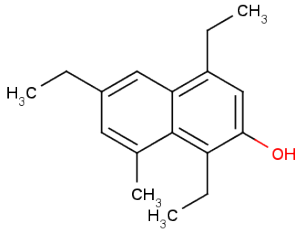
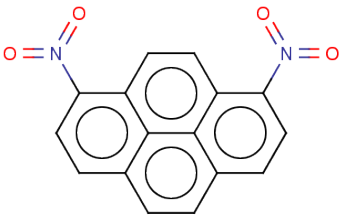
The *enforced QSAR selection of critical PAC* (60 compounds) are listed in in Table 21 to Table 24. Most of these semipolar PAC (38 of 69) are not identifiable by a CAS-number.

The semipolar PAC originating from the enforced QSAR selection procedure were subsequently screened with respect to their occurrence in the analysed profiles. The following results were obtained:

- Four out of the 60 compounds originate from the KORA/LAWA list and could not be identified in the profiles analysed.
- All of the remaining compounds are not frequently found in the evaluated profiles (predominantly 1-2-fold, maximum: Methylbenzo(b)-naphtho(2,1-d)thiophene in 6 profiles).
- Several compounds originated from catalytically cracked petroleum vacuum residues, which are produced as fuel and therefore they are not relevant under the scope of this project. The same holds true for candidates identified only in ambient air or urban dust.
- Most of the remaining critical semipolar PAC from the enforced QSAR selection have been determined only qualitatively. In several profiles quantitative information is reported in parallel to quantitative values for other compounds, indicating the presence of the respective critical semipolar PAC in only very low amounts. 10 compounds have been determined quantitatively in relevant amounts; they are highlighted in bold in the following tables. The PBT list contains only one compound (Triphenyleno(4,5-bcd)thiophene) quantitatively determined in carbon black (30 mg/kg). 6 quantitatively determined compounds originate from the BT-list (not P) and one from the TP (not B) lists. 5 of these have been identified in parallel in a single sample of anthracene oil (same profile), with a concentration range of 200-1400 mg/kg. The other two have been identified in a sample of gasworks soil (17 mg/kg) and a pitch sample (1170 mg/kg), respectively. These yields cannot be regarded as representative for the respective matrices because the data are based on a single profile, each. However the relevance of these compounds could be possibly substantiated by the analysis of products derived from e.g. anthracene oils. From the vPvB list, further 2 substances (isomeric mixtures, each) were quantified in a sample of anthracene oil (100 and 64 mg/kg, respectively).
- Several groups of closely structurally related compounds were identified (Table 20):

Table 20 Groups of closely structurally related compounds identified within *enforced QSAR selection of critical PAC*

Number of related structures - group	Structural representative
3 - Methylbenzo(b)-naphthothiophene isomers	
4 - alkylated Benzo(b)-naphtho(2,1-d)thiophenes with a varying grade of alkyl substitution	
5 - alkylated Phenanthro(4,5-bcd)thiophenes with a varying grade of alkyl substitution	
5 - Chryseno(4,5-bcd)thiophene core and 4 alkylation isomers with a varying grade of alkyl substitution	
2 - Benzopyrenodibenzothiophene isomers	
4 - alkylated Dibenzothiophenes with a varying grade / pattern of alkyl substitution	

Number of related structures - group	Structural representative
2 - alkylated (1)Benzothieno(3,2-b)(1)benzothiophenes with a varying grade of alkyl substitution	
2 - alkylated Acenaphthenols with a varying grade of alkyl substitution	
3 - alkylated Naphtholes with a varying grade of alkyl substitution and	
4 – Nitropyrene (1) and dinitropyrene (3) isomers	

The compounds of the enforced QSAR selection of critical semipolar PAC are summarized in detail in Table 21 to Table 24 according to the predicted properties.

Table 21: Compounds predicted to fulfil PBT-properties according to enforced QSAR selection criteria (28 compounds) with their frequency of occurrence in analysed profiles (quantitatively determined compounds are highlighted in bold in the following table)

Name	CAS	SMILES	Frequency of occurrence in profiles	Remarks
Methylbenzo(b)-naphtho(1,2-d)thiophene	84258-62-8	CC1=CC=CC2=C1C1=C(SC3=C1C=CC=C3)C=C2	0	from KORA/LAWA list, not occurring in analysed profiles
Methylbenzo(b)-naphtho(2,1-d)thiophene	4567-41-3	CC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	6	5 profiles only qualitative (pitch, tar, tar eluate and distillate), quantified only in fuel (60 mg/kg)
Methylbenzo(b)-naphtho(2,3-d)thiophene	36821-08-6	CC1=CC=CC2=C1SC1=C2C=C2C=CC=CC2=C1	0	from KORA/LAWA list, not occurring in analysed profiles
Phenanthro(4,5-bcd)thiophene, -ethyl; (Phenanthro(4,5-bcd)thiophene, C2-substituted, different isomers)	--	CCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	1	identified only in fuel ((oil fraction, hydrotreated 57 mg/kg)
Phenanthro(4,5-bcd)thiophene, -propyl; (Phenanthro(4,5-bcd)thiophene, C3-substituted, different isomers)	--	CCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	1	identified only in fuel(oil fraction, hydrotreated 95 mg/kg)
Phenanthro(4,5-bcd)thiophene, -butyl; (Phenanthro(4,5-bcd)thiophene, C4-substituted, different isomers)	--	CCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	1	identified only in fuel(oil fraction, hydrotreated 120 mg/kg)
Phenanthro(4,5-bcd)thiophene, -pentyl; (Phenanthro(4,5-bcd)thiophene, C5-substituted, different isomers)	--	CCCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	1	identified only in fuel(oil fraction, hydrotreated 92 mg/kg)
Phenanthro(4,5-bcd)thiophene, -Hexyl; (Phenanthro(4,5-bcd)thiophene, C6-substituted, different isomers)	--	CCCCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	1	identified only in fuel(oil fraction, hydrotreated 7 mg/kg)
Benzo(b)-naphtho(2,1-d)thiophene, -ethyl; (benzo(b)-naphtho(2,1-d)thiophene, C2-substituted, different isomers)	--	CCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	3	qualitative in tar and tar distillates, quantified only in fuel (185.3 mg/kg)
Benzo(b)-naphtho(2,1-d)thiophene, -propyl; (benzo(b)-naphtho(2,1-d)thiophene, C3-substituted, different isomers)	--	CCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	1	identified only in fuel (oil fraction, hydrotreated 573 mg/kg)

Name	CAS	SMILES	Frequency of occurrence in profiles	Remarks
Benzo(b)-naphtho(2,1-d)thiophene, -butyl; (benzo(b)-naphtho(2,1-d)thiophene, C4-substituted, different isomers)	--	CCCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	1	identified only in fuel (oil fraction, hydrotreated 328 mg/kg)
Benzo(b)-naphtho(2,1-d)thiophene, -pentyl; (benzo(b)-naphtho(2,1-d)thiophene, C5-substituted)	--	CCCCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	1	identified only in fuel (oil fraction, hydrotreated 5 mg/kg)
Chryseno(4,5-bcd)thiophene, C1-substituted	--	CC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	1	identified only in fuel (oil fraction, hydrotreated 10 mg/kg)
Chryseno(4,5-bcd)thiophene, C2-substituted, different isomers	--	CCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	1	identified only in fuel(oil fraction, hydrotreated 290 mg/kg)
Chryseno(4,5-bcd)thiophene, C3-substituted, different isomers	--	CCCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	1	identified only in fuel(oil fraction, hydrotreated 590 mg/kg)
Chryseno(4,5-bcd)thiophene, C4-substituted, different isomers	--	CCCCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	1	identified only in fuel(oil fraction, hydrotreated 200 mg/kg)
Benzophenanthrothiophene (Benzo(2,3)phenanthro(4,5-bcd)thiophene)	72072-20-9	C1=CC=C2C(=C1)C=C3C=CC4=C5C3=C2SC5=CC=C4	3	qualitative in pitch, tar and tar distillates
6-Nitrobenzo(a)pyrene	63041-90-7	O=N(=O)c2c1ccccc1c3ccc4cccc5ccc2c3c45	2	qualitative in air and Carbon Black (CB)
Dinaphthofurans	68518-20-7	c1ccc2cc3c(cc2c1)oc1cc2ccccc2cc31	2	qualitative in gasworks soil and urban dust
Benzo(def)naphthobenzothiophene (= Chryseno(4,5-bcd)thiophene)	72076-98-3	c15c3c4ccc5c2c(cc1sc3ccc4)cccc2	2	qualitative in CB and pitch

Name	CAS	SMILES	Frequency of occurrence in profiles	Remarks
Methylbenzophenanthro(4,5-bcd)thiophene (SMILES for 2-Methyl isomer)	--	CC1=CC2=C3C(SC4=C5C=CC=CC5=CC(C=C2)=C34)=C1	2	qualitative in tar and tar distillates
Dibenzo(c,g)phenanthro(12,9-bcd)thiophene = Benzo(6,7)perylo(1,12-bcd)thiophene (only defined by structure, isomers existing)	--	S1C2=C3C4=C1C=CC1=C4C4=C(C=C1)C=CC1=C4C3=C(C=C2)C=C1	1	qualitative in CB
Phenanthro(7,6,5,4b,4a,4:12,12a,12b,1,2,3)peryleno(6,7-bcd)thiophene	--	S1C2=C3C4=C1C=CC1=C4C4=C5C(C=CC(C=C2)=C35)=CC2=C4C3=C(C=CC=C13)C=C2	1	qualitative in CB
Tribenzo(2,1,4,5,6,7)chryseno(10,11,bcd)thiophene	--	S1C2=C3C4=C1C1=C5C(C=CC6=C5C4=C(C=C6)C4=C3C(C=C2)=CC=C4)=CC=C1	1	qualitative in CB
Benzo(9,10)pyreno(3,4,5,6-jklma)dibenzothiophene	--	S1C2=CC=C3C=CC4=C5C=CC=CC5=C5C=CC6=C7C(=C1C=C6)C2=C3C4=C57	1	qualitative in CB
Benzo(1,2)pyreno(3,4,5,6-jklma)dibenzothiophene	--	S1C2=CC=C3C4=CC=CC=C4C4=CC=C5C=CC6=C7C(=C1C=C6)C2=C3C4=C57	1	qualitative in CB
Coroneno(2,3,4-bcd)benzothiophene	--	S1C2=CC=CC3=C2C2=C4C5=C(C=CC6=C5C5=C(C=C6)C=CC6=C5C4=C3C=C6)C=C12	1	qualitative in CB
Triphenyleno(4,5-bcd)thiophene	68558-73-6	S1C2=CC=CC3=C4C=CC=CC4=C4C=CC=C1C4=C23	1	30 mg/kg in CB

Table 22: Compounds predicted to fulfil BT-properties according to enforced QSAR selection criteria (22 compounds) with their frequency of occurrence in analysed profiles (quantitatively determined compounds are highlighted in bold in the following table)

Name	CAS	SMILES	Frequency of occurrence in profiles	Remarks
Propyldibenzothiophene; (Dibenzothiophene, C3-substituted, different isomers)	79313-23-8	c12c3c(cccc3)sc1cccc2CCC	1	identified only in fuel (oil fraction, hydrotreated 293 mg/kg)
Butyldibenzothiophene; (Dibenzothiophene, C4-substituted, different isomers)	79313-25-0	c12sc3ccccc3c2cccc1C(CCC)	1	identified only in fuel (oil fraction, hydrotreated 115 mg/kg)
Dibenzothiophene, -butylmethyl; (Dibenzothiophene, C5-substituted, different isomers)	--	CCCCC1=CC=CC2=C1C1=C(S2)C=C(C)C=C1	1	identified only in fuel (oil fraction, hydrotreated 25 mg/kg)
(1)Benzothieno(3,2-b)(1)benzothiophene, C3-substituted, different isomers	--	CCCC1=CC2=C(C=C1)C1=C(S2)C2=CC=CC=C2S1	1	identified only in fuel (oil fraction, hydrotreated 47 mg/kg)
(1)Benzothieno(3,2-b)(1)benzothiophene, C4-substituted	--	CCCCC1=CC2=C(C=C1)C1=C(S2)C2=CC=CC=C2S1	1	identified only in fuel (oil fraction, hydrotreated 10 mg/kg)
Naphthobenzothiophane, C3-substituted	--	CCCC1=CC2=C(C=C1)C1C=CC3=C(C=CC=C3)C1S2	1	identified only in fuel (oil fraction, hydrotreated 3 mg/kg)
Dinaphthothiophene, C2-substituted	--	CCC1=C2SC3=C(C=CC4=C3C=CC=C4)C2=CC2=C1C=CC=C2	1	identified only in fuel(oil fraction, hydrotreated 110 mg/kg)
Dibenzo(b,def)chrysene-7,14-quinone	128-66-5	O=C(c(c(c(c1c(c(c(c(C2=O)ccc3)c3)cc4)c2c5)c5)ccc6)c6)c14	1	qualitative in air
Dinaphtho(2,1-b:1',2'-d)thiophene	194-65-0	S(c(ccc1cc2)c-3c1cc2)c(ccc4cc5)c3c4cc5	4	qualitative in gasworks soil, pitch, tar and tar distillates
Perylo(1,12-bcd)thiophene (4 isomers)	31473-75-3	S1C2=CC=C3C=CC=C4C5=CC=CC6=CC=C1C(C2=C34)=C56	3	qualitative in CB, tar and tar distillates
C3-Dibenzothiophenes (Trimethyldibenzothiophenes)	70021-48-6	Cc3ccc(c2c3sc1c2cc(cc1)C)C	1	Qualitative in anthracene oil, hydrocarbon fraction

Name	CAS	SMILES	Frequency of occurrence in profiles	Remarks
Dibenzanthracenone (7H-Dibenz(de,j)anthracene-7-one 80440-44-4)	80440-44-4	O=C1C2=C3C(C=CC=C3C3=C1C1=C(C=CC=C1)C=C3)=CC=C2	2	quantitative in gasworks soil (17 mg/kg), qualitative in urban dust
Methylnaphthobenzofurans (Methylbenzo(b)naphtho(2,3-d)furan)	84540-55-6	CC1=C2C(OC3=C2C=CC=C3)=CC2=C1C=CC=C2	1	qualitative in anthracene oil and pitch, quantitative in pitch (1170 mg/kg)
Dibenzonaphthofuran or isomers (SMILES for Anthra(2,1-b)benzo(d)furan)	--	O1C2=CC=CC=C2C2=C1C=CC1=C2C=C2C=CC=CC2=C1	1	qualitative in pitch
C3-Hydroxyanthracenes or -phenanthrenes (SMILES for 1,9,10-Trimethylanthracene-2-ol)	--	CC1=C2C=CC=CC2=C(C)C2=C(C)C(O)=CC=C12	1	qualitative in anthracene oil
C5-Acenaphthenols (3,4,5,6,8-Pentamethylacenaphthene-1-ol)	--	CC1=CC(C)=C2C(O)CC3=C(C)C(C)=C(C)C1=C23	1	quantitative in anthracene oil (200 mg/kg)
C4-Acenaphthenols (SMILES for 3,5,6,8-Tetramethylacenaphthene-1-ol)	--	CC1=CC(C)=C2CC(O)C3=C(C)C=C(C)C1=C23	1	quantitative in anthracene oil (1200 mg/kg)
Dimethylbenzonaphthofuran (SMILES for 9,11-dimethylbenzo(b)naphtho(2,3-d)furan)	--	CC1=CC2=C(C=C1)C=C1OC3=C(C=CC=C3)C1=C2C	1	qualitative in pitch
Dimethyldibenzonaphthofurans or isomers (SMILES for 3,7-Dimethylanthra(2,1-b)benzo(d)furan)	--	CC1=CC=C2C(OC3=C2C2=C(C=C4C=CC=CC4=C2)C(C)=C3)=C1	1	qualitative in pitch
C7-Naphtholes (SMILES for 8-Methyl-1,4,6-triethyl-2-naphthol)	--	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C(C)=C1	1	quantitative in anthracene oil (200 mg/kg)
C8-Naphtholes (SMILES for 1,4,6,8-Tetraethyl-2-naphthol)	--	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C(CC)=C1	1	qualitative in anthracene oil
C6-Naphtholes (SMILES for 1,4,6-Triethyl-2-naphthol)	--	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C=C1	1	quantitative in anthracene oil (300 mg/kg)

Table 23: Compounds predicted to fulfil TP-properties (not B) according to enforced QSAR selection criteria (1 compound) with their frequency of occurrence in analysed profiles (quantitatively determined compounds are highlighted in bold in the following table)

Name	CAS	SMILES	Frequency of occurrence in profiles	Remarks
Naphthoindoles (SMILES for 1H-Naphtho(1,2-g)indole	--	N1C=CC2=C1C1=C(C=C2)C2=C(C=CC=C2)C=C1	1	quantitative in anthracene oil (1400 mg/kg)

Table 24: Compounds predicted to fulfil vPvB-properties according to enforced QSAR selection criteria (9 compounds) with their frequency of occurrence in analysed profiles (quantitatively determined compounds are highlighted in bold in the following table)

Name	CAS	SMILES	Frequency of occurrence in profiles	Remarks
Dimethylbenz(c)acridine	2381-40-0	Cc4ccc3c(C)c2ccc1ccccc1c2nc3c4	0	from KORA/LAWA list, not occurring in analysed profiles
1-Nitropyrene	5522-43-0	O=N(=O)c(c(c(c(c(c1)ccc2)c2cc3)c3c4)c1)c4	5	qualitative in ambient air and CB
1,6-Dinitropyrene	42397-64-8	O=N(=O)c1ccc2ccc3c(ccc4ccc1c2c34)N(=O)=O	2	qualitative in CB
1,8-Dinitropyrene	42397-65-9	O=N(=O)c1ccc2ccc3ccc(N(=O)=O)c4ccc1c2c34	2	qualitative in CB
1,3-Dinitropyrene	75321-20-9	O=N(=O)c4c2c3c(c(c4)N(=O)=O)ccc1c3c(ccc1)cc2	2	qualitative in CB
3-Nitrofluoranthene	892-21-7	O=N(=O)c2ccc3c1ccccc1c4ccc2c34	1	quantitative in ambient air
4H-Naphtho(1,2,3,4-def)carbazole or isomer	--	N1C2=CC=CC3=C4C=CC=CC4=C4C=CC=C1C4=C23	1	qualitative in coal tar pitch
Dimethyl- or ethyl-azafluoranthenes/pyrenes or azabenzofluorenes (SMILES for 1,6-Dimethyl-4-azapyrene)	--	CC1=CC=C2N=CC3=C(C)C=CC4=CC=C1C2=C34	1	quantitative in anthracene oil (100 mg/kg)

Name	CAS	SMILES	Frequency of occurrence in profiles	Remarks
Trimethyl- or methylethylbenzo(def)carbazoles or dimethylindenoindoles (Trimethylbenzo(def)carbazole = Trimethylphenanthro(bcd)pyrrole (SMILES for 1,7,8--trimethylphenanthro(bcd)pyrrole)	--	CC1=CC2=C(C)C=CC3=C2C2=C(N3)C=CC(C)=C12	1	quantitative in anthracene oil (64 mg/kg)

8.5 Screening of PBT candidates with respect to frequency of occurrence in profiles

Due to the rare occurrence of the *enforced QSAR selection of critical PAC* (60 compounds) in the analysed profiles, an alternative approach was used: The extended QSAR selection of 154 critical PAC (94 PBT, 57 BT and 3 PB compounds) from the initial, less restrictive QSAR selection procedure (see section 6 for details) was filtered by their frequency of occurrence in profiles and only compounds with the highest occurrence were considered (cut-off criterion: ≥ 6, the maximum frequency reached by the enforced selection procedure described above). Eleven compounds were selected applying this cut-off criterion on the *extended QSAR selection of critical PAC*: 9 compounds predicted to be PBT and 2 predicted to be BT. All of them are identifiable by a CAS-number. They are listed in the following Table 25. With methylbenzo(b)-naphtho(2,1-d)thiophene one compound is included which was predicted to have PBT properties also according to the enforced QSAR selection criteria outtlined above.

Table 25: Most frequently occurring compounds of *extended QSAR selection of critical PAC* (154 compounds)

PBT screening results	Name (CAS-number)	Frequency of occurrence in profiles	Remarks
PBT	Benzo(b)naphtho[2,1-d]thiophene (239-35-0)	19	qualitative in bitumen, 2 tars, 2 tar distillates, aqueous tar eluate, pitch, 2 gasworks soils and CB quantitative in bitumen: 2,34-15,06 mg/kg (7 samples) pitch: 2390 mg/kg CB: 5 mg/kg
PBT	Benz(c)acridine (225-51-4)	12	qualitative in 2 anthracene oils and pitch quantitative in anthracene oils: 150-418 mg/kg (4 samples) creosote: 547 mg/kg tar: 47 mg/kg pitch: 730 mg/kg and in ambient air and sediment
PBT	Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline = Benzo(a)phenanthridine, 195-29-9)	12	qualitative in 2 anthracene oils and 2 pitches quantitative in anthracene oils: 31-385 mg/kg (4 samples) pitch: 620-1060 mg/kg (2 samples) tar: 38 mg/kg and in ambient air

PBT	Benzo(b)naphtho(1,2-d)furan (205-39-0)	10	qualitative in pitch, creosote, 2 tars, tar distillate, aqueous tar eluate, gasworks soil and CB quantitative in pitch: 2570 mg/kg and in urban dust
PBT	Dibenz(aj)acridine (224-42-0)	6	qualitative in pitch, creosote and gasworks soil quantitative in creosote: 3 mg/kg and in ambient air and sediment
PBT	Methylbenzo(b)-naphtho(2,1-d)thiophene (4567-41-3)	6	qualitative in 2 tars, in a tar fraction from coal tar distillates, in the volatile fraction from coal tar pitch quantitative in oil fraction hydrotreated (60 mg/kg) and in environmental aqueous eluate from tar
PBT	Phenanthro(4,5-bcd)thiophene (30796-92-0)= Benzo(def)dibenzothiophene	11	qualitative in 2 tars, tar distillate, aqueous tar eluate, pitch, gasworks soil and 2 CBs quantitative in pitch: 610 mg/kg CB: 90 mg/kg and in ambient air
PBT	(Benzo(b)naphtho(2,3-d)furan) (243-42-5)	7	qualitative in 2 tars, aqueous tar eluate, tar distillate quantitative in anthracene oil: 2900 mg/kg tar: 2000 mg/kg and in urban dust
PBT	Benzo(b)naphtho(2,3-d)thiophene (243-46-9)	6	qualitative in tar, tar distillate, aqueous tar eluate, pitch and gasworks soil quantitative in pitch: 1390 mg/kg
BT	Methyldibenzothiophenes (2-Methyldibenzothiophene 20928-02-3)	8	qualitative in 2 tars, tar distillate, anthracene oil, aqueous tar eluate and gasworks soil and in fuel and ambient air
BT	Benz(de)anthracene-7-one (82-05-3)	6	qualitative in soil, contaminated by gas works or wood impregnation quantitative in gasworks soils: 12-22 mg/kg (2 samples) and in ambient air (2 samples) and urban dust

8.6 Comparison with other QSAR-models

The results of the PBT screening by the EPI suite models (enforced QSAR selection criteria) were compared with the predicted persistency of the CATALOGIC QSAR-Model, based on the predicted Biological Oxygen Demand (BOD). This model run was kindly performed by Ms Böhnhardt, UBA.

The comparison of the results of our candidate list with the CATALOGIC output yielded the following results:

- All the 27[21] PBT candidates were also predicted to be persistent
- 21 of the 22 BT candidates (by definition not persistent) were predicted to be persistent by CATALOGIC (based on ultimate half-lives), but mostly only marginally persistent based on primary half-lives (up to about 2 months), what is close to the predictions of the EPI suite model. Only 2 substances were predicted to be highly persistent with primary half-lives of several years. These were Dinaphtho(2,1-b:1',2'-d)thiophene and Dibenzonaphthofuran or isomers (A first search in eChemPortal did not yield any experimental result with respect to biodegradability).

These partially contradictory results are discussed later (see section 9.4.2). In general it seems that CATALOGIC tends to estimate a higher persistency of compounds compared to the EPI suite BIOWIN modules.

[21] The number refers to the state of the project at the time of the CATALOGIC model run and has meanwhile slightly changed (see other chapters)

9 Complementation and Amendment of the List of Priority Semipolar PAC Derived from QSAR-Screening and Prevalence in Profiles

9.1 Additional priority compounds from REACH Annex XIV support documents on relevant UVCBs and their IUCLID-datasets

For the following possibly relevant UVCBs being part of the candidate list for authorization, Annex XIV-support documents as well as the respective IUCLID-data sets (as far as available from the ECHA web site, information on constituents not identical) were checked for semipolar polyaromatic compounds contained in the UVCB:

- **Anthracene oil, anthracene-low**
- **Anthracene oil, anthracene paste, distn. lights**
- **Anthracene oil, anthracene paste, anthracene fraction**
- **Anthracene oil, anthracene paste**
- **Anthracene oil**
- **Anthracene**
- **Coal tar pitch, high temperature**

Of all listed constituents of these UVCBs only 8 are semipolar polyaromatic compounds in the sense of this project. Of these, all are contained in the *pool of identified semipolar PAC* (443 compounds) but none is contained in the *extended QSAR selection of critical PAC* (154 compounds) and consequently within the 11 priority compounds resulting from the combined selection on substance properties and occurrence in profiles.

Therefore, from this source of information no further priority semipolar polyaromatic compounds could be derived. This however does not exclude the presence of further semipolar polyaromatic compounds in these UVCBs, as details on substance identity are probably incomplete.

9.2 Additional priority compounds according to availability of experimental results on PBT-properties and further indicators for priority

As we assume that compounds with several experimental data points available may be of certain relevance concerning their occurrence in the environment or their uses, such compounds might be chosen to broaden the list of 11 priority compounds gained so far by QSAR screening (extended QSAR selection criteria) combined with weighing according to occurrence in profiles (≥6). To gather experimental data, first and foremost the OECD QSAR Toolbox was used. Besides its functions regarding QSARs and grouping approaches, this software instrument also retrieves experimental data from several relevant data bases and thus is an easy measure to gain an overview regarding availability of PBT-relevant data

The inquiry encompassed the complete list of 154 compounds of the *extended QSAR selection of critical PAC* applying the more moderate criteria (log Kow ≥4.0, $LC/EC_{50} \leq 1$ mg/L, persistence according to combined evaluation of BIOWIN 2,3 and 6-results). Input occurred via CAS-numbers as far as available and as far as deposited in the QSAR Toolbox data base. For all other compounds, SMILES codes were used for the inquiry. Data retrieval was restricted to the following categories: log K_{OW}, bioaccumulation aquatic and terrestrial, biodegradation, ecotoxicological information, i.e. human health hazards were excluded.

Generally, via OECD QSAR Toolbox experimental data for only 15 compounds were available, including 6 compounds already contained in the list of 11 priority compounds. For 8 compounds, only one data point could be retrieved (for 7 cases log K_{OW}, in one instance LC_{50} value). For 5 compounds only, more than 2 experimental data points were determined (2x 5 data points, 2x 6 data points, 1x 21 data points). Concerning bioconcentration or accumulation in fish, often several data points relate to this endpoint (e.g. BCF, lipid content, biotransformation rate). As such, the total number of endpoints is lower as the data count. Table 26

lists only those compounds which are not already contained in the priority list of 11 compounds described above. Furthermore, compounds were only listed if a minimum of 2 experimental data points from the QSAR Toolbox were available.

As further means to ascertain the list of priority compounds, UBA checked REACH registration dossiers for the *extended QSAR selection of critical PAC* (154 compounds) relating to relevance as constituents of UVCBs or MCS or impurities in substances. However, no information on these substances was contained in the dossiers. Furthermore, UBA checked a database developed in an earlier project (Nendza, et al., 2010) including a multitude of different substance lists with different scope and relevance. As a result of this, 2 compounds of the *extended QSAR selection of critical PAC* (154 compounds) were within the list of Washington state PBT compounds (State of Washington, 2006) compiled using similar but more moderate criteria than those according to REACH (see annotation to Table 26). Consequently, these are included in Table 26. Further 5 compounds were found in other lists of the database, qualifying them as not relevant or at least not relevant for the current project (e.g. *possibly carcinogenic to humans*).

To check for possibly relevant substances overlooked so far the comprehensive data base of bioaccumulation and bioconcentration data established by Arnot and Gobas (2006, supplementary information) encompassing 5317 bioconcentration factors and 1656 bioaccumulation factors for 842 organic chemicals rated for validity was checked for entries relevant for compounds of the *pool of identified semipolar PAC* (443 compounds). For 13 compounds BCF-values were available: For 10 compounds 2 to 3 BCF-values with rating 1 (acceptable), for 2 compounds one acceptable value, each and for one compound BCF-values of rating 3 (low confidence). Only two compounds were amongst the *extended QSAR selection of critical PAC* (154 compounds), one already part of the list of 11 priority compounds (Benz(de)anthracene-7-one), one already part of Table 26 because of experimental data from the OECD QSAR Toolbox (dibenz(a,h)acridine). In general, compounds with BCF-values above 1000 according to the data of Arnot and Gobas are captured at the same time by QSAR-screening on log Kow larger or equal to 4.0. Therefore, no additional priority compounds resulted from the data of Arnot and Gobas.

As for dibenzothiophene, a substance dismissed in the QSAR-screening because of failing the toxicity criterion (EC/LC_{50} 1.37 mg/L– cut off: ≤1 mg/L) and the persistence criterion (but fulfilling the criterion for B with log K_{OW} 4.38 – cut off ≥4.0), three reliable BCF-values from the data base established by Arnot and Gobas (2006, supplementary information) spanning from 1122-1820 were available and therefore being close to the criterion for B according to REACH (BCF > 2000), plausibility of dismissing this compound was examined. Therefore, OECD QSAR Toolbox was checked for experimental data on toxicity and degradability of dibenzothiophene. NOEC values for fish (2 species) and Daphnia magna are reported to be between 0.032 and 0.054 mg/L, thus being close to but failing the T-criterion according to REACH (< 0,01 mg/L). EC/LC_{50}-values are between 0.18 and 1.4 mg/L, which is again close to but failing the cut-off for the screening criterion on T according to REACH guidance R.11 (<0.1 mg/L). Relating to persistence, only one experimental value (reliable) on ready biodegradability was available, with 0.5% biodegradation (BOD) within 28d. Thus, dibenzothiophene is not readily biodegradable however no information on inherent biodegradability is available via the QSAR Toolbox. These results of the plausibility check corroborate the assessment of dibenzothiophene by our QSAR screening approach. The compound is however a borderline case being close to criteria for B and T and being at least not readily biodegradable. As it furthermore occurred 42 times in profiles of 9 different categories dibenzothiophene might be suitable as an indicator substance for semipolar PAC.

Because IULID datasets including experimental results are available from ECHA for already registered substances[22], the list of registered substances available from ECHA in MS Excel format[22] was checked for compounds being part of the *pool of identified semipolar PAC* (443 compounds). This was meant as further plausibility check for the applied QSAR screening approach by comparison of QSAR-results with experimental data. In effect, 12 substances were amongst the REACH registered substances, however none of these was part of the *extended QSAR selection of critical PAC* (154 compounds) in agreement with the above mentioned results by UBA. According to QSAR-results, not any of these 12 compounds did fulfil any one of the three properties P, B or T. To compare this with experimental data, we had a closer look on IUCLID 5 data sets of these 12 substances. For some of the compounds available information was more or less restricted to classification and labelling information and no experimental data could be retrieved (probably compounds of low tonnage band and classification as CMR 1/2 or N, R50/53 according to directive 67/548/EEC). As regards compounds where IUCLID-data sets contained experimental data, for only one compound (2-hydroxybiphenyl, actually no polycyclic aromatic compound but a degradation product thereof) the T-criterion according to REACH (chronic NOEC < 0,01 mg/L) is fulfilled by a NOEC for reproduction (Daphnia magna) of 0,009 mg/L, but at the same time experimental data proved that neither B nor P were fulfilled. None of the compounds fulfilled the B criterion. A borderline case is mercaptobenzothiazole (149-30-4), barely missing the REACH criterion for T with a chronic NOEC for fish of 0,041 mg/L. Being not readily biodegradable (≤ 2.5% biodegradation), the P criterion could be fulfilled. However, the compound is clearly not bioaccumulative (log K_{OW} 2.3-2.5). It was detected only once in a rubber profile. To summarize the examination of REACH registration dossiers available so far[23], no information could be gained as to broaden the range of priority semipolar PAC but the experimental data retrieved corroborate the QSAR screening approach applied earlier.

Complementation of the list of 11 priority compounds

Because of the generally very poor experimental data base a compound was rated relevant due to existence of experimental data if besides log Kow one further data point concerning toxicity or bioaccumulation was available. Additionally, a listing among Washington state PBT compounds (State of Washington, 2006) was also deemed as a hint for relevance (Dibenz(a,h)acridine and 7H-Dibenzo(c,g)carbazole). Therefore, all the compounds listed in Table 26 are to be considered for complementing the list of 11 priority compounds. For all compounds in Table 26 with experimental data from the Toolbox REACH criteria for P, B or T-properties and screening criteria according to REACH guidance R.11, respectively, are fulfilled or nearly fulfilled (bold and underlined data, respectively, in column “Comment on exp. Data”) with the exception of BCF-values determined in fish of about 100 for Benz(a)acridine and Dibenz(a,h)acridine. However, as the latter is also rated as PBT by the Washington state list and the former shows relevant toxicity in Daphnia pulex and especially Chironomus riparius, both are retained within the list of additional priority compounds. 7H-Dibenzo(c,g)carbazole retrieved no data by the QSAR Toolbox query but is listed nonetheless within the Washington state PBT compounds. Therefore, also this compound seems to be of environmental relevance and is included in the complementing list.

From the compounds of Table 26 the following ones are isomers of substances already contained within the 11 priority compounds:

[22] http://apps.echa.europa.eu/registered/registered-sub.aspx

[23] As of June 30th, 2011

- **Benzo[b]naphtho[1,2-d]thiophene (Benzo[b]naphtho[2,1-d]thiophene)**
- **Benz(a)acridine (Benz(c)acridine)**
- **Dibenz(a,h)acridine (Dibenz(a,j)acridine)**

One compound from Table 26 is also part of the compounds selected from the *pool of identified semipolar PAC* by the enforced QSAR selection criteria, namely 1-Nitropyrene (vPvB according to QSAR-results).

As such, the resulting list of priority semipolar polyaromatic compounds is enlarged from 11 to 18 substances in total, listed together in Table 27. The next step is an extensive literature research for experimental data corroborating or falsifying the relevance of these compounds.

Table 26: Additional priority compounds according to experimental results available via OECD QSAR-Toolbox and other indications for relevance

Source	CAS	Name	SMILES	Properties (moderate criteria)	Comment	Data Toolbox[1]	Comment on exp. Data: bold, if REACH-criteria or screening criteria (guidance) are fulfilled, underlined, if slightly above these criteria	Occurrence in profiles	Other indications for relevance
KORA / LAWA	205-43-6	Benzo[b]naphtho [1,2-d]thiophene	c1ccc3c(c1)ccc4sc2ccccc2c34	PBT		2	LC 50 Daphnia m. (0.22 mg/L) log K_{ow} 5.19	5	
KORA / LAWA	225-11-6	Benz(a)acridine	c4ccc3nc2ccc1ccccc1c2cc3c4	PBT		5	LC50 Daphnia pulex (0.449 mg/L); LC50 Chironomus (0.0153 mg/L); BCF (Pimephales prom., 7d): 106 log K_{ow} 4.48	5	
KORA / LAWA	226-36-8	Dibenz(a,h)acridine	c1ccc4c(c1)ccc5nc2c(ccc3ccccc23)cc45	PBT		6	All data related to bioconcentration: BCF (Pimephales prom., 96h): 100 - metabolic biotransformation rate constant: 0.951/d; log K_{ow} 5.73	2	Washington state PBT[2]
Profiles	239-64-5	13H-Dibenzo(a,i)carbazole	c12c(c3ccc4c(c3[nH]2)cccc4)ccc2c1cccc2	PBT		2	BCF Daphnia pulex (60h) 7130 log K_{ow} 6.4	1	
KORA / LAWA	194-59-2	7H-Dibenzo(c,g)carbazole	c12c3c4c(ccc3[nH]c2ccc2c1cccc2)cccc4	PBT		0		5	Washington state PBT[2]
Profiles	5522-43-0	1-Nitropyrene	O=N(=O)c(c(c(c(c(c1)ccc2)c2cc3)c3c4)c1)c4	PBT	According to enforced screening criteria vPvB	6	Daphnia Repro (NOEC 0.054 mg/L)+ Algae (NOEC & EC50 < 0.01 mg/L) log K_{ow} 5,06	5	
Profiles	892-21-7	3-Nitrofluoranthene	O=N(=O)c2ccc3c1ccccc1c4cccc2c34	PBT		5	Algae NOEC (0.045 mg/L) Daphnia Repro NOEC (0.082 mg/L)	1	

(1) Data Toolbox: The OECD QSAR Toolbox was used to retrieve experimental data relating to PBT properties (log Kow, bioaccumulation aquatic and terrestrial, biodegradation, ecotoxicological information, i.e. not human health hazard) from several databases.

(2) Washington state PBT (State of Washington, 2006): Rated in (Nendza, et al., 2010) as priority 2 (high priority for P and B (P >60d in all media; BCF >1000 and log Kow >5, respectively) with priority 1 (very high priority) for aquatic T (acute NOEC < 1.0 mg/L chronic NOEC < 0.1 mg/L)

Table 27: List of 18 priority semipolar polyaromatic compounds as deduced from QSAR screening for PBT-properties together with occurrence in profiles (11 compounds) and prevalence of experimental data or classification in relevant priority list (7 compounds)

CAS-NO	Name	SMILES (incl. substituents, however not specific for each isomer)
225-11-6	Benz(a)acridine	c4ccc3nc2ccc1ccccc1c2cc3c4
225-51-4	Benz(c)acridine	c4ccc3nc1c(ccc2ccccc12)cc3c4
205-43-6	Benzo[b]naphtho[1,2-d]thiophene	c1ccc3c(c1)ccc4sc2ccccc2c34
239-35-0	Benzo(b)naphtho(2,1-d)thiophene	c1ccc3c(c1)ccc4c2ccccc2sc34
243-42-5	Benzo(b)naphtho (2,3-d)furan	c1ccc2c(c1)oc1cc3ccccc3cc21
243-46-9	Benzo(b)naphtho(2,3-d)thiophene	c1ccc2c(c1)sc1cc3ccccc3cc21
226-36-8	Dibenz(a,h)acridine	c1ccc4c(c1)ccc5nc2c(ccc3ccccc23)cc45
224-42-0	Dibenz(a,j)acridine	c1ccc4c(c1)ccc5nc3ccc2ccccc2c3cc45
20928-02-3	Methyldibenzothiophene, 2- (SMILES for 4-methyl-is.)	Cc3cccc2c3sc1c2cccc1
4567-41-3	Methylbenzo(b)-naphtho(2,1-d)thiophene	CC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2
195-29-9	Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline = Benzo(a)phenanthridine, 195-29-9)	c12c3c(cccc3)cnc2ccc2c1cccc2
205-39-0	2,3-Benzodiphenylene oxide (3 CAS, Benzo(b)naphtho(1,2-d)furan, 205-39-0;	c1ccc2c(c1)oc1ccc3ccccc3c21
82-05-3	Benz(de)anthracene-7-one	O=C(c(c(c(c1c(ccc2)cc3)c3)ccc4)c4)c12
30796-92-0	Phenanthro(4,5-bcd)thiophene	c1cc2sc3cccc4ccc(c1)c2c34
239-64-5	13H-Dibenzo(a,i)carbazole	c12c(c3ccc4c(c3[nH]2)cccc4)ccc2c1cccc2
5522-43-0	1-Nitropyrene	O=N(=O)c(c(c(c(c(c1)ccc2)c2cc3)c3c4)c1)c4
892-21-7	3-Nitrofluoranthene	O=N(=O)c2ccc3c1ccccc1c4cccc2c34
194-59-2	7H-Dibenzo(c,g)carbazole	c12c3c4c(ccc3[nH]c2ccc2c1cccc2)cccc4

9.3 Modification of the list of priority compounds based on preliminary evaluation of literature survey

The literature retrieved so far (see section 10 for details) already afforded an amendment of the list of priority compounds gained from QSAR-results and occurrence in profiles.

For 1-nitropyrene and 3-nitrofluoranthene detailed information on sources of human and environmental exposure are available in EHC 229 (WHO, 2003). Accordingly, nitro-PAH are formed either from PAH in the atmosphere (radical reaction) or during combustion processes and are only very rarely produced as intermediates (only nitro-naphthalenes and nitro-acenaphthenes).

Formerly, nitropyrenes were found in carbon black and toners (carbon black B) due to the production process then applied. Due to its modification however, nitropyrenes were substantially reduced in carbon black since the 1980s (see also IARC, 2010).

1-Nitropyrene is the primary marker of Diesel exhaust and mainly particle bound, whereas 2-nitropyrene originates from hydroxyl radical reaction in the atmosphere.

3-Nitrofluoranthene is found in Diesel exhaust, whereas 2-nitrofluoranthene is found in ambient air particle bound and generated by hydroxyl radical reaction.

As we have presently no indications that 1-nitropyrene or 3-nitrofluoranthene are part of fossil raw materials (coal, oil, bitumen) or products derived thereof, we disregard them as priority semipolar PAC.

Similarly, we also disregard benz(de)anthracene-7-one (benzanthrone). In frame of profile evaluation within this project it was solely found in environmental matrices. Detailed information on production, processing, application and environmental emissions is available in BUA Report 251 (BUA, 2006). Accordingly, it is (was) produced for use as dye intermediate (vat dyes). Besides BASF no other European producers were known in 2004. It was a low production volume compound and handled as intermediate in closed systems with very low emission. BASF discontinued production in May 2003 (BUA, 2006). Benzanthrone is currently not registered under REACH[24].

Environmental benzanthrone is generated first and foremost by fossil fuel combustion and other combustion processes or e.g. pyrolysis of automobile tyres and is mainly particle bound (BUA, 2006). We have no hints on presence of benzanthrone in fossil raw materials (coal, oil, bitumen) or products derived thereof.

As such, the list of priority semipolar PAC is reduced by three compounds. Only for the remaining 15 compounds further literature research was carried out and experimental data retrieved by literature research were evaluated in detail only for the remaining 15 priority compounds.

9.4 Discussion

9.4.1 Complex mixtures of structural isomers – handling for QSAR-Screening

Substance profiles for UVCBs from petrol oils or coal tar oils often contain complex mixtures of alkylated core heterocyclic structures, most often only described as e.g. “Dibenzothiophene, C5-substituted, different isomers”. Moreover, these UVCBs often contain isomers of HET-PAC regarding the position of an amino-substituent, the position of a hetero-atom in the ring structure or isomers regarding the assembly of heterocyclic and homocyclic aromatic rings to the core structure. As these isomers frequently cannot be described by a CAS-number, a SMILES code has to be generated.

As it was unclear to what detail PBT properties depend on the discrete isomeric properties examples for these special cases of isomerism were gathered from substances contained in the *pool of identified semipolar PAC* (443 compounds). Where necessary, missing links were filled with structures from the scratch with SMILES codes generated by MarvinSketch 5.3.6 contained in ChemIDplus Advanced[25]. For results and conclusions - accounted for when generating SMILES for QSAR-screening on PBT properties - the reader is referred to chapter 14 (structure-activity relationships).

[24] http://apps.echa.europa.eu/registered/registered-sub.aspx, assessed 19.07.2011

[25] http://chem.sis.nlm.nih.gov/chemidplus/

9.4.2 Discussion of Screening Results

Pool of identified semipolar PAC

The final *pool of identified semipolar PAC* used for the screening for PBT-properties by QSAR amounts to 443 substances / isomeric mixtures on the basis of 118 analysed profiles listed in the Annex (Annex I: Profile list). It is obvious that this does not represent the complete number of semipolar PAC present in these samples. An extensive analysis of these polycyclic aromatic compounds is sophisticated and has not been performed by all authors. Especially minor components are difficult to identify. As already mentioned, the analytical limitations are reflected by peaks in the chromatograms, which cannot be allocated to a single compound, but consist of isomeric mixtures of closely structurally related compounds (e.g. multiple methylated and/or ethylated core structures). In these cases a representative isomer was selected for the identification of candidates.

An overview of the selection process is summarised in the following Figure 3.

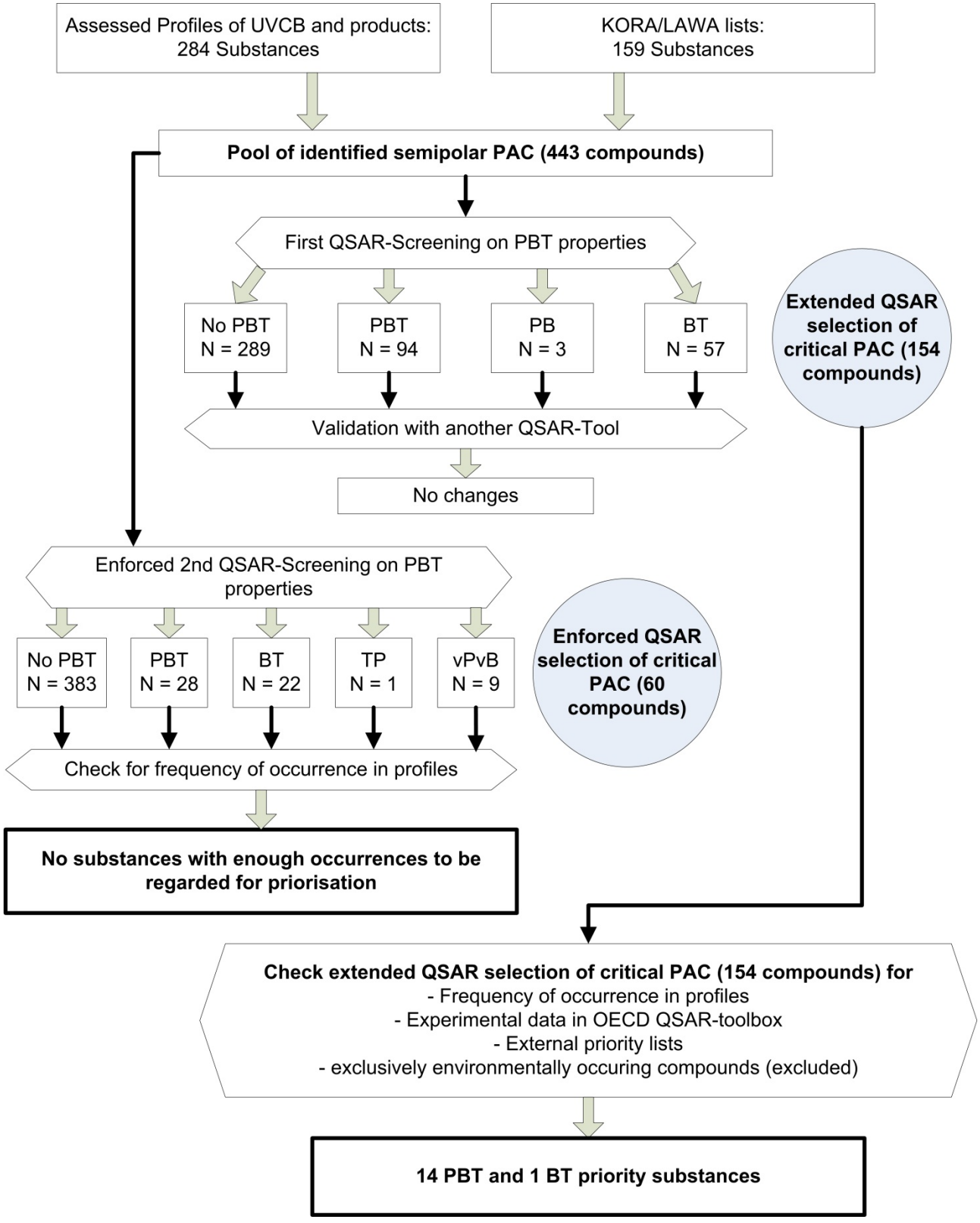


Figure 3: Selection process of PBT relevant semipolar PAC under consideration of their relevance of occurrence in analysed sample profiles (for details, see section 5.2.1 to 9)

Occurrence of critical semipolar PAC in profiles – critical semipolar PAC selected based on other criteria

The *enforced QSAR selection of critical PAC* (60 compounds, for criteria see section 6.1) are complex heteroaromatics with at least 4 aromatic rings, often with alkyl substituents. They have been detected only in a few profiles (with a maximum frequency of 6 for methylbenzo(b)-naphtho(2,1-d)thiophene). This does not mean they are not contained in the other profile samples, but rather they have not been identified by most of the analysis methods used: only few authors performed such an extensive analysis as to identify these compounds, and for this reason they might have been detected only in the corresponding profiles. It therefore cannot be excluded that these compounds are in general present in samples related to the analysed profiles. However this finding may also have been obtained by chance and cannot be taken as a representative result. Therefore it could be probably helpful to analyse products with suspected occurrence of compounds from the *enforced QSAR selection of critical PAC* with analytical methods capable of identifying these substances. This would apply to products derived from pitch, tar, anthracene oil and carbon black. Adding further to the uncertainty concerning the relevance of these rarely detected compounds is the fact that for only one of these 60 compounds a negative result is reported (i.e. it has been looked for within the analysis but was not detected). This was the case for dinaphtho(2,1-b:1',2'-d)thiophene (194-65-0), detected in four profiles, not detected in one profile).

Because of this uncertainty, the subsequent approach was to grade the 154 compounds of the *extended QSAR selection of critical PAC* with respect to their occurrence in the analysed profiles. The cut-off criterion of a minimum of 6 occurrences of a certain substance in different profiles (the maximum observed in the selection procedure according to enforced QSAR selection criteria as described above) yielded 11 compounds, which are expected to occur in samples of tar and several tar-derived fractions or products, environmental samples and bitumen (only Benzo[b]naphtho[2,1-d]thiophene). Although these compounds were the most frequently occurring substances with (estimated) PBT properties, the data base is still lean. Nevertheless the higher reliability of these results raises concern with respect to the use of tar- and bitumen-derived products.

To increase the probability of capturing the most important semipolar PAC within the list of priority compounds, beyond others, REACH Annex XIV support documents were checked for relevant substances (no further priority compounds), REACH registration dossiers were checked for compounds from the *extended QSAR selection of critical PAC* (n=154), prevalence of experimental data available via OECD QSAR-toolbox was taken as a hint on importance as well as classification as PBT from external priority lists (together 7 additional priority compounds). On the other hand, exclusively environmentally occurring compounds were excluded (3 compounds, one from the list of 11 and two from the list of 7 additional compounds). Thus, the final number of priority compounds comprises 15 compounds, 10 from combined screening on PBT-properties and occurrence in profiles and 5 due to prevalence of experimental data or specification within validated external priority lists as PBT. These priority semipolar PAC shall be subject of a more comprehensive search on experimental data on (eco)toxic effects and physico-chemical properties determining the environmental behaviour of them.

Comparison with other QSAR-models

The comparison of the results of the EPI suite model and the CATALOGIC model revealed a general higher percentage of compounds predicted to be persistent by the latter. Several factors may be the reason for this discrepancy:

EPI suite focusses on the fast (ready) biodegradability with models 2 and 6. Very often predicted fast biodegradation probability is low or 0 (no fast biodegradability). In these cases the outcome of the persistency screening depends on BIOWIN model 3 results. This model is based on a library of compounds evaluated by experts in terms of the time required to reach ultimate biodegradation. From the evaluated dataset molecular fragments with potential impact on biodegradation were derived. Together with molecular weight these molecular descriptors are used for evaluation of new substances. CATALOGIC is based on mechanistical models taking into account genetic operon organization of important microbial enzymes for biodegradation including the degradation of metabolites (ultimate biodegradation). Considering the multitude of organisms taking part in biodegradation reactions, this approach must be limited to a range of important organisms for ready biodegradability reactions (restricted on models for a range of OECD 301 tests). As such, the outcome of CATALOGIC is rather comparable to results of BIOWIN 2 and 6 models than BIOWIN 3 model results because the latter model is especially focussing on long term biodegradability potential.

Another aspect is the applicability domain. Only one of the substances of the *enforced QSAR selection of critical PAC* lies within the structural domain defined by the CATALOGIC model, the others are outside of the applicability domain. This is a general restriction of the validity of the corresponding CATALOGIC results. As currently the EPI suite applicability domain is not clearly defined and cannot be checked automatically also these results must be interpreted with care. Moreover, there are inconsistencies within the CATALOGIC results, as the primary half-lives of substances are in several cases longer than the ultimate half-lives (which are expected to be longer than the primary half-lives due to the inclusion of the metabolites' degradation). Furthermore, all substances are listed with observed ultimate half-lives of more than 10 years, but it can be expected that there are data gaps of reported values. Possibly these problems arise from the fact of the limited applicability domain of the CATALOGIC model and may also be a cause for the observed discrepancies between the models' predictions.

The CATALOGIC results should however be regarded as supportive to our results, as most of the persistency predictions of the EPI suite model are corroborated by the CATALOGIC predictions.

9.4.3 Relevance of substances derived from KORA/LAWA lists for semipolar PAC priority compounds

As can be derived from the flow chart of Figure 3 159 substances from KORA/LAWA lists with relevance for contaminated sites were introduced in our *pool of identified semipolar PAC* (443 compounds).

To give an indication to which extent PAC relevant for contaminated sites show at the same time PBT-properties (as deduced from QSAR) and are of potential relevance as impurities or constituents of products, this short section compares the different lists so far generated as to the fraction of KORA/LAWA contained therein.

From 159 compounds of KORA/LAWA-lists 29 are part of the *extended QSAR selection of critical PAC* containing 154 compounds with combinations of P, B, and T-properties as deduced from QSAR (94 PBT, 3 PB, 57 BT). In other words, from 159 compounds of KORA/LAWA, 130 do not fulfil the applied screening criteria (EC_{50} $LC_{50} \leq 1$ mg/L for T; log $K_{ow} \geq 4.0$; persistency according to combined evaluation of BIOWIN 2, 3 and 6 according to REACH guidance R.11). As many of these compounds were detected in ground water, in most cases the solubility in water may be too high (and concurrently the octanol solubility too low) to

show a significant potential for bioaccumulation. However, no in depth analysis was conducted to investigate this further.

But the majority (20) of the 29 compounds stemming from KORA/LAWA-lists and fulfilling the applied screening criteria on P-, B-, and T-properties was indeed detected in the analysed profiles. 7 of these 20 compounds were even found in ≥ 6 different profiles and are therefore part of the list of 15 priority semipolar PAC selected by the combination of screening criteria on PBT-properties and occurrence in a minimum of 6 profiles (see flow chart of Figure 3).

In conclusion, due to their substance properties only about 18% of substances from the KORA/LAWA-lists were relevant in regard to PBT. As ground water contamination was the main focus in these projects this is not surprising. However, 20 of 29 compounds (70%) fulfilling the screening criteria on combinations of P, B and T-properties were detected in the analysed profiles. Thus, at least this lipophilic fraction seems to stem not predominantly from transformation processes but is contained also in original UVCB-material from coal and petrol refinery or products based on these sources.

10 Retrieval and Evaluation of Experimental Data on PBT-Properties for 15 Priority Semipolar PAC

15 priority semipolar PAC were selected based on the established QSAR-screening methodology on PBT-properties (see sections 5.2.1 and 0) and occurrence in profiles (see section 8.5). The next important step was to try to corroborate or falsify QSAR-predictions by experimental data on PBT-properties. Therefore, an extensive literature research was performed, experimental data analysed and compared to QSAR-results. Finally, for each compound experimental data on toxicity, bioaccumulation and persistence (degradability) were summarized (ecotoxicological profiles).

10.1 Literature Research and Evaluation for Experimental Data on Priority Compounds regarding PBT properties

Initial literature research encompassed the initially 18 priority semipolar PAC as listed in Table 27 plus 1-methyldibenzofuran. The latter compound was positively screened for BT due to a wrong SMILES structural representation (see chapter 8.2) and dismissed later. For the STN-SEARCH (16 compounds, see section 10.1.2), 3 compounds of the list of 18 plus 1-methyldibenzofuran were dismissed due to literature information (see chapter 9.3).

10.1.1 Methodological description of literature research

The following hierarchy for literature research was followed:

1. Check relevant databases for entries:
    1. OECD QSAR-toolbox[26], incorporating experimental results from several other databases
    2. ESIS (http://esis.jrc.ec.europa.eu/) for Risk Assessment Reports (RAR) or IUCLID 4-Data
       Result: negative for all compounds
    3. KEMI-Riskline (http://apps.kemi.se/riskline/index.htm)
       Result: Information concerning carcinogenicity assessment or other human toxicity for some compounds
    4. Ecotoc-DB (US-EPA, http://cfpub.epa.gov/ecotox/)
       Information for 10 out of 19 compounds available
    5. Teratox-DB (Benzene derivatives, http://www.vet.utk.edu/TETRATOX/index.php)
       No information for any of the 19 compounds available
    6. ETOX-DB (Umweltbundesamt, Germany;
       http://webetox.uba.de/webETOX/index.do?language=de&language=en)
       Information for one compound available
    7. INERIS-DB (Portail Substances Chimiques; http://www.ineris.fr/substances/fr/)
       No information for any of the 19 compounds available
    8. CHRIP Japan (Chemical Risk Information Platform; http://www.safe.nite.go.jp/english/db.html)
       Information for three out of 19 compounds available
2. Check internet locators:

[26] http://www.oecd.org/document/54/0,3343,en_2649_34379_42923638_1_1_1_1,00.html

1. eChemPortal (http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en)
   Links on information sources (e.g. EHC, ACTOR,INCHEM, Envichem) for 7 out of 19 compounds
2. ChemIDplus lite (http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)
   Internet locators checked: E.g. SRC DTALOG (available for 16 compounds), SRC CHEMFATE (available for 3 compounds) and SRC BIODEG (one compound). Other targets included the Hazardous Substances Databank (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB) or priority lists classifying certain compounds as PBT or carcinogen.

3. Check monographs via the FoBiG reference software (check.enl)
   BUA-report (251) for Benzanthrone (82-05-3) available
4. ToxSeek (http://toxseek.nlm.nih.gov, a meta-search and clustering engine provided by U.S. NLM) was tried for 15 of 19 compounds and discontinued as no reasonable results could be retrieved.
5. TSCATS search for human health, environmental fate and ecotoxicity studies under the U.S. Toxic Substances Control Act (http://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm) was performed without results for any of the 19 compounds.
6. Check bibliographic databases
   1. PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed):
      For most compounds search by CAS-number and name without any constraints, as usually rather sparse information could be retrieved and the risk of missing relevant information by constraints had to be avoided. Examples for typical searches:

      Dibenz*a,h*acridine OR 226-36-8 [rn]: 44 results

      3-Nitrofluoranthene OR 892-21-7 [rn]: 69 results

      As for nitropyrene, a high number of hits resulted (>500) and the following constraints were set: (1-Nitropyrene OR 5522-43-0 [rn]) AND ((daphnia* OR *alga* OR *fish) OR ecotoxic* OR bioaccumul* OR bioconcentration* OR aquatic OR terrestr*) as well as (1-Nitropyrene OR 5522-43-0 [rn]) AND *degrad*.
   2. Toxline (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE, without inclusion of hits from PubMed)
      For most compounds search by CAS-number without any constraints, as usually rather sparse information could be retrieved and the risk of missing relevant information by constraints had to be avoided. Most often simultaneous search of several CAS-numbers to avoid multiple selection of hits common to 2 or more compounds. Examples for typical searches:

      #1 243-46-9 [rn] [not] PubMed [org] [not] pubdart [org] - 35 hits

      #2 224-42-0 [rn] [not] PubMed [org] [not] pubdart [org] – 140 hits

      …

      #1 OR #2 OR #3 … - 358 hits

      Where the number of hits was well above 500, the following constraints were set:

      [search results] AND (ecotoxic* OR aquatic OR sediment OR terrestr* OR *daphnia OR alga* OR fish* OR (tetrahymena) OR plankton) NOT PubMed [org] NOT pubdart [org]

      as well as [search results] AND *degrad* and

      [search results] AND (bioaccumul* OR bioconcentration*) to include also these topics.

7. Perform Google SCHOLAR-search with following search criteria:

"Genaue Wortgruppe" = CAS-Nr.; also more general name search has been tried for "methyldibenzothiophene", yielding no reasonable additional results.

"mit irgendeinem der Wörter": ecotoxic aquatic sediment terrestr daphnia alga fish tetrahymena plankton degrada bioaccumul bioconcentration

This search resulted in about 16 journal articles with possible relevance (ordered) together with some freely available reports for the 19 compounds.

To broaden the search for compounds found in isomeric mixtures, further searches for "Methyldibenzofuran", "Methylbenzo(b)-naphtho(2,1-d)thiophene", Phenanthro(1,2-b)thiophene / Phenanthro(4,3-b)thiophene (found together with Benzo(b)naphtho(2,1-d)thiophene), other benzonaphthofurans (76724-40-8, 239-30-5) and dibenzoquinolines (together with benzo(a)phenanthridine) combined with "mit irgendeinem der Wörter": ecotoxic "aquatic toxicity" degrada bioaccumul bioconcentration mineraliz mineralis was performed, however yielded only one additional result for methyldibenzofuran.

8. Perform Google search with following search criteria:

   ecotoxic OR aquatic OR sediment OR terrestr OR daphnia OR alga OR fish OR tetrahymena OR plankton OR degrada OR bioaccumul OR bioconcentration AND "CAS-number"

   Results of this search were much more unspecific compared to SCHOLAR search, however, one additional journal article with possible relevance and 5 to 6 free possibly useful documents were found for the 19 compounds.

10.1.2 Additional literature research via STN Karlsruhe

At STN Karlsruhe[27], for the 16 priority compounds and some additional close isomers databases BIOSIS, POLLUAB and WATER were searched for literature. A CAS-number based query was conducted implying the following numbers (in disjunction):

- 225-11-6, 225-51-4, 205-43-6, 239-35-0, 243-42-5, 243-46-9, 224-42-0, 226-36-8, 20928-02-3, 194-59-2, 7320-50-5, 4567-41-3, 195-29-9, 205-39-0, 239-64-5 and 30796-92-0 (16 priority compounds)
- 76724-40-8, 239-30-5, 30995-64-3, 16587-52-3, 7320-51-6, 7320-52-7, 7320-53-8, 4567-43-5, 4567-45-7, 4567-47-9, 4567-49-1, 4567-51-5, 85509-92-8, 194-03-6 (additional close isomers)

These were used in conjunction with the following, partly truncated terms (in disjunction): bioaccumul? OR bioconcentration? OR ecotoxic? OR daphni? OR alga? OR fish OR biodegrad? OR mineraliz? OR mineralis?

Of the resulting 6 publications, 2 publications had been already found by the preceding searches. The remaining 4 publications were ordered for assessment.

[27] https://stnweb.fiz-karlsruhe.de/html/english/, STN International, online service for research and patent information in science and technology

10.2 Evaluation of experimental data for the 15 Priority Compounds

10.2.1 Evaluation of Literature retrieved for Priority Semipolar PAC

The focus of evaluation was on persistence (P) with priority on biodegradation, on bioaccumulation (B) and on toxicity (T) with restriction on ecotoxicity. Due to their limited or uncertain evidence in vitro-results were generally not considered. The quality of studies was not generally assessed in detail but restricted on special cases:

- **When experimental result was in contradiction to QSAR-result**
- **Conflicting experimental information**
- **Obvious flaws**

The confirmation of QSAR results was generally restricted on one solid experimental study as far as available. Further studies were evaluated for results inconsistent with QSAR-data or if inconclusive study results afforded a weight of evidence approach.

Data retrieved from all available resources without limits regarding publication date were carefully evaluated regarding their impact on PBT assessment and a possible concurrent need for adaption of QSAR-derived classification. Predictivity of the applied QSAR-screening procedure was tentatively assessed.

10.2.2 Available experimental data: Impact on tentative PBT-assessment

Table 28 shows the 15 priority semipolar PAC with their molecular descriptors, PBT screening-assessment based on QSAR and tentative assessment based on available experimental data. The second last column concludes, if these data justify disregard of a substance due to insufficient fulfilment of PBT-properties, the last column identifies urgent need for generation of test results as a prerequisite for PBT-evaluation based on real data.

Entries T-R, B-R or P-R relate to direct fulfilment of REACH criteria on PBT according to annex XIII or according to REACH guidance R.11 on PBT-assessment (screening-criteria), i.e.

- T-R, if long-term NOEC / EC_{10} < 0.01 mg/L or $L(E)C_{50}$ < 0.1 mg/L
- B-R, if BCF > 2000 or experimental log K_{ow} > 4.5
- P-R, if $t_{1/2}$ > 40 d (freshwater) or >120 d (freshwater sediment or soil) **or** tests on ready biodegradability (or other short time tests) with no or very low biodegradation **and** QSAR-estimation based on BIOWIN-models is P
- Likely P-R, if read across to a very similar structural isomer with the hetero-atom in a better accessible position was possible and this isomer was P-R from experimental data **or** (special case, only for 1-Methylbenzo(b)-naphtho(2,1-d)thiophene) the non-alkylated core is P-W and further experimental data show a relatively poorer biodegradability of the alkylated structure compared to the core.
- T-W (weak), if acute experimental toxicity in-between 1.0 and 0.1 mg/L
- B-W (weak), if BCF ≥ 1000 or exp. log K_{ow} ≥ 4.0
- P-W, if QSAR-estimation based on BIOWIN-models is P and data on biodegradation showing some extent of biodegradability are available. However, experimental setup is too far from environmental conditions leaving doubt on adequacy of these data and / or not high enough under optimized experimental conditions to be conclusive for natural environments.

- Likely not P, special case for Phenanthro(4,5-bcd)thiophene: The only experimental data available report 77% degradation within 29d, however degradability in natural environment questionable (optimized experimental conditions)
- not T, B or P, if all the above conditions are not fulfilled
- DM = data missing
- Suffix "(2-1)" means that from 2 available experimental results 1 supports the assessment (e.g. T-R). "(2s-2s)" relates on supplemental information (experimental setup not fit for direct comparison with criteria) from 2 literature sources supporting (more or less indirectly) the assessment.

General remarks on B: If data for fish and e.g. daphnia were available and fish BCF-data were below the respective limit for B-R or B-W in contrast to Daphnia because of fish-specific metabolism of the xenobiotic, classification was based on bioaccumulation in daphnia, but negative fish data mentioned. Contrasting data are discussed in section 10.2.6

Table 28: Modification of QSAR-based PBT-assessment based on available experimental data. Please see text body for further details on assessment criteria and abbreviations.

Compound descriptors		QSAR-Screen on PBT	Assessment according experimental data on				Testing necessary?
CAS	Name		T	B	P	Disregard	Yes / No
225-11-6	Benz(a)acridine	PBT	T-R (4-1)	Not B (4-4) (some accumulation of metabolites)	P-R (1-1)	No	No
225-51-4	Benz(c)acridine	PBT	T-R (2-2)	B-W (log K_{ow})	P-R (2-2)	No	No
195-29-9	Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline = Benzo(a)phenanthridine, 195-29-9)	PBT	DM	DM	DM	No	Yes
224-42-0	Dibenz(a,j)acridine	PBT	Not T (1-1)	B-R (log K_{ow})	P-R (2-2)	No	Yes
226-36-8	Dibenz(a,h)acridine	PBT	DM	B-R (2-1) (for Daphnia, **not** fish)	P-R (2-2)	No	Yes
239-64-5	13H-Dibenzo(a,i)carbazole	PBT	DM	B-R (2-2)	Likely P-R (read across to 194-59-2)	No	Yes
194-59-2	7H-Dibenzo(c,g)carbazole and other dibenzocarbazoles	PBT	T-W, likely T-R (1-1) (EC_{50} < 0,4 mg/L)	B-R (log K_{ow}; 2s-2s; read across to 239-64-5)	P-R (3-2)	No	No

Compound descriptors		QSAR-Screen on PBT	Assessment according experimental data on				Testing necessary?
CAS	Name		T	B	P	Disregard	Yes / No
205-43-6	Benzo(b)naphtho(1,2-d)thiophene	PBT	T-W (1-1)	B-R (log K_{ow}; read across from 239-35-0 and 243-46-9)	P-W (2)	No	Yes
239-35-0	Benzo(b)naphtho(2,1-d)thiophene	PBT	Not T (1-1) (limit dose 0.22 mg/L?)*	B-R (1-1, daphnia) and read across from 243-46-9	P-W (3)	No*	Yes
4567-41-3	1-Methylbenzo(b)-naphtho(2,1-d)thiophene, several methylation isomers	PBT	DM	B-R (log K_{ow}; read across from 239-35-0 and 243-46-9)	Likely P-R (1-1)	No	Yes
243-46-9	Benzo(b)naphtho(2,3-d)thiophene	PBT	DM	B-R (1-1)	P-W (1)	No	Yes
30796-92-0	Phenanthro(4,5-bcd)thiophene	PBT	DM	B-R (log K_{ow})	Likely not P (1-1)	No	Yes
20928-02-3	Methyldibenzothiophene, 2- (several isomers)	BT	T-W (non-methylated core, 3-2)	DM	Not P (6-5)	**Yes**	--
205-39-0	Benzo(b)naphtho(1,2-d)furan, several Benzonaphthofurans	PBT	T-W (2-1)	B-R (log K_{ow})	P-W (1)	No	Yes
243-42-5	Benzo(b)naphtho (2,3-d)furan and other naphthobenzofurans	PBT	DM	B-R (log K_{ow})	P-W (1)	No	Yes

(*) T-W cannot be excluded as limit dose unclear (probably ≥ 0.22 mg/L)

Experimental data assessed for the 15 priority semipolar PAC might lead to disregard of certain compounds due to falsification of QSAR-prediction or failure of fulfilment of REACH criteria (according to annex XIII or guidance R.11). The latter is not equal to the first, as QSAR-screening criteria were deliberately chosen weaker to avoid false negatives.

As experimental data are often fragmentary (e.g. toxicity data for only one species of one trophic level and most often only acute values), a dogmatic exclusion based on REACH criteria seems not to be justified. However, as with QSAR-screening, at least two of the PBT-properties must be basically fulfilled. Under one of the following criteria a compound was deemed a priority compound:

- If two criteria according to REACH or REACH guidance (extension –R) were fulfilled.
- If one criterion according to REACH or REACH guidance (extension –R) and the other two criteria according to the weaker thresholds (extension –W) were fulfilled
- Irrespective of the third criterion if at least two criteria with missing data (DM) **or** one criterion with missing data (DM) together with the two other criteria fulfilling the weaker thresholds. For missing data we assume fulfilment of REACH-criteria for P, B or T according to the QSAR screening results as a conservative approach.

Only one compound could be dismissed without too much uncertainty, namely 2-methyldibenzothiophene (and isomers). This isomer group is clearly not persistent (5 out of 6 records) and toxicity of the core (benzothiophene) is below REACH screening criteria: LC_{50} (48h) fish embryo toxicity test = 0.33 mg/L; EC_{50} (24h) Daphnia magna = 0.2 mg/L; EC_{50} (72h) Desmodesmus subspicatus (green alga) = 4.0 mg/L. The lowest effect dose according to QSAR is LC_{50} (Daphnid) 0.53 mg/L for 2-methyldibenzothiophene. Data on bioaccumulation and log K_{ow} are however lacking, the calculated log K_{ow} of 4.71 points to a bioaccumulation potential. As B and T-W combined with not P are not enough to give high concern, this compound could formally be dismissed from the set of priority semipolar PAC. As however compound specific data on toxicity are lacking and monitoring data may raise concern (see sections 10.2.6), the compound is further assessed within the 15 priority semipolar PAC (see also section 10.3).

For 11 out of 15 compounds data are missing or not sufficiently conclusive for PBT-assessment and testing is urgently needed on one or more of the three PBT-criteria. As relevance cannot be excluded, these compounds are retained within the priority semipolar PAC in agreement with the criteria explained above.

For tests, one drawback is that the higher the log Kow, the higher the potential for bioaccumulation (valid up to a maximum for log Kow of slightly below 6 (Arnot, J. A. and Gobas, 2003; Arnot, J. A. and Gobas, 2006), the lower however water solubility. This implies, that for the more lipophilic compounds acute toxicity tests will often result in no observable effects due to the low concentration in the test water and only chronic tests or tests with benthic organisms (due to sediment sorption) would possibly detect toxic effects. These tests however are lengthy and expensive to perform. In this respect it is interesting to note that the fish embryo toxicity test (FET), for which an ISO-guideline (DIN 38415-6) and an OECD-draft guideline are available, may be performed in a modified way to test toxicity of sediments (Hollert, et al., 2003; Rocha, et al., 2011).

In conclusion, mostly due to missing data there is not one compound with experimental data clearly demonstrating fulfilment of all three properties P, B and T at the same time. Concomitantly, only one compound could be formally dismissed from the list due to experimental data indicating failure of PBT-criteria. However, also in this case data are insufficient for a final statement on PBT-properties. However, for the actual SVHC-assessment including also data on genotoxicity and carcinogenicity as well as QSAR-derived data (where experimental data are lacking), see chapter 12. Structural representations and information on occurrence for all 15 priority semipolar PAC are given in Table 56 of Annex III.

10.2.3 Predictivity of the applied QSAR-screening procedure

To conclude on the applicability of the applied QSAR screening methodology to select for compounds with PBT properties, assumptions on substance properties were compared with our PBT-classification according to available experimental results. Please refer to Table 28 for substance specific information on missing data (DM), the number of available studies, comments on reasoning, possibly applied read across or ambiguous experimental data.

For evaluation of QSAR results according to Table 29 the following rules were applied:

- **Only compounds with data for the respective criterion were included in the statistics**
- **As QSAR-screening procedure applied conservative (weak, "W") thresholds for classification on T (< or = 1.0 mg/L) and B (log K_{ow} > or = 4.0) both experimental results leading to "T-R" / "B-R" and "T-W" / "B-W" were rated as confirming QSAR T-classification.**
- **Results "P-W" for compounds with experimental data demonstrating a potential for biodegradation however under experimental conditions leaving doubt whether in natural environments degradability half-lives would fall below REACH criteria on P are rated as confirming QSAR P-classification.**
- **Results "Likely P-R" and "Likely not P" are rated "P and "not P" in respect to evaluation of QSAR-classification**

Table 29: Evaluation of applied QSAR screening methodology on available experimental data for the 15 priority compounds

Categorization in relation to experimental-results	Number of compounds for criteria		
	T	B	P
Without any experimental data (DM)	7	2	1
Rated T-R, B-R or P-R confirming QSAR	2	11	7
Rated Not T, Not B or Not P confirming QSAR	0	0	1
Rated Not T, Not B or Not P contradictory to QSAR	2	1	1
Rated T-W, B-W or P-W confirming QSAR	4	1	5
Checksum	15	15	15
% confirming QSAR of compounds with experimental data	75.0%	92.3%	92.9%
% contradictory to QSAR of compounds with experimental data	25.0%	7.7%	7.1%

Evaluation of QSAR T-prediction

For only 8 from 15 compounds experimental data on T were available. 2 “T-R”- and 4 “T-W”- results are confirming QSAR T-classification. For 2 compounds, QSAR-classification as T was not confirmed by experimental data, i.e. QSAR overestimated toxicity. In terms of a screening procedure for identification of possible PBT-candidates this is a less severe error compared to a possible underestimation of toxicity. The latter however could not be the case for the 15 priority compounds as all 15 were QSAR-rated “T”. Therefore, the T-prediction of QSAR was confirmed by 75% of compounds with experimental data. As no systematic data retrieval was accomplished for compounds QSAR-rated “not T”, no judgment in this respect is possible.

However, only for the non-methylated core structure of one compound (2-methyldibenzothiophene) data on T for all three trophic levels could be retrieved; for all other compounds a provisional assessment based on data for one or two trophic levels was carried out and the result is compared to the QSAR-screening result. While one result below the thresholds is sufficient for a (positive) T-classification, for a classification as “Not T” at least data from three trophic levels are needed. Thus it cannot be excluded that the two compounds rated “Not T” conflicting with QSAR-results would in fact be “T-W” or even “T-R” in respect to the trophic levels lacking experimental data. 75% confirmation of QSAR T-prediction therefore rather underestimates QSAR predictivity.

A more detailed comparison of predicted effect values with experimental ones was not performed. And it is questionable, if this would be worthwhile: Of the effect values predicted by QSAR all but one (this compound without experimental data) are above 0.1 mg/L, i.e. would correspond to the “T-W” classification applied for experimental data. As specific target toxicity is generally not predicted by ECOSAR (rather log K_{OW} dependent baseline toxicity improved by correction factors for molecular fragments) especially for highly toxic chemicals toxicity will be rather underestimated by this program. Within the applied QSAR-screening procedure on PBT properties the T-prediction for the 15 priority compounds proved however to be very useful.

Evaluation of QSAR B-prediction

For only 2 out of 15 priority compounds no experimental data were available. From the remaining 13 substances 12 (92.3%) were correctly classified as B (see Table 29). As all priority compounds chosen based on occurrence in profiles and QSAR PBT-screening were predicted to be B, experimental data not fitting QSAR prediction naturally demonstrated non-bioaccumulative properties. This was due to metabolic transformation for both of these compounds (dibenz(a,h)acridine rated B-R due to bioaccumulation in Daphnia, however no bioaccumulation in fish due to metabolism). Again, for a screening procedure for identifying possible PBT-candidates this is a less severe error compared to a possible underestimation of bioaccumulative properties.

As pointed out in section 6.2, the lower cut-off for log K_{OW} of > or = 4.0 compared to REACH regulation was chosen to avoid false negatives (misses).

As REACH guidance allows for screening assessment on B by conclusion from experimental log K_{OW} (>4.5), this was applied for classifying priority compounds whenever other experimental data were missing or in combination with read across to structural isomers. As already experimental log K_{OW}-values from the PhysProp-DB (SRC) were used for the screening assessment as far as available and log K_{OW}-prediction by KOWWIN is quite sophisticated (see interim report 2, section 3.2.1) good correlation of QSAR B-prediction with experimental data on log K_{OW} is not surprising for the 5 compounds with only experimental log K_{OW}-values (all B or B-W, as predicted). Thus the high percentage of correct QSAR-prediction on B (more than 90%, see Table 29) is somewhat misleading. Excluding these compounds, for only 8 from 15 compounds could experimental data on bioaccumulation be retrieved or a read across to other closely related compounds was possible. Taking these compounds as basis for evaluation, 88% were correctly QSAR-classified as B with the 12% (one compound) not correctly assigned being “Not B” because of metabolic transformation.

Evaluation of QSAR P-prediction

For all but one compound experimental data on P were available. QSAR correctly identified 13 (93%) of these 14 compounds as P. The one substance of the 15 priority compounds rated “Not P” according to QSAR (2-Methyldibenzothiophene) in fact proved to be “Not P” according to experimental data. This compound was thus the only one of the 15 priority compounds where experimental data could have proven a PBT-property (P) not predicted by QSAR-screening. Some uncertainty is associated with thiophene and furan containing PAC. For all of these compounds data exist showing more or less biodegradation extent. As in general experimental setup was far from natural environments or generally accepted test guidelines, these compounds were rated “P-W” if not exceptionally high (“Likely not P”, the one compound not correctly predicted by QSAR) or low (“Likely P-R”) rates of biodegradation were reported.

Conclusion

As the current evaluation of QSAR data is based on just 14 compounds with existing experimental data for at least two properties (one compound without any data on T, B or T) it may give a rough impression only on usability of the applied QSAR screening methodology. With correct prediction of 75% on T, 85% on B and 93% on P the method seems useful in identifying PBT-properties. Evaluation against a higher number of compounds with experimental data would be desirable.

10.2.4 Additional Evaluation of QSAR-T-Screening Using QSAR-Based “Advisory Classifications”

To strengthen the above résumé, “Advisory Classifications” of the Danish Environmental Protection Agency[28] (dated '28/6-2010), based on QSARs were evaluated (data not shown). Classification as N, R50 or N, R50/53 equals an E(L)C_{50} of ≤ 1 mg/L, i.e. the cut-off-criterion applied for our T-screening using ECOSAR. For 164 substances of the *pool of identified semipolar PAC* (443 compounds) “Advisory Classifications” were available. Additionally for 9 substances legal classifications according to regulation 2172/2008/EC existed. One of these 9 classified compounds was at the same time part of the advisory list. According to legal

[28] http://www.mst.dk/Virksomhed_og_myndighed/Kemikalier/Stoflister+og+databaser/Vejledende+liste+til+selvklassificering+af+farlige+stoffer/

classifications, 3 compounds of the *pool of identified semipolar PAC* dismissed by ECOSAR (effect level > 1 mg/L) had R50 assigned, i.e. would have been rated T according to screening criteria. As regards „Advisory Classifications“, 51 compounds dismissed by ECOSAR were assigned R50. For a scenario assuming correct predictions for the “Advisory Classifications“ of Danish EPA combined with legal classifications available, from 172 classified compounds (9 legal plus 163 advisory) 54 compounds (31%) would be false negatives, i.e. dismissed by ECOSAR in spite of an effect concentration ≤ 1.0 mg/L. Vice versa, selecting those compounds screened as T according to ECOSAR ($E(L)C_{50} \leq 1{,}0$) and at the same time were not assigned R50 or R50/53 according to the “Advisory Classifications”, 10 compounds resulted, including 1 PBT and 2 BT-compounds (i.e. 6% false positives). It must be emphasized here that also “Advisory Classifications“ are based on QSARs, however several QSAR-models are implied. Nonetheless they may as well be faulty as it is the case with the “Advisory Classification” for the one substance (quinoline) at the same time legally classified. According to Danish QSAR-based classification R50 is assigned, whereas R51/53 is assigned according to the legal classification (i.e. $10 \text{ mg/L} \geq E(L)C_{50} > 1 \text{ mg/L}$).

Would an complementary T-screening based on „Advisory Classifications“ have led to critical compounds in addition to the 154 compounds of the *extended QSAR selection of critical PAC* or even additional priority compounds? One additional compound would have been added to the BT-list and 2 additional compounds to the PT-list of the *extended QSAR selection of critical PAC* applying the R50-criterion on the „Advisory Classifications“. However, no additional PBT-compounds would result. From the legally classified compounds, no compound fulfils in addition to the T-criterion screening-criteria for B or T. Furthermore, selecting for priority compounds and thus applying the criterion of equal or larger 6 occurrences in different profiles, no further priority compounds would have resulted. As regards the actually selected priority compounds according to Table 28, “Advisory Classifications“ are available for 8 of these 15 substances. There are no discrepancies between our QSAR-screening results and the “Advisory Classifications“ for these compounds.

In conclusion, the described approach emphasises the importance to keep in mind false negatives potentially missed by QSAR-screening albeit it was a comparison of QSAR and QSARs, not of QSAR and reality (leaving behind the legally classified compounds). However, this different QSAR based approach leading essentially to the same results gained from our approach corroborates our QSAR-based selection strategy.

10.2.5 Ecotoxicological Profiles (experimental data)

In this section all available data on ecotoxicity, bioaccumulation and persistence for the 15 priority semipolar PAC are summarized in substance profiles. For structural representations and information on occurrence for all 15 priority semipolar PAC see Table 56 of Annex III. Furthermore, short substance profiles summarizing the data from the current section and including data on mutagenicity and carcinogenicity from section 11 can be found in section 12. In section 12, also provisional PNECs for the aquatic environment, provisional SVHC-classifications including data on mutagenicity and carcinogenicity, provisional environmental classifications, and a summary of occurrence in relevant matrices can be found.

Benz(a)acridine

Toxicity

Data from two acute studies in aquatic invertebrates, one study on marine algae and one acute study on sediment dwelling invertebrates are available (Chironomus riparius larvae). The test on Chironomus was however performed in water only (no sediment present, chemical dissolved in water) and is therefore categorized as aquatic toxicity test. The determined acute toxicity effect value on C. riparius larvae is below the REACH guidance screening value of < 0.1 mg/L and thus points to fulfilment of the T-criterion (classified as “T-R”).

According to a database entry (UBA Etox-DB[29]) EC_{50} for Daphnia sp. equalled 0.36 mg/L (no information on data source, exposure duration and experimental conditions). This effect value is in the same order of magnitude as the LC_{50} (24h) for Daphnia pulex of 0.45 mg/L (Southworth, et al., 1978) according to OECD QSAR Toolbox-entry; original data not verified).

The marine flagellate alga Dunaliella tertiolecta was tested under white light conditions (16/8 h l/d) including a fraction of UV-A-irradiation sufficiently high for photo enhanced toxicity of acridine (Wiegman, et al., 2001). The determined EC_{50} (72h, growth rate) of 0.11 mg/L was close to the REACH guidance screening value of < 0.1 mg/L.

Acute toxicity to larvae of the midge Chironomus riparius (water only) was determined (Bleeker, et al., 1998). The EC_{50} (96h) of 0.015 mg/L (measured, 95% confidence interval: 0.0138-0.0169) was clearly below the REACH guidance screening value of < 0.1 mg/L (classification as “T-R”).

Bioaccumulation

The experimental data set on bioaccumulation is comparably large and conclusive. The BCF was determined in fish (7d, Pimephales promelas; K1/K2-determination, radioactive labelled parent compound clearly discerned from metabolites, reliable study) to be 106 L/kg wet weight (Southworth, et al., 1981). This is clearly below REACH criteria on B (BCF > 2000). However, BCF was approximately 1500 in relation to metabolites, which were largely retained in the fish. Details on metabolites and their possible toxicity are not given (Southworth, et al., 1981).

Further data on fish relate to the half-life in minnow (Pimephales sp.) which was determined to be smaller than 1 day (Niimi, 1987), corroborating the above results.

A BCF of 352 was determined in Daphnia pulex at an initial concentration in water of 18 µg/L (Verschueren, 1983).

As such, in fish as well as in Daphnia bioconcentration was far below the REACH threshold of BCF 2000. Therefore, the B-criterion is not fulfilled (classification as “not B”). However, bioconcentration of metabolites in fish was close to this value (1500) and a closer examination of this phenomenon common also to some PAH (Southworth, et al., 1981) would be desirable.

Persistence

Degradability of benz(a)acridine was tested with a Pseudomonas fluorescens strain able to degrade chrysene and benzo(b)naphtho(1,2-d)thiophene isolated from exhausted oil-polluted soil (Caldini, et al., 1995). Within 8 days at 25°C no biodegradation of benz(a)acridine was found (0%).

As no further experimental data are available and also QSAR-screening points to persistence, the compound is classified as “P-R”.

[29] http://webetox.uba.de/webETOX/index.do?language=de&language=en

Benz(c)acridine

Toxicity

Data from one acute study on marine algae and one acute study on sediment dwelling invertebrates are available (Chironomus riparius larvae). The test on Chironomus was however performed in water only (no sediment present, chemical dissolved in water) and is therefore categorized as aquatic toxicity test. The determined acute toxicity effect value on C. riparius larvae is far below the REACH guidance screening value of < 0.1 mg/L (i.e. < 0.01 mg/L) and thus Benz(c)acridine definitely fulfils the T-criterion ("T-R").

The marine flagellate alga Dunaliella tertiolecta was tested under white light conditions (16/8 h l/d) including a fraction of UV-A-irradiation sufficiently high for photo-enhanced toxicity of acridine (Wiegman, et al., 2001). The determined EC_{50} (72h, growth rate) of 0.025 mg/L was clearly below the REACH guidance screening value of < 0.1 mg/L.

Acute toxicity to sediment dwelling larvae of the midge Chironomus riparius (water only) was determined (Bleeker, et al., 1998). The EC_{50} (96h) of 0.0069 mg/L (measured concentration, 95% confidence interval: 0.0067-0.0072) was clearly below the REACH guidance screening value of < 0.1 mg/L and the compound classified as "T-R".

Bioaccumulation

No experimental data on bioaccumulation could be found. As BCF of benz(a)acridine depends on the high biotransformation rate in fish, simple read across to benz(c)acridine is associated with doubts as steric hindrance might result in slower or no biotransformation. Therefore, based on log K_{ow} of 4.48 determined for Benz(a)acridine and application of the screening criteria of REACH guidance R.11 the compound is classified as "B-W".

Persistence

Degradability of benz(c)acridine was tested with the fungus Cunninghamella elegans able to S-oxidize dibenzothiophene. No metabolism could be found (Holland, et al., 1986).

In a further study the BOD (6 days) was determined at a substrate concentration of 500 mg/L and a sludge concentration of 2500 mg/L (sludges of different sewage treatment plants tested). No biodegradation could be found (approx. 0% theoretical oxygen demand). For two sludges, even slightly inhibitory effects were observed (Lutin, et al., 1965). The compound is therefore classified as "P-R".

Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline, 195-29-9)

No experimental data relating to toxicity, bioaccumulation or persistence could be found.

Dibenz(a,j)acridine

Toxicity

Only data from one acute study on the fresh water alga Pseudokirchneriella subcapitata were available. The determined EC_{50} (biomass, 6d) was larger than the exposure concentration of 0.4 mg/L. The study was conducted under cool white light (16/8 h l/d) with significant fraction of UV-A irradiation enabling phototoxicity of benzo(a)pyrene. After 6 days, 96% of control cells at 400 µg/L (70% day 1, 89% day 2, 94% day 4) were quantified (Warshawsky, et al., 1995a). The determined effect value is therefore higher than the REACH guidance screening value on T of < 0.1 mg/L and the compound tentatively classified as "not T".

However, no analytical determination of the test item was performed in this study and experimentally determined water solubility (0.16 mg/L, see section 15) is considerably lower than the exposure concentration, potentially leading to adsorption losses and underestimation of toxicity. As data on other trophic levels are missing, final conclusion on T from experimental data is not possible.

Bioaccumulation

Experimental data are restricted to log K_{ow}, which was determined to be 5.63 (Helweg, et al., 1997 as cited in SRC-database). According to screening criteria to REACH guidance R.11, the B-assumption would result. If however metabolism in fish would be like with Dibenz(a,h)acridine, BCF in fish could be comparably low. As experimental data on BCF are lacking the compound is classified as “B-R” applying the screening criteria based on log K_{ow}.

Persistence

Mineralization in different soils within 64 days was tested by Gosser et al. (1995). No mineralization above heat treated control soils could be found. Based on these data, the half-life in soils was calculated to be >160 days (Aronson, et al., 1998).

The BOD (6 days) was determined at a substrate concentration of 500 mg/L and a sludge concentration of 2500 mg/L (sludges of different sewage treatment plants tested). Only very low biodegradation could be found (less than 6% theoretical oxygen demand). For two sludges, inhibitory effects >5% were observed (Lutin, et al., 1965).

As such, persistence criteria according to REACH are fulfilled (half-life >120 days in soils) and the compound classified as “P-R”.

Dibenz(a,h)acridine

Toxicity

Experimental data on toxicity are missing.

Bioaccumulation

The BCF was determined in fish (96h, Pimephales promelas; k1/k2-determination; metabolism/excretion clearly shown; reliable study according to Arnot-Gobas-evaluation (Arnot, J. A. and Gobas, 2006)) to be 107.2 L/kg wet weight (Southworth, et al., 1980). This is clearly below REACH criteria on B (BCF > 2000) and a result of metabolism/excretion in fish. However, BCF for Daphnia pulex reported in the same study was 3500 and thus well above the REACH threshold.

Further data are restricted to the experimentally determined log K_{ow} of 5.73 (Helweg C. et al., 1997 as cited in SRC-database).

Fish is commonly regarded as the most relevant organism for determination of bioconcentration. However, as also bioconcentration in other aquatic organisms is relevant (REACH Annex XIII) the compound is still classified as “B-R“.

Persistence

The BOD (6 days) was determined at a substrate concentration of 500 mg/L and a sludge concentration of 2500 mg/L (sludges of different sewage treatment plants tested). Only very low biodegradation could be

found (less than 6% theoretical oxygen demand). For two sludges, inhibitory effects >5% were observed (Lutin, et al., 1965).

In a similar experiment, the BOD (6 days) was determined at a substrate concentration of 500 mg/L and a high sludge concentration of 5000 mg/L (sludges of three different sewage treatment plants used). Only low biodegradation could be found with a minimum of 5% and a maximum of 17.3% theoretical oxygen demand (Malaney, et al., 1967).

As such, according to REACH screening criteria the substance may be regarded as P ("P-R").

13H-Dibenzo(a,i)carbazole

Toxicity

Experimental data on toxicity are missing.

Bioaccumulation

The BCF was determined in fish (48h, Poecilia reticulata) to be 7060 L/kg wet weight (de Voogt, et al., 1991) using the unlabelled compound. Concluding from aqueous concentrations, equilibrium was already reached within 4 hours. The fat content was 9% lipid (not lipid normalized). Bioconcentration factor was calculated from concentration in the fish and concentration in the water at equilibrium.

Furthermore, a BCF for Daphnia pulex (60h; k1/k2-method) of 7126 L/kg wet weight was determined in a reliable study (Southworth, et al., 1979). Exposure concentration was measured and declined from initially 10.5 µg/L to 5.5 µg/L at the end of the uptake phase. This decline was compensated for by a two-stage procedure to determine uptake and elimination kinetics. The study was rated reliable in terms of carbazole (analysed in parallel) by Arnot and Gobas (2006).

Thus, BCF-values for 13H-Dibenzo(a,i)carbazole in both fish and Daphnia are even above REACH criteria on vB (very bioaccumulative, BCF > 5000). The compound is therefore classified as "B-R".

Persistence

No experimental data available. However, read across to the closely related isomer 7H-Dibenzo(c,g)carbazole may be possible, as this isomer proved to be recalcitrant in biodegradation tests and its aromatic nitrogen is sterically better accessible than in the 13H-Dibenzo(a,i)carbazole isomer.

Concluding from read-across the substance is likely to be persistent according to REACH criteria ("likely P-R").

7H-Dibenzo(c,g)carbazole

Toxicity

Only data from one acute study on the fresh water alga Pseudokirchneriella subcapitata were available. The determined EC_{50} (biomass, 6d) was less than the exposure concentration of 0.4 mg/L. The study was conducted under cool white light (16/8 h l/d) with significant fraction of UV-A irradiation enabling phototoxicity of benzo(a)pyrene. After 6 days, 13% of control cells at 400 µg/L (48% day 1, 25% day 2, 13% day 4) were quantified (Warshawsky, et al., 1995a). As no lower concentrations had been tested, it is uncertain if the effect value is below the screening value on T of REACH guidance R.11 (0.1 mg/L). In any case it is close to it ("TW, likely T-R"). Furthermore, no analytical determination of the test item was performed and experimentally determined water solubility (0.010-0.063 mg/L, see section 15) is considerably lower than the

exposure concentration, potentially leading to adsorption losses and underestimation of toxicity. As data on other trophic levels are missing, final conclusion on T from experimental data is not possible. Supporting data on T from fish species are available (see section *Bioaccumulation*).

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. However, the following literature information is available supporting the QSAR screening-assessment on T and B:

DNA-adduct formation in English Sole liver was observed after single intermuscular injection (100 µmol/kg body weight) more rapidly and at consistently higher levels for 7H-Dibenzo(c,g)carbazole compared to Benzo(a)pyrene. After an initial decline from maximum adduct level of 2000 nmol/mol to about 700 within 28d (apparent half-life 13d), no apparent further decline till day 84 was observed. This points to a potential for genotoxicity and bioaccumulation (Stein, et al., 1993).

Similarly, DNA-adduct formation in Northern Pike was determined after 5 times of oral feeding (25 µmol/kg body weight, interval 12-14 days) and monitored till day 128. Levels declined in intestine to about 29% of maximum value, were persistent however in liver, brain and gills. Also this result points to a potential for genotoxicity and accumulation during long term exposure is likely (Ericson, et al., 1999).

Furthermore, read across to 13H-Dibenzo(a,i)carbazole may be possible although the aromatic nitrogen is sterically better accessible with 7H-Dibenzo(c,g)carbazole compared to the 13H-Dibenzo(a,i)carbazole isomer possibly leading to a higher metabolism rate. 13H-Dibenzo(a,i)carbazole is very bioaccumulative (vB) according to REACH criteria.

In conclusion, from experimentally determined log K_{ow} (6.4) (Hansch, et al., 1995 as cited in KOWWIN data reference), supporting data and read across to 13H-Dibenzo(a,i)carbazole 7H-Dibenzo(c,g)carbazole is regarded as B according to REACH (“B-R”).

Persistence

Mineralization in different soils within 64 days was tested by Gosser et al. (1995). No mineralization above heat treated control soils could be found. Based on these data, the half-life in soils was calculated to be >160 days (Aronson, et al., 1998).

Bohonos et al. (1977) tried the development of degrading cultures by an enrichment process from water during 6 weeks. However no degradation was observed with and without co-substrates.

For UV-induced photodegradation in water under sunlight (with 0,1% Acetonitrile as co-solvent) a half-life of 30 minutes was determined (Mill, et al., 1981).

As such, persistence criteria according to REACH are fulfilled (half-life >120 days in soils, “P-R”). A (primary) degradation may be possible under direct sunlight in surface near water layers.

Benzo(b)naphtho(1,2-d)thiophene

Toxicity

Only data from one reliable (reliability category 2) acute study (static design) on Daphnia magna were available using acetone (0.5 ml/L) as carrier and Triton X-100 (0.5 mg/L) as surfactant. The determined EC_{50} (48h, mobility) was 0.22 mg/L (P95-CI: 0.091-0.380) and filtered and autoclaved natural river water was used (Eastmond, et al., 1984). It is close to albeit higher than the screening value on T of REACH guidance

R.11 (0.1 mg/L). Benzo(b)naphtho(1,2-d)thiophene is therefore T according to the weaker criteria applied for QSAR screening (≤ 1 mg/L), i.e. “T-W”.

As data on other trophic levels are missing, final conclusion on T from experimental data is not possible.

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. However, according to the experimentally determined log K_{ow} of 5.19 (Andersson and Schräder, 1999 as cited in SRC-database) the compound is regarded as B according to PBT-screening criteria of REACH guidance R.11 (“B-R”).

Furthermore, read across to the structural isomers benzo(b)naphtho(2,1-d)thiophene (B-R in Daphnia) and benzo(b)naphtho(2,3-d)thiophene (B-R in fish) corroborate the screening assessment from log K_{ow} (“B-R”).

Persistence

Two studies were available demonstrating a potential for biodegradation for benzo(b)naphtho(1,2-d)thiophene.

Caldini et al. (1995) used a strain of Pseudomonas fluorescens isolated from a site heavily contaminated by exhausted lubricating oils. This strain was able to grow in minimal medium on chrysene and other PAC including benzo(b)naphtho(1,2-d)thiophene. Detailed experimental conditions are however only given for chrysene (1 mg/L, solubility enhanced by cosolvent acetonitrile 25 mg/L). Most probably also growth on benzo(b)naphtho(1,2-d)thiophene was supported by applying this cosolvent to achieve higher solubility in the growth medium. Within 5 days Pseudomonas fluorescens strain grew on benzo(b)naphtho(1,2-d)thiophene from optical density 7.1 x 10^4/ml to 6.1 x 10^6/ml and degraded 94.6% of the initial concentration (initial concentration not given). As no other carbon source was present, the observed bacterial growth may implement biodegradation events beyond primary biodegradation if not ultimate biodegradation (no information given).

A second study (Lundstedt, et al., 2003) used a contaminated gas work soil-water slurry plus nutrients to investigate the relative degradation rates of polycyclic aromatic compounds (PAC) and to determine if persistent oxidation products would be formed during the degradation process. The contaminated soil was aerobically processed (at 27°C, reactor, agitation and aeration) with cultures designed for PAH-degradation in soil (Deutsche Montan Technologie, Essen, Germany) and a balanced liquid nutrient mixture containting also complex oranic constituents besides nitrate and phosphate. On five occations during the treatment (day 0, 3, 7, 24, 29) samples were taken, separated from water by filtration (0.45 µm) and dissolved contaminants in water extracted by solid phase extraction disk. Soil samles and disks were extracted by pressurized liquid extraction, fractionated by HPLC followed by GC and PAC detected by mass spectrometry (full-scan mode for identification and single ion mode for quantification). Identification proceeded by comparison of retention time data and mass spectra with reference materials and literature data. More than 100 PAC were identified. General observations were that low molecular weight PAC degraded faster than high molecular weight compounds and unsubstituted ring systems faster than correspoinding alkylated ones. Nitrogen heteroxycles degraded slower than their PAH counterparts.

Benzo(b)naphtho(1,2-d)thiophene was part of the contaminated gas work soil used in the experiment and was degraded by 61% within 29 days (initial concentration not given).

Both studies concordantly show the potential for biodegradation. However, due to the poor water solubility (0.056 mg/L estimated by WATERNT-program v. 1.01 of US-EPA EPI suite 4.1) and the high adsorption potential (log K_{oc} 5.0 as estimated by KOCWIN program v. 2.00 of US-EPA EPI suite 4.1) of ben-

zo(b)naphtho(1,2-d)thiophene it is doubtable how far these results are applicable to assess environmental degradation. Although Lundstedt et al. (2003) used no cosolvent to enhance water solubility (like Caldini, et al., 1995), using an especially developed mixture of microorganisms capable of PAH degradation and addition of a special nutrient mixture (including complex organic constituents) are also optimized non-standard conditions. Even if the test according to Lundstedt et al. (2003) is regarded as a kind of inherent biodegradability test regime, as monitoring was performed by GC-MS most probably predominantly primary biodegradation is recognized. To use the result of an inherent biodegradability test under REACH to waive persistence at least 70% mineralization must be reached while primary degradation products must be separately assessed for PBT-properties.

As such, as a preliminary and conservative assessment the compound is classified as weakly persistent ("P-W") however with some potential for inherent biodegradability. A direct comparison to REACH criteria is not possible due to the non-standard experimental setup.

Benzo(b)naphtho(2,1-d)thiophene

Toxicity

Only data from one reliable (reliability category 2) acute study on Daphnia magna (static design) were available where filtered and autoclaved natural river water was used. The reported result of a 48 h-test to determine LC_{50} using acetone (0.5 ml/L) as carrier and Triton X-100 (0.5 mg/L) as surfactant was "non-toxic" giving no further details. As LC_{50} for the isomeric benzo(b)naphtho(1,2-d)thiophene is reported as 0.22 mg/L, LC_{50} for benzo(b)naphtho(2,1-d)thiophene is most probably > 0.22 mg/L (Eastmond, et al., 1984). This value would be above the screening value on T of REACH guidance R.11 (0.1 mg/L). Benzo(b)naphtho(1,2-d)thiophene is therefore preliminary assessed as "not T".

As the upper tested concentration is not given, "T-W" ($\leq$ 1 mg/L) cannot be excluded. Moreover, as data on other trophic levels are missing, final conclusion on T from experimental data is not possible.

Bioaccumulation

Eastmond et al. (1984) assessed bioconcentration in Daphnia magna (reliability category 2). The BCF peaked after 15 hours at approximately 8000 dpm tissue / dpm water and then declined with concomitant increase of radioactivity in medium (water). After 70 hours of incubation BCF was approximately 2300 dpm tissue / dpm water (taking into account 5% volatilization) and reached a kind of steady state. According to the authors the observed decline was due to metabolism and excretion. The observed bioconcentration in Daphnia manga is above the REACH-threshold for B (BCF 2000) and the compound is therefore classified as "B-R".

Furthermore, read across to the structural isomer benzo(b)naphtho(2,3-d)thiophene (B-R in fish) corroborate the result from Eastmond et al. (1984) for Daphnia (B-R).

Persistence

Three studies were available demonstrating a potential for biodegradation for benzo(b)naphtho(2,1-d)thiophene.

Desulfurization (12 h) to 1-hydroxy-2-phenyl-naphthalene was assessed by use of Rhodococcus erythropolis H-2 whole cell assay using high concentration of lyophilized cells (40g/L) at 30°C (Ohshiro, et al., 1996). The strain grows on dibenzothiophene as sole sulphur source in the presence of hydrocarbons (30 °C). 90% of initial concentration (1 mM) was desulfurized after 12 hours. Growth (cell density) on ben-

zo(b)naphtho(2,1-d)thiophene as sole sulphur source (4d) and degradation in whole cell assay (2h) was approximately 30% and 20% (of initial rate), respectively of dibenzothiophene.

In a similar assay, applying Mycobacterium G3 desulfurization was assessed at 37°C for 17 h at 1 mM benzo(b)naphtho(2,1-d)thiophene in DMF (1% final). Again a highly concentrated cell suspension (72 OD / L) was used. Degradation (primary) led to hydroxy-biphenyl derivatives which were partly methoxylated. At the end of the experiment only a minor substrate peak was detectable by GC-MS (only qualitative data) (Okada, et al., 2002).

Both results may be regarded as a hint for inherent primary degradability. However the studies were designed as a step to biotechnological removal of organic sulphur compounds from petroleum and experimental conditions are far from environmental relevance.

In a more relevant third study (Lundstedt, et al., 2003) a contaminated gas work soil-water slurry plus nutrients was used to assess (primary) aerobic degradability. For a critical evaluation of this study see section Benzo(b)naphtho(1,2-d)thiophene. Benzo(b)naphtho(2,1-d)thiophene was part of the contaminated gas work soil used in the experiment and was degraded by 53% within 29 days (initial concentration not given). Compared to this structural isomer which was degraded in the same assay by 61% Benzo(b)naphtho(2,1-d)thiophene was more resistant to biodegradation.

While these experimental data show some potential for primary biodegradation (desulphurisation), a direct comparison of these results to REACH criteria is not possible due to the non-standard experimental setup. According to Lundstedt et al. (2003) benzo(b)naphtho(2,1-d)thiophene is less biodegradable than benzo(b)naphtho(1,2-d)thiophene already classified as P-W. No experimental data are available demonstrating ultimate biodegradability of benzo(b)naphtho(2,1-d)thiophene. The compound is therefore also classified as “P-W” (persistent with some hints for potential inherent biodegradability).

1-Methylbenzo(b)-naphtho(2,1-d)thiophene

Toxicity

Experimental data on toxicity are missing.

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. However, according to the calculated log K_{ow} of 5.89 (experimental log K_{ow} for aromatic core available, see above) the compound is regarded as B according to PBT-screening criteria of REACH guidance R.11 (“B-R”).

Furthermore, read across to the non-methylated structural isomers benzo(b)naphtho(2,1-d)thiophene (B-R in Daphnia) and benzo(b)naphtho(2,3-d)thiophene (B-R in fish) corroborates the screening assessment from log K_{ow} (“B-R”).

Persistence

Cleavage of a methylation isomer, 10-methyl-benzo(b)naphtho(2,1-d)thiophene was assessed using engineered Mycobacterium strain MR65. Reported desulfurization activity was only 26% of dibenzothiophene and 50% of non-methylated form (aromatic core structure) (Watanabe, et al., 2003). As benzo(b)naphtho(2,1-d)thiophene itself seems to be hardly degradable (P-W), the methylated form is rather P. It was therefore tentatively assessed as “likely P-R”, i.e. most probably persistent according to REACH. Further experimental data are needed however for a final assessment.

Benzo(b)naphtho(2,3-d)thiophene

Toxicity

Experimental data on toxicity are missing.

Bioaccumulation

The BCF was determined in fish (48h, Poecilia reticulata) to be 14900 L/kg wet weight (de Voogt, et al., 1991) using the unlabelled compound. A high standard error (SE +/- 34500) is associated with this value due to high uncertainty of depuration rate constant k2. The authors did not comment on that. However, taking the lower limit of the uptake rate constant (i.e. 70.8-14.0 =56.8, k1) and the higher limit of the depuration rate constant (i.e. 0.005+0.011 =0.016, k2) still gives a BCF of 3550 (k1/k2). The method applied was according to Banerjee et al. (1984), i.e. from depletion rate from water. The fat content was 9% lipid (not lipid normalized). A potential drawback of this method is that tissue concentrations are not analysed (metabolism generally not assessed). While control experiments provided corrections against losses from water during uptake phase (e.g. instability, evaporation or adsorption), adsorption to fish surface would be rated as uptake. However, in absence of more reliable data a high bioaccumulation potential is most probably associated with this compound (“B-R”).

Moreover, read across to the structural isomer benzo(b)naphtho(2,1-d)thiophene which bioconcentrated highly in Daphnia (“B-R”) as well as a high experimentally determined log K_{ow} (5.34, SRC-database) corroborates the result on bioconcentration from fish.

Persistence

Lundstedt et al. (2003) used a contaminated gas work soil-water slurry plus nutrients to assess (primary) aerobic degradability. For a critical evaluation of this study see section Benzo(b)naphtho(1,2-d)thiophene. Benzo(b)naphtho(2,3-d)thiophene was part of the contaminated gas work soil used in the experiment and was degraded by 53% within 29 days (initial concentration not given). Compared to this structural isomer which was degraded in the same assay by 61% and classified as P-W, benzo(b)naphtho(2,3-d)thiophene was more resistant to biodegradation and was degraded to the same extent as benzo(b)naphtho(2,1-d)thiophene. This might be due to the similar accessibility of the sulphur group, which is better accessible in the benzo(b)naphtho(1,2-d)thiophene isomer.

No experimental data are available demonstrating ultimate biodegradability of benzo(b)naphtho(2,1-d)thiophene. Like the two other structural isomers the compound is therefore classified as ”P-W “(persistent with some hints for potential inherent biodegradability).

Phenanthro(4,5-bcd)thiophene

Toxicity

Experimental data on toxicity are missing.

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. However, according to the experimentally determined log K_{ow} of 4.95 (Andersson and Schräder, 1999 as cited in SRC-database) the compound is regarded as B according to PBT-screening criteria of REACH guidance R.11 (“B-R”).

Persistence

Lundstedt et al. (2003) used a contaminated gas work soil-water slurry plus nutrients to assess (primary) aerobic degradability. For a critical evaluation of this study see section Benzo(b)naphtho(1,2-d)thiophene. Phenanthro(4,5-bcd)thiophene was part of the contaminated gas work soil used in the experiment and was degraded by 77% within 29 days (initial concentration not given). Compared to this structural isomer which was degraded in the same assay by 61% and classified as P-W, phenanthro(4,5-bcd)thiophene was biodegraded to a pronouncedly higher extent. Therefore, in spite of the lack of data demonstrating ultimate biodegradability for phenanthro(4,5-bcd)thiophene and the limitation of the study in regard to relevance for environmental conditions, the high percentage of biodegradation observed points to a potential for biodegradation also under natural environmental conditions and the compound is classified as “likely not P”. For a final assessment more relevant data would be needed.

2-Methyldibenzothiophene and methylation isomers

Toxicity

Experimental data on toxicity are only available for the non-methylated core (i.e. dibenzothiophene). Studies for all three trophic levels are available and the determined effect levels agree with a classification as “T-W” (1 mg/L $\geq$ E(L)C_{50} $\geq$ 0.1 mg/L) consistent also with the QSAR-estimation for the methylated core (0.53 mg/L for daphnids).

The EC_{50}(24 h) was determined for Daphnia magna as 0.2 mg/L (Sagner, 2009).

In the same publication acute fish toxicity was determined by application of the fish embryo toxicity test (FET, DIN 38415-6 and OECD draft guideline available). LC_{50} (48 h, Danio rerio) was determined as 0.33 mg/L.

Algal toxicity was determined on Desmodesmus subspicatus resulting in an EC_{50} (72 h) of 4 mg/L (Eisentraeger, et al., 2008).

Bioaccumulation

There are no experimental data on BCF, BAF or log K_{ow} for this compound.

Persistence

Lundstedt et al. (2003) used a contaminated gas work soil-water slurry plus nutrients to assess (primary) aerobic degradability. For a critical evaluation of this study see section Benzo(b)naphtho(1,2-d)thiophene. Different methylation isomers of methyldibenzothiophene were part of the contaminated gas work soil used in the experiment and were degraded by 91% within 29 days (initial concentration not given).

The degradability was qualitatively assessed for different methylation isomers of methyldibenzothiophene using cold marine inoculum (4°C, 28 days). All isomers proved to be degradable, the relative degradability of isomers is reported as 2-/3-methyl-DBT > 1- and 4-methyl-DBT (Wang, Z. and Fingas, 1995).

Further studies demonstrate high percentages of at least primary biodegradation: 90% biodegradation as part of weathered oil in marine water after 6d at 5 g/L oil mousse (2/3-methyl-isomers) (Fayad and Overton, 1995); desulphurisation (qualitative) of dibenzothiophene (non-methylated) and 4,6-dimethyldibenzothiophene within 17 hours by concentrated cell suspension of Mycobacterium G3 to mainly hydroxybiphenyl-derivatives and to a lower extent methoxybiphenyl-derivatives (Okada, et al., 2002); desul-

phurisation of different methylation isomers by Rhodococcus erythropolis between 68% and 95% (depending on the isomer) to hydroxybiphenyl-derivatives within 24 h (Onaka, et al., 2000).

Taking all this into consideration, persistence of methyldibenzothiophene isomers is highly improbable. The compound group is therefore classified as “not P” agreeing also with the QSAR-prediction.

Benzo(b)naphtho(1,2-d)furan

Toxicity

Maas (1990, as cited in US EPA ECOTOX-DB) determined aquatic toxicity for invertebrates and fish with static test design:

LC_{50} (48 h, Daphnia magna) = 4.0 mg/L (nominal, unmeasured);

LC_{50} (96h, Poecilia reticulata) = 0.85 mg/L (nominal, unmeasured);

The compound is therefore preliminary classified as “T-W”. For final assessment at least acute data for all three trophic levels would be needed (no data on algal toxicity). Additionally, the original publication could not be retrieved in spite of being cited in several data bases. As the title is rather misleading (“Toxicity research with thiourea”), a bug propagated through several databases cannot be excluded.

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. However, according to the experimentally determined log K_{ow} for the structural isomer Benzo(b)naphtho(2,3-d)furan of 5.05 (SRC-database) the compound is regarded as B according to PBT-screening criteria of REACH guidance R.11 (“B-R”).

Persistence

Lundstedt et al. (2003) used a contaminated gas work soil-water slurry plus nutrients to assess (primary) aerobic degradability. For a critical evaluation of this study see section Benzo(b)naphtho(1,2-d)thiophene. Different benzonaphthofuran isomers were part of the contaminated gas work soil used in the experiment and were degraded by 68% within 29 days (initial concentration not given). No other experimental data on biodegradation are available.

As most probably primary degradation was assessed, REACH requirements for inherent ultimate degradation would be at least 70% and the experimental conditions were favouring biodegradation and are not fully comparable to guideline studies, there are doubts if under natural environmental conditions the half-life limits according to REACH can be met. Accordingly the compound is classified as “P-W”. Further experimental data are needed for a final assessment.

Benzo(b)naphtho(2,3-d)furan

Toxicity

No experimental data on toxicity are available.

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. However, according to the experimentally determined log K_{ow} of 5.05 (SRC-database[30]) the compound is regarded as B according to PBT-screening criteria of REACH guidance R.11 ("B-R").

Persistence

Lundstedt et al. (2003) used a contaminated gas work soil-water slurry plus nutrients to assess (primary) aerobic degradability. For a critical evaluation of this study see section Benzo(b)naphtho(1,2-d)thiophene. Different benzonaphthofuran isomers were part of the contaminated gas work soil used in the experiment and were degraded by 68% within 29 days (initial concentration not given). No other experimental data on biodegradation are available.

As most probably primary degradation was assessed, REACH requirements for inherent ultimate degradation would be at least 70% and the experimental conditions were favouring biodegradation and are not fully comparable to guideline studies, there are doubts if under natural environmental conditions the half-life limits according to REACH can be met. Accordingly the compound is classified as "P-W". Further experimental data are needed for a final assessment.

10.2.6 Data from Bio-Monitoring

Results from bio-monitoring may corroborate a potential for bioaccumulation (especially if respective environmental concentrations were determined at the same time) and may give hints on metabolic capabilities of different organisms. Therefore, identified studies with results on the 15 priority semipolar PAC are summarized in this section.

Evaluating US-Environmental Protection Agency (EPA) *Screening Procedures for Bioconcentratable Organic Chemicals in Effluents and Sediments*, Burkhard and Sheedy (1995) found around 200 tentatively identified chemicals in effluents of 2 coke plants located at the same river (Five Mile Creek). Sampling sites for Crayfish, Sunfish and sediment included one site downstream of both discharges. Results for priority semipolar PAC included in the substance list of tentatively identified chemicals are summarized in Table 30.

High concentrations in Crayfish and Sunfish compared to effluent concentrations (concentrations in river water not given) were determined especially for 3-methyl-dibenzothiophene. Effluent concentrations of the core compound dibenzothiophene were at least comparable (see also sediment concentration), however concentrations in biota were markedly lower. Similarly, concentrations of the 4-ring compounds benzo(b)naphtho(1,2-d)thiophene, Benzo(b)naphtho(2,1-d)thiophene and Benzo(b)naphtho(2,3-d)furan in biota were lower than methyldibenzothiophene while effluent- and sediment-concentrations were comparable or higher. This could be due to more rapid partitioning to the sediment for these compounds, but results are not discussed in such detail by Burkhard and Sheedy. Generally, lower concentrations in Sunfish compared to Crayfish are due to the higher metabolic capacity of vertebrates (fish) compared to invertebrates (arthropods), as the authors note.

[30] SRC PhysProp Database, http://www.syrres.com/what-we-do/databaseforms.aspx?id=386

Table 30: Detected effluent concentrations of some priority semipolar PAC of 2 coke plants at the Five Mile Creek and corresponding concentration in Crayfish, Sunfish and sediment sampled downstream of both plants. Data compiled from Burkhard and Sheedy (1995). Data on dibenzothiophene are given for comparison to methyl-derivative only.

CAS-No	Tetatively identified chemical	Effluent concentration [ng/L]		Crayfish [µg/kg]	Sunfish [µg/kg]	Sediment [mg/kg]
		Plant 1	Plant 2	Station 3	Station 3	Station 3
16587-52-3	Dibenzothiophene, 3-methyl-	349	329	1153	1010	12.79
132-65-0	Dibenzothiophene	829	43.3	178	476	33.4
205-43-6	Benzo(b)naphtho(1,2-d)thiophene	804	2.367	75.6	15.17	28.43
239-35-0	Benzo(b)naphtho(2,1-d)thiophene	2700	8.29	264.9	11.06	137.8
243-42-5	Benzo(b)naphtho(2,3-d)furan	2317	18.85	358.4	44.05	230.7

Some monitoring data on semipolar PAC in marine organisms exist, pointing to relevance of alkylated core structures for accumulation in biota. King et al. found (1993) in digestive glands of lobsters besides benzo(b)naphtho(2,1-d)thiophene (190 µg/kg), benzo(b)naphtho(2,3-d)thiophene (50 µg/kg) and dibenz(a,h)acridine (10 µg/kg) a comparably high concentration of methyldibenzothiophene (150 µg/kg), but not the core compound dibenzothiophene. This corroborates the obviously higher potential for bioaccumulation of the methylated compound according to the data of Burkhard and Sheedy (1995).

Similar observations are reported by Hale (1988) for hepatopancreas, muscle and ovaries of blue crabs (qualitative). Besides benzo(b)naphtho(2,1-d)thiophene and dibenzothiophene three isomers of methyldibenzothiophene, five isomers of C2-dibenzothiophene and three isomers of C3-dibenzothiophene were detected. Baumard et al. (1999) studied the accumulation of methyl dibenzothiophenes compared to parent dibenzothiophene. Relative to sediment concentrations, concentrations of alkylated isomers found in mussels were three times greater than the parent compound. In another study Baumard analysed concentrations of 1-, 2/3- (combined isomers) and 4-methyldibenzothiophene together with the non-alkylated parent in mussels and sediment (Baumard, et al., 1998). In sediments, no systematic differences in concentrations between these groups could be found. In mussels however, concentrations of 4-methyldibenzothiophene were pronouncedly higher than those of the parent and the other isomers (highest detected concentration: 0.1 mg/kg dry weight). This fits with observations of Wang and Fingas reporting a higher biodegradability for the 2- and 3-isomers compared to the 1- and 4-methyl-isomers (1995). As differences in isomer concentrations in sediment were not pronounced, this points to a lower potential for metabolic transformation in mussel for the 4-methyl-isomer.

In their review on condensed thiophenes found in petroleum, Kropp and Fedorak (1998) summarize bio-monitoring data. After an oil spill C1-, C2 and C3-substituted dibenzothiophenes were found in Flat and Japanese oysters. Some of the higher substituted isomers persisted up to three years after the spill. Similarly, an enrichment of alkylated dibenzothiophenes relative to the un-substituted parent was observed after an oil spill in clams and mussels.

In summary, data on bio-monitoring for the 15 priority semipolar PAC are very scarce. No conclusion as regards quantitative aspects of bioaccumulation may be drawn. However, metabolic capacity seems to be generally higher in vertebrates (fish) compared to invertebrates (crayfish). At least for dibenzothiophene, bioaccumulation seems to be considerably more pronounced for the alkylated core structures. This might be due to higher lipophilicity and / or lower metabolism caused by the alkyl residue(s). The results on 4-methyldibenzothiophene in mussels indicate that at least in part the latter might have some importance.

10.2.7 Known Aerobic Degradation Pathways

Knowing degradation pathways enables one to conclude on possible recalcitrant intermediates prone to accumulate in contaminated areas. These should then be additionally monitored and their properties in regard to PBT assessed. Thus, identified literature for the 15 priority semipolar PAC containing mechanistic information on biodegradation was evaluated and is summarized in this chapter.

Priority semipolar PAC were selected to be persistent, bioaccumulative and toxic (PBT) or to cover at least two of these properties. All but one (2-methyldibenzothiophene) of the 15 compounds are predicted to be persistent, experimental data for 7 compounds (all aza-arenes) confirm their recalcitrance to biodegradation (P-R) and further experimental data for 5 compounds show some potential for biodegradation, however under circumstances that are probably too far from environmentally relevant conditions to be predictive for efficient environmental biodegradability (P-W). These latter compounds are benzonaphthothiophenes (3) and benzonaphthofurans (2). For 2-methyldibenzothiophene experimental data sufficiently show biodegradability, also phenanthro(4,5-bcd)thiophene might be biodegradable to some extent (likely not P). For one compound (benzo(a)phenanthridine) no experimental data are available.

As studies on biodegradation pathways depend on a certain extent on biodegradability and not all classes of compounds were extensively studied, information is largely restricted on condensed thiophenes.

Degradation pathways for condensed thiophenes

Two major pathways for the degradation of (methyl)dibenzothiophenes were described: The “Kodama pathway”, which was shown to be cometabolic at least for Pseudomonas janii, and the desulfuration by the so-called “4S-pathway” (Bressler, et al., 1998; Kropp and Fedorak, 1998). The latter will only be relevant if sulfur is limiting due to repression of the responsible *dsz* genes by sulfate and sulfur-containing amino acids and is found in many mesophilic microorganisms (Díaz and García, 2010). The relevance of the first is for oil-contaminated environments providing sufficient cometabolites while sulfur is not limiting (Kropp and Fedorak, 1998) and is found in different Pseudomonas strains (Díaz and García, 2010).

The “Kodama pathway” involves oxidation of one of the benzene rings to yield 3-hydroxy-2-formylbenzothiophene if dibenzothiophene is the substrate (Figure 4). This results in the liberation of pyruvate which serves as a carbon source. 3-hydroxy-2-formylbenzothiophene is often described as the end product using pure cultures of Pseudomonas strains. In mixed cultures further transformation was reported however liberation of sulfate was not observed, pointing to incomplete mineralization. Other observed dead end products with Pseudomonas strains are dibenzothiophene sulfoxide (several isolates) and dibenzothiophene sulfone (Pseudomonas putida).

Further oxidation of 3-hydroxy-2-formylbenzothiophene may yield benzothiophene-2,3-dione in acidic cultures, which is in equilibrium with 2-mercaptophenylglyoxylate (dominating at neutral conditions) (Bressler, et al., 1998).

The different isomers of methyl-dibenzothiophene are preferentially oxidized at the non-substituted ring and corresponding methylated 3-hydroxy-2-formylbenzothiophene- and benzothiophene-2,3-dione-derivatives are formed. Susceptibility of dimethyl-dibenzothiophenes depends on positioning of methyl groups. Oxidation of the 4,6-isomer (both homocyclic rings substituted) was not observed in pure cultures, while the 3,4-isomer resulted in 6,7-dimethylbenzothiophene-2,3-dione (Bressler, et al., 1998; Kropp and Fedorak, 1998).

Degradation of higher condensed thiophenes (more than three rings) was not observed via the “Kodama pathway”.

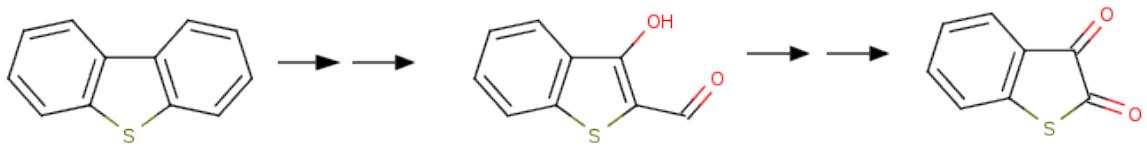


Figure 4: Important intermediates of dibenzothiophene biotransformation following the „Kodama pathway“: A stepwise oxidaton of one of the benzene rings liberizes pyruvate and yields 3-hydroxy-2-formylbenzothiophene. Further oxidation may result in benzothiophene-2,3-dione (Bressler, et al., 1998; Kropp and Fedorak, 1998).

The “4S pathway” in contrast to the “Kodama pathway” does not lead to C-C bond cleavage. Instead, the S atom of the thiophene moiety is selectively oxidized to from the sulfoxide, the sulfone, sulfonate, and finally release it as sulfate (Figure 5).

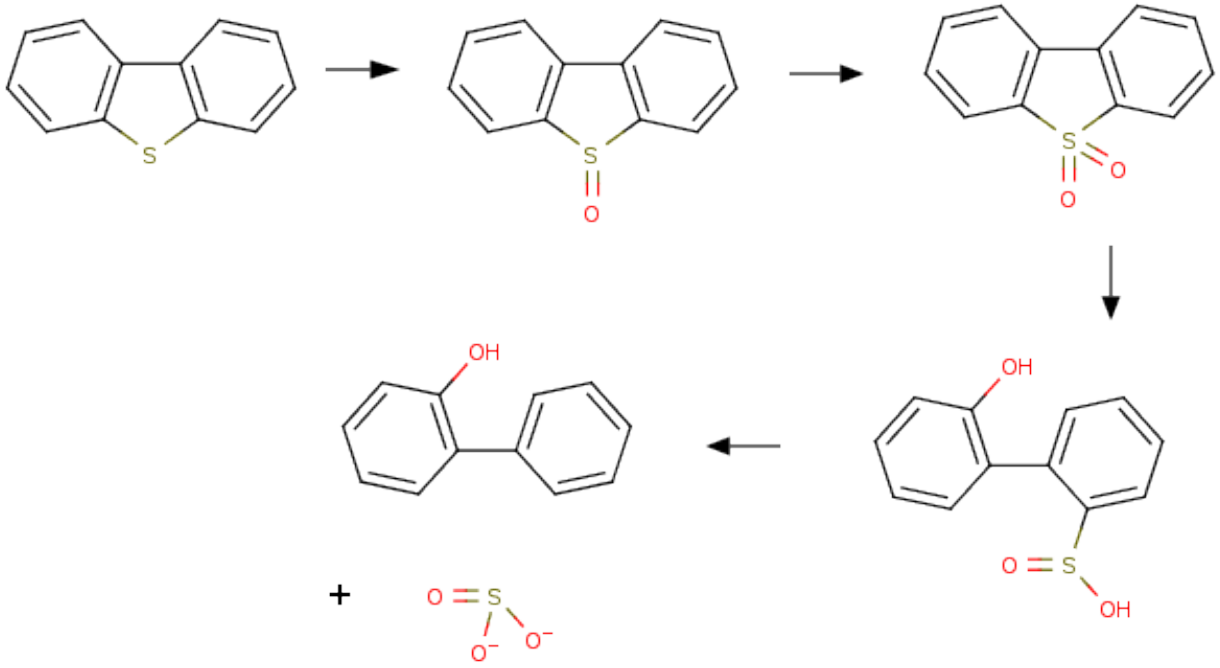


Figure 5: Modified 4S-Pathway of desulfuration of dibenzothiophene (DBT): The pathway proceeds via the stepwise oxidation of sulfur leading to formation of DBT-sulfoxide, DBT-sulfone, 2’-hydroxybiphenyl-2-sulfinic acid and 2-hydroxybiphenyl and sulfate as final products (Bressler, et al., 1998).

The “4S pathway” proved to be versatile in that Rhodococcus erythropolis strains were shown to desulfurize also several alkylated dibenzothiophenes as well as higher condensed thiophenes including the priority compound benzo(b)naphtho(2,1-d)thiophene and a methylated derivative, 10-methyl-benzo(b)naphtho(2,1-d)thiophene (Ohshiro, et al., 1996; Watanabe, et al., 2003). The main end products were α-hydroxy-β-phenylnaphthalene for the first and 1-hydroxy-2-(3-methylphenyl)naphthalene. To a minor extent also the other possible isomer resulting from sulfone ring cleavage 2-(2-hydroxy-3-methylphenyl)naphthalene was detected. Some portion of both fractions was also found to be methoxylated. While both, Pseudomonas and Rhodococcus strains are able to perform this pathway, the former are restricted to absorption from the water phase while the latter my directly access compounds from oil (Díaz and García, 2010).

Further degradation pathways with some relevance for priority semipolar PAC

Mechanistic details are available for the degradation of dibenzofuran and carbazole (Díaz and García, 2010; Reineke and Hollender, 2007). After angular dioxygenation yielding a hemiacetal or hemiaminal, upon spon-

taneous ring cleavage 2,3-dihydroxy-2´-hydroxypenyl and 2,3-dihydroxy-2´-aminobiphenyl is formed, respectively. After further oxidation the final products are salicylate or anthranilate and 2-hydroxy-2,4-pentadienoic acid which all can be further metabolized (Díaz and García, 2010; Reineke and Hollender, 2007).

However concerning priority compounds, only one study reports some biodegradability of benzonaphthofurans without however analyzing pathways or degradation products (Lundstedt, et al., 2003). No biodegradation was observed for dibenzocarbazoles. While the above described pathway is at least theoretically compatible with degradation of benzonaphthofurans (described reaction sequence could proceed on the benzyl ring), it is not with dibenzocarbazoles and no biodegradation was observed for 7H-dibenzo(c,g)carbazole.

In summary, information on pathways of degradation for the 15 priority semipolar PAC is scarce and reliable information limited to thiaarenes. Possibly hardly degradable intermediates from isomers of methyldibenzothiophenes are methyl-derivatives of 3-hydroxy-2-formylbenzothiophene and benzothiophene-2,3-dione (Kodama-pathway). From the “4S pathway” methyl-derivatives of 2-hydroxybiphenyl are resulting as final products not further degraded by this route at least. Relevant intermediates from higher condensed thiophenes can only be expected from the “4S pathway”. Benzonaphthothiophenes will probably be metabolized mainly to α-hydroxy-β-phenylnaphthalene and isomers, depending on the positioning of the naphthalene moiety in the relevant benzonaphthothiophene isomer. It is beyond the scope of this project to analyze further possible PBT-properties of the mentioned, possibly recalcitrant intermediates.

10.3 Discussion on Experimental Data on PBT-Properties for the 15 Priority Compounds

For the 15 priority compounds determined from QSAR-screening results combined with relevance in profiles (for structural representations and information on occurrence see Table 56 of Annex III.) an exhaustive literature research yielded experimental results on P, B or T-properties for 14 compounds. However, for 11 out of 15 compounds important data for PBT-assessment are missing or not sufficiently conclusive and testing is urgently needed on one or more of the three PBT-criteria. Thus, mainly due to data gaps no compounds fulfilling P, B and T-criteria at the same time could be determined from available experimental data for the 15 priority compounds. Benz(a)acridine was the only compound for which clear conclusions on all three properties were possible. It was identified as toxic and persistent according to REACH criteria, but not to be bioaccumulative in fish (due to metabolism) nor in Daphnia pulex (BCF 350). However, for the actual SVHC-assessment including also data on genotoxicity and carcinogenicity as well as QSAR-derived data (where experimental data are lacking), see chapter 12. Structural representations and information on occurrence for all 15 priority semipolar PAC are given in Table 56 of Annex III.

Because of fragmentary data (e.g. predominantly acute toxicity data mostly for less than three trophic levels, often inadequate data on degradability), compounds were kept in the list of priority semipolar PAC basically if at least two of the PBT-properties were fulfilled (for exact criteria see section 10.2.2). Counting missing data together with a positive QSAR-screening result for the respective property as fulfilment of REACH criteria, all but one compound are to be retained in the list. For this compound (2-methyldibenzothiophene) exclusion from the list of priority compounds would probably be justified as degradability is clearly shown and at least for the non-methylated core structure acute effect concentrations for three trophic levels fail to fulfil the criterion according to REACH guidance R.11 ($E(L)C_{50}$ < 0.1 mg/L). As however no experimental toxicity data for the actual compound, i.e. the methylated core, are available and also experimental data on bioaccumulation are lacking, even this exclusion would be associated with uncertainty. Furthermore, monitoring data might nonetheless indicate a potential for bioaccumulation even if no quantitative conclusions may be drawn (see section 10.2.6). Thus, the compound is further assessed within the 15 priority semipolar PAC.

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

Retrieved experimental data were used to check for predictivity of the applied QSAR-screening procedure. As all priority compounds were screened as T and B and all but one compound as P, besides this single case only false positives could be identified by comparison with experimental data. With correct prediction of 75% on T, 85% on B and 93% on P the method seems useful in identifying PBT-candidates. As this evaluation of QSAR data is based on just 14 compounds with existing experimental data for at least two properties (one compound without any data on T, B or T) it may however give only a rough impression on usability of the applied QSAR screening methodology.

In respect to false negatives regarding QSAR-T-screening, ECOSAR-based screening-results for compounds of the of the *extended QSAR selection of critical PAC* (443 compounds) were compared to legal classifications available for 9 compounds and QSAR-based classification by Danish EPA ("Advisory Classifications") available for a further 163 of the 443 compounds from the *extended QSAR selection of critical PAC*. Taking the Danish data (implying different QSAR models but still being only predictions) together with legal classifications as a reference (with R50 or R50/53 as criterion for T), screening implying ECOSAR would result in 31% false negatives and 6% false positives. Thus, the Danish approach seems to be more conservative compared to ECOSAR based screening. Evaluating the data further it can be concluded, that even an additional screening on T implying "Advisory Classifications" where available would not have resulted in additional or other priority compounds. „Advisory Classifications" existing for 8 of the 15 priority compounds were fully in line with our QSAR-screening-results. This different QSAR based approach emphasises on the one hand the potential importance of false negatives in QSAR-screening (to be evaluated by experimental data). On the other hand the essentially identical results gained from our approach corroborate our QSAR-based selection strategy.

From the literature research, also some publications on bio-monitoring were identified. Monitoring data on semipolar PAC 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 determinations in profiles and even less in regard to ecotoxicological tests are available for alkylated het-PAC. Therefore it would be important to close this knowledge gap.

11 Genotoxicity / Carcinogenicity Assessment for 15 Priority Semipolar PAC

In principal, assessment of human toxicity was not included in the scope of this project. As toxicity data for the 15 priority semipolar PAC are often insufficient or lacking and PBT-criteria according to REACH include carcinogenicity and germ cell mutagenicity, a literature research for these endpoints was performed. However, the search was restricted to the following bibliographic databases and internet resources: A literature research was performed in PubMed (NLM, 2012b), Toxline (NLM, 2012c) and the Genetox data base (NLM, 2012a) with CAS numbers, in PubMed and Toxline combined with the terms "(genotox* OR mutagen* OR cancer OR carcinogen*)". The search was expanded with the substance names in PubMed to identify all relevant hits which did not contain CAS numbers as keywords. A subsequent Google-Scholar search was restricted by these terms only when too many (about > 50 hits) were obtained with the individual CAS numbers. Dr. Albrecht Seidel from BIU kindly provided the available BIU inhouse literature regarding genotoxicity and carcinogenicity, which basically corroborated the results emerging from our literature search but did not yield further relevant information.

11.1 Available Classification and Labeling ragarding Genotoxicity / Carcinogenicity

No harmonized classifications for the 15 priority semipolar PAC were available as of January 2012[31]. Furthermore, notified classifications for the 15 compounds were checked as contained in the CLP Database of ECHA[32] (as of January 2012). No notified classifications regarding genotoxicity or carcinogenicity were available.

11.2 Genotoxic and carcinogenic properties

Genotoxic and carcinogenic properties of benz(a)acridine

The substance was tested in an Ames test with metabolic activation, but only with Salmonella typhimurium TA98. The result was inconclusive (IARC, 1983). A weak mutagenic response was also obtained in strains TA98 and TA100 by Karcher et al. (1984). A genotoxicity test in saccharomyces cerevisiae FF18984 and a SOS chromotest in Salmonella typhimurium yielded both positive results (Bartoš, et al., 2006).

A mouse study with dermal exposure showed no tumorigenic effects, but the study was considered to be inadequate due to a small number of test animals (IARC, 1983).

The IARC (1983; 1987) classified the substance as carcinogen of group 3, this was confirmed by Lauby-Secretan et al. (2011).

Genotoxic and carcinogenic properties of benz(c)acridine

The substance was tested in two Ames tests with metabolic activation, but performed only with strain Salmonella typhimurium TA98. Both tests produced a positive response (IARC, 1983). A weak mutagenic response was obtained in strains TA98 and TA100 by Karcher et al. (1984). A genotoxicity test in saccharomyces cerevisiae FF18984 and a SOS chromotest in Salmonella typhimurium yielded both negative results (Bartoš, et al., 2006). The substance induced sister chromatid exchange in transformed Chines hamster lung

[31] http://esis.jrc.ec.europa.eu/index.php?PGM=cla

[32] http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database

cells (Tucker, et al., 1993) and DNA-fragmentation in human HL60 cells in vitro at cytotoxic doses (Sakagami, et al., 1995).

In an initiation/promotion carcinogenicity study benz(c)acridine did not act as dermal tumour initiator. Implantation in wax pellets into the bladder of rats produced an increased incidence of bladder tumours. A mouse study with dermal exposure showed a low incidence of skin tumours, but the study was considered to be inadequate due to a small number of test animals (IARC, 1983). Newborn mice, injected intraperitoneal with benz(c)acridine developed lung and liver tumours (Chang, et al., 1984). No lung tumours were induced after implantation of low amounts (1 mg) in wax pellets into rat lungs (Deutsch-Wenzel, et al., 1983). In contrast to the described lacking initiator activity, a further initiation/promotion protocol reported the substance to be a weak tumour initiator in mice (Levin, et al., 1983).

The IARC (1983; 1987) classified the substance as carcinogen of group 3, this was confirmed by Lauby-Secretan et al. (2011).

Genotoxic and carcinogenic properties of mixtures of dibenzoquinolines (dibenzo(c,f)quinoline = benzo(a)phenanthridine)

No studies with experimental data concerning mutagenicity or carcinogenicity were identified.

Genotoxic and carcinogenic properties of dibenz(a,j)acridine

Positive results were obtained in standard Ames tests with Salmonella typhimurium strains TA98 and TA100 with metabolic activation. No induction of unscheduled DNA synthesis was observed in primary rat hepatocytes in vitro (IARC, 1983). Only a weak mutagenic response was obtained in strains TA98 and TA100 by Karcher et al. (1984). A further Ames test with Salmonella typhimurium strains TA98 and TA100 (with metabolic activation) produced a negative response in TA98 and a positive response in TA100 (Bonin, et al., 1989). The same authors tested the substance in V79 hamster cells in vitro and observed a dose-related increase in gene mutations. A genotoxicity test in saccharomyces cerevisiae FF18984 and a SOS chromotest in Salmonella typhimurium yielded negative results (Bartoš, et al., 2006). Dibenz(a,j)acridine induced no chromosomal aberrations in Chinese hamster lung cells (Serra, et al., 2003), but micronuclei in human lymphocytes in vitro (Warshawsky, et al., 1995b). The substance provoked DNA adducts, predominantly in skin after topical application to mice, in lower amounts also in the lung, but not other organs (Talaska, et al., 1995; Warshawsky, et al., 1996).

Dermal exposure induced skin tumours and subcutaneous application produced local sarcomas in mice. The substance increased the incidence of lung tumours after subcutaneous administration in a susceptible mouse strain. No increased incidences of tumours were observed after oral exposure of mice, but the test was considered to be inadequate due to a small number of test animals (IARC, 1973; NTP, 2011; Warshawsky, et al., 1994; Warshawsky, et al., 1996) No lung tumours were induced after implantation of low amounts (1 mg) in wax pellets into rat lungs (Deutsch-Wenzel, et al., 1983).

The IARC (1983; 1987) classified the substance as carcinogen of group 2B, which was recently altered to class 2A (probably carcinogenic to humans) due to strong mechanistic evidence (genotoxicity) contributing to the overall evaluation (Lauby-Secretan, et al., 2011). The National Toxicology Program considered the substance as “reasonably anticipated to be a human carcinogen (NTP, 2011), Nesnow et al. (1986) as “sufficient positive” for carcinogenicity.

A potency equivalency factor of 0.1 was derived in relation to benzo(a)pyrene as reference substance (Collins, et al., 1998).

Genotoxic and carcinogenic properties of dibenz(a,h)acridine

Several Ames tests are reported, all with metabolic activation. One tested the substance with five strains (including Salmonella typhimurium TA100), resulting in a negative response. Another mutagenicity test in Salmonella typhimurium TA100 came to a positive result (IARC, 1983). However, only a weak mutagenic response was obtained in strains TA98 and TA100 by Karcher et al. (1984). A genotoxicity test in saccharomyces cerevisiae FF18984 and a SOS chromotest in Salmonella typhimurium yielded both negative results (Bartoš, et al., 2006). No induction of chromosomal aberrations was observed in Chinese hamster lung cells in vitro (Serra, et al., 2003). The substance provoked in vivo DNA adducts, sister chromatid exchange and micronuclei in rat lung cells after intratracheal instillation (Whong, et al., 1994).

Dermal exposure of mice induced skin tumours. Subcutaneous application induced local sarcomas and increased the incidence of lung tumours in mice (IARC, 1973). The intrapulmonary implantation of dibenz(a,h)acridine-containing pellets produced lung carcinomas in rats (Deutsch-Wenzel, et al., 1983).

The IARC (1983; 1987) classified the substance as carcinogen of group 2B, this was confirmed by Lauby-Secretan et al. (2011). The National Toxicology Program evaluated the substance as “reasonably anticipated to be a human carcinogen (NTP, 2011), Nesnow et al. (1986) as “sufficient positive” for carcinogenicity.

A potency equivalency factor of 0.1 was derived for carcinogenicity in relation to benzo(a)pyrene as reference substance (Collins, et al., 1998).

Genotoxic and carcinogenic properties of 13H-dibenzo(a,i)carbazole

No mutagenic responses were observed in bacteria (Salmonella G46, TA98, TA100, TA 1535, TA1537, and TA1538 and E.coli WP2uvrA- with or without metabolic activation) and no unscheduled DNA synthesis was induced in primary rat hepatocytes in vitro (Probst, et al., 1981). Dibenzo(a,i)carbazole caused DNA-adducts in mouse liver. The route of application was not provided in the abstract published (Saito, et al., 1988).

The substance is reported to be a weak skin carcinogen in mice after topical application (Warshawsky, 1992).

Genotoxic and carcinogenic properties of 7H-dibenzo(c,g)carbazole

Two Ames tests in Salmonella typhimurium strain TA100 (with metabolic activation) yielded negative and inconclusive results (IARC, 1983). Further tests showed negative outcomes in TA98 and TA100, a positive result in Salmonella typhimurium FM677 and a mutagenic response in a rat liver/hamster embryonic cell cocultivation system (Schoeny and Warshawsky, 1987; Warshawsky, 1992). A genotoxicity test in saccharomyces cerevisiae FF18984 and a SOS chromotest in Salmonella typhimurium yielded both negative results (Bartoš, et al., 2006). The substance induced micronuclei in human lymphocytes in vitro (Warshawsky, et al., 1995b). DNA strand-breaks, micronuclei, and DNA adducts were induced in a human keratinocyte cell line expressing CYP1A1 (Valovicová, et al., 2012). No DNA adducts were formed in V79 hamster cells in vitro (no mono-oxygenase activity present), but in genetically modified cells expressing human CYP1A1 (Gábelová, et al., 2004). The substance caused DNA adducts and mutations in human fibroblasts in vitro in presence of human liver hepatoma cells as exogenous metabolizing system (Parks, et al., 1986). Mutagenicity was also observed in another cocultivation system with rat liver and epithelial cell lines (Warshawsky, 1992).

Dermal application (single or repeated exposures) as well as single oral or subcutaneous administration produced DNA adducts predominantly in the liver. All three exposure routes caused adducts (to a lesser extend) also in skin, lung and kidney of mice (Dorchies, et al., 2001; Schurdak and Randerath, 1989; Talaska, et al.,

1994; Warshawsky, 1992). Topical application produced also low amounts of DNA adducts also in spleen, pancreas and brain (Schurdak and Randerath, 1985).

The substance caused liver and forestomach tumours after oral exposure of mice, tumours in the respiratory system after intratracheal instillation in hamsters and lung tumours in susceptible strains of mice after subcutaneous, intravenous or intraperitoneal administration. Dermal or subcutaneous exposure of mice also caused liver tumours with the same potency as benzo(a)pyrene. Local skin tumours were observed in mice and rats after dermal application or subcutaneous injection, and injection into the urinary bladder produced tumours in dogs (IARC, 1973; NTP, 2011; Warshawsky, 1992; Warshawsky and Barkley, 1987; Warshawsky, et al., 1996).

The IARC (1983; 1987) classified the substance as carcinogen of group 2B, this was confirmed by Lauby-Secretan et al. (2011). The National Toxicology Program evaluated the substance as “reasonably anticipated to be a human carcinogen (NTP, 2011), Nesnow et al. (1986) as “sufficient positive” for carcinogenicity.

A potency equivalency factor of 1 was derived in relation to benzo(a)pyrene as reference substance (Collins, et al., 1998).

Genotoxic and carcinogenic properties of benzo(b)naphtho(1,2-d)thiophene

Mutagenicity tests in Salmonella typhimurium TA98, TA 100, TM667 and E. coli PKM101 with metabolic activation produced negative results, both with standard and pre-incubation protocols. Negative results were also obtained in TA1535 and TA1537 (Jacob, 1990; Kaden, et al., 1979; McFall, et al., 1984; Pelroy, et al., 1983; Pool, et al., 1989).

Genotoxic and carcinogenic properties of benzo(b)naphtho(2,1-d)thiophene

Mutagenicity tests in Salmonella typhimurium TA98, TA 100 and E. coli PKM101 with standard protocols in the presence of a metabolic activation system produced negative results. Negative results were also obtained in TA1535 and TA1537 (Jacob, 1990; Karcher, et al., 1981; McFall, et al., 1984; Pelroy, et al., 1983). However, a mutagenic response was obtained in a standard protocol (strain TA98, with metabolic activation) by Pool et al. (1989) and TA100 with metabolic activation in a pre-incubation assay (Misra and Amin, 1990). A gene mutagenicity test with human h1A1v2 cells in vitro yielded a negative result (Durant, et al., 1996).

Injection into the lungs of rats produced a local carcinogenic effect (Wenzel-Hartung, et al., 1990). Croisy et al. (1984) reported benzo(b)naphtho(2,1-d)thiophene to be a carcinogen after subcutaneous injection to mice, but no details are provided.

The IARC recently classified the substance as carcinogen of group 3 (Lauby-Secretan, et al., 2011).

Genotoxic and carcinogenic properties of 1-methylbenzo(b)-naphtho(2,1-d)thiophene (and other methylation isomers)

A mutagenicity test in Salmonella typhimurium TA98 with metabolic activation produced a positive result. The 6-methylated compound was also active, but not the other isomers with methylation in position 2 to 10 of the core structure (McFall, et al., 1984).

Genotoxic and carcinogenic properties of benzo(b)naphtho(2,3-d)thiophene

Mutagenicity tests in Salmonella typhimurium TA98 and TA100 with metabolic activation produced negative results, both with standard and pre-incubation protocols. Negative results were also obtained in TA1535 and TA1537 (Jacob, 1990; McFall, et al., 1984; Pelroy, et al., 1983).

Genotoxic and carcinogenic properties of phenanthro(4,5-bcd)thiophene

A negative result was obtained in standard Ames tests with Salmonella typhimurium TA98, TA 100 and E. coli PKM101 (Karcher, et al., 1981).

Genotoxic and carcinogenic properties of 2-methyldibenzothiophene (and other methylation isomers)

A mutagenicity tests in Salmonella typhimurium TA98 with metabolic activation produced a negative result, the other methylated isomers were also negative (McFall, et al., 1984).

Genotoxic and carcinogenic properties of benzo(b)naphtho(1,2-d)furan

No studies with experimental data concerning mutagenicity or carcinogenicity were identified.

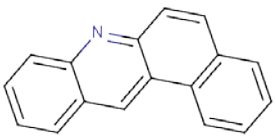
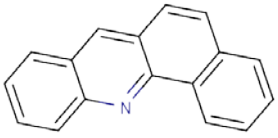
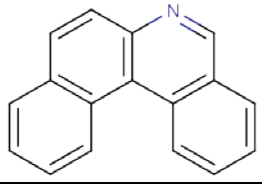
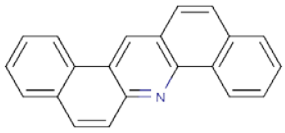
Genotoxic and carcinogenic properties of benzo(b)naphtho(2,3-d)furan

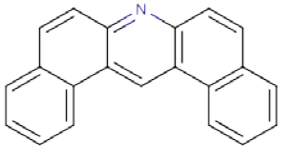
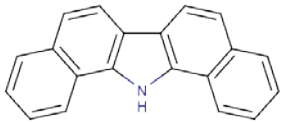
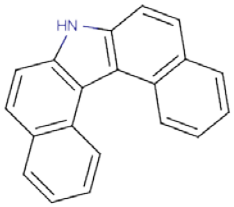
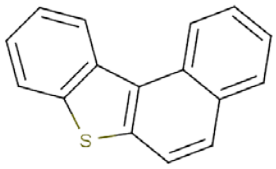
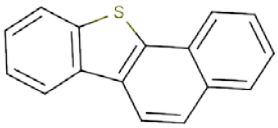
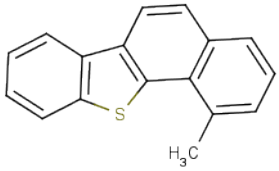
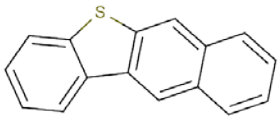
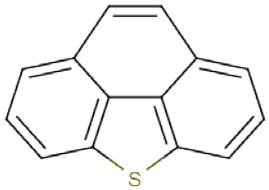
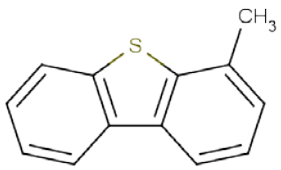
No studies with experimental data concerning mutagenicity or carcinogenicity were identified.

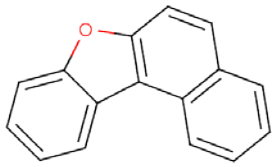
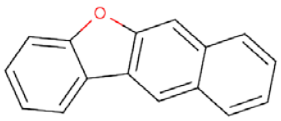
11.2.1 Summary of identified mutagenic and carcinogenic properties of 15 high priority semipolar PAC

The following table preliminarily summarises the available experimental results for mutagenicity and carcinogenicity.

Table 31: NSO-heterocyclic priority compounds, summary of mutagenic and carcinogenic properties

CAS-No.	Chemical name	Structure	Summary of mutagenic properties [1)]	Summary carcinogenic properties [1)]
225-11-6	Benz(a)acridine		in vitro: +? in vivo: nd	-?
225-51-4	Benz(c)acridine		in vitro: + in vivo: nd	+?
195-29-9	Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline = Benzo(a)phenanthridine, 195-29-9)		in vitro:nd in vivo: nd	nd
226-36-8	Dibenz(a,j)acridine		in vitro: +? in vivo: +	+

CAS-No.	Chemical name	Structure	Summary of mutagenic properties [1)]	Summary carcinogenic properties [1)]
224-42-0	Dibenz(a,h)acridine		in vitro: -? in vivo: +	+
239-64-5	13H-Dibenzo(a,i)carbazole		in vitro: - in vivo: +	+?
194-59-2	7H-Dibenzo(c,g)carbazole and other dibenzocarba-zoles		in vitro: + in vivo: +	+
205-43-6	Benzo(b)naphtho(1,2-d)thiophene		in vitro: - in vivo: nd	nd
239-35-0	Benzo(b)naphtho(2,1-d)thiophene		in vitro: +? in vivo: nd	+?
4567-41-3	1-Methylbenzo(b)-naphtho(2,1-d)thiophene, several methylation isomers		in vitro: + in vivo: nd	nd
243-46-9	Benzo(b)naphtho(2,3-d)thiophene		in vitro: - in vivo: nd	nd
30796-92-0	Phenanthro(4,5-bcd)thiophene		in vitro: - in vivo: nd	nd
20928-02-3	Methyldibenzothiophene, 2- (several isomers, SMILES for 4-methyl-isomer)		in vitro: - in vivo: nd	nd

CAS-No.	Chemical name	Structure	Summary of mutagenic properties [1)]	Summary carcinogenic properties [1)]
205-39-0	Benzo(b)naphtho(1,2-d)furan, several Benzonaphthofurans		in vitro: nd in vivo: nd	nd
243-42-5	Benzo(b)naphtho (2,3-d)furan and other naphthobenzofurans		in vitro: nd in vivo: nd	nd

1): -: negative; +?: questionable positive; -?:questionable negative; +: positive; nd: no data

Remark: The reader shall bear in mind that this table summarises only the preliminary conclusion based on the available experimental results for the individual chemical compounds, but not the final results after considering the structure activity relationship analysis (see below).

11.2.2 Structure-Activity Relationship (SAR) analysis

The semipolar PAC priority substances were subjected to a SAR analysis with respect to their mutagenic and carcinogenic properties (Toxtree-software33), which is based on the categorization of structural domains of molecules allocated with common mechanisms in genotoxicity and carcinogenicity (Benigni, et al., 2008; Benigni, et al., 2009). The 15 semipolar PAC priority compounds all contain the structural alert “heterocyclic polycyclic aromatic hydrocarbon” (could not be differentiated within the used algorithms), which was considered to be a relevant determinant for mutagenicity in the in vivo micronucleus assay as well as for genotoxic carcinogenicity.

11.2.3 Summary and Discussion on Genotoxicity / Carcinogenicity

The data base for the priority semipolar PAC is very heterogeneous with respect to mutagenicity and carcinogenicity.

IARC (Lauby-Secretan, et al., 2011) classified some of the compounds as carcinogenic (class 2A/B: probably/possibly carcinogenic to humans): dibenz(a,j)acridine, dibenz(a,h)acridine, 7H-dibenzo(c,g)carbazole. Collins et al. (1998) derived potency equivalency factors of 0.1, 0.1 and 1, respectively, for these three substances in relation to benzo(a)pyrene as reference substance. These results have direct implications on the toxicity assessment in the course of evaluation for PBT-properties (see section 12).

Benz(a)acridine, benz(c)acridine, benzo(b)naphtho(2,1-d)thiophene were considered to be “not classifiable as to its carcinogenicity” (IARC group 3) by Lauby-Secretan et al. (2011) due to data limitations. No potency equivalency factors were derived for these substances.

These different classifications are mainly a consequence of the varying quality of the available data for an individual chemical compound and are not per se indicative of different carcinogenic properties.

The other semipolar hydrocarbons were not classified by IARC, and only few or no data were published for them.

[33] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

Benzo(b)naphtho(1,2-d)thiophene and its methyl isomers, benzo(b)naphtho(2,3-d)thiophene, phenanthro(4,5-bcd)thiophene, 2-methyldibenzothiophene are characterized by Ames tests only. Consistent negative results in different Ames tests have been obtained with benzo(b)naphtho(1,2-d)thiophene and benzo(b)naphtho(2,3-d)thiophene, both in standard and pre-incubation protocols. The 1- and 6-methyl isomers of benzonaphthothiophenes were positive (only one test available), the other methyl isomers negative. Phenanthro(4,5-bcd)thiophene and 2-methyldibenzothiophene gave a negative result (only one test available).

The information emerging solely from in vitro mutagenicity tests is difficult to interpret. Conflicting results in comparable tests cannot be explained on this level of characterisation within the scope of this project. In general, it cannot necessarily be gathered from negative results in such tests that the substance is actually non genotoxic. This applies particularly to standard Ames tests (without pre-incubation, used by most of the researchers mentioned above) with negative outcome, e.g. contradicted by positive results in tests with a pre-incubation protocol (which seem to be more sensitive than the standard procedure). This holds true also for cellular test systems as the V79 gene mutation test (with no relevant expression of mono-oxygenases). Appropriate metabolizing systems may turn the negative result into a positive one, as the insensitivity of this cellular system can be overcome by the introduction of a cytochrome gene into the genome (Gábelová, et al., 2004) or co-cultivation systems of target cells and liver cells as exogenous metabolizing system (e.g. Parks et al., 1986; Warshawsky, 1992).

No experimental data at all are available for the dibenzoquinolines and benzonaphthofurans.

A Structural Alert Relation (SAR) analysis revealed that all the semipolar PAC priority compounds contain the structural alert “heterocyclic polycyclic aromatic hydrocarbon” (could not be differentiated within the used algorithms), which was considered to be a relevant determinant for mutagenicity in the in vivo micronucleus assay as well as for genotoxic carcinogenicity.

These estimates are corroborated by the experimental results of all of the substances from the priority list with a more extensive data base. Therefore, the other semipolar PAC with data lacks should be suspected to be mutagens (and possibly carcinogens), including those with negative results emerging only from Ames tests.

12 Summary and Conclusion on 15 Priority Semipolar PAC

In the following sections 12.2 to 12.16, most important results from experimental ecotoxicity tests (described in detail in section 10.2.5) as well as data on mutagenicity / carcinogenicity (section 11) are summarized per compound, data gaps are provisionally closed by QSAR-results. If data on bio-monitoring were identified (see section 10.2.6), these are also mentioned.

Based on available data supported by QSAR for missing data provisional PNECs are derived, provisional SVHC classifications are suggested (also including data on genotoxicity and carcinogenicity), and provisional classifications for environmental hazards according to CLP regulation (1272/2008/EC) are performed.

As data are often missing for certain endpoints (QSAR results used associated with considerable uncertainty) or not sufficiently reliable or inconclusive (further studies would be needed to draw definitive conclusions) PNECs, SVHC- and CLP-classifications are only intended to mirror the presently available data in a condensed form and guide for prioritization in regard to filling data gaps as well as possible regulatory activities further on.

Furthermore, data on occurrence are summarized. With regard to literature data, a number in brackets following a given matrix (e.g. “tar (1)”) denotes the number of occurrences within this matrix.

12.1 Provisional SVHC-Assessment – Summary on Results

Deviating from the SVHC-based assessment performed in chapter 10, actual SVHC-classifications performed in the following sections rely also on genotoxicity and carcinogenicity data as far as available and in cases where experimental data are lacking QSAR-data are used for a provisional assessment. Furthermore, following REACH guidance R.11 experimental data on biodegradation are interpreted conservatively especially if other SVHC-properties were fulfilled. This pertains to studies far from current guidelines as well as far from prevailing natural environmental conditions where no clear conclusions regarding cut-off values for degradation half-lives as given in REACH Annex XIII could be drawn. Altogether, and especially due to consideration of genotoxicity and carcinogenicity data a new perspective on SVHC-properties of priority semipolar PAC is provided.

Briefly summarized, the following compounds were provisionally classified as PBT:

- Dibenz(a,j)acridine
- Dibenz(a,h)acridine
- 7H-Dibenzo(c,g)carbazole
- 1-Methylbenzo(b)-naphtho(2,1-d)thiophene (T-assessment based on QSAR-data only)

And one further compound was provisionally classified as vPvB:

13H-Dibenzo(a,i)carbazole

Details on assessment are given in the following substance-specific sections.

12.2 Benz(a)acridine

Ecotoxicity

Data from two acute studies in aquatic invertebrates, one study on marine algae and one acute study on sediment dwelling invertebrates (Chironomus riparius larvae, tested in aquatic phase only) are available. From

these studies, highest toxicity was observed on dwelling larvae of the midge C. riparius (Bleeker, et al., 1998) with an EC_{50} (96h) of 0.015 mg/L (measured, reliable study).

Bioaccumulation

Reported bioconcentration factors in fish (Southworth, et al., 1981) and Daphnia (Verschueren, 1983) are far below 2000 (106 L/kg wet weight and 352, respectively), the data on fish being confirmed by other publications. However, BCF was approximately 1500 in relation to metabolites, which were largely retained in the fish. Details on metabolites and their possible toxicity are not given (Southworth, et al., 1981).

Persistence

No degradation of benz(a)acridine was found within 8 days at 25°C using a Pseudomonas fluorescens strain able to degrade chrysene and benzo(b)naphtho(1,2-d)thiophene (Caldini, et al., 1995). No other experimental data are available and QSAR-screening also points to persistence.

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were questionable positive *in vitro*, in vivo tests are not available. Tests on carcinogenicity were questionable negative. From SAR-analysis (Toxtree-software[34]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Based on these data the IARC (1983; 1987) classified the substance as carcinogen of group 3, this was confirmed by Lauby-Secretan et al. (2011).

Provisional $PNEC_{aqua}$-Derivation

Acute toxicity data on Daphnia, Chironomus riparius larvae and marine alga are available. The calculated LC_{50} for fish is above the experimentally determined values for the other species. From the LC_{50} of the most sensitive species Chironomus riparius of 0.015 mg/L and an assessment factor of 1000 for acute test data only the following PNEC is derived:

$PNEC_{aqua}$ = 15 ng/L

This low value seems to be very conservative. However, according to the “exposure threshold of no concern” approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology - an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) and reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). Specifically acting chemicals resulted in even lower $ETNC_{aq}$ between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L. Because specific toxicity cannot be excluded for this chemical, the corresponding $ETNC_{aq}$ of 5 ng/L is still lower than the supposed PNEC and does not warrant a less conservative approach.

Provisional SVHC-Classification

The T-criterion is presumably fulfilled (<0.1 mg/L for acute data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) based on ecotoxicity. The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

[34] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

The B-criterion is not fulfilled (BCF << 2000).

The P-criterion is assumed to be fulfilled.

Thus, according to REACH regulation the compound is neither PBT nor vPvB.

Provisional Environmental Classification

Category Acute 1, M-factor 10; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (1), tar fractions (2) and the environment (2).

Data from analytics section of the project (for details see section 16): Benz(a)acridine was determined in coal tar pitch (1149.1 mg/kg), spare tyre (61.9 mg/kg), corresponding spare tube (36.9 mg/kg), flip-flop (0.71 mg/kg), bitumen (0.07 mg/kg), rubber boots (0.05 mg/kg), furnace black (0.03 mg/kg), MES (0.03) and RAE (0.01). Concentrations in DAE and TDAE were below LOQ.

12.3 Benz(c)acridine

Ecotoxicity

Data from one study on marine algae and one acute study on sediment dwelling invertebrates (Chironomus riparius larvae, tested in aquatic phase only) are available. From these studies, highest toxicity was observed on dwelling larvae of the midge C. riparius (Bleeker, et al., 1998) with an EC_{50} (96h) of 0.0069 mg/L (measured, reliable study).

Bioaccumulation

No experimental data on bioaccumulation could be found. Log K_{ow} of 4.48 was experimentally determined for benz(a)acridine and is believed to be valid also for benz(c)acridine.

Persistence

No biodegradation could be found with the fungus Cunninghamella elegans (Holland, et al., 1986) and in BOD (6 days) studies with several sludges (Lutin, et al., 1965).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were positive *in vitro*, in vivo tests are not available. Tests on carcinogenicity were questionable positive. From SAR-analysis (Toxtree-software[35]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Based on these data the IARC (1983; 1987) classified the substance as carcinogen of group 3, this was confirmed by Lauby-Secretan et al. (2011).

[35] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

Provisional $PNEC_{aqua}$-Derivation

Acute toxicity data on Chironomus riparius larvae and marine alga are available. The calculated $L(E)C_{50}$ values for fish and daphnia are above the experimentally determined values for the other two species. From the LC_{50} of the most sensitive species Chironomus riparius of 0.0069 mg/L and an assessment factor of 1000 for acute test data only the following PNEC is derived:

$PNEC_{aqua}$ = 6.9 ng/L

This low value seems to be very conservative. However, according to the “exposure threshold of no concern” approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology - an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) and reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). Specifically acting chemicals resulted in even lower $ETNC_{aq}$ between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L. Because specific toxicity cannot be excluded for this chemical, the corresponding $ETNC_{aq}$ of 5 ng/L is still slightly lower than the supposed PNEC and does not warrant a less conservative approach.

Provisional SVHC-Classification

The T-criterion is definitely fulfilled (<0.01 mg/L for acute data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) based on ecotoxicity. The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

The screening-criterion on B (log K_{ow} >4.5) is just barely missed with a log K_{ow} of 4.48.

The P-criterion is assumed to be fulfilled.

Thus, according to REACH regulation the compound is neither PBT nor vPvB. However, as the screening criterion on B is just barely missed an experimental determination of BCF resulting in BCF >2000 would lead to a classification as PBT.

Provisional Environmental Classification

Category Acute 1, M-factor 100; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (1), tar fractions (7), pitch (2) and the environment (2).

Data from analytics section of the project (for details see section 16): Benz(c)acridine was determined in coal tar pitch (985.0 mg/kg), spare tyre (81.0 mg/kg), corresponding spare tube (55.9 mg/kg), flip-flop (0.63 mg/kg) and bitumen (0.07 mg/kg), while concentrations in rubber boots, furnace black, MES, RAE, DAE and TDAE were below LOQ.

12.4 Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline = Benzo(a)phenanthridine, 195-29-9)

Ecotoxicity

No experimental data on ecotoxicity are available. Lowest calculated effect concentration (ECOSAR from US-EPA EPI Suite) for three trophic levels is LC_{50} (48h) for Daphnid of 0.92 mg/L (ECOSAR class neutral organics).

Bioaccumulation

No experimental data on bioaccumulation could be found. Log K_{ow} of 4.49 was estimated by KOWWIN (US-EPA EPI Suite).

Persistence

No experimental data on persistence could be found. According to combined evaluation of BIOWIN 2, 3 and 6-results according to ECHA[36]-guidance on Information requirements and Chemical Safety Assessment, part R11 the substance is predicted to be persistent (P1P2).

Genotoxicity and Carcinogenicity

No studies with experimental data concerning mutagenicity or carcinogenicity were identified. From SAR-analysis (Toxtree-software[37]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Provisional $PNEC_{aqua}$-Derivation

No experimental data are available. Based on the lowest calculated LC_{50} value of three trophic levels for Daphnid of 0.92 mg/L and an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.92 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*). However, according to the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology - an $ETNC_{aq}$ for specifically acting chemicals resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005). Considering ecotoxicity of benz(a)acridine and benz(c)acridine specific toxicity can however not be ruled out for this chemical.

Provisional SVHC-Classification

The T-criterion based on QSAR-data is not fulfilled (>0.1 mg/L for acute data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) based on ecotoxicity. However, as the QSAR model is based on narcotic action, actual ecotoxicity may be considerably underestimated and experimental data are required for conclusion on T.

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[36] European Chemicals Agency, http://echa.europa.eu/web/guest/home

[37] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

No data on genotoxicity and carcinogenicity are available to decide on T in regard to germ cell mutagenicity or carcinogenicity. Structure-activity relationships (SAR) resulted in the structural alert “heterocyclic polycyclic aromatic hydrocarbon” pointing to mutagenic and genotoxic potential.

The screening-criterion on B (log K_{ow} >4.5) is just barely missed with a predicted log K_{ow} of 4.49.

The P-criterion is assumed to be fulfilled based on QSAR estimations.

Thus, according to REACH regulation the compound is neither PBT nor vPvB. However, as the screening criterion on B is just barely missed an experimental determination of BCF could result in BCF >2000. If the chemical is a specifically acting toxicant toxicity could be considerably underestimated by QSAR and experimental data could prove the T-criterion to be fulfilled. Gathering from that, properties fulfilling PBT-criteria according to REACH cannot be excluded and experimental data are mandatory.

Provisional Environmental Classification

Classification is based on QSAR-data only:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (1), tar fractions (6), pitch (4) and the environment (1).

Data from analytics section of the project (for details see section 16): Benzo(a)phenanthridine was determined in coal tar pitch (22.1 mg/kg) and spare tyre (1.8 mg/kg) while concentrations in corresponding spare tube, flip-flop, bitumen, rubber boots, furnace black, MES, RAE, DAE and TDAE were below LOQ

12.5 Dibenz(a,j)acridine

Ecotoxicity

The only experimental data available is one acute study on the fresh water alga Pseudokirchneriella subcapitata (Warshawsky, et al., 1995a). The determined EC_{50} (biomass, 6d) was larger than the exposure concentration of 0.4 mg/L. The study was conducted under cool white light (16/8 h l/d) with significant fraction of UV-A irradiation enabling phototoxicity of benzo(a)pyrene. However, no analytical determination of the test item was performed and experimentally determined water solubility (0.16 mg/L) is considerably lower than the exposure concentration, potentially leading to adsorption losses and underestimation of toxicity. QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) resulted in generally lower L(E)C_{50}-values with an LC_{50} (48h) for the most sensitive trophic level (Daphnid) of 0.123 mg/L (ECOSAR class neutral organics).

Bioaccumulation

Experimental data are restricted to log K_{ow}, which was determined to be 5.63 (Helweg, et al., 1997 as cited in SRC-database).

Persistence

No relevant biodegradation was observed in soils within 64 days (Grosser, et al., 1995) and determinations of BOD (6 days) using different sludges (Lutin, et al., 1965). From the data of Gosser et al. a half-life in soils of be >160 days was calculated (Aronson, et al., 1998).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were questionable positive *in vitro* and positive *in vivo*. Tests on carcinogenicity were positive.

The IARC (1983; 1987) classified the substance as carcinogen of group 2B, which was recently altered to group 2A due to strong mechanistic evidence (genotoxicity) contributing to the overall evaluation (Lauby-Secretan, et al., 2011). The National Toxicology Program considered the substance as "reasonably anticipated to be a human carcinogen (NTP, 2011). A potency equivalency factor of 0.1 was derived in relation to benzo(a)pyrene as reference substance (Collins, et al., 1998)

Provisional $PNEC_{aqua}$-Derivation

Experimental results are only available for alga and QSAR-results are generally lower with an LC_{50} (48h) for the most sensitive species (Daphnid) of 0.123 mg/L. Using an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.12 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*). However, according to the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology - an $ETNC_{aq}$ for specifically acting chemicals resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005). Considering ecotoxicity of benz(a)acridine as well as mutagenic and carcinogenic properties of dibenz(a,j)acridine itself, specific toxicity can however not be ruled out for this chemical.

Provisional SVHC-Classification

The T-criterion based on QSAR-data is just barely missed (>0.1 mg/L for acute data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) based on ecotoxicity. However, as the QSAR model is based on narcotic action, actual ecotoxicity may be considerably underestimated and experimental data are required for conclusion on T.

The IARC-classification as carcinogen of group 2A supported by the conclusion of the National Toxicology Program justifies a classification as T in regard to carcinogenicity according to REACH Annex XIII.

The screening-criterion on B (log K_{ow} >4.5) is met with an experimentally determined log K_{ow} of 5.63.

The P-criterion is assumed to be fulfilled based on QSAR estimations.

Thus, according to REACH regulation in combination with REACH guidance the compound is provisionally classified as PBT.

Provisional Environmental Classification

Classification is based on QSAR-data:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar fractions (2), pitch (1), and the environment (3).

Data from analytics section of the project (for details see section 16): Dibenz(a,j)acridine was determined in coal tar pitch (310.8 mg/kg), spare tyre (10.4 mg/kg), corresponding spare tube (4.9 mg/kg), while concentrations in flip-flop, bitumen, rubber boots, furnace black, MES, RAE, DAE and TDAE were below LOQ.

12.6 Dibenz(a,h)acridine

Ecotoxicity

No experimental data on ecotoxicity are available. Lowest calculated effect concentration (ECOSAR from US-EPA EPI Suite) for three trophic levels is LC_{50} (48h) for Daphnid of 0.123 mg/L (ECOSAR class neutral organics).

Bioaccumulation

The BCF was determined in fish in a reliable study to be 107.2 L/kg wet weight due to metabolism/excretion in fish (Southworth, et al., 1980).

BCF for Daphnia pulex determined in the same study was 3500.

Persistence

Only low to very low biodegradation could be found by determining the BOD (6 days) using different sludges and concentrations thereof (Lutin, et al., 1965; Malaney, et al., 1967). This is corroborated by combined evaluation of BIOWIN 2, 3 and 6-results according to ECHA[38]-guidance on Information requirements and Chemical Safety Assessment, part R11 qualifying the substance as persistent (P1P2).

Bio-Monitoring

Dibenz(a,h)acridine has been detected in digestive glands of lobsters. No other data were identified (see section 10.2.6).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were questionable negative *in vitro* and positive *in vivo*. Tests on carcinogenicity were positive.

The IARC (1983; 1987) classified the substance as carcinogen of group 2B, this was confirmed by Lauby-Secretan et al. (2011). The National Toxicology Program evaluated the substance as “reasonably anticipated to be a human carcinogen” (NTP, 2011). A potency equivalency factor of 0.1 was derived for carcinogenicity in relation to benzo(a)pyrene as reference substance (Collins, et al., 1998).

[38] European Chemicals Agency, http://echa.europa.eu/web/guest/home

Provisional $PNEC_{aqua}$-Derivation

No experimental data are available. Based on the lowest calculated LC_{50} value of three trophic levels for Daphnid of 0.123 mg/L and an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.12 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*). However, according to the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology - an $ETNC_{aq}$ for specifically acting chemicals resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005). Considering ecotoxicity of benz(c)acridine as well as mutagenic and carcinogenic properties of dibenz(a,h)acridine itself, specific toxicity can however not be ruled out for this chemical.

Provisional SVHC-Classification

The T-criterion based on QSAR-data is just barely missed (>0.1 mg/L for acute data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) based on ecotoxicity. However, as the QSAR model is based on narcotic action, actual ecotoxicity may be considerably underestimated and experimental data are required for conclusion on T.

The IARC-classification as carcinogen of group 2B underpinned by the conclusion of the National Toxicology Program qualifying the substance as "reasonably anticipated to be a human carcinogen" justifies a classification as T in regard to carcinogenicity according to REACH Annex XIII.

The REACH criterion for B (BCF >2000) is met in case of Daphnia pulex (3500) however failed for fish. As also bioconcentration in other aquatic organisms than fish is relevant (REACH Annex XIII) the compound is still classified as B.

The P-criterion is assumed to be fulfilled based experimental data and QSAR estimations.

Thus, according to REACH regulation in combination with REACH guidance the compound is provisionally classified as PBT.

Provisional Environmental Classification

Classification is based on QSAR-data only:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in environmental matrices only (2).

Data from analytics section of the project (for details see section 16): Dibenz(a,h)acridine was determined in coal tar pitch (517.7 mg/kg), spare tyre (24.7 mg/kg), corresponding spare tube (17.4 mg/kg), flip-flop (0.13 mg/kg), furnace black (0.043 mg/kg), bitumen (0.02 mg/kg), rubber boots (0.02 mg/kg), RAE (0.01 mg/kg), while concentrations in MES, DAE and TDAE were below LOQ.

12.7 13H-Dibenzo(a,i)carbazole

Ecotoxicity

No experimental data on ecotoxicity are available. Lowest calculated effect concentration (ECOSAR from US-EPA EPI Suite) for three trophic levels is LC_{50} (48h) for Daphnid of 0.139 mg/L (ECOSAR class neutral organics).

Bioaccumulation

The BCF was determined in fish (48h, Poecilia reticulata) to be 7060 L/kg wet weight (de Voogt, et al., 1991).

This is supported by a BCF of 7126 L/kg wet weight for Daphnia pulex determined in a reliable study (Southworth, et al., 1979).

Persistence

No experimental data available. However, the closely related isomer 7H-Dibenzo(c,g)carbazole proved to be recalcitrant in biodegradation tests and its aromatic nitrogen is sterically better accessible than in the 13H-Dibenzo(a,i)carbazole isomer.

This is corroborated by combined evaluation of BIOWIN 2, 3 and 6-results according to ECHA[39]-guidance on Information requirements and Chemical Safety Assessment, part R11 qualifying the substance as persistent (P1P2).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were negative *in vitro* and positive *in vivo*. Tests on carcinogenicity were questionable positive. From SAR-analysis (Toxtree-software[40]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Provisional $PNEC_{aqua}$-Derivation

No experimental data are available. Based on the lowest calculated LC_{50} value of three trophic levels for Daphnid of 0.139 mg/L and an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.14 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*) and this may also hold true for reactive toxicity following the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology. Here, an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) *and* reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). However, in case of specifically acting chemicals $ETNC_{aq}$ resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005).

[39] European Chemicals Agency, http://echa.europa.eu/web/guest/home

[40] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

Provisional SVHC-Classification

The T-criterion based on QSAR-data is just barely missed (>0.1 mg/L for acute data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) based on ecotoxicity. However, as the QSAR model is based on narcotic action, actual ecotoxicity may be considerably underestimated and experimental data are required for conclusion on T.

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

The REACH criterion for vB according to Annex XIII (BCF >5000) is met in case of fish (7060) and Daphnia (7126).

The P-criterion is assumed to be fulfilled based on experimental data on the closely related isomer 7H-Dibenzo(c,g)carbazole and QSAR estimations.

According to REACH regulation and REACH guidance documents the compound is not PBT. However, as the screening criterion on T is just barely missed an experimental determination of ecotoxicity could very well result in classification as PBT, as the other two criteria are fulfilled.

According to REACH regulation and REACH guidance documents the compound is provisionally classified as vPvB. While experimental or monitoring data pointing to half-lives ≤ 60 days are missing, vP may be assumed.

Provisional Environmental Classification

Classification is based on QSAR-data only:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in a tar fraction (1).

Data from analytics section of the project (for details see section 16): Dibenzo(a,i)carbazole was determined in coal tar pitch (187.6 mg/kg), spare tyre (11.3 mg/kg), corresponding spare tube (7.3 mg/kg), flip-flop (0.13 mg/kg), bitumen (0.06 mg/kg) and RAE (0.03), while concentrations in rubber boots, furnace black, MES, DAE and TDAE were below LOQ.

12.8 7H-Dibenzo(c,g)carbazole

Ecotoxicity

The only experimental data available is one acute study on the fresh water alga Pseudokirchneriella subcapitata (Warshawsky, et al., 1995a). The determined EC_{50} (biomass, 6d) was less than the exposure concentration of 0.4 mg/L. The study was conducted under cool white light (16/8 h l/d) with significant fraction of UV-A irradiation enabling phototoxicity of benzo(a)pyrene. However, no analytical determination of the test item was performed and experimentally determined water solubility (0.010-0.063 mg/L) is considerably lower than the exposure concentration, potentially leading to adsorption losses and underestimation of toxicity. QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) resulted in L(E)C_{50}-values generally lower than 0.4 mg/L with an LC_{50} (48h) for the most sensitive trophic level (Daphnid) of 0.139 mg/L (ECOSAR class neutral organics).

Bioaccumulation

There are no experimental data on BCF or BAF for this compound besides an experimentally determined log K_{ow} of 6.4 (Hansch, et al., 1995 as cited in KOWWIN data reference). However, the closely related isomer 13H-Dibenzo(a,i)carbazole proved to be very bioaccumulative (vB) according to REACH criteria. Supporting data show persistent DNA-adduct formation in English Sole liver (Stein, et al., 1993) and in liver, brain and gills of Northern Pike (Ericson, et al., 1999) which point to a potential for bioaccumulation (besides the potential for genotoxicity).

Persistence

From mineralization experiments in soils where no mineralization within 64 days could be found (Grosser, et al., 1995) half-life in soils was calculated to be >160 days (Aronson, et al., 1998). No development of degrading microbial cultures during 6 weeks by an enrichment process from water could be obtained by Bohonos et al. (1977).

For UV-induced photodegradation in water under sunlight (with 0,1% Acetonitrile as co-solvent) a half-life of 30 minutes was determined (Mill, et al., 1981).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were positive *in vitro* and *in vivo*. Tests on carcinogenicity were positive. The IARC (1983; 1987) classified the substance as carcinogen of group 2B, this was confirmed by Lauby-Secretan et al. (2011). The National Toxicology Program evaluated the substance as “reasonably anticipated to be a human carcinogen (NTP, 2011). A potency equivalency factor of 1 was derived in relation to benzo(a)pyrene as reference substance (Collins, et al., 1998).

Provisional $PNEC_{aqua}$-Derivation

Experimental results are only available for alga and QSAR-results are generally lower with an LC_{50} (48h) for the most sensitive species (Daphnid) of 0.139 mg/L. Using an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.14 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*) and this may also hold true for reactive toxicity following the “exposure threshold of no concern” approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology. Here, an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) *and* reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). However, in case of specifically acting chemicals $ETNC_{aq}$ resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005). Considering the mutagenic and carcinogenic properties of the compound, specific toxicity can however not be ruled out for this chemical.

Provisional SVHC-Classification

The T-criterion based on QSAR-data is just barely missed (>0.1 mg/L for acute data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) based on ecotoxicity. However, as the QSAR model is based on narcotic action, actual ecotoxicity may be considerably underestimated and (further) experimental data are required for conclusion on T.

The IARC-classification as carcinogen of group 2B underpinned by the conclusion of the National Toxicology Program qualifying the substance as “reasonably anticipated to be a human carcinogen” and the determined potency equivalency factor of 1 in relation to benzo(a)pyrene justify a classification as T in regard to carcinogenicity according to REACH Annex XIII.

Concluding from log K_{ow} of 6.4 and the closely related isomer 13H-Dibenzo(a,i)carbazole (vB according to REACH) the compound fulfills at least the REACH criterion for B with a potential for vB, the latter however would have to be confirmed by experimental data.

The P-criterion is assumed to be fulfilled. However, as UV-induced photodegradation in water under sunlight has been shown, primary degradation in surface near water layers is expected.

According to REACH regulation and REACH guidance documents the compound is provisionally classified as PBT.

Provisional Environmental Classification

Classification is based on QSAR-data supported by an experimental result for alga:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in pitch (1), tar fractions (2) and the environment (2).

Data from analytics section of the project (for details see section 16): Dibenzo(c,g)carbazole was determined in coal tar pitch (73.3 mg/kg), spare tyre (9.3 mg/kg), corresponding spare tube (6.9 mg/kg), while concentrations in flip-flop, bitumen, rubber boots, furnace black, MES, RAE, DAE and TDAE were below LOQ.

12.9 Benzo(b)naphtho(1,2-d)thiophene

Ecotoxicity

The only experimental data available is one reliable acute study on Daphnia magna with a determined EC_{50} (48h, mobility) of 0.22 mg/L (P95-CI: 0.091-0.380) (Eastmond, et al., 1984). QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) resulted in an LC_{50} (48h) for Daphnid of 0.192 mg/L (ECOSAR class neutral organics) which is very close to the experimental value. According to QSAR the acute most sensitive organism is Daphnia.

Bioaccumulation

There are no experimental data on BCF or BAF for this compound besides an experimentally determined log K_{ow} of 5.19 (Andersson and Schräder, 1999 as cited in SRC-database).

For the structurally similar isomer benzo(b)naphtho(2,1-d)thiophene the BCF for Daphnia was determined as 2300 (reliable study; Eastmond, et al., 1984). For another structurally similar isomer, benzo(b)naphtho(2,3-d)thiophene, a BCF in fish of 14900 L/kg wet weight (de Voogt, et al., 1991) is reported (data not reliable). For details see compound specific sections.

Persistence

Caldini et al. (1995) used a strain of Pseudomonas fluorescens isolated from a site heavily contaminated by exhausted lubricating oils and showed a potential for biodegradation. Also Lundstedt et al (2003) demonstrated a potential for at least primary biodegradation of the compound as part of a contaminated gas work soil-water slurry supplemented with nutrients and cultures designed for PAH-degradation in soil.

Bio-Monitoring

Benzo(b)naphtho(1,2-d)thiophene has been detected in biota, namely crayfish and sunfish. No other data on bio-monitoring were identified (see section 10.2.6).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were negative *in vitro* and not determined *in vivo*. Tests on carcinogenicity are not available. From SAR-analysis (Toxtree-software[41]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Provisional $PNEC_{aqua}$-Derivation

The only experimental result available is a reliable EC_{50} (48h) for Daphnia magna of 0.22 mg/L. From QSAR prediction Daphnia is the acute most sensitive species. The QSAR-derived value is close to the experimental value. Based on the experimentally determined LC_{50} using an assessment factor of 1000 for acute test data only the following QSAR-supported PNEC is derived:

$PNEC_{aqua}$ = 0.22 µg/L

This value can be considered to be protective in regard to Daphnia and protective as relates to narcotic and reactive toxicity in regard to the other two trophic levels according to the “exposure threshold of no concern” approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology. Here, an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) *and* reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). However, in case of specifically acting chemicals $ETNC_{aq}$ resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005). Currently no indications for a toxicity based on specific interactions are available.

Provisional SVHC-Classification

The T-criterion based on an acute experimental EC_{50} for Daphnia magna and QSAR-data for the other trophic levels is missed (>0.1 mg/L for acute data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) based on ecotoxicity. However, ecotoxicity is close to the screening criterion and further (preferably chronic) toxicity tests are necessary to reliably exclude T[42].

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

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[41] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

[42] T-criterion according to REACH Annex XIII for chronic data: NOEC < 0.01 mg/L

Concluding from log K_{ow} of 5.19 and bioconcentration factors determined for the structurally similar isomers benzo(b)naphtho(2,1-d)thiophene with Daphnia of 2300 (reliable study) and benzo(b)naphtho(2,3-d)thiophene with fish of 14900 (data not reliable) a bioconcentration potential for benzo(b)naphtho(1,2-d)thiophene is probable and a classification as B warranted from the screening criterion of REACH guidance R.11 (log K_{ow} > 4.5).

The P-criterion is assumed to be fulfilled. The design of available studies demonstrating some potential for biodegradation is too far from guideline studies as well as natural environmental conditions to invalidate QSAR-derived persistency predictions (P1P2 based on BIOWIN modules of US-EPA´s EPI Suite).

According to REACH regulation and REACH guidance documents the compound is not classified as SVHC as the T-criterion is not fulfilled based on the inadequate data base.

Provisional Environmental Classification

Classification is based on experimental EC_{50} for Daphnia magna supported by QSAR-data for the other two trophic levels:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (1), a tar fraction (1), pitch (2) and the environment (1).

Data from analytics section of the project (for details see section 16): Benzo(b)naphtho(1,2-d)thiophene was determined in coal tar pitch (380.2 mg/kg), spare tyre (126.6 mg/kg), corresponding spare tube (105.9 mg/kg), DAE (1.9 mg/kg), flip-flop (1.3 mg/kg), TDAE (0.21 mg/kg), MES (0.08 mg/kg), RAE (0.07 mg/kg), rubber boots (0.04 mg/kg), bitumen (0.03 mg/kg) and furnace black (0.02 mg/kg).

12.10 Benzo(b)naphtho(2,1-d)thiophene

Ecotoxicity

The only experimental data available is one reliable acute study on Daphnia magna where no toxicity was observed (48 h). The limit concentration is however unclear and it can only be guessed that it was at least as high as the determined EC_{50} of 0.22 mg/L for the structural isomer benzo(b)naphtho(1,2-d)thiophene determined in the same study (Eastmond, et al., 1984), i.e. EC_{50} (48h) for Daphnia magna >0.22 mg/L. QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) resulted in an LC_{50} (48h) for Daphnid of 0.192 mg/L (ECOSAR class neutral organics) as the most sensitive species and slightly higher values for fish (0.21 mg/L) and algae (0.39 mg/L).

Bioaccumulation

Eastmond et al. (1984) assessed bioconcentration in Daphnia magna using radiolabeled compound (reliability category 2). The BCF determined after 70 hours of incubation was approximately 2300 dpm tissue / dpm water (taking into account 5% volatilization) and reached a kind of steady state. The experimentally determined log K_{ow} is 5.19 (Andersson and Schräder, 1999 as cited in SRC-database).

Persistence

In vitro desulfurization (12 h) to 1-hydroxy-2-phenyl-naphthalene was demonstrated by of Rhodococcus erythropolis H-2 whole cell assay using high concentration of lyophilized cells (Ohshiro, et al., 1996).

Using highly concentrated cell suspensions of Mycobacterium G3 again desulfurization was demonstrated at 37°C in 1% DMF with partly methoxylated hydroxy-biphenyl derivatives as products (Okada, et al., 2002).

Lundstedt et al (2003) demonstrated a potential for at least primary biodegradation of the compound as part of a contaminated gas work soil-water slurry supplemented with nutrients and cultures designed for PAH-degradation in soil.

Bio-Monitoring

Benzo(b)naphtho(2,1-d)thiophene has been detected in biota, namely crayfish, lobster and sunfish. No other data on bio-monitoring were identified (see section 10.2.6).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were questionable positive *in vitro*, in vivo tests are not available. Tests on carcinogenicity were questionable positive. From SAR-analysis (Toxtree-software[43]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

The IARC recently classified the substance as carcinogen of group 3 (Lauby-Secretan, et al., 2011).

Provisional $PNEC_{aqua}$-Derivation

The only experimental result available points to an EC_{50} (48h) for Daphnia magna of >0.22 mg/L. From QSAR prediction Daphnia is the acute most sensitive species with an LC_{50} (48h) for Daphnid of 0.192 mg/L. Based on the experimentally determined acute limit concentration for Daphnia magna using an assessment factor of 1000 for acute test data only the following QSAR-supported PNEC is derived:

$PNEC_{aqua}$ = 0.22 µg/L

This value can be considered to be protective in regard to Daphnia and protective as relates to narcotic and reactive toxicity in regard to the other two trophic levels according to the “exposure threshold of no concern” approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology. Here, an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) *and* reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). However, in case of specifically acting chemicals $ETNC_{aq}$ resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005).

Provisional SVHC-Classification

The T-criterion based on an acute experimental data on Daphnia magna (EC_{50} > limit concentration of probably ≥ 0.22 mg/L) and QSAR-data for the other trophic levels is missed (>0.1 mg/L for acute data according

[43] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11) in relation to ecotoxicity. Further tests are necessary to reliably exclude T[44].

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

Concluding from log K_{ow} of 5.19 and a bioconcentration factor determined for Daphnia of 2300 (reliable study) classification as B is warranted according to REACH Annex XIII and screening criteria of REACH guidance R.11 (log $K_{ow} > 4.5$).

The P-criterion is assumed to be fulfilled. The design of available studies demonstrating some potential for desulfuration or biodegradation is too far from guideline studies as well as natural environmental conditions to invalidate QSAR-derived persistency predictions (P1P2 based on BIOWIN modules of US-EPA´s EPI Suite).

According to REACH regulation and REACH guidance documents the compound is not classified as SVHC as the T-criterion is not fulfilled based on the inadequate data base.

Provisional Environmental Classification

Classification is based on QSAR-data for all three trophic levels:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (2), a tar fractions (2), pitch (2), carbon black (2), bitumen (8) and the environment (3).

Data from analytics section of the project (for details see section 16): Benzo(b)naphtho(2,1-d)thiophene was determined in coal tar pitch (2141.8 mg/kg), spare tyre (615.7 mg/kg), corresponding spare tube (519.9 mg/kg), DAE (6.2 mg/kg), flip-flop (6.8 mg/kg), TDAE (1.0 mg/kg), MES (0.41 mg/kg), RAE (0.32 mg/kg), rubber boots (0.12 mg/kg), bitumen (0.07 mg/kg) and furnace black (0.09 mg/kg).

12.11 1-Methylbenzo(b)-naphtho(2,1-d)thiophene

Ecotoxicity

No experimental data on ecotoxicity are available. Lowest calculated effect concentrations (ECOSAR from US-EPA EPI Suite) for three trophic levels are LC_{50} (48 h) for Daphnid of 0.073 mg/L and LC_{50} (96 h) for fish of also 0.073 mg/L (ECOSAR class neutral organics).

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. The log K_{ow} was calculated as 5.89 (KOWWIN of US-EPA´s EPI Suite; experimental log K_{ow} for aromatic core available, see above). For the non-methylated core (benzo(b)naphtho(2,1-d)thiophene) a BCF in Daphnia of 2300 had been determined.

[44] T-criterion according to REACH Annex XIII for chronic data: NOEC < 0.01 mg/L

Persistence

Cleavage of a methylation isomer, 10-methyl-benzo(b)naphtho(2,1-d)thiophene was assessed using engineered Mycobacterium strain MR65. Reported desulfurization activity was only 50% of non-methylated core (Watanabe, et al., 2003), pointing to an even lower biodegradation potential for the methylated isomers.

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were positive *in vitro* for the 1- and 6-, but not for the 2- and 10-isomers. *In vivo*-tests are not available, neither are tests on carcinogenicity . From SAR-analysis (Toxtree-software[45]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Provisional $PNEC_{aqua}$-Derivation

No experimental data are available. Based on the lowest calculated LC_{50} values of three trophic levels for Daphnid and fish of 0.073 mg/L and an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.073 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*) and this may also hold true for reactive toxicity following the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology. Here, an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) *and* reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). However, in case of specifically acting chemicals $ETNC_{aq}$ resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005).

Provisional SVHC-Classification

T is presumably fulfilled based on QSAR data and T screening-criteria of REACH guidance on information requirements and chemical safety assessment, chapter R.11 (< 0.1 mg/L for acute effect data) as regards ecotoxicity. However, experimental data are required to reliably conclude on T.

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

Concluding from calculated log K_{ow} of 5.89 and a bioconcentration factor determined with the non-methylated core for Daphnia of 2300 (reliable study) classification as B is warranted according to REACH Annex XIII and screening criteria of REACH guidance R.11 (log K_{ow} > 4.5).

The P-criterion is assumed to be fulfilled. An available study demonstrates an even lower desulfuration potential than for the non-methylated core and thus corroborates QSAR-derived persistency predictions (P1P2 based on BIOWIN modules of US-EPA´s EPI Suite).

According to REACH regulation and REACH guidance documents the compound is provisionally classified as PBT relying solely on QSAR-data for ecotoxicity.

[45] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

Provisional Environmental Classification

Classification is based on QSAR-data only:

Category Acute 1, M-factor 10; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (2), a tar fraction (1), pitch (1), a hydrotreated oil fraction (1) and the environment (1).

Data from analytics section of the project (for details see section 16): 1-methyl-benzo(b)naphtho(2,1-d)thiophene was determined in spare tube (9.7 mg/kg), corresponding spare tyre (8.3 mg/kg), DAE (2.3 mg/kg), flip-flop (0.98 mg/kg), TDAE (0.42 mg/kg), RAE (0.17 mg/kg), MES (0.11 mg/kg), rubber boots (0.03 mg/kg), bitumen (0.017 mg/kg), while it was below LOQ in coal tar pitch and furnace black.

The rather unexpected finding that 1-methyl-benzo(b)naphtho(2,1-d)thiophene was below LOQ in coal tar pitch fits with observations that in contrast to petrogenic PAH, where often alkyl PAH are more abundant than the parent compounds, in pyrogenic samples the parent PAH dominate in relation to alkyl PAH and alkylation extent is much more limited (Neff, et al., 2005).

12.12 Benzo(b)naphtho(2,3-d)thiophene

Ecotoxicity

No experimental data on ecotoxicity are available. QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) resulted in an LC_{50} (48h) for Daphnid of 0.192 mg/L (ECOSAR class neutral organics) as the most sensitive species and slightly higher values for fish (0.21 mg/L) and algae (0.39 mg/L).

Bioaccumulation

An experimental log K_{ow} of 5.34 is reported for the compound (SRC-Database[46]). The BCF was determined in fish (48h, Poecilia reticulata) to be 14900 L/kg wet weight (de Voogt, et al., 1991) using the unlabelled compound and the method of Banerjee et al. (1984). A high standard error is associated with this value due to high uncertainty of depuration rate constant k2. However, taking the lower limit of the uptake rate constant and the higher limit of the depuration rate constant still gives a BCF of 3550 (k1/k2). A further drawback of the study is that potential metabolism is not assessed.

For the structural isomer benzo(b)naphtho(2,1-d)thiophene a BCF in Daphnia magna of 2300 had been determined.

Persistence

Lundstedt et al (2003) demonstrated a potential for at least primary biodegradation of the compound as part of a contaminated gas work soil-water slurry supplemented with nutrients and cultures designed for PAH-degradation in soil.

[46] SRC PhysProp Database, http://www.syrres.com/what-we-do/databaseforms.aspx?id=386

Bio-Monitoring

Benzo(b)naphtho(2,3-d)thiophene has been detected in digestive glands of lobsters. No other data on bio-monitoring were identified (see section 10.2.6).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were negative *in vitro* and not determined *in vivo*. Tests on carcinogenicity are not available. From SAR-analysis (Toxtree-software[47]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Provisional $PNEC_{aqua}$-Derivation

No experimental data are available. Based on the lowest calculated LC_{50} value of three trophic levels for Daphnid of of 0.192 mg/L and an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.19 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*) and this may also hold true for reactive toxicity following the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology. Here, an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) *and* reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). However, in case of specifically acting chemicals $ETNC_{aq}$ resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005). Currently no indications for a toxicity based on specific interactions are available.

Provisional SVHC-Classification

The T-criterion is missed in relation to ecotoxicity. Assessment is based solely on QSAR-data and the limit value of 0.1 mg/L for acute effect data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11. The predicted value is close to the screening limit and experimental determination of ecotoxicity (preferably chronic data) is crucial to reliably exclude T[48].

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

Concluding from log K_{ow} of 5.34 and a bioconcentration factor determined for fish of 14900 (lower limit 3550, potential metabolism not assessed) classification as B is warranted according to REACH Annex XIII and screening criteria of REACH guidance R.11 (log K_{ow} > 4.5). Taking the reported BCF for fish of 14900 vB would result. The study however is not reliable enough for such a conclusion.

The P-criterion is assumed to be fulfilled. The design of the available studies demonstrating some potential for at least primary biodegradation is too far from guideline studies as well as natural environmental condi-

[47] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

[48] T-criterion according to REACH Annex XIII for chronic data: NOEC < 0.01 mg/L

tions to invalidate QSAR-derived persistency predictions (P1P2 based on BIOWIN modules of US-EPA´s EPI Suite).

According to REACH regulation and REACH guidance documents the compound is not classified as SVHC as the T-criterion is not fulfilled based on QSAR derived ecotoxicity data only.

Provisional Environmental Classification

Classification is based on QSAR-data only:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (1), a tar fraction (1), pitch (2) and the environment (2).

Data from analytics section of the project (for details see section 16): Benzo(b)naphtho(2,3-d)thiophene was determined in coal tar pitch (691.0 mg/kg), spare tyre (209.4 mg/kg), corresponding spare tube (178.6 mg/kg), flip-flop (1.6 mg/kg), DAE (1.0 mg/kg), RAE (0.17 mg/kg), TDAE (0.14 mg/kg), MES (0.04 mg/kg), bitumen (0.04 mg/kg), furnace black (0.04 mg/kg) and rubber boots (0.02 mg/kg).

12.13 Phenanthro(4,5-bcd)thiophene

Ecotoxicity

No experimental data on ecotoxicity are available. QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) resulted in an LC_{50} (48h) for Daphnid of 0.514 mg/L (ECOSAR class neutral organics) as the most sensitive species and slightly higher values for fish (0.61 mg/L) and algae (0.81 mg/L).

Bioaccumulation

There are no experimental data on BCF or BAF for this compound besides an experimentally determined log K_{ow} of 4.95 (Andersson and Schräder, 1999 as cited in SRC-database).

Persistence

Lundstedt et al (2003) demonstrated a potential for at least primary biodegradation of the compound as part of a contaminated gas work soil-water slurry supplemented with nutrients and cultures designed for PAH-degradation in soil. Phenanthro(4,5-bcd)thiophene was degraded by 77% within 29 days (initial concentration not given).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties were negative *in vitro*. *In vivo*-tests are not available, neither are tests on carcinogenicity. From SAR-analysis (Toxtree-software[49]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

[49] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

Provisional $PNEC_{aqua}$-Derivation

No experimental data are available. Based on the lowest calculated LC_{50} value of three trophic levels for Daphnid of of 0.514 mg/L and an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.51 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*) and this may also hold true for reactive toxicity following the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology. Here, an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) *and* reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005). However, in case of specifically acting chemicals $ETNC_{aq}$ resulted in considerably lower values between 0.4 (based on chronic data) and 5.0 (based on extrapolated acute data) ng/L (de Wolf, et al., 2005). Currently no indications for a toxicity based on specific interactions are available.

Provisional SVHC-Classification

The T-criterion is missed in relation to ecotoxicity. Assessment is based solely on QSAR-data and the limit value of 0.1 mg/L for acute effect data according to screening criterion of REACH guidance on information requirements and chemical safety assessment, chapter R.11. The predicted value is quite close to the screening limit and experimental determination of ecotoxicity (preferably chronic data) is crucial to reliably exclude T[50].

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

Concluding from log K_{ow} of 4.95, classification as B is warranted according to screening criteria of REACH guidance R.11 (log K_{ow} > 4.5).

Fulfillment of the P-criterion is questionable. The available study reported 77% biodegradation within 28 days, which is the highest value out of the 4 5-ringed structural thiophene-based isomers investigated (see compound related sections). However, obviously only primary biodegradation was assessed and the study design is too far from guideline studies as well as natural environmental conditions to invalidate QSAR-derived persistency predictions (P1P2 based on BIOWIN modules of US-EPA´s EPI Suite) without further support, e.g. bio-monitoring results.

According to REACH regulation and REACH guidance documents the compound is not classified as SVHC as the T-criterion is not fulfilled based on QSAR derived ecotoxicity data only.

Provisional Environmental Classification

Classification is based on QSAR-data only:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

[50] T-criterion according to REACH Annex XIII for chronic data: NOEC < 0.01 mg/L

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (2), a tar fraction (1), pitch (2), carbon black (3) and the environment (3).

Data from analytics section of the project (for details see section 16): Phenanthro(4,5-bcd)thiophene was determined in coal tar pitch (426.3 mg/kg), spare tyre (153.7 mg/kg), corresponding spare tube (141.7 mg/kg), furnace black (7.4 mg/kg), flip-flop (1.1 mg/kg), TDAE (0.10 mg/kg), DAE (0.08 mg/kg), RAE (0.08 mg/kg), rubber boots (0.04 mg/kg), MES (0.03 mg/kg) and bitumen (0.02 mg/kg).

12.14 2-Methyldibenzothiophene and methylation isomers

Ecotoxicity

Experimental data on toxicity are only available for the non-methylated core (i.e. dibenzothiophene). Most sensitive species was Daphnia magna with an EC_{50}(24 h) of 0.2 mg/L (Sagner, 2009). Lower acute toxicity was determined for fish (0.33 mg/L) and alga (4 mg/L). According to QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) for 2-methyldibenzothiophene the most sensitive species is again Daphnia, however the predicted LC_{50} (48h) for Daphnid of 0.528 mg/L (ECOSAR class neutral organics) is higher than the experimentally determined value for the non-methylated core, pointing to an underestimation of toxicity by QSAR. This is corroborated by comparison of the experimentally determined toxicity to daphnia to the QSAR-derived value for the non-methylated core (1.37 mg/L).

Thus, considering the inadequate data base key value for acute ecotoxicity of 2-methyldibenzothiophene is EC_{50}(24 h, Daphnia magna) of 0.2 mg/L determined for the non-methylated core.

Bioaccumulation

There are no experimental data on BCF, BAF or log K_{ow} for this compound. The log K_{ow} was calculated as 4.71 (KOWWIN of US-EPA´s EPI Suite; experimental log K_{ow} for aromatic core available, see above).

Persistence

Lundstedt et al (2003) demonstrated a potential for at least primary biodegradation of the compound as part of a contaminated gas work soil-water slurry supplemented with nutrients and cultures designed for PAH-degradation in soil. 2-methyldibenzothiophene was degraded by 91% within 29 days (initial concentration not given). This is corroborated by several other studies.

Bio-Monitoring

Several methyldibenzothiophenes have been detected in biota, namely crayfish, lobster, sunfish and mussels. There are indications that methyldibenzothiophenes are more relevant in terms of bioaccumulation than is the non-methylated aromatic core (see section 10.2.6).

Genotoxicity and Carcinogenicity

Tests on mutagenic properties for several methylation isomers including 2-methyldibenzothiophene were negative *in vitro*. *In vivo*-tests are not available, neither are tests on carcinogenicity. From SAR-analysis

(Toxtree-software[51]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Provisional $PNEC_{aqua}$-Derivation

The PNEC-derivation is based on the EC_{50}(24 h, Daphnia magna) of 0.2 mg/L determined for non-methylated dibenzothiophene supported by QSAR-estimations. With an assessment factor of 1000 for acute test data only the following provisional PNEC is derived:

$PNEC_{aqua}$ (dibenzothiophene) = 0.2 µg/L

This value can be considered to be protective a priori only for non-methylated dibenzothiophene. However, following the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology, an $ETNC_{aq}$ for inert (baseline toxicity including polar narcosis) *and* reactive chemicals of 0.1 µg/L was derived (de Wolf, et al., 2005).

Thus, as long as experimental data for 2-methyldibenzothiophene are missing, a $PNEC_{aqua}$ based on the $ETNC_{aq}$ of 0.1 µg/L is suggested.

Provisional SVHC-Classification

The T-criterion is missed in relation to ecotoxicity regardless if QSAR-data for the compound are used or experimental data for the non-methylated core (effect concentrations > limit value of 0.1 mg/L for acute effect data according to screening criterion of REACH guidance R.11). The experimentally determined acute Daphnia toxicity for the non-methylated core is however quite close to the screening limit. Therefore experimental determination of ecotoxicity (preferably chronic data) for 2-methyldibenzothiophene would be crucial to reliably exclude T[52].

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

Concluding from calculated log K_{ow} of 4.71, classification as B is warranted according to screening criteria of REACH guidance R.11 (log K_{ow} > 4.5).

The P-criterion is not fulfilled. All available biodegradability studies demonstrated degradation to mostly high degrees. Also the QSAR-derived persistency prediction (BIOWIN modules of US-EPA´s EPI Suite) resulted in "not P".

According to REACH regulation and REACH guidance documents the compound is not classified as SVHC as neither the P nor the T-criterion (data missing) are fulfilled.

Provisional Environmental Classification

Classification is based on QSAR-data and data for the non-methylated core:

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

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[51] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

[52] T-criterion according to REACH Annex XIII for chronic data: NOEC < 0.01 mg/L

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (2), a tar fraction (2), a hydrotreated oil fraction (1) and the environment (3).

Data from analytics section of the project (for details see section 16): 2-Methyldibenzothiophene was determined in spare tyre (36.8 mg/kg), corresponding spare tube (36.3 mg/kg), coal tar pitch (36.4 mg/kg), TDAE (2.2 mg/kg), flip-flop (0.82 mg/kg), MES (0.53 mg/kg), RAE (0.36 mg/kg), DAE (0.27 mg/kg), rubber boots (0.06 mg/kg), bitumen (0.02 mg/kg), while concentration in furnace black was below LOQ.

12.15 Benzo(b)naphtho(1,2-d)furan

Ecotoxicity

Experimental data for daphnia and fish are available from Maas (1990, as cited in US EPA ECOTOX-DB): LC_{50} (48 h, Daphnia magna) 4.0 mg/L and LC_{50} (96h, Poecilia reticulata) 0.85 mg/L. These data are not reliable (not assignable) as the original publication to the database entry could not be retrieved and the title is rather misleading ("Toxicity research with thiourea"). According to QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) Daphnia is the most sensitive species with an LC_{50} (48h) of 0.416 mg/L (ECOSAR class neutral organics) and slightly higher values for fish (0.48 mg/L) and algae (0.69 mg/L). As QSAR data are more conservative than the not assignable experimental data, these will be used for further assessment.

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. The log K_{ow} was experimentally determined as 5.05 (SRC-database[53]) for the structural isomer Benzo(b)naphtho(2,3-d)furan.

Persistence

Lundstedt et al (2003) demonstrated a potential for at least primary biodegradation of the compound as part of a contaminated gas work soil-water slurry supplemented with nutrients and cultures designed for PAH-degradation in soil. Benzonaphthofurans were degraded by 68% within 29 days (initial concentration not given).

Genotoxicity and Carcinogenicity

No studies with experimental data concerning mutagenicity or carcinogenicity were identified. From SAR-analysis (Toxtree-software[54]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Provisional $PNEC_{aqua}$-Derivation

Based on the lowest calculated LC_{50} value of three trophic levels for Daphnid of 0.416 mg/L and an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.42 µg/L

[53] SRC PhysProp Database, http://www.syrres.com/what-we-do/databaseforms.aspx?id=386

[54] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*). According to the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology - the $ETNC_{aq}$ covering also reactive chemicals is 0.1 µg/L (de Wolf, et al., 2005) and thus is still somewhat lower than the QSAR-based PNEC.

Provisional SVHC-Classification

The T-criterion is missed in relation to ecotoxicity regardless if QSAR-data for the compound are used or available not assignable (reliability category 4) experimental data (effect concentrations > limit value of 0.1 mg/L for acute effect data according to screening criterion of REACH guidance R.11). Experimental determination of ecotoxicity (preferably chronic data) for benzo(b)naphtho(1,2-d)furan would be crucial to reliably exclude T[55].

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

Concluding from experimentally determined log K_{ow} of 5.05, classification as B is warranted according to screening criteria of REACH guidance R.11 (log K_{ow} > 4.5).

The P-criterion is regarded to be fulfilled. This should however be confirmed as the available study reported 68% biodegradation within 28 days. But obviously only primary biodegradation was assessed and the study design is too far from guideline studies as well as natural environmental conditions to invalidate QSAR-derived persistency predictions (P1P2 based on BIOWIN modules of US-EPA´s EPI Suite).

According to REACH regulation and REACH guidance documents the compound is not classified as SVHC as the T-criterion is not fulfilled based on the inadequate data base.

Provisional Environmental Classification

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (2), a tar fraction (2), pitch (2), carbon black (1) and the environment (3).

Data from analytics section of the project (for details see section 16): Benzo(b)naphtho(1,2-d)furan was determined in coal tar pitch (474.9 mg/kg), spare tyre (73.4 mg/kg), corresponding spare tube (61.0 mg/kg), flip-flop (0.89 mg/kg), DAE (0.37 mg/kg), rubber boots (0.08 mg/kg), TDAE (0.04 mg/kg), MES (0.01 mg/kg), RAE (0.01 mg/kg), while concentrations in furnace black and bitumen were below LOQ.

[55] T-criterion according to REACH Annex XIII for chronic data: NOEC < 0.01 mg/L

12.16 Benzo(b)naphtho(2,3-d)furan

Ecotoxicity

No experimental data were identified. According to QSAR prediction of ecotoxicity (ECOSAR from US-EPA EPI Suite) Daphnia is the most sensitive species with an LC_{50} (48h) of 0.416 mg/L (ECOSAR class neutral organics) and slightly higher values for fish (0.48 mg/L) and algae (0.69 mg/L).

Bioaccumulation

There are no experimental data on BCF or BAF for this compound. The log K_{ow} was experimentally determined as 5.05 (SRC-database[56]).

Persistence

Lundstedt et al (2003) demonstrated a potential for at least primary biodegradation of the compound as part of a contaminated gas work soil-water slurry supplemented with nutrients and cultures designed for PAH-degradation in soil. Benzonaphthofurans were degraded by 68% within 29 days (initial concentration not given).

Bio-Monitoring

Benzo(b)naphtho(2,3-d)furan has been detected in biota, namely crayfish and sunfish. No other data on bio-monitoring were identified (see section 10.2.6).

Genotoxicity and Carcinogenicity

No studies with experimental data concerning mutagenicity or carcinogenicity were identified. From SAR-analysis (Toxtree-software[57]) a general suspicion of mutagenicity and/or genotoxic carcinogenicity exists (Benigni, et al., 2008; Benigni, et al., 2009).

Provisional $PNEC_{aqua}$-Derivation

Based on the lowest calculated LC_{50} value of three trophic levels for Daphnid of 0.416 mg/L and an assessment factor of 1000 for acute test data only the following QSAR based PNEC is derived:

$PNEC_{aqua}$ = 0.42 µg/L

This value can be considered to be protective if baseline toxicity is the relevant mechanism of action (ECOSAR class *neutral organics*). According to the "exposure threshold of no concern" approach for freshwater systems ($ETNC_{aq}$) - an approach analogous to the TTC-concept in human toxicology - the $ETNC_{aq}$ covering also reactive chemicals is 0.1 µg/L (de Wolf, et al., 2005) and thus is still somewhat lower than the QSAR-based PNEC.

[56] SRC PhysProp Database, http://www.syrres.com/what-we-do/databaseforms.aspx?id=386

[57] http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

Provisional SVHC-Classification

The T-criterion is missed for the compound in relation to ecotoxicity based on QSAR-data (effect concentrations > limit value of 0.1 mg/L for acute effect data according to screening criterion of REACH guidance R.11). Experimental determination of ecotoxicity (preferably chronic data) for benzo(b)naphtho(2,3-d)furan would be crucial to reliably exclude T[58].

The data on genotoxicity and carcinogenicity are insufficient to decide on T in regard to germ cell mutagenicity or carcinogenicity.

Concluding from experimentally determined log K_{ow} of 5.05, classification as B is warranted according to screening criteria of REACH guidance R.11 (log K_{ow} > 4.5).

The P-criterion is regarded to be fulfilled. This should however be confirmed as the available study reported 68% biodegradation within 28 days. But obviously only primary biodegradation was assessed and the study design is too far from guideline studies as well as natural environmental conditions to invalidate QSAR-derived persistency predictions (P1P2 based on BIOWIN modules of US-EPA´s EPI Suite).

According to REACH regulation and REACH guidance documents the compound is not classified as SVHC as the T-criterion (data missing) is not fulfilled (based on QSAR-data only).

Provisional Environmental Classification

Category Acute 1, M-factor 1; H400: Very toxic to aquatic life

Category Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Occurrence in Relevant Matrices

Literature data (only qualitative): The compound was found in tar (3), a tar fraction (2), and the environment (2).

Data from analytics section of the project (for details see section 16): Benzo(b)naphtho(2,3-d)furan was determined in coal tar pitch (895.6 mg/kg), spare tyre (112.2 mg/kg), corresponding spare tube (92.0 mg/kg), flip-flop (1.1 mg/kg), DAE (0.14 mg/kg) and rubber boots (0.07 mg/kg), while concentrations in TDAE, MES, RAE, furnace black and bitumen were below LOQ.

[58] T-criterion according to REACH Annex XIII for chronic data: NOEC < 0.01 mg/L

13 Other Structural Representatives of Semipolar PAC

Applying our extended QSAR-selection methodology, with the resulting *extended QSAR selection of critical PAC* 154 compounds had been determined fulfilling at least 2 of three SVHC-properties (P, B, T). From these, 15 priority compounds were selected manly based on their occurrence in profiles. So far, the remaining 139 compounds of the list were not further analyzed.

In this section a plausibility check was undertaken to determine if there were indications that important classes of compounds had been missed by our selection strategy leading to the 15 priority semipolar PAC. To this end, chemical expertise and knowledge on formation and occurrence as well as biological activities of PAC (mainly in regard to genotoxicity) was applied. Dr. Albrecht Seidel from BIU gave valuable input in this regard. This leads to the following approach:

- Structural groups were formed and those selected which were regarded as being not yet sufficiently represented by the 15 semipolar PAC
- Knowledge on formation and occurrence was applied to judge on relevance (e.g. low relevance if environmentally formed by combustion processes).
- Relevance based on T as regards functional groups or known aromatic core systems was assessed, in some cases also implying literature sources identified in the project.
- QSAR-predictions on P and B are assumed to be sufficiently reliable and these properties were not further accounted for. However, some theoretical considerations on fate were employed to judge on relevance especially in regard to solubility.

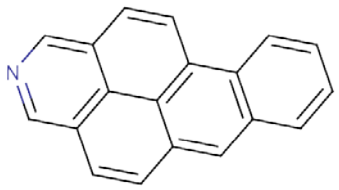
As most often no ecotoxicity data were available, predominantly considerations in regard to genotoxicity were decisive as regards T.

13.1 Azabenzo(a)pyrenes

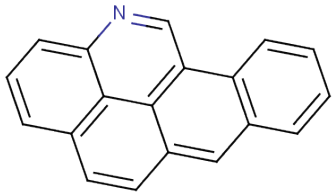
Benzo(a)pyrene is highly carcinogenic and one of 16 PAH of US EPA which are used as reference compounds for risk assessment of complex PAH mixtures. From a list of 10 reference compounds compiled by German MAK-commission (Greim, 2008) and devised toxic equivalency factors (TEF) from 0.01 to 1, Benzo(a)pyrene was assigned a TEF of 1. From structure-activity relationships, aza-benzo(a)pyrenes are assumed to be of similar carcinogenicity provided the aza-residue is not at positions 7,8,9,10 (important residues for diol-epoxide activation of benzo(a)pyrene).

The following azabenzo(a)pyrenes are part of the *extended QSAR selection of critical PAC* (154 compounds):

- 2-Azabenzo(b)pyrene = 2-Azabenzo(a)pyrene (CAS 189-90-2)
  Occurrence in profiles: 2, environment & pitch
  Screening result: PBT



- 12-Azabenzo(a)pyrene = Dibenzo[i,lmn]phenanthridine (CAS 24496-65-9)
  Occurrence in profiles: 1, pitch
  Screening result: PBT



To verify ecotoxicological relevance of these two compounds, several mainly ecotoxicological databases have been checked for data. Data sources and results are summarized in Table 32.

No information could be retrieved for 2-azabenzo(a)pyrene, even the CAS-number seems to be not well defined. In contrast, for 12-azabenzo(a)pyrene two databases (Oasis-DB assessed via QSAR-toolbox and CCRIS-DB) report positive in vitro gene mutation data. However, also for this compound obviously no ecotoxicity data exist.

Table 32: (Eco)Toxicological information on 2 azabenzopyrenes of the *extended QSAR selection of critical PAC*: Inquired databases and retrieved results

Data base	**189-90-2**	**24496-65-9**
QSAR Toolbox: All databases and inventories selected; Endpoints: Environmental fate, Ecotoxicity, Genetic toxicity	CAS not found Structure search: No results No other CAS-number available (PubChem, ChemID, WebSearch: structure and name)	CAS found Single entry Gene mutation: Positive in vitro ("Gene mutation I"), Kazius J, McGuire R., Bursi R: Journal of Medicinal Chemistry 48 (1), pp. 312-32, 25 [OASIS-DB, Genotoxicity]
CHRIP Japan	No results (name and CAS)	No results (name and CAS)
ECETOC Auatic toxicity database	No results (name and CAS)	No results (name and CAS)
ECOTOX-DB, US-EPA	No results (name and CAS)	No results (name and CAS)
ETOX-DB	No results (name and CAS)	No results (name and CAS)
INERIS-DB	No results (name and CAS)	No results (name and CAS)
ChemIDplus Lite – File and Internet Locators	No results (CAS)	Mutagenicity data (CCRIS-DB: Ames positive) TANGA,MJ, MIAO,RM AND REIST,EJ; SYNTHESIS AND TESTING OF SOME AZA-AROMATIC HYDROCARBONS; 9TH INT. SYMP.,POLYNUCL. AROMAT. HYDROCARBONS: CHEM. CHARACT. CARCINOG. 901-915, 1986)
EchemPortal	No results (name and CAS)	No results (name and CAS)

In conclusion, no specific information exists that these substances are of special relevance in regard to occurrence in matrices or (eco)toxicity.

From results of the analytics section of the project (see chapter 16) it was learned that also 10-azabenzo(a)pyrene is part of coal tar pitch and spare tyre (approximately 1% and 0.3%, respectively of totally determined semipolar PAC). However, in the other matrixes analyzed it was below LOQ.

13.2 Quinones and Ketones

Quinones are generally redox cycler and thus potential mutagens. Three quinone compounds are contained within the *pool of identified semipolar PAC* which are derived from mutagenic PAHs and thus most probably also mutagens. However, these quinones were only found in ambient air (environmental matrix) and are

probably most often formed by particle bound oxidation processes. Thus they may well be of environmental relevance but not relevant in regard to the current project. The following three quinones are part of the *pool of identified semipolar PAC*:

- Dibenzo(b,def)chrysene-7,14-quinone, CAS 128-66-5
- Benzo(a)pyrene-1,6-quinone, CAS 3067-13-8
- Benzo(a)pyrene-3,6-quinone, CAS 3067-14-9

Several PAH derived ketones are part of *pool of identified semipolar PAC.* Gathering from structure-activity relationships, ketones from mutagenic PAHs are prone to be also mutagenic. The mutagenic ketones benzo(cd)pyren-6-one (CAS 3074-00-8) and benz(de)anthracen-7-one (CAS 82-05-3) were found as depositions on several environmental matrices including biota (Brorström-Lundén, et al., 2010). Like quinones, ketones are most often formed environmentally by particle bound oxidation processes and additionally during combustion. Thus, some ketones are also found in carbon black and might be of relevance also in the frame of this project. However, from the *extended QSAR selection of critical PAC* (154 compounds) the only ketone found in carbon black was benzo(cd)pyren-6-one.

13.3 Alkylated Semipolar PAC

As a general rule, ecotoxicity seems to increase with increasing alkylation extent which is mirrored by QSAR derived toxicity data (see section 14). Similarly, methylated PAHs are believed to be in most cases at least not less active than the non-alkylated core structures. On the contrary, examples like chrysene and 5-methylchrysene or pyrene and 1-methylpyrene are known where the methylated form is a stronger carcinogen than the non-methylated core. As such, all core structures from the *extended QSAR selection of critical PAC* being itself relevant in terms of PBT properties might be at least as relevant in their alkylated forms. However, as solubility decreases with increasing alkylation extent and the higher condensed semipolar PAC are mostly already of very poor solubility, it is questionable if sufficiently high aquatic concentrations are reached for bioconcentration. Bioaccumulation via food however might be an alternative route to be ruled out for these compound groups. The *enforced QSAR selection of critical PAC* (60 compounds) contains many alkylated core structures of possible relevance as can be gathered from section 8.3. The list of 15 priority semipolar PAC also contains two methylated compounds, namely 1-methylbenzo(b)-naphtho(2,1-d)thiophene (CAS 4567-41-3) and 2-methyldibenzothiophene (CAS 20928-02-3).

In summary, there are indications that alkylated semipolar PAC may be of ecotoxicological relevance and 2 compounds represent this group within the 15 priority semipolar PAC. However, as nearly no experimental results on this compound group exist no firm conclusions can be drawn.

13.4 Nitrile Derivatives of PAHs

The *extended QSAR selection of critical PAC* contains 4 cyano-PAHs (nitriles) detected in carbon black and / or in coal tar fractions. These are not represented by the list of 15 priority semipolar PAC. To our knowledge there is not much known in regard to those higher molecular weight nitriles. Cyano-PAHs of lower molecular weight are metabolically reduced by methemoglobin to benzylamines. By elimination of ammonia carboxaldehydes can be formed which are known to be cytotoxic. If core PAHs are mutagenic, most probably also cyano-PAHs will be. The four compounds or rather compound groups (no exact structural determination) of the *extended QSAR selection of critical PAC* are

- Cyanopyrenes or -fluoranthenes (1-Cyanopyrene, 4107-64-6)

- Cyanofluoranthene or -pyrene or azabenzo(ghi)fluoranthene (SMILES for Fluoranthene-1-carbonitrile: N#CC1=C2C3=CC=CC=C3C3=C2C(C=C1)=CC=C3)
- Cyanopentapyrenes or -benzo(ghi)fluoranthenes (SMILES for Benzo(ghi)fluoranthene carbonitrile: N#CC1=CC2=C3C(=C1)C1=CC=CC4=C1C3=C(C=C4)C=C2)
- Chrysenenitriles (SMILES for Chrysene-6-carbonitrile: N#CC1=CC2=C3C=CC=CC3=CC=C2C2=CC=CC=C12).

13.5 Further Thiaarenes

The 15 priority semipolar PAC encompass 3- and 4-ring thiaarenes. Several further thiophene derived thiaarenes are contained within the *extended QSAR selection of critical PAC*. Besides structures of 4 to 5 ringed systems higher condensed structures of up to 9 rings were found within the analyzed profiles. However, according to Jacob (1990) regarding mutagenic activity of PAC (aromatic fractions) defined as revertants per mg of fraction (Salmonella typhimurium strain TA 98 +/- S9) peaks within the 4- and 5-ring fractions and declines for PAC with more than 5 rings. On the other hand, as regards weight % of fractions in a synfuel a decline with increasing ring number (degree of condensation) is observed (weight-% of 2-, 3-, 4-, and 5-ring fractions 33.7, 8, 2.7 and 0.6%, respectively). Concluding from this, the higher condensed structures with more than 5 rings may be less active and at the same time by far less abundant than the lower weight structures. In addition, already the 4-ring thiophene derivatives are of very low solubility (calculated values around 50 µg/L) and will even further decline with growing ring number. This might render them less relevant in regard to ecotoxicity. However, three thiaarenes with 4 to 5 rings might be relevant semipolar PAC. These compounds together with their occurrence and mutagenic activity as reported by Jacob (1990) are listed in Table 33.

Table 33: Potentially relevant thiaarenes not covered by the 15 priority semipolar PAC

CAS	Name	Ring number	Occurrence (Jacob, 1990)	Mutagenic activity (Jacob, 1990)
227-86-1	Anthra(1,2-b)thiophene	4	coal tar, coal oil, solvent refined coal materials	Weak mutagenicity with S9
72076-98-3	Benzo(def)naphthobenzothiophene (= Chryseno(4,5-bcd)thiophene)	5	coal tar, coal derived products, shale oil and carbon black	Isoster to benzo(a)pyrene, mutagenic activity even exceeding that of benzo(a)pyrene
68558-73-6	Triphenyleno(4,5-bcd)thiophene (= Triphenyleno(1,12-bcd)thiophene)	5	carbon black, coal tar related products, crude oil	Not mutagenic

13.6 Hydroxylated PAHs and Xanthenes

Several hydroxylated PAHs are within the *extended QSAR selection of critical PAC*. These were all found in one profile of anthracene oil and one of these compounds also in tar contaminated ground water and thus might stem from the same source. Further research would be necessary to determine their relevance in regard to occurrence and ecotoxicity.

Also two xanthene derivatives are in the *extended QSAR selection of critical PAC*. As xanthene itself was reported to be toxic in the fish embryo toxicity test with Danio rerio with a LC_{50} (48h) of 0.38 mg/L (Sagner, 2009), also these higher molecular weight structures might be of ecotoxicological relevance (B and T according to QSAR):

- Benzo(kl)xanthene, CAS 200-23-7
- 2-(9H-Xanthene-9-yl)-1H-indene-1,3(2H)-dione, CAS 87688-44-6

The first compound was detected in coal tar derived (pitch, tar fraction) and environmental (n=2) matrices, the second one in creosote contaminated ground water (n=1).

13.7 Conclusions

Activities outlined above aimed to scrutinize, if selection of our 15 priority compounds could have been biased leaving behind important groups of semipolar PAC. In summary, there are no clear indications that important compounds / compound groups have been missed. Rather, with the current knowledge on occurrence and substance properties the 15 semipolar PAC seem to include or represent the most important compounds / groups of compounds. However, some uncertainty remains as no systematic analysis could be performed within the scope of the project and conclusions are often based on theoretical considerations.

As a possible exception from the conclusions stated above, Chryseno(4,5-bcd)thiophene could be a relevant compound judging from mutagenic activity and occurrence as reported by Jacob (1990). From our analysis of profiles, occurrence in pitch (n=1) and carbon black (n=1) was found.

14 Structure-Activity Relationships with Particular Regard to QSAR-Results

Kern et al. (2008) reported some correlations between structural determinants of semipolar PAC (e.g. the increase in ecotoxicity of compounds with increasing ring number on the one hand and with increasing degree of alkylation on the other hand). These and similar possible structure-activity relationships shall be examined. To this end, predictions of the EPI suite models for members of the *pool of identified semipolar PAC* (443 compounds) are analysed and results compared to the few identified experimental data.

More specific questions arise when SMILES codes must be generated for difficult substances. As outlined in section 9.4.1, substance profiles for UVCBs from e.g. petrol oils or coal tar oils often contain complex mixtures of alkylated core heterocyclic structures, most often only described as e.g. “Dibenzothiophene, C5-substituted, different isomers”. Moreover, these UVCBs often contain isomers of HET-PAC regarding the position of an amino- or hydroxy-substituent, the position of a hetero-atom within the ring structure or isomers regarding the assembly of heterocyclic and homocyclic aromatic rings to the overall structure. As these isomers frequently cannot be described by a CAS-number, a SMILES code has to be generated which should ideally be representative for the group (substances not exactly defined) but must be of sufficient specificity to capture substance properties predicted by QSAR (EPI Suite models), i.e. log Kow, ecotoxicity and biodegradability. Examples for these special cases of isomerism were gathered from substances contained in the *pool of identified semipolar PAC* (see Figure 6 for core structures). Where necessary, missing links were filled with structures from the scratch with SMILES codes generated by MarvinSketch 5.3.6 contained in ChemIDplus Advanced[59].

In addition, results from different QSAR software (COSMOtherm) capable to yield compound specific log Kow values even for closely related isomers treated together by KOWWIN (EPI suite) are compared to values calculated by the latter.

14.1 Alkylation extent

14.1.1 Analysis Based on QSAR

For quinoline, alkylation in the 3-position was elongated in a stepwise manner with methyl groups from 3-methyl- to 3-butyl-quinoline (see Figure 6 for core structures and Table 34 for QSAR-results).

Regarding the persistency assessment, this was without significant effect; all isomers are predicted to be not persistent.

The log K_{ow}, however, rises from 2.69 for 3-methyl-quinoline to 4.16 for 3-butyl-quinoline, i.e. 0.49 log-units per methyl group.

As the log K_{ow} is one descriptor for toxicity-estimation by ECOSAR, elongation of the alkyl chain from 3-methyl- to 3-butyl-quinoline results in an increase in aquatic toxicity, as the EC_{50} (green algae, 96h) declines from 10.9 to 1.68 mg/L, i.e. about 0.27 log-units per methyl group .

As a second example a different core structure, phenanthro(4,5-bcd)thiophene (one of the 15 priority semipolar PAC) was chosen. Starting from the non-alkylated core structure, the 9-position was alkylated in a stepwise manner by methyl-units till 9-hexyl- phenanthro(4,5-bcd)thiophene (see Figure 6 for core structures and Table 34 for QSAR-results).

[59] http://chem.sis.nlm.nih.gov/chemidplus/

Regarding the persistence assessment, this was without significant effect; all isomers are predicted to be persistent (P1P2).

The log K_{ow}, however, rises from 4.74 for the non-alkylated form to 7.76 for 9-hexyl-phenanthro(4,5-bcd)thiophene, i.e. 0.5 log-units per methyl group.

As the log K_{ow} is one descriptor for toxicity-estimation by ECOSAR, elongation of the alkyl chain from the non-alkylated form to 9-hexyl- phenanthro(4,5-bcd)thiophene results in an increase in aquatic toxicity, as the EC_{50} (daphnid, 48h) declines from 0.51 to 0.003 mg/L, i.e. about 0.4 log-units per methyl group .

Conclusion from QSAR-Results:

- The alkylation extent seems to have no significant effect on biodegradability, however a slight decrease in probability for degradation is observed up to C3 followed by a marked increase for C4, such that BIOWIN3-score for 9-butyl-phenanthro(4,5-bcd)thiophene is higher than for the non-alkylated core. This is due to the fragment contribution method applied (see section 6.2.2 for details on methodology).
- The alkylation extent has a systematic positive effect on log K_{ow}, about 0.5 log-units increase per methyl group for both examples. This is due to the fragment contribution method applied (for KOWWIN: Log $K_{OW} = \Sigma(f_i * n_i) + \Sigma (c_j * n_j) + 0.229$, where f_i are fragment contributions derived from regression analysis and n_i are the number of times the respective fragment occurs in a given structure; and c_j are the correction factor coefficients derived from regression analysis wit n_j being the number of times the specific correction factor applies on the given molecule).
- The alkylation extent has a systematic negative effect on L(E)C_{50}: As log K_{ow} is the one determinant for the calculation of aquatic toxicity by ECOSAR via linear regression, the absolute contribution is depending on the regression equation (the applied ECOSAR class for both examples is Neutral Organics; however, for example 1 the equation for algae, for example 2 the equation for fish was used). As the regression equations relate to mmol/L, the difference in molecular weight has to be accounted for in the two examples (143.19 versus 208.28 g/mol). Normalizing by molecular weight, a decrease per methyl group of 1.89 x 10^{-3} log units for example 1 and 1.92 x 10^{-3} log units for example 2 results, the difference being due to the different organisms (and therefore different regression equations).

⇨ Different SMILES codes have to be generated for isomers differing in alkylation extent.

14.1.2 Critical verification by Experimental Data

Persistency

Identified results point to a decrease of biodegradability upon methylation:

Cleavage of 10-methyl-benzo(b)naphtho(2,1-d)thiophene was assessed using engineered Mycobacterium strain MR65. Reported desulfurization activity was only 50% of non-methylated core (Watanabe, et al., 2003), pointing to a lower biodegradation potential for the methylated isomers. This is corroborated by further data from Lundstedt et al (2003). Analysing (at least primary) biodegradation of different PAC as part of a contaminated gas work soil-water slurry supplemented with nutrients and cultures designed for PAH-degradation in soil the following results are reported:

While from dibenzothiophene-fraction 19% was left after 7 days, from 2-methyldibenzothiophene fraction still 62% was present. However, after 29 days the difference was only marginal (4.1% vs. 8.7%). Similar re-

sults are reported for carbazole (29% after 7 days, 18% after 29 days) and methylcarbazoles (52% after 7 days, 14% after 29 days) as well as benzoacridine (73% after 24 days, 68% after 29 days) and methylbenzoacridine (100% after 24 days and 79% after 29 days).

For dibenzothiophene was further shown that the higher the alkyl substitution, the slower proceeded biodegradation, i.e. dibenzothiophene (DBT) > C1-DPT > C2-DBT > C3-DBT (Kropp and Fedorak, 1998).

In conclusion, methylation of the core structure seems to decrease biodegradability, however mostly not to a very high extent. This may also be deduced by the exact output of BIOWIN modules for the two examples above. Further experimental results indicate growing impact on biodegradability with increasing alkyl substitution (unclear if longer alkyl chain length or multiple methyl substitution). BIOWIN modules output of degradation probability tends to decrease up to propyl-substitution and increases significantly for butyl-substitution. This observation is contra-intuitive, experimental data to further investigate these predictions are not available.

Ecotoxicity

Reviewing data from KORA-project, Kern et al. (2008) state that ecotoxicity increases with increasing alkylation extent. Ecotoxicity data on alkylated PAC are very scarce. Peddinghaus et al. (2012) corroborate this statement with data for quinoline and 6-methylquinoline. Nominal LC_{50}-values (48 h) determined in the fish embryo toxicity (FET) test on Danio rerio of 17 and 5.26 mg/L, respectively, are reported. For both compounds also toxicity in algae (EC_{50} 72 h, Desmodesmus subspicatus, measured) of 60.9 mg/L and 33.2 mg/L, respectively and Daphnia magna (EC_{50} 24 h, measured) of 14.7 mg/L and 8.6 mg/L were determined (Eisentraeger, et al., 2008) and are in agreement with the expected higher toxicity for the metyl-isomer. Within the same work toxicities for 2-methylpyridine and 2,4,6-trimethylpyridine were determined (Eisentraeger, et al., 2008). The respective toxicities determined in algae (EC_{50} 72 h, Desmodesmus subspicatus, measured) are 132.4 mg/L and 20.4 mg/l and those determined for Daphnia magna (EC_{50} 24 h, measured) are reported as 107.1 mg/L and 76.5 mg/L.

A further example from the work of Peddinghaus et al. (2012) is the series benzofuran, 2-methylbenzofuran and 2,3-dimethylbenzofuran. Observed ecotoxicity for these compounds in the FET test (LC_{50}-values (48 h)) were 15.98 mg/L, 14.03 mg/L and 7.34 mg/L, respectively. For these latter compounds however considerable substance loss was observed, such that results have to be treated with care.

In conclusion, these (limited) data are in agreement with QSAR-based predictions of an increase of ecotoxicity upon increasing alkylation extent of semipolar PAC core structures.

14.2 Positioning of Alkyl Residue

14.2.1 Analysis Based on QSAR

To investigate the effect of position of a linear alkyl group, the 7 possible isomers of butyl-quinoline were analyzed for their calculated PBT-properties (see Figure 6 for core structures and Table 34 for QSAR-results).

Position had neither any effect on persistence, nor on log K_{ow} or toxicity. This is due to the fragment contribution method applied for determining log Kow and biodegradability (see sections 14.1.1 and 6.2.2, respectively). As toxicity is essentially estimated based on log K_{ow}, results also indirectly depend on the group contribution method.

Conclusion from QSAR-Results:

As the ring-position of an alkyl group seems to have no effects regarding PBT-properties, possible isomers may be assessed as a group and one SMILES code is representative for all possible isomers.

14.2.2 Critical verification by Experimental Data

Experimental data were only identified in regard to biodegradability and are in conflict with QSAR-results:

Observations on biodegradability of different isomers of methyldibenzothiophene of Wang and Fingas (1995) are in contrast to QSAR-predictions: They report higher biodegradability for the 2- and 3-isomers compared to the 1- and 4-methyl-isomers.

Similarly, differences regarding biodegradation of dimethyl-dibenzothiophene isomers via the Kodama-pathway were observed: While oxidation of the 4,6-isomer (both homocyclic rings substituted) was not observed in pure cultures, the 3,4-isomer resulted in 6,7-dimethylbenzothiophene-2,3-dione (Bressler, et al., 1998; Kropp and Fedorak, 1998).

14.3 Position of hydroxyl substitution on the ring structure

14.3.1 Analysis Based on QSAR

While *hydroxy-quinoline isomers* 4-hydroxy-quinoline and 8-hydroxyquinoline yield identical QSAR-results for T (LC_{50} (48 h, daphid) = 11.8 mg/L), 2-hydroxyquinoline differs (LC_{50} (48 h, daphid) = 4.4 mg/L). For this isomer, the hydroxyl-group is positioned vicinal to the aza-nitrogen (see Figure 6 for structure). Therefore, other as in case of alkyl residues, ECOSAR recognizes a hydroxy-substituent in vicinal position to the aza-heteroatom.

Furthermore, 2-hydroxy-, 4-hydroxy- and 8-hydroxyquinoline differ considerably in their log K_{OW}-values (2.41, 1.66 and 2.02, respectively). Therefore, other as with alkyl-substituent position, the position of a hydroxyl-substituent relative to the hetero-atom of an aromatic ring system may influence the magnitude of log K_{OW}.

However, results for all three BIOWIN-modules (persistency screening) for these three hydroxy-quinoline isomers are identical.

1- and 2-*indanol isomers* (see Figure 6 for structure) yield similar (but not identical) results by QSAR-Screening for T as regards ECOSAR class “Neutral Organics” (EC_{50} (green algae, 96h) 28.5 and 30.7 mg/L, respectively). As however 1-indanol is recognized as *benzyl alcohol* and therefore assigned also to the group “Benzyl Alcohols”, toxicity of 1-indanol according to this class is pronouncedly higher than that of 2-indanol with the only class “Neutral Organics” (LC_{50} Daphnid, 48h 9.75 mg/L for 1-indanol).

Whereas for 1- and 2-indanol the hydroxyl-group is located in non-aromatic position, location on the aromatic ring holds true for 4- and 5-indanol. Both are assigned to the ECOSAR group “Phenols” and results for T-screening are identical in this case. However, with a LC_{50} (Daphnid, 48h) of 1.9 mg/L these are regarded considerably more toxic than 1- and 2-indanol.

Thus, for mixed ring systems with aromatic and non-aromatic rings substitution by hydroxy groups is recognized by ECOSAR differently depending on the kind of ring system actually hydroxylated. Even more, with the “Benzyl Alcohol” class of ECOSAR, hydroxylations on non-aromatic Cα to the phenyl group are assessed separately.

As regards log Kow, 1- and 2-indanol are differing in magnitude considerably from 4- and 5-indanol (1.93 versus 2.99). However, no further differences are observed. The impact of these substitution patterns on P-

screening results are less pronounced (impact on BIOWIN 6-results, but only marginally on BIOWIN 2 and 3).

Conclusion from QSAR-results with hydroxy-quinoline- and indanol-isomers

As regards the positioning of a hydroxyl-substituent on heterocyclic or mixed aromatic/nonaromatic ring systems, in contrast to alkyl-substituents special care has to be taken while considering treatment of isomeric mixtures as a group. Thus, whenever clear substance definitions were reported in profiles, structural isomers in regard to positioning of hydroxyl substituents were handled separately, i.e. specific SMILES codes were generated, where not available from ChemIDplus (see section 8.1.1). In cases where only a rough description of the isomer group detected was available due to analytical limitations (e.g. “Dimethyl- or ethylhydroxypyrenes or –fluoranthenes”), from the group of compounds one representative was chosen, either arbitrary, or based on additional information as far as available. Only this representative was included in the QSAR screening procedure on PBT-properties and no additional evaluations on potentially more or less toxic isomer representatives took place. In these cases we cannot exclude that the real compound(s) actually present in the profile were in fact more or less toxic, bioaccumulative or persistent.

14.3.2 Critical verification by Experimental Data

No experimental data were identified. However, no specific literature search was performed in this regard, as no hydroxylated compounds are within the 15 priority semipolar PAC.

14.4 Position of Amino-substituent

14.4.1 Analysis Based on QSAR

To investigate the effect of position of an amino-substituent on PBT-properties of a HET-PAC, the 4 different isomers possible for amino-dibenzothiophene were analyzed by QSAR properties (see Figure 6 for core structures and Table 34 for QSAR-results).

Position had neither any effect on persistence, nor on log K_{ow} or toxicity. This is due to the fragment contribution method applied for determining log Kow and biodegradability (see sections 14.1.1 and 6.2.2, respectively). As toxicity is essentially estimated based on log K_{ow}, results also indirectly depend on the group contribution method.

Conclusion from QSAR-Results

As the ring-position of an amino-group seems to have no effects regarding PBT-properties of amino-substituted HET-PAC, possible isomers may be assessed as a group and one SMILES code is representative for all possible isomers.

14.4.2 Critical verification by Experimental Data

No experimental data were identified. However, no specific literature search was performed in this regard, as no amines are within the 15 priority semipolar PAC.

14.5 Alkylation pattern

14.5.1 Analysis Based on QSAR

Impacts on PBT-properties could result from different alkylation patterns, i.e. four separate methyl groups at distinct positions of the ring system instead of one linear butyl chain, vicinal alkylation of heteroatoms or alkylation of a heteroatom of the ring-system itself.

Therefore, properties of the 7 butyl-isomers of quinoline were compared to 2,3,4,5-tetramethyl-quinoline (see Figure 6 for core structure and Table 34 for QSAR-results).

In respect to persistence, still the assessment result is *not P*, however a slight reduction in the degradation score for ultimate degradation (Biowin 3) was observed (2.49 versus 3.01 for the butyl-isomers).

As regards bioaccumulation potential, log K_{ow} is slightly higher compared to the butyl-isomers (4.33 versus 4.16). This results in a slightly higher acute toxicity (1.01 mg/L versus 1.39 mg/L LC50 for daphnids).

As a second example, 3,6-diisopropyl-phenanthro(4,5-bcd)thiophene, which is alkylated at both vicinal positions of the sulphur heteroatom of the ring-system, was compared to 9-hexyl- phenanthro(4,5-bcd)thiophene (see Figure 6 for core structure and Table 34 for QSAR-results).

In respect to persistence, for the diisopropyl-isomer probability for biodegradation is lower (BIOWIN3-score 1.6 versus 1.98 for the hexyl-isomer) while classification as *P* (P1P2) is naturally unchanged as already 9-hexyl-isomer is classified as *P*.

As regards bioaccumulation potential, log K_{ow} is even slightly lower compared to the hexyl-isomer (7.67 versus 7.76). The resulting effect on acute toxicity is however negligible (0.002 mg/L versus 0.0019 mg/L LC50 for fish).

The third example is 4,6-di-isopropyl-(1-Aminodibenzothiophene), also alkylated at both vicinal positions of the sulphur-heteroatom of the ringsystem, compared to the non-alkylated parent (see Figure 6 for core structure and Table 34 for QSAR-results). From mechanistic considerations, a negative impact on biodegradability would be expected from vicinal and bulky substitution to S.

As regards persistence assessment, the alkylated form is still predicted to be *not P*, however Biowin 3-score for ultimate biodegradability is indeed reduced from 2.62 to 2.29 for the di-alkylated compound. This is quite close to the cut-off value of 2.25 (a score lower than that would lead to the *P*-prediction, as both models for fast biodegradability give probabilities near zero).

As expected, log K_{ow} of the alkylated form is pronouncedly higher (6.29 versus 3.37) and correspondingly the EC_{50} for daphnids lower (0.30 versus 1.01).

As a last example, the effect of N-methylation of the heterocyclic nitrogen of indole was assessed, especially in regard to biodegradability.

Biodegradability prediction did however not change significantly due to N-methylation. The compound is still assessed a *not P*.

As log K_{ow} is increased for the N-methylated form by 0.55 log units (2.6 versus 2.05), acute fish toxicity is also higher for this compound (LC_{50} 0.42 versus 0.75 for the non-methylated parent).

Conclusions from QSAR-Results

- The alkylation pattern (linear chain versus methyl or isopropyl-groups) affects the log K_{ow}, however not in a uniform manner (higher for tetra-methyl compared to butyl, example 1; lower for di-

isopropyl versus hexyl, example 2) and to a much lower extent than the addition of extra methyl-units. As such, in regard to log K_{ow}, the alkylation pattern can be neglected and the isomers be treated as a group.

- Correspondingly, aquatic toxicity is only marginally affected by the alkylation pattern corroborating the group approach for these isomers.
- The result of QSAR based persistency assessment was overall unchanged for the four examples investigated. Examples one to three, however, showed reductions in Biowin3-scores by 0.33 to 0.52 and the highest reduction was observed for 2,3,4,5-tetramethyl-quinoline, being vicinal alkylated to the N-heteroatom and additionally alkylated at three further ring-positions. As such, also the persistency assessment argues for the possibility to assess isomers in alkylation pattern as a group. However, if close to the cut-off value for Biowin3, a closer look for isomers with several substituted ring positions (especially if including vicinal positions to hetero-atoms) might be necessary.

14.5.2 Critical verification by Experimental Data

No experimental data were identified exactly fitting to the above mentioned examples. However, biodegradability results described above for dimethyl-dibenzothiophene isomers via the Kodama-pathway points to an underestimation of the influence of alkylation pattern on substance properties, at least in regard to biodegradation. For dimethyl-dibenzothiophenes, no oxidation of the 4,6-isomer (both homocyclic rings substituted) was observed in pure cultures while the 3,4-isomer resulted in 6,7-dimethylbenzothiophene-2,3-dione (Bressler, et al., 1998; Kropp and Fedorak, 1998).

14.6 Isomers in Position of Hetero-Atom

14.6.1 Analysis Based on QSAR

To investigate a possible influence of the position of a hetero-atom in an aromatic ring system, the four possible N-isomers of azadibenzothiophene were evaluated.

1-, 2-, 3- and 4-azadibenzothiphene all were indistinguishable with regard to QSAR-results affecting PBT-properties (see Figure 6 for core structure and Table 34 for QSAR-results).

Conclusion from QSAR-Results

Het-PAC isomers in ring-position of a heteroatom may be assessed together as a group.

14.6.2 Critical verification by Experimental Data

The marine flagellate alga Dunaliella tertiolecta was tested under white light conditions (16/8 h l/d) (Wiegman, et al., 2001). The determined EC_{50}-values (72h, growth rate) for the isomers quinoline and isoquinoline were 571 µM (73.8 mg/L) and 464 µM (59.9 mg/L). The slightly higher toxicity of isoquinoline to algae is confirmed by Van Vlaardingen et al. (1996), who determined 96 hours EC_{50}-values (Chlorophyll a) in Scenedesmus acuminatus of >10 mg/L and 8.8 mg/L for quinoline and isoquinoline, respectively. Corresponding data for Daphnia magna reviewed in the same publication report LC_{50} (48 h)-values of 28.5 mg/L and 4.1 mg/L. While algal toxicity is similar for both compounds, daphnia toxicity of isoquinoline is considerably higher compared to the structural isomer.

From structure-activity relationships, differences in biological properties are expected depending on the positioning of a hetero-atom in the aromatic system: E.g. in regard to cancerogenicity of benzo(a)pyrene and aza-derivatives thereof, aza-benzo(a)pyrenes would be assumed to be of similar carcinogenicity provided the

aza-residue is not at positions 7,8,9,10, which are important residues for diol-epoxide activation of benzo(a)pyrene.

The latter example applies to specific toxicity only. QSAR-prediction is based on baseline toxicity, thus for PAC acting via narcotic mechanism there may indeed be no pronounced difference in ecotoxicity depending on ring-positioning of the heteroatom, as seen for algal toxicity. As such, toxicity to daphnia exhibited by isoquinoline may follow a more specific mechanism.

See also the following chapter on relative positioning of aromatic ring systems, as phenanthridine, benzo(f)quinoline and benzo(h)quinoline may also be regarded as the same structural backbone with altering position of the N-heteroatom.

14.7 Het-PAC Isomers in relative Position of the Aromatic Ring Systems

14.7.1 Analysis Based on QSAR

Acridine (benzo(b)quinoline), phenanthridine (benzo(c)quinoline), benzo(f)quinoline, benzo(h)quinoline are different only in the positioning of the third benzylic aromatic ring relative to the diaromatic quinoline ring system.

All four compounds were indistinguishable with regard to QSAR-results affecting PBT-properties (see Figure 6 for core structure and Table 34 for QSAR-results).

Conclusion from QSAR-Results

Het-PAC isomers in relative position of the aromatic ring systems may be assessed together as a group.

14.7.2 Critical verification by Experimental Data

The QSAR-derived statement that Het-PAC isomers in relative positioning of the aromatic ring systems are not significantly different in properties relating to T, B or P are partly contradicted by experimental results:

The marine flagellate alga Dunaliella tertiolecta was tested under white light conditions (16/8 h l/d) including a fraction of UV-A-irradiation sufficiently high for photo enhanced toxicity of acridine (Wiegman, et al., 2001). The determined EC_{50} (72h, growth rate) of 2.1 µM (0.38 mg/L) and 14.7 µM (2.64 mg/L) for acridine and phenanthridine are pronouncedly different. This was explained by a HOMO-LUMO-gap of 7.53 eV for acridine (8.37 eV for phenanthridine) which is within the HOMO-LUMO gap window of PAHs known to exhibit photoenhanced toxicity (7.2 ± 0.4 eV). These results are corroborated by Van Vlaardingen et al. (1996), who determined 96 hours EC_{50}-values (cell number) in Scenedesmus acuminatus of 0.3 mg/L and 5.2 mg/L for acridine and phenanthridine, respectively. The QSAR-prediction of ECOSAR for algae EC_{50} (96 h) for all 4 isomers of 5.5 mg/L nearly exactly corresponds to experimentally determined phenanthridine toxicity which mirrors baseline toxicity. Also toxicity of Benzo(h)quinoline with 96 hours EC_{50}-value of 6.6 mg/L (Van Vlaardingen, et al., 1996) would have been correctly predicted by ECOSAR. Like phenanthridine, HOMO-LUMO-gap of 7.95 eV would be out of window for photoenhanced toxicity. However, in spite of benzo(f)quinoline being out of the HOMO-LUMO-gap window (7.86 eV), algal 96 hours EC_{50}-value of 1.6 mg/L is still considerably lower compared to both, benzo(h)quinoline and phenanthridine. This is however not further commented by the authors.

Similarly, toxicity to Dunaliella tertiolecta (EC_{50} growth rate, 72 h) determined for benz(a)acridine and benz(c)acridine of 0.5 µM (0.115 mg/L) and 0.11 µM (0.025 mg/L), respectively, mirror the HOMO-LUMO-gap of 7.6 eV and 7.5 e.V and may be explained due to photoenhanced toxicity (Van Vlaardingen, et al., 1996).

Concluding from these experimental results, the QSAR-derived rule that het-PAC isomers in relative positioning of the aromatic ring systems (or positioning of a heteroatom within a given ring system) are of comparable toxicity seem to be corroborated as far as narcotic action is involved. As soon as specific or reactive toxicity is involved (as it is the case for the examples where photoactivation is assumed), this rule does not longer hold and toxicity between isomers may deviate considerably.

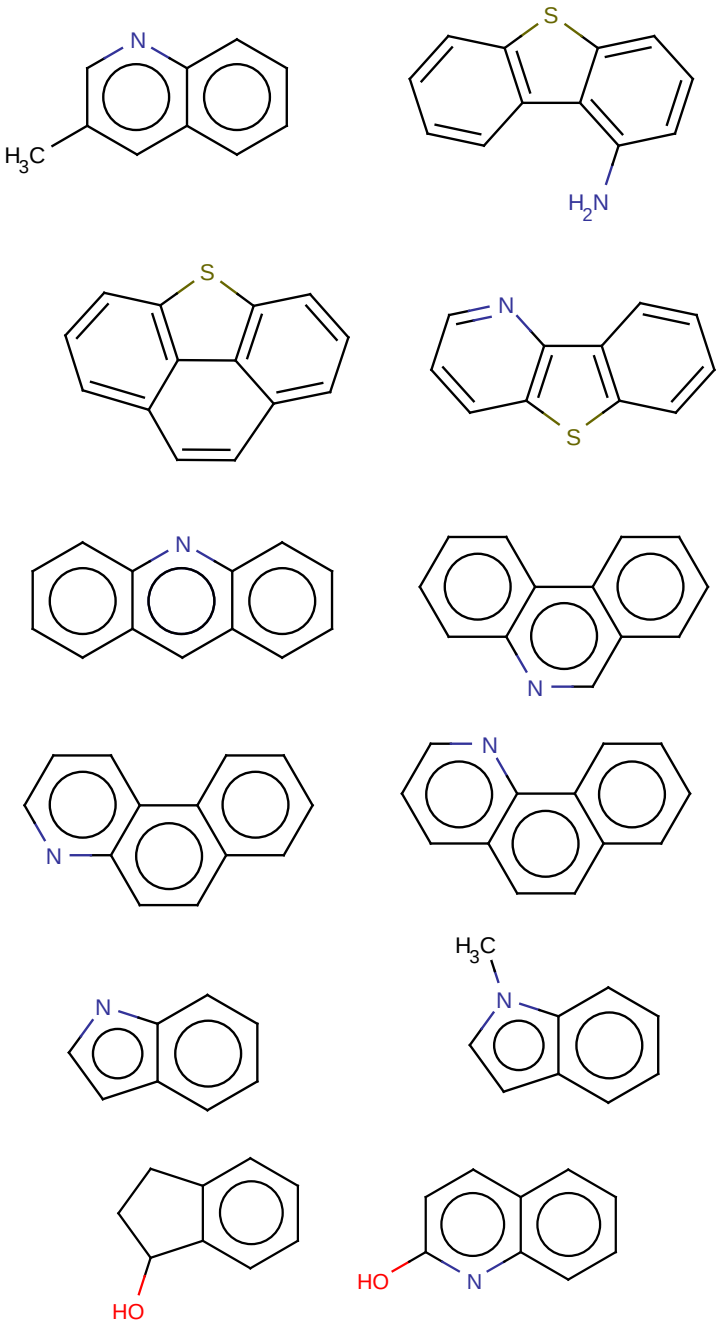


Figure 6: Core structures of the examples of section 14 and Table 34, from upper left to lower right: 3-methyl-Quinoline; 1-Aminodibenzothiophene; Phenanthro(4,5-bcd)thiophene; 1-Azadibenzothiophene; Acridine (benzo(b)quinoline); Phenanthridine (benzo(c)quinoline); Benzo(f)quinoline; Benzo(h)quinoline; Indole; N-methyl-Indole; 1-Indanol; 2-Hydroxyquinoline.

Table 34: Different forms of isomerisms and their effects on PBT-properties as determined by EPI Suite 4.1 (EPA, 2011). P-assessment according to REACH guidance R.11 (Biowin 2/3 <0.5/≤2,25 (P1) or 6/3 <0.5/≤2,25 (P2)); if the most sensitive organism changed during one topical row, the lowest effect value is given under "Organism".

Topic	Chemical name	SMILES	Persis-tency assess-ment	Esti-mated Log K_{ow}	BIOWIN 2	BIO-WIN3	BIOWIN 6	ECOSAR L(E)C50 [mg/L]	Organism
Alkylation, extent;	Quinoline, 3-methyl-	Cc2cnc1ccccc1c2	not P	2.69	0.83	2.81	0.36	10.90	Green algae, NO
Alkylation, extent;	Quinoline, 3-ethyl-	n1c2c(cccc2)cc(c1)CC	not P	3.18	0.79	2.78	0.20	5.89	Green algae, NO
Alkylation, extent;	Quinoline, 3-propyl-	n1cc(cc2ccccc12)CCC	not P	3.67	0.76	2.75	0.20	3.16	Green algae, NO
Alkylation, extent;	Quinoline, 3-butyl-	CCCCC1=CC2=CC=CC=C2N=C1	not P	4.16	0.94	3.01	0.21	1.68	Green algae, NO; Daphnid, NO: 1,39
Alkylation, positi-on	Quinoline, 2-butyl	CCCCC1=NC2=CC=CC=C2C=C1	not P	4.16	0.94	3.01	0.21	1.39	Daphnid, NO
Alkylation, positi-on	Quinoline, 4-butyl	CCCCC1=CC=NC2=CC=CC=C12	not P	4.16	0.94	3.01	0.21	1.39	Daphnid, NO
Alkylation, positi-on	Quinoline, 5-butyl	CCCCC1=C2C=CC=NC2=CC=C1	not P	4.16	0.94	3.01	0.21	1.39	Daphnid, NO
Alkylation, positi-on	Quinoline, 6-butyl	n1cccc2c1ccc(CCCC)c2	not P	4.16	0.94	3.01	0.21	1.39	Daphnid, NO
Alkylation, positi-on	Quinoline, 7-butyl	CCCCC1=CC2=NC=CC=C2C=C1	not P	4.16	0.94	3.01	0.21	1.39	Daphnid, NO
Alkylation, positi-on	Quinoline, 8-butyl	CCCCC1=CC=CC2=CC=CN=C12	not P	4.16	0.94	3.01	0.21	1.39	Daphnid, NO
Alkylation, pattern	Quinoline, 2,3,4,5-tetramethyl	CC1=CC=CC2=NC(C)=C(C)C(C)=C12	not P	4.33	0.94	2.49	0.23	1.01	Daphnid, NO
Amination, positi-on	1-Aminodibenzothiophene	NC1=C2C(SC3=C2C=CC=C3)=CC=C1	not P	3.37	0.15	2.62	0.03	1.01	Daphnid, AN

Semipolar polycyclic aromatic hydrocarbons

Topic	Chemical name	SMILES	Persistency assessment	Estimated Log K_{ow}	BIOWIN 2	BIOWIN3	BIOWIN 6	ECOSAR L(E)C50 [mg/L]	Organism
Amination, position	2-Aminodibenzothiophene	NC1=CC=C2SC3=C(C=CC=C3)C2=C1	not P	3.37	0.15	2.62	0.03	1.01	Daphnid, AN
Amination, position	3-Aminodibenzothiophene	NC1=CC=C2C(SC3=C2C=CC=C3)=C1	not P	3.37	0.15	2.62	0.03	1.01	Daphnid, AN
Amination, position	4-Aminodibenzothiophene	NC1=C2SC3=C(C=CC=C3)C2=CC=C1	not P	3.37	0.15	2.62	0.03	1.01	Daphnid, AN
Degradation: Alkylation vicinal to S	1-Aminodibenzothiophene, 4,6-di-isopropyl	CC(C)C1=CC=CC2=C1SC1=C(C=CC(N)=C21)C(C)C	not P	6.29	0.15	2.29	0.00	0.38	Daphnid, AN; Fish, AN: 0,24
Alkylation, extent;	Phenanthro(4,5-bcd)thiophene	S1C2=C3C4=C1C=CC=C4C=CC3=CC=C2	P1P2	4.75	0.00	1.94	0.07	0.51	Daphnid, NO
Alkylation, extent;	Phenanthro(4,5-bcd)thiophene, 9-methyl	CC1=CC4=CC=CC3=C4C2=C(S3)C=CC=C12	P1P2	5.30	0.00	1.83	0.06	0.20	Daphnid, NO
Alkylation, extent;	Phenanthro(4,5-bcd)thiophene, 9-ethyl	CCC1=CC4=CC=CC3=C4C2=C(S3)C=CC=C12	P1P2	5.79	0.00	1.80	0.03	0.08	Daphnid, NO;
Alkylation, extent;	Phenanthro(4,5-bcd)thiophene, 9-propyl	CCCC1=CC4=CC=CC3=C4C2=C(S3)C=CC=C12	P1P2	6.28	0.00	1.77	0.03	0.04	Daphnid, NO; Fish, NO: 0,03
Alkylation, extent;	Phenanthro(4,5-bcd)thiophene, 9-butyl	CCCCC1=CC4=CC=CC3=C4C2=C(S3)C=CC=C12	P1P2	6.77	0.00	2.04	0.03	0.02	Daphnid, NO; Fish, NO: 0,01
Alkylation, extent;	Phenanthro(4,5-bcd)thiophene, 9-pentyl	CCCCCC1=CC4=CC=CC3=C4C2=C(S3)C=CC=C12	P1P2	7.26	0.00	2.01	0.03	0.01	Daphnid, NO; Fish, NO: 0.01
Alkylation, extent;	Phenanthro(4,5-bcd)thiophene, 9-hexyl	CCCCCCC1=CC4=CC=CC3=C4C2=C(S3)C=CC=C12	P1P2	7.76	0.00	1.98	0.03	0.00	Daphnid, NO; Fish, NO: 0,00
Degradation: Alkylation vicinal to S	Phenanthro(4,5-bcd)thiophene, 3,6-di-isopropyl	CC(C)C1=CC=C2C=CC3=CC=C(C(C)C)C4=C3C2=C1S4	P1P2	7.67	0.00	1.60	0.01	0.00	Daphnid, NO; Fish, NO: 0,00
Position of Heteroatom	1-Azadibenzothiophene	S1C3=C(C=CC=C3)C2=C1C=CC=N2	not P	3.14	0.59	2.79	0.12	7.38	Green algae, NO

Semipolar polycyclic aromatic hydrocarbons

Topic	Chemical name	SMILES	Persistency assessment	Estimated Log K_{ow}	BIOWIN 2	BIOWIN3	BIOWIN 6	ECOSAR L(E)C50 [mg/L]	Organism
Position of Heteroatom	2-Azadibenzothiophene	S1C3=C(C=CC=C3)C2=C1C=CN=C2	not P	3.14	0.59	2.79	0.12	7.38	Green algae, NO
Position of Heteroatom	3-Azadibenzothiophene	S1C3=C(C=CC=C3)C2=C1C=NC=C2	not P	3.14	0.59	2.79	0.12	7.38	Green algae, NO
Position of Heteroatom	4-Azadibenzothiophene	S1C3=C(C=CC=C3)C2=C1N=CC=C2	not P	3.14	0.59	2.79	0.12	7.38	Green algae, NO
Relative ring position	Acridine (benzo[b]quinoline)	n(c(c(ccc1)cc2cccc3)c1)c23	not P	3.32	0.61	2.80	0.17	5.51	Green algae, NO
Relative ring position	Phenanthridine (benzo[c]quinoline)	c1ccc2c(c1)cnc3ccccc23	not P	3.32	0.61	2.80	0.17	5.51	Green algae, NO
Relative ring position	Benzo[f]quinoline	c1ccc2c(c1)ccc3ncccc23	not P	3.32	0.61	2.80	0.17	5.51	Green algae, NO
Relative ring position	Benzo[h]quinoline	c1ccc2c(c1)ccc3cccnc23	not P	3.32	0.61	2.80	0.17	5.51	Green algae, NO
Degradation: N-Alkylation	Indole	c1ccc2ccnc2c1	not P	2.05	0.79	2.94	0.47	0.75	Fish, PP
Degradation: N-Alkylation	N-Methylindole	c1ccc2ccn(C)c2c1	not P	2.60	0.76	2.91	0.37	0.42	Fish, PP

Abbreviations of ECOSAR classes: NO neutral organics; AN Anilines; PP Pyrazoles/Pyrroles

14.8 COSMOTHERM – Evaluation of an Alternative QSAR Method for Calculation of log Kow-values for Structural Isomers

To further evaluate applied QSAR predictions with regard to calculated log Kow-values which are the basis for ecotoxicity estimation using ECOSAR and decisive for classification as B (bioaccumulative), UBA performed calculations with COSMOtherm for the different types of structural isomers analyzed exemplary in the sections above (listed in Table 34).

COSMOtherm is a program for the quantitative calculation of solvation mixture thermodynamics based on quantum chemistry[60], i.e. essentially independent from experimental input values and thus not restricted to these. Therefore, even for close structural isomers, specific log Kow-values are calculated as presented in Table 35. In this table, values are compared to those calculated using KOWWIN v.1.68 (part of US EPA EPI Suite) and analyzed in regard to differences observed. Furthermore, calculated values are compared to available experimental values from the PhysProp Database[61] (accessed automatically from KOWWIN in case of data availability). In the following sections results are discussed in detail.

1. The most important difference between results from both calculation methods is of general nature: Nearly all values from COSMOtherm are lower compared to corresponding values from KOWWIN. To determine if the generally lower values from COSMOtherm are justified, a in depth analysis of applied methods and validation procedures would be required which cannot be provided within the scope of this project. However, some experimental values are available from the PhysProp DB. These are without exception higher than the predicted values from COSMOtherm. Actually, the differences between experimental values and COSMOtherm calculated values are of similar magnitude as are the differences between COSMOtherm and KOWWIN calculated values. Taken for granted the validity of the experimental data, this would point to KOWWIN estimation being closer to reality than COSMOtherm.
2. The difference a methyl group makes in regard to the size of log Kow (in light green in Table 35, dark green for N-methylation) is according to both methods approximately 0.5 log units. Thus, both methods agree very well in this respect.
3. Pronounced differences (quantitatively and also according to observed trends) are obvious in regard to the “Shift compared to mono-substituted equi-molecular weight isomer” (colored purple in Table 35). In case of 2,3,4,5-tetramethylquinoline and the butylquinoline-isomers, COSMOtherm predicts a much lower log Kow for the former compared to the mean log Kow for the seven mono-substituted butylquinolines (difference of 0.56 log units). In contrast, KOWWIN predicts a slightly higher value for 2,3,4,5-tetramethylquinoline (difference of 0.17 log units). In case of the second example the COSMOtherm predicted value for 3,6-diisopropylphenanthro[4,5-bcd]thiophene is higher than the predicted value for mono-substituted 9-hexyl phenanthro[4,5-bcd]thiophene (difference of 0.37 log units) and thus opposite in trend to the result from the first example. As in the first example, KOWWIN predicts a slightly higher value (0.09 log units) for the di-substituted equimolecular weight isomer, compared to the mono-substituted isomer. However, these observed differences between the

[60] http://www.cosmologic.de

[61] http://www.syrres.com/what-we-do/databaseforms.aspx?id=386

results of both programs are of lower importance compared to the more general difference in magnitude of calculated log Kow-values described under point 1.

4. Isomeric effects not reflected in KOWWIN-calculated values (shaded blue in Table 35): The maximum difference observed within one group of homologous isomers (three examples given) is 0.57 log units for the example butylquinoline (7 isomers). Maximum differences for the other examples were 0.2 log units (aminodibenzothiophene) and 0.41 log units (benzoquinoline).

The most important conclusion is therefore, that KOWWIN generally gives more conservative values for log Kow than COSMOtherm. Given the experimental values available from the PhysChem DB, KOWWIN calculated values are closer to reality than those from COSMOtherm. This general difference between both QSARs prevail quantitatively compared to the further differences observed.

Further, COSMOtherm is able to discern isomeric effects for which KOWWIN provides identical values. In this regard, the value from KOWWIN is approximately equal to or slightly higher than the highest value for a single isomer of one isomeric group delivered by COSMOtherm. Interestingly, experimental values are available for all 4 benzoquinoline isomers for which KOWWIN predicts identical log Kow-values whereas values are different according to COSMOtherm. According to these experimental data, isomeric differences are lower than predicted from COSMOtherm (experimental values between 3.40 and 3.48; COSMOtherm values between 2.67 and 3.07). Moreover, there is no uniform rank when sorting compounds to ascending experimental or COSMOtherm calculated values. As the experimental database match within KOWWIN clearly specifies the specific isomer (acridine = benzo[b]quinoline; phenanthridine = benzo[c]quinoline; benzo[f]quinoline; benzo[h]quinoline), an erroneous allocation of experimental values is a priori not to be expected.

To give an impression to what extent differences in predicted log Kow influence predicted toxicity by ECOSAR an exemplary calculation for butylquinoline-isomers was performed. Using the log Kow from the isomer with the highest predicted value from COSMOtherm (8-butylquinoline, log Kow 4.24) is resulting in a LC_{50}(48 hours) of 1.2 mg/L for daphnids calculated by ECOSAR, whereas the lowest value (3.68 for 4-butylquinoline) is yielding a LC_{50}(48 hours) of 3.44 mg/L.

In conclusion, KOWWIN predictions of log Kow are more conservative than COSMOtherm predictions and available experimental data are without exception in favor of values predicted by KOWWIN. However, also experimental values may be faulty and a more in-depth analysis is needed for final conclusions. Without further data in support for COSMOtherm at hand, the obviously more conservative nature of KOWWIN reflects the aim of the QSAR-screening approach applied within this project: A conservative tool for prioritization of compounds for further experimental evaluations in later steps.

Table 35: Comparison of calculated log Kow-values from KOWWIN v.1.68 (EPI) and COSMOtherm (COSMO) for different structural isomers (color code for groups of isomer-types): With available experimental values from PhysProp-DB (exp), molecular weight (MW), ECOSAR-Class and exemplary toxicity estimation using ECOSAR based on lowest and highest predicted COSMOtherm log Kow value for butylquinoline-isomers.

Name	MW	log Kow exp	log KOW (COSMO)	log KOW (EPI)	Δ log Kow EPI-Cosmo	Δ log Kow Exp-Cosmo	ECOSAR-Class*	Δ log Kow (methyl) Cosmo	Δ log Kow (methyl) EPI	Comment	ECOSAR with log Kow from Cosmo	Comment
Quinoline,3-methyl	143.19	2.53	2.30	2.69	0.39	0.23	NO	0.50	0.49			
Quinoline,3-ethyl	157.22	----	2.79	3.18	0.39		NO	0.52	0.49			
Quinoline,3-propyl	171.24	----	3.31	3.67	0.36		NO	0.53	0.49			
Quinoline,3-butyl	185.27	----	3.84	4.16	0.32		NO	3.68	MIN Cosmo			
Quinoline,2-butyl	185.27	----	3.93	4.16	0.23		NO	4.24	MAX Cosmo			
Quinoline,4-butyl	185.27	----	3.68	4.16	0.48		NO	**0.57**	**Δ Min-Max**		3.44	LC50 (Daphnid, 48h, mg/L)
Quinoline,5-butyl	185.27	----	3.70	4.16	0.46		NO					
Quinoline,6-butyl	185.27	----	3.80	4.16	0.36		NO					
uinoline,7-butyl	185.27	----	3.80	4.16	0.36		NO					
Quinoline,8-butyl	185.27	----	4.24	4.16	-0.08		NO				1.2	LC50 (Daphnid, 48h, mg/L)
Quinoline,2,3,4,5-tetramethyl	185.27	----	3.29	4.33	1.04		NO	-0.56	0.17	Shift compared to mono-substituted equi-molecular weight isomer		
1-Aminodibenzothiophene	199.27	----	2.94	3.37	0.43		AN	2.74	MIN Cosmo			
2-Aminodibenzothiophene	199.27	----	2.74	3.37	0.63		AN	2.94	MAX Cosm0			
3-Aminodibenzothiophene	199.27	----	2.81	3.37	0.56		AN	**0.20**	**Δ Min-Max**			
4-Aminodibenzothiophene	199.27	----	2.92	3.37	0.45		AN					
1-Aminodibenzothiophene,4,6-di-isopropyl	283.43	----	5.49	6.29	0.80		AN					
Phenanthro[4,5-bcd]thiophene	208.28	4.95	4.14	4.75	0.61	0.81	NO	0.44	0.55			
Phenanthro[4,5-bcd]thiophene,9-methyl	222.31	----	4.59	5.3	0.71		NO	0.45	0.49			
Phenanthro[4,5-bcd]thiophene,9-ethyl	236.33	----	5.04	5.79	0.75		NO	0.52	0.49			
Phenanthro[4,5-bcd]thiophene,9-propyl	250.36	----	5.56	6.28	0.72		NO	0.51	0.49			
Phenanthro[4,5-bcd]thiophene,9-butyl	264.39	----	6.07	6.77	0.70		NO	0.51	0.49			

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Name	MW	log Kow exp	log KOW (COSMO)	log KOW (EPI)	Δ log Kow EPI-Cosmo	Δ log Kow Exp-Cosmo	ECOSAR-Class*	Δ log Kow (methyl) Cosmo	Δ log Kow (methyl) EPI	Comment	ECOSAR with log Kow from Cosmo	Comment
Phenanthro[4,5-bcd]thiophene,9-pentyl	278.41	----	6.59	7.26	0.67		NO	0.52	0.50			
Phenanthro[4,5-bcd]thiophene,9-hexyl	292.44	----	7.11	7.76	0.65		NO					
Phenanthro[4,5-bcd]thiophene,3,6-di-isopropyl	292.44	----	6.74	7.67	0.93		NO	0.37	0.09	Shift compared to mono-substituated equi-molecular weight isomer		
1-Azadibenzothiophene	185.24	----	2.93	3.14	0.21		NO	2.57	MIN Cosmo			
2-Azadibenzothiophene	185.24	----	2.57	3.14	0.57		NO	2.93	MAX Cosm0			
3-Azadibenzothiophene	185.24	----	2.62	3.14	0.52		NO	**0.36**	**Δ Min-Max**			
4-Azadibenzothiophene	185.24	----	2.90	3.14	0.24		NO					
Benzo[b]quinoline	179.22	3.40	2.82	3.32	0.50	0.58	NO	2.67	MIN Cosmo			
Benzo[c]quinoline	179.22	3.48	2.71	3.32	0.61	0.77	NO	3.07	MAX Cosm0			
Benzo[f]quinoline	179.22	3.43	2.67	3.32	0.65	0.76	NO	**0.41**	**Δ Min-Max**			
Benzo[h]quinoline	179.22	3.43	3.07	3.32	0.25	0.36	NO					
Indole	117.15	2.14	1.87	2.05	0.18	0.27	PP	0.68	0.55	N-Methylation		
N-Methylindole	131.18	2.72	2.55	2.6	0.05	0.17	PP			N-Methylation		

(*) ECOSAR Class: NO = neutral organics; AN = Anilines; PP = Pyrazoles/Pyrroles

14.9 Dependence of Ecotoxicity on Ring Number

Reviewing data from KORA-project, Kern et al. (2008) state that ecotoxicity increases with increasing PAH-ring number in a given heterocyclic system. This is easily confirmed by QSAR and caused by a considerable increase of log K_{ow} with each phenylic ring. It is also experimentally confirmed by several publications, e.g. Wiegman et al. (2001) for the series quinoline, phenanthridine and benz(a)acridine (EC_{50} 72 h, Dunaliella tertiolecta: 73.8 mg/L, 2.64 mg/L and 0.12 mg/L, respectively) or Eastmond et al. (1984) for the series benzo(b)thiophene, dibenzothiophene and benzo(b)naphtho(1,2-d)thiophene (LC_{50} Daphnia magna: 63.7 mg/L, 0.466 mg/L and 0.22 mg/L, respectively). As outlined in section 14.7 toxic action other than narcosis has to be taken into account separately.

14.10 Dependence of Ecotoxicity on Hetero Atom

Reviewing data from KORA-project, Kern et al. (2008) state that ecotoxicity is especially high for S-heterocycles. From a data compilation derived from the *pool of identified semipolar PAC* (see Table 57 of the Annex) the following conclusions have been drawn:

Compared to N-heterocycles of the pyrrol type, log K_{ow} seems to be generally higher for S-heterocycles of the thiophene type. Thus higher toxicity is predicted by ECOSAR for the latter group (ECOSAR class neutral organics) and this might generally hold true as long as only narcotic mechanisms of action are involved. The similar is the case for O-heterocycles of the furan-type compared to S-heterocycles of the thiophene type, the latter obviously being more hydrophobic (higher log K_{ow}) and thus higher toxicity is predicted by ECOSAR and confirmed by the experimental data reviewed by Kern et al. (2008).

These rules explained simply by polarity issues will however most probably not be valid as soon as reactive or specific toxicity mechanisms are involved.

15 Recommendations for Ecotoxicity Testing in Follow-Up Projects

A list 15 priority compounds had been selected as target compounds for analytical determinations in several matrices in the analytics section of the project (see chapater 16). This selection was based on the compounds prevalence in substance profiles (literature) and ecotoxicological relevance (QSAR-screening approach, validation by experimental data retrieval). This list (see Table 56 in the Annex for structural representations) consists of three classes of compounds:

- 7 Azaarenes
- 6 Thiaarenes and
- 2 Oxaarenes

As often experimental data on ecotoxicity are missing or not sufficient for assessment, further ecotoxicity tests are recommended. In Table 56 target compounds are ranked in regard to the need for ecotoxicity tests. A low rank (0 = no tests recommended) was assumed for compounds most probably not fulfilling P- and B-criteria. Substances requiring data for all three trophic levels are given lower priority than those with data for at least one trophic level available. Further parameters were prevalence in profiles and – for thiaarenes – concentrations found in process oils analysed in the analytics section of the project.

Further explanations to Table 36 regarding experimental data on PBT-properties (column 3 – further details in section 10.2.2):

- Extension "–DM": Data missing, only QSAR-Result
- Extension "-R": Strict criteria according to REACH or REACH guidance
- Extension "-W": weaker criteria as applied for QSAR screening or some doubts regarding publications on potential biodegradability.
- Number in brackets for T-criterion, e.g. T-W (2): toxic to weak criteria (≤ 1 mg/L and > 0.1 mg/L), but only data on 2 trophic levels.

Due to limitations regarding capabilities for testing the four compounds with highest ranking of 3 according to Table 36 were chosen for future ecotoxicity testing (highlighted in bold). These tests are intended to be performed by UBA in a future project with external support by BIU ("*FKZ 3712 65 415/02 Nachweis des PBT Status von Chemikalien – unterstützende experimentelle Datenermittlung für die Erstellung von Annex XV Dossiers: Daten zur Löslichkeit und begleitende chemische Analytik für ökotoxikologische Tests semipolarer PAK*") :

- Dibenz(a,j)acridine
- 7H-Dibenzo(c,g)carbazole
- Benzo(b)naphtho(2,1-d)thiophene and
- Benzo(b)naphtho(1,2-d)furan

As becomes already clear from Table 36, solubility for all of these four compounds is very poor (calculated by Water NT-program of US-EPA´s EPI Suite or experimental value as far as available). This becomes obvious expressing water solubility as percent of the lowest predicted effect concentration out of three trophic levels (ECOSAR, column 5 of Table 36): Only for dibenz(a,j)acridine water solubility corresponds to more than 100% of the predicted E(L)C_{50}. For the other compounds water solubility approaches only 7 to 55%. As solubility in water is one of the most critical paramters for ecotoxicity testing, additionally several calculation methods were applied. Applied methods and results are listed in Table 37. QSAR models were either in-

tegrated in the OECD QSAR Toolbox or ChemProp (Chemical´s Properties Estimation Program System) version 5.2.5[62] As derived water solubilities deviated considerably depending on the method applied gemometric mean values were calculated. Experimental data were only available for dibenz(a,j)acridine and dibenzo(c,g)carbazole. For the latter compound, experimental data and the geometric mean calculated value fit quite well, which is not the case for Dibenz(a,j)acridine. For this compound, experimental water solubility is higher by a factor of 15 than the geometric mean calculated value. Because pyridine-type azaarenes are more basic and thus more polar than carbazole-type compounds and mean calculated values are nearly identical for both azaarenes, the experimental value is closer to what would be expected from a chemical point of view.

Table 36: Priority semipolar PAC: Ranking for ecotoxicity tests. 3 = highest ranking; *calculated* solubility in water in % of *predicted* effect concentration (ECOSAR).

CAS-No.	Chemical name	PBT-Properties exp.	Rank	Solubility [% effect conc.]	Solubility [mg/L], WaterNT	Reasoning for Priority Score
225-11-6	Benz(a)acridine	T-R Not B P-R	0	4336%	39.893	Toxicity data sufficient for assessment
225-51-4	Benz(c)acridine	T-R B-W P-R	0	4336%	39.893	Toxicity data sufficient for assessment
195-29-9	Mixture of Dibenzo-quinolines (Diben-zo(c,f)quinoline = Ben-zo(a)phenanthridine, 195-29-9)	T-DM B-DM P-DM	0	4336%	39.893	No experimental data on P and B Full dataset would be needed (fish, alga, daph-nia)
224-42-0	**Dibenz(a,j)acridine**	**Not-T (1)** **B-R** **P-R**	**3**	129%	0.159 (exp.)	P and B most probably fulfilled Medium to high prevalence in profiles (6) Data needed: **fish, daphnia**
226-36-8	Dibenz(a,h)acridine	T-DM B-R P-R	1	129%	0.159 (exp.)	P and B fulfilled Prevalence in profiles low (2) Full dataset would be needed (fish, alga daph-nia)
239-64-5	13H-Diben-zo(a,i)carbazole	T-DM B-R P-R (RA)	1	7%	0.0104 (exp.)	P and B fulfilled Prevalence in profiles low (1) Full dataset would be needed (fish, alga daph-nia)

[62] UFZ Department of Ecological Chemistry 2012. ChemProp 5.2.6.1. http://www.ufz.de/index.php?en=6738

CAS-No.	Chemical name	PBT-Properties exp.	Rank	Solubility [% effect conc.]	Solubility [mg/L], WaterNT	Reasoning for Priority Score
194-59-2	**7H-Dibenzo(c,g)carbazole and other dibenzocarbazoles**	**T-W (1)** **B-R** **P-R**	**3**	7%	0.0104 (exp.)	P and B fulfilled Medium prevalence in profiles (5) Data needed: **fish, daphnia** – optional data on algae (EC50 < 0.4 mg/L)
205-43-6	Benzo(b)naphtho(1,2-d)thiophene	T-W (1) B-R P-W	1	29%	0.0558	P and B most probably fulfilled Medium prevalence in profiles (5) Low relative amount in process oils Data needed: **fish, alga**
239-35-0	**Benzo(b)naphtho(2,1-d)thiophene**	**Not-T (1)*** **B-R** **P-W**	**3**	29%	0.0558	P and B most probably fulfilled High prevalence in profiles (19) High relative amount in process oils Data needed: **fish, alga**
4567-41-3	1-Methylbenzo(b)-naphtho(2,1-d)thiophene, several methylation isomers	T-DM B-R P-R	2	24%	0.0176	P and B fulfilled Medium to high prevalence in profiles (6) Medium relative amount in process oils Comparison to 239-35-0 would be interesting Full dataset would be needed (**fish, alga, daphnia**)
243-46-9	Benzo(b)naphtho(2,3-d)thiophene	T-DM B-R P-W	1	29%	0.0558	P and B most probably fulfilled Medium to high prevalence in profiles (6) Low relative amount in process oils Full dataset would be needed (**fish, alga, daphnia**)
30796-92-0	Phenanthro(4,5-bcd)thiophene	T-DM B-R Not P (likely)	0	38%	0.2328	Likely not P High prevalence in profiles (11) Low relative amount in process oils Full dataset would be needed (**fish, alga, daphnia**)
20928-02-3	Methyldibenzothiophene, 2- (several isomers)	T-W (3, core**) B-DM Not P	0	150%	0.7946	Not P, no data on B High prevalence in profiles (8) High relative amount in process oils Full dataset would be needed (**fish, alga, daphnia**)
205-39-0	**Benzo(b)naphtho(1,2-d)furan, several Benzonaphthofurans**	**T-W (2)** **B-R** **P-W**	**3**	55%	0.2273	P and B most probably fulfilled High prevalence in profiles (10) Data needed: **alga, daphnia, optional fish** (data source daphnia & fish enigmataic, according to this source fish more sensitive than daphnia)
243-42-5	Benzo(b)naphtho(2,3-d)furan and other naphthobenzofurans	T-DM B-R P-W	2	55%	0.2273	P and B most probably fulfilled High prevalence in profiles (7) Full dataset would be needed (**fish, alga, daphnia**)

(*) T-W cannot be excluded as limit dose unclear (probably ≥ 0.22 mg/L)

(**) Toxicity data only for non-methylated core!

Thus, dibenz(a,j)acridine and benzo(b)naphtho(1,2-d)furan would be the less insoluble compounds with a solubility in the 100 µg/L-range compared to 7H-dibenzo(c,g)carbazole and benzo(b)naphtho(2,1-d)thiophene with solubilities around 50 µg/L. However, as calculated values deviate considerably and it is out of scope of this project to further analyze benefits and weaknesses of the applied QSAR-models, these

results can only give a hint on the order of magnitude of water solubility and experimental determinations are mandatory for aquatic ecotoxicity tests to be performed. Water solubilities might be influenced by pH and solute concentrations in the test media.

The following relevant topics for ecotoxicity tests with these 4 semipolar PAC were discussed by FoBiG with UBA and Dr. Albrecht Seidel from BIU:

Analytical determination and treatment of test items

Because of the low water solubilities and high hydrophobicity of the compounds, very sensitive detection methods are needed and GC-MS is recommended. To prevent adsorption losses, only glass ware is appropriate for aquatic solutions and plastics must not be used. To enable correction against adsorption losses a sufficient frequency of analytical monitoring during tests should be determined.

Table 37: Selected set of 4 compounds for ecotoxicity testing - water solubilities calculated by different QSAR models and experimental data, where available.

CAS	NAME	Data [mg/L]	Exp. / QSAR	Software / Source	Model / Reference	Domain
224-42-0	Dibenz(a,j)acridine	0.1590	Exp.	Epi Suite	PEARLMAN,RS ET AL. (1984)	
		0.0016	QSAR	Toolbox	WS (Multicase) for 224-42-0	Undefined
		0.0861	QSAR	Toolbox	WS (fragments) (EPISUITE) for 224-42-0	Undefined
		0.0160	QSAR	Toolbox	WS (EPISUITE) for 224-42-0	Undefined
		0.0022	QSAR	ChemProp	Sw ACF-based model selection, from structure 2 - Hou et al.	
		0.0344	QSAR	ChemProp	Sw by LSER (Abraham et al.)	
		0.0094	QSAR	ChemProp	Sw via read across from ACF (UFZ set)	1= border out
Geometric mean of calculated		**0.0107**				
194-59-2	7h-Dibenzo(c,g)carbazole	0.0104	Exp.	Epi Suite	BANWART,WL ET AL. (1982)	
		0.0630	Exp.	Toolbox / Epi Suite	SMITH,JH ET AL. (1978)	
		0.0038	QSAR	Toolbox	WS (Multicase) for 194-59-2	Undefined
		0.0106	QSAR	Toolbox	WS (fragments) (EPISUITE) for 194-59-2	Undefined
		0.0223	QSAR	Toolbox	WS (EPISUITE) for 194-59-2	Undefined
		0.0013	QSAR	ChemProp	Sw ACF-based model selection, from structure 2 - Hou et al.	
		0.1263	QSAR	ChemProp	Sw by LSER (Abraham et al.)	

CAS	NAME	Data [mg/L]	Exp. / QSAR	Software / Source	Model / Reference	Domain
		0.0104	QSAR	ChemProp	Sw via read across from ACF (UFZ set)	1= border out
Geometric mean of calculated		**0.0107**				
239-35-0	Benzo(b)naphtho(2,1-d)thiophene	0.0115	QSAR	Toolbox	WS (Multicase) for 239-35-0	Undefined
		0.0544	QSAR	Toolbox	WS (fragments) (EPISUITE) for 239-35-0	Undefined
		0.0480	QSAR	Toolbox	WS (EPISUITE) for 239-35-0	Undefined
		0.0302	QSAR	ChemProp	Sw ACF-based model selection, from structure 2 - Hou et al.	
		0.0639	QSAR	ChemProp	Sw by LSER (Abraham et al.)	
		0.5291	QSAR	ChemProp	Sw via read across from ACF (UFZ set)	1= border out
Geometric mean of calculated		**0.0559**				
205-39-0	Benzo[b]naphtho[1,2-d]furan	0.0603	QSAR	Toolbox	WS (Multicase) for N/A	Undefined
		0.0511	QSAR	Toolbox	WS (fragments) (EPISUITE) for N/A	Undefined
		0.0941	QSAR	Toolbox	WS (EPISUITE) for N/A	Undefined
		0.0242	QSAR	ChemProp	Sw ACF-based model selection, from structure 5 - Klopman and Zhu	
		0.2827	QSAR	ChemProp	Sw by LSER (Abraham et al.)	
		0.7360	QSAR	ChemProp	Sw via read across from ACF (UFZ set)	1= border out
Geometric mean of calculated		**0.1065**				

Critical parameters for the selection / adaption of ecotoxicity tests

As all the compounds are of low solubility with several compounds being critically low with regard to predicted effect concentrations, compounds are expected to adsorb and furthermore expected to bioconcentrate over time, in general chronic tests are recommended and semistatic tests would be preferable over static tests. As capabilities for the performance of ecotoxicity tests at UBA are limited, the following prolonged / chronic tetsts were supposed:

- At least prolonged test (7 days) with Daphnia magna if reproduction tests are not possible
- Prolonged test (96 hours) with algae (Desmodesmus subspicatus)

- Prolonged fish embryo toxicity test (FET) with Danio rerio (similar to OECD draft guideline or ISO-guideline DIN 38415-6) up to 6 days to enable bioconcentration, chronic effects and prevent the chorion barrier which seems to be higher for lipophilic substances (Braunbeck, et al., 2005)
- Tests with sediment organisms are recommended, as in the envirionment partitioning to sediment is expected. Tests with Caenorhabditis elegans (ISO 10872) could possibly be performed.

General test design:

A limit test with saturated solution should be the first step. In case of positive results, a range finding test sould be performed before the final test with test item monitoring in all tests.

Photoactivation by UV could enhance toxicity, at the same time photolysis could occur. This could be rouled out in pre-experiments with different light sources.

For stock solutions DMSO (dimethyl sulfoxide) is recommended. The four compounds should all be very well soluble in this solvent and a concentration of < 100 µg/L in the final test solution is neither expected to be toxic itself nor to alter toxicity of test compounds (ECETOC, 1996; Hutchinson, et al., 2006).

16 Analytical Determination of 15 Priority Semipolar PAC in Consumer Products, Extender Oils, Carbon Black, Bitumen and Coal Tar Pitch

The analytical work outlined in this section was performed by private lecturer Dr. Albrecht Seidel from Biochemical Institute for Environmental Carcinogens (BIU), Prof. Dr. Gernot Grimmer-Foundation, Lurup 4, D-22927 Grosshansdorf, Germany.

Intentions of this work was

- to see if literature information on occurrence used for selection of priority semipolar PACs could be confirmed by testing of relevant matrices in the sense of this project. This was especially important as information on occurrence was generally very limited and then information on articles was completely missing. Would our selection be confirmed by quantification in relevant matrices?
- to compare concentrations of PAH in these matrices with concentrations of priority semipolar PAC: Would there be obvious trends which could be of relevance in regard to regulatory actions?

16.1 Target Compounds

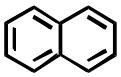
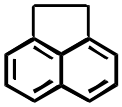
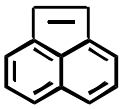
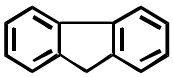
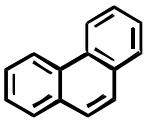
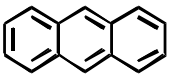
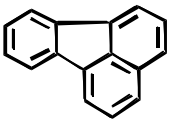
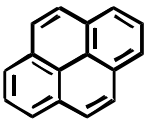
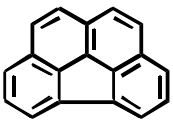
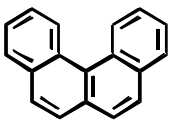
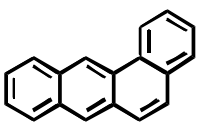
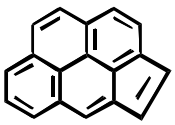
A list of 15 priority compounds had been selected as target compounds for analytical determinations based on their prevalence in substance profiles (literature) and ecotoxicological relevance (QSAR-screening approach, validation by experimental data retrieval, see sections above). This list (see Table 56 in Annex III) consist of three classes of compounds:

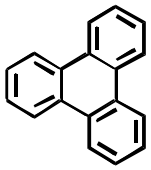
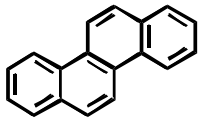
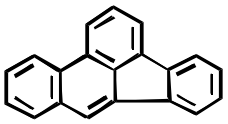
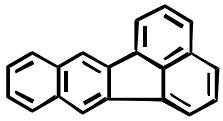
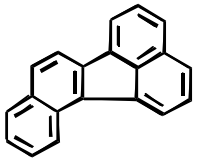
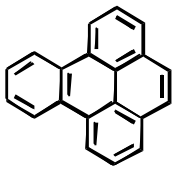
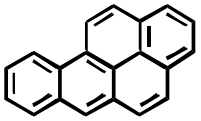
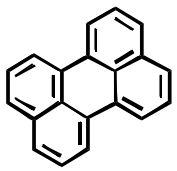
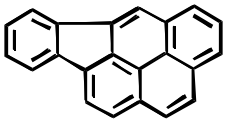
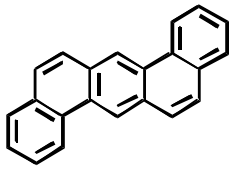
- 7 Azaarenes
- 6 Thiaarenes and
- 2 Oxaarenes

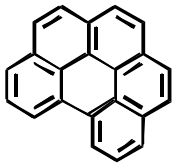
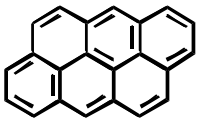
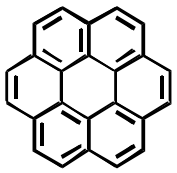
For these classes of compounds different clean-up procedures are needed for their determination in a variety of different matrices.

An important aspect of the project is the question of interdependency of the prevalence of semipolar PAC and (rather nonpolar) PAH. Therefore, besides the semipolar NSO-heterocyclic compounds described above the following PAHs are determined in parallel (if not earlier investigation on PAHs were available):

Table 38: Polycyclic aromatic hydrocarbon compounds for analytical determinations in matrices of UVCBs and products (EPA + Grimmer PAH)

CAS-No.	Chemical name	Structure
91-20-3	Naphthalene *	
208-96-8	Acenaphthylene *	
83-32-9	Acenaphthene *	
86-73-7	Fluorene *	
85-01-8	Phenanthrene *	
120-12-7	Anthracene *	
206-44-0	Fluoranthene *	
1718-52-1	Pyrene *	
203-12-3	Benzo[*ghi*]fluoranthene	
195-19-7	Benzo[*c*]phenanthrene	
56-55-3	Benzo[*a*]anthracene *,**	
27208-37-3	Cyclopenta[*cd*]pyrene	

217-59-4	Triphenylene	
218-01-9	Chrysene *,**	
205-99-2	Benzo[*b*]fluoranthene *,**	
207-08-9	Benzo[*k*]fluoranthene *,**	
205-82-3	Benzo[*j*]fluoranthene **	
192-97-2	Benzo[*e*]pyrene **	
50-32-8	Benzo[*a*]pyrene *,**	
198-55-0	Perylene	
193-39-5	Indeno[1,2,3-*cd*]pyrene *	
53-70-3	Dibenzo[*a*,*h*]anthracene *,**	

191-24-2	Benzo[*ghi*]perylene *	
191-26-4	Anthanthrene	
191-07-1	Coronene	

* EPA 16 priority PAH; ** EU 8 priority PAH indicated in Commission Regulation (EC) 552/2009

16.2 Selection of Test Samples

Modern extender oils used for tyre production in the European Community generally are reduced in PAH content and have to keep the limit values of 1 ppm for benzo[*a*]pyrene and 10 ppm for the sum of 8 priority EU PAH according to Commission Regulation (EC) 552/2009 amending Regulation (EC) 1907/2006 (REACH). This value is regarded as kept if the PCA extract is less than 3% (IP 346 method, DMSO-extract). At the same time the extender oils are probably largely depleted from het-PAC. As however a clear demonstration of the concomitant depletion of semipolar PAC with PAH reduction is missing thus far it is interesting to analyse modern extender oils of European production in comparison to high aromatic oils still used outside EC for production of e.g. rubber containing products. For analysed oils see Table 39. The TDAE sample was purchased from a company in Thailand. DAE, RAE, and MES were products from European companies.

Table 39: Rubber process oils (RPO) investigated in the present study

RPO	DAE (distillate aromatic extract)	TDAE (treated distillate aromatic extract)	RAE (residual aromatic extract)	MES (mildly extracted solvate)
Source	Germany	Thailand	Germany	Europe

Bitumen (CAS 8052-42-4, not oxidised) was chosen based on its high volume use and concomitant relevance for humans and the environment while at the same time analytical data on the prevalence of heterocyclic PAC are very scarce.

Coal tar pitch contains PAHs and het-PAC to a large extent. Therefore, this material serves as a kind of positive reference material where most of or all of the priority semipolar PAC are expected to be found. Coal tar pitch and bitumen (CAS 8052-42-4, not oxidised) were obtained from German producers.

From the analysis of literature profiles earlier in this project three of the 15 priority compounds were reported for carbon black. These however were partly older investigations and more recent data are lacking. Because of this and the fact that carbon black is widely used in a multitude of applications investigation of this matrix was regarded to be of interest. Carbon black (furnace black) was obtained from a German trader.

Table 40: PAH-rich materials investigated in the present study

Material	Coal tar pitch	Bitumen	Carbon black (Furnace black)
Source	Germany	Germany	Germany

Especially rubber based products are manufactured using extender oils and these oils might not adhere to European standards if produced outside the European Community. Therefore, products were included in the analysis showing high PAH-levels in earlier investigations (see Table 41 for analysed products). The spare tyre and tube from the do-it-yourself (DIY) market were from the repository of BIU purchased from German suppliers (commercial products purchased from German supplier in first quarter of 2009). The flip-flop (bought in 2008) were analyzed in an earlier project (Kalberlah, et al., 2011) due to their intense and unpleasant odor. High concentrations of PAH had been determined. Children´s rubber boots were reported to contain comparably high concentrations of PAH in a chemical analysis performed on behalf of ÖKO-TEST magazine, Germany[63]. The rubber boots were bought in 2011 from a German shop but were manufactured in China. A retained sample was kindly provided by ÖKO-TEST for analysis within this project.

Table 41: Rubber products investigated in the present study

Rubber product	Tyre from DIY market*	Tube of the tyre from DIY market*	Flip-flop	Children´s Rubber-boots
Source	Product of Asia, bought in Germany	Product of Asia, bought in Germany	Place of production unclear, bought in Germany	Made in China, bought in Germany

* spare tyre for garden tools purchased from a do-it-yourself (DIY) market

16.3 Reference materials and internal standards

2-Methyldibenzothiophene was purchased from Prof. Andersson (University of Muenster, Germany). Benzo[*c*]acridine, benzo[*a*]phenanthridine, benzo[*a*]acridine, 10-azabenzo[*a*]pyrene, dibenzo[*a,h*]acridine, dibenzo[*a,j*]acridine, 13H-dibenzo[*a,i*]carbazole, 7H-dibenzo[*c,g*]carbazole, benzo[*b*]naphtho[1,2-*d*]furan (1,2-BNF), benzo[*b*]naphtho[2,3-*d*]furan (2,3-BNF), phenanthro[4,5-*bcd*]thiophene and the three BNT isomers were taken from the BIU repository of reference materials. The purity of all *N*-PAC, the two BNF isomers, and all thiaarenes was proven to be better than 98% based on GC-FID analysis. 1-Me-2,1-BNT was synthesized at BIU as described under chapter 4.1.

In the present study 10-azabenzo[*a*]pyrene was determined in addition to the selected priority azaarenes to clarify whether it could be used as internal standard as reported previously (Grimmer, 1983; Grimmer and Naujack, 1985). Initial experiments indicate that 10-azabenzo[*a*]pyrene is not suitable to serve in general as internal standard for azaarene determination at low levels. Thus [D_6]-dibenzo[*a,h*]acridine (TRC, D416902) was used instead as internal standard for the determination of *N*-PAH by GC-MS. It was purchased from Toronto Research Chemicals (Canada). [D_{10}]-pyrene, [D_{10}]-phenanthrene, and [D_{12}]-benzo[*a*]anthracene used as internal standards for PAH, *O*- and *S*-PAH determination were all products of Chemical Isotope Laboratories Inc. purchased from LGC Standards GmbH (Wesel, Germany).

[63] ÖKO-TEST 09/2011; Test Gummistiefel; Online: http://www.oekotest.de/cgi/index.cgi?artnr=98316;bernr=07;co=;suche=Gummistiefel, as of 2012-09-19

16.3.1 Synthesis of 1-methylbenzo[*b*]naphtho[2,1-*d*]thiophene (1-Me-2,1-BNT)

Because reference material of 1-Me-2,1-BNT was pivotal for the performance of its analytical determination and no commercial source could be identified, a targeted synthesis of this compound was developed and conducted in the in-house synthesis laboratory of BIU. The new synthesis is illustrated in Figure 7 and allowed for the first time the preparation of 1-Me-2,1-BNT free of other isomers. Hitherto only a mixture of the 1- and 3-methyl-2,1-BNT isomers have been synthesised from which it is difficult get both isomers in pure form (Jacob, 1990).

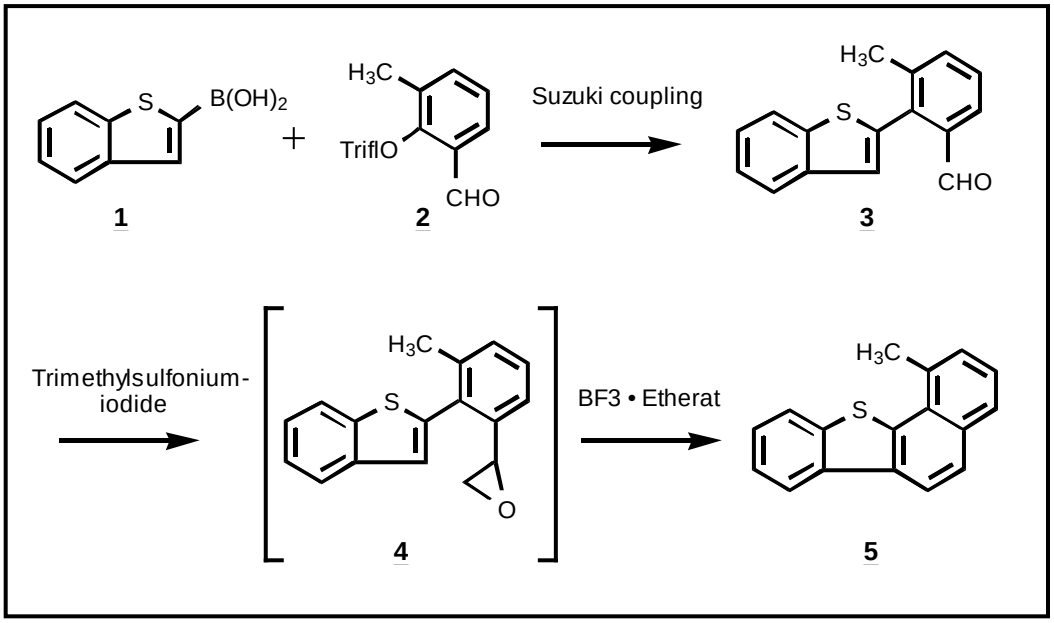


Figure 7: Pathway for the regioselective synthesis of 1-methylbenzo[*b*]naphtho[2,1-d]thiophene **5**.

In brief, a modified Suzuki coupling using benzothiophene boronic acid 1 and the triflate building block 2 gave the biaryl aldehyde 3 which was transformed with trimethylsulfonium iodide into the styrene oxide derivative 4. This intermediate compound was in situ cyclyzed to 1-methylbenzo[b]naphtho[2,1-d]thiophene 5 under BF3 catalysis.

16.4 Methodology for determination of PAHs, Thia-, Oxa- and Azaarenes according to the Grimmer method

16.4.1 Methodology for determination of PAHs, Thia-, and Oxaarenes

In principal, the determination of the PAH profile according to the Grimmer method is based on a stable isotope dilution methodology (use of max. 11 deuterated PAHs and indeno[1,2,3-cd]fluoranthene as internal standards) with a quantification by GC-MS(SIM) (Grimmer, et al., 1997). Due to the great similarities of the physico-chemical properties between PAHs, thia- and oxaarenes all three classes of compounds can be determined together in one profile analysis.

For sample preparation, an aliquot of each liquid matrix was dissolved in cyclohexane either at room temperature or under reflux. In case of a solid matrix an aliquot was crushed in a mill under cooling with liquid nitrogen and subsequently extracted with toluene in a soxhlet apparatus. The solvent of the resulting extract was exchanged to cyclohexane. After extraction of the cyclohexane solution with a mixture of dimethylformamide and water the obtained mixture was re-extracted with cyclohexane and subsequently concentrated under vacuum. Further removal of matrix components from the sample was achieved by SPE with partially

deactivated silica gel. After solvent exchange to toluene quantification was performed with GC-MS(SIM). A three point calibration was performed with each individual reference material of PAHs, thia-, and oxaarenes.

16.4.2 Methodology for determination of Azaarenes

For the determination of *N*-PAHs the Grimmer method was further developed including a deuterated internal standard. An aliquot of the sample is dissolved in cyclohexane or extracted as already described (vide supra). The extraction and re-extraction is performed with a mixture of DMF and aqueous ammonia solution (0.01 N). The concentrated cyclohexane solution is subjected to a preconditioned SPE column (Bond Elute®PRS) and fractionated by consecutive elution with cyclohexane and a mixture of methanol/NH_3 (33%) (9:1, v/v). The collected fractions were combined, concentrated and the obtained residue was dissolved in toluene. An aliquot was used for quantification with GC-MS(SIM) using a VF-200ms capillary (0.25 µm; 30m x 0.25mm). A three-point calibration was performed with each individual reference material of azaarenes.

16.4.3 Limits of quantification (LOQ) and limits of detection (LOD)

The limit of quantification (LOQ = 0.01 mg/kg) for the thiaarenes, oxa- and azaarenes was roughly estimated using the signal to noise method (S/N, 10/1). Values smaller than LOQ are reported as < 0.01. The exact determination of LOQ and LOD must be performed for each individual NOS-PAH and each investigated matrix and is beyond this project.

16.5 Results

16.5.1 Thiaarenes, oxaarenes, and azaarenes in high aromatic DAE process oil and modern rubber process oils (TDAE, RAE, and MES) with low PAH levels

Table 42: Thiaarene (*S*-PAH), oxaarene (*O*-PAH), and azaarene (*N*-PAH) concentrations determined in DAE (high aromatic extender oil), and modern rubber process oils (TDAE, RAE, MES).

mg/kg *	DAE	TDAE	RAE	MES
***S*-PAH**				
2-Methyldibenzothiophene	0.27	2.18	0.36	0.53
Phenanthro[4,5-*bcd*]thiophene	0.08	0.10	0.08	0.03
Benzo[*b*]naphtho[2,1-*d*]thiophene	6.17	1.03	0.32	0.41
Benzo[*b*]naphtho[1,2-*d*]thiophene	1.85	0.21	0.07	0.08
Benzo[*b*]naphtho[2,3-*d*]thiophene	1.04	0.14	0.17	0.04
1-Methylbenzo[*b*]naphtho[2,1-*d*]thiophene	2.31	0.42	0.17	0.11
***O*-PAH**				
Benzo[*b*]naphtho[1,2-*d*]furan	0.37	0.04	0.01	0.01
Benzo[*b*]naphtho[2,3-*d*]furan	0.14	< 0.01	< 0.01	< 0.01
***N*-PAH**				
Benzo[*c*]acridine	< 0.01	< 0.01	< 0.01	< 0.01
Benzo[*a*]phenanthridine	< 0.01	< 0.01	< 0.01	< 0.01
Benzo[*a*]acridine	< 0.01	< 0.01	0.01	0.03
10-Azabenzo[*a*]pyrene	< 0.01	< 0.01	< 0.01	< 0.01
Dibenzo[*a*,*h*]acridine	< 0.01	< 0.01	0.01	< 0.01
Dibenzo[*a*,*j*]acridine	< 0.01	< 0.01	< 0.01	< 0.01
13H-Dibenzo[*a*,*i*]carbazole	< 0.01	< 0.01	0.03	< 0.01

mg/kg *	DAE	TDAE	RAE	MES
7H-Dibenzo[*c*,*g*]carbazole	< 0.01	< 0.01	< 0.01	< 0.01

* < 0.01 = < LOQ, limit of quantification

16.5.2 PAH concentrations in high aromatic DAE process oil and modern rubber process oils (TDAE, RAE, and MES) with low PAH levels

Table 43: PAH concentrations determined in DAE (high aromatic extender oil), and modern rubber process oils (TDAE, RAE, MES).

mg/kg	DAE	TDAE	RAE	MES
Naphthalene	0.04	0.05	0.03	0.16
Acenaphthylene	<0.001	<0.001	0.01	<0.001
Acenaphthene	0.02	0.13	0.10	0.05
Fluorene	0.15	0.27	0.16	0.12
Phenanthrene	1.90	3.80	0.27	0.56
Anthracene	0.24	0.37	0.05	0.02
Fluoranthene	0.58	0.26	0.02	0.01
Pyrene	4.30	1.89	0.11	0.07
Benzo[*ghi*]fluoranthene	0.68	0.04	0.02	0.01
Benzo[*c*]phenanthrene	0.61	0.03	0.02	0.01
Benzo[*a*]anthracene	3.59	0.75	0.03	0.02
Cyclopenta[*cd*]pyrene	0.11	<0.001	<0.001	<0.001
Triphenylene	17.3	0.92	0.13	0.13
Chrysene	12.8	0.91	0.06	0.07
Benzo[*b*]fluoranthene	14.3	0.37	0.23	0.04
Benzo[*k*]fluoranthene	3.07	0.06	0.05	0.01
Benzo[*j*]fluoranthene	2.71	0.06	0.03	0.01
Benzo[*e*]pyrene	81.5	0.90	1.33	0.14
Benzo[*a*]pyrene	11.7	0.78	0.14	0.02
Perylene	18.2	0.19	0.05	0.01
Indeno[1,2,3-*cd*]pyrene	10.9	0.06	0.06	<0.001
Dibenzo[*a*,*h*]anthracene	5.40	0.05	0.05	0.01
Benzo[*ghi*]perylene	60.9	1.47	1.80	0.12
Anthanthrene	5.94	0.11	0.21	0.02
Coronene	5.50	0.180	0.31	0.03

16.5.3 Thiaarenes, oxaarenes, and azaarenes in coal tar pitch, bitumen, and carbon black

Table 44: Thiaarene (*S*-PAH), oxaarene (*O*-PAH), and azaarene (*N*-PAH) concentrations determined in coal tar pitch, bitumen, and carbon black (furnace black).

mg/kg *	Coal tar pitch	Bitumen	Carbon black
***S*-PAC**			
2-Methyldibenzothiophene	36.4	0.02	< 0.01
Phenanthro[4,5-*bcd*]thiophene	426.3	0.02	7.43
Benzo[*b*]naphtho[2,1-*d*]thiophene	2141.8	0.07	0.09
Benzo[*b*]naphtho[1,2-*d*]thiophene	380.2	0.03	0.02
Benzo[*b*]naphtho[2,3-*d*]thiophene	691.0	0.04	0.04
1-Methylbenzo[*b*]naphtho[2,1-*d*]thiophene **	< 0.01	0.02	< 0.01
***O*-PAC**			
Benzo[*b*]naphtho[1,2-*d*]furan	474.9	< 0.01	< 0.01
Benzo[*b*]naphtho[2,3-*d*]furan	895.6	< 0.01	< 0.01
***N*-PAC**			
Benzo[*c*]acridine	985.0	<0.01	<0.01
Benzo[*a*]phenanthridine	22.1	<0.01	<0.01
Benzo[*a*]acridine	1149.1	0.07	0.03
10-Azabenzo[*a*]pyrene	80.4	<0.01	<0.01
Dibenzo[*a*,*h*]acridine	517.7	0.02	0.04
Dibenzo[*a*,*j*]acridine	310.8	<0.01	<0.01
13H-Dibenzo[*a*,*i*]carbazole	187.6	0.06	<0.01
7H-Dibenzo[*c*,*g*]carbazole	73.3	<0.01	<0.01

* * < 0.01 = < LOQ. limit of quantification ; ** other isomers are probably coeluting with 1-Me-2.1-BNT

16.5.4 PAH in coal tar pitch. bitumen. and carbon black

Table 45: PAH concentrations determined in coal tar pitch, bitumen, and carbon black (furnace black).

mg/kg	Coal tar pitch	Bitumen	Carbon black
Naphthalene	6792.3	0.12	1.14
Acenaphthylene	41.8	0.02	0.28
Acenaphthene	4036.4	0.04	<0.001
Fluorene	2722.3	0.09	0.04
Phenanthrene	18539.4	0.17	23.3
Anthracene	2919.5	0.53	0.95
Fluoranthene	23281.3	0.05	168.5
Pyrene	16468.0	0.18	402.5
Benzo[*ghi*]fluoranthene	919.8	0.01	38.2
Benzo[*c*]phenanthrene	718.9	0.01	1.55
Benzo[*a*]anthracene	12052.8	0.09	2.70
Cyclopenta[*cd*]pyrene	135.0	<0.001	105.4
Triphenylene	2241.6	0.10	0.83
Chrysene	10718.5	0.11	6.76
Benzo[*b*]fluoranthene	10820.5	0.11	13.0
Benzo[*k*]fluoranthene	4261.8	0.02	2.65
Benzo[*j*]fluoranthene	6051.0	0.03	4.89
Benzo[*e*]pyrene	8051.0	0.55	41.2
Benzo[*a*]pyrene	11739.3	0.25	42.4
Perylene	2752.1	0.82	9.15
Indeno[1,2,3-*cd*]pyrene	10149.9	0.33	95.4
Dibenzo[*a*,*h*]anthracene	1539.5	0.10	<0.001
Benzo[*ghi*]perylene	6604.4	2.78	187.4
Anthanthrene	1348.5	0.45	83.4
Coronene	742.2	1.72	75.6

16.5.5 Thiaarenes. oxaarenes. and azaarenes in several consumer products

Table 46: Thiaarene (*S*-PAH), oxaarene (*O*-PAH), and azaarene (*N*-PAH) concentrations determined in a tyre from a DIY market, the corresponding tyre tube, flip-flop, and rubber-boots.

mg/kg *	Tyre from DIY market	Tyre tube from DIY market	Flip-flop	Rubber-boots
***S*-PAC**				
2-Methyldibenzothiophene	36.8	36.3	0.82	0.06
Phenanthro[4,5-*bcd*]thiophene	153.7	141.7	1.05	0.04
Benzo[*b*]naphtho[2,1-*d*]thiophene	615.7	519.8	6.84	0.12
Benzo[*b*]naphtho[1,2-*d*]thiophene	126.6	105.9	1.29	0.04
Benzo[*b*]naphtho[2,3-*d*]thiophene	209.4	178.6	1.64	0.02
1-Methylbenzo[*b*]naphtho[2,1-*d*]thiophene **	8.34	9.71	0.98	0.03
***O*-PAC**				
Benzo[*b*]naphtho[1,2-*d*]furan	73.4	61.0	0.89	0.08
Benzo[*b*]naphtho[2,3-*d*]furan	112.2	91.9	1.11	0.07
***N*-PAC**				
Benzo[*c*]acridine	81.0	55.9	0.63	<0.01
Benzo[*a*]phenanthridine	1.80	<0.01	<0.01	<0.01
Benzo[*a*]acridine	61.9	36.9	0.71	0.05
10-Azabenzo[*a*]pyrene	4.33	<0.01	<0.01	<0.01
Dibenzo[*a*,*h*]acridine	24.7	17.4	0.13	0.02
Dibenzo[*a*,*j*]acridine	10.4	4.90	<0.01	<0.01
13H-Dibenzo[*a*,*i*]carbazole	11.3	7.29	0.13	<0.01
7H-Dibenzo[*c*,*g*]carbazole	9.35	6.90	<0.01	<0.01

* < 0.01 = < LOQ (limit of quantification)

16.5.6 PAH in several consumer products

Table 47: PAH concentrations determined in a tyre from a DIY market, the corresponding tyre tube, flip-flop, and rubber-boots.

mg/kg	Tyre from DIY market	Tyre tube from DIY market	Flip-flop	Rubber-boots
Naphthalene	39.2	9.71	1.05	0.19
Acenaphthylene	30.4	22.4	6.44	0.10
Acenaphthene	23.2	21.5	7.66	0.22
Fluorene	351.8	391.7	58.5	1.14
Phenanthrene	4368.0	4246.1	118.1	2.62
Anthracene	752.6	716.5	24.1	0.80
Fluoranthene	3268.2	2813.1	39.4	0.49
Pyrene	1976.1	1651.8	47.9	1.20
Benzo[*ghi*]fluoranthene	108.6	83.2	1.50	0.07
Benzo[*c*]phenanthrene	83.9	68.9	1.85	0.03
Benzo[*a*]anthracene	1207.5	954.0	26.1	0.11
Cyclopenta[*cd*]pyrene	4.71	3.92	0.18	0.07
Triphenylene	313.7	242.1	15.4	0.69
Chrysene	1027.6	816.5	26.6	0.20
Benzo[*b*]fluoranthene	917.0	645.0	8.22	0.11
Benzo[*k*]fluoranthene	326.4	237.5	1.94	0.01
Benzo[*j*]fluoranthene	449.6	291.6	2.66	0.02
Benzo[*e*]pyrene	892.6	594.5	19.1	0.31
Benzo[*a*]pyrene	964.8	669.7	12.8	0.05
Perylene	256.2	164.8	2.44	0.03
Indeno[1,2,3-*cd*]pyrene	950.0	581.7	6.32	0.03
Dibenzo[*a,h*]anthracene	163.0	115.21	1.25	0.01
Benzo[*ghi*]perylene	587.5	329.0	7.83	0.14
Anthanthrene	177.4	100.8	0.48	0.02
Coronene	68.6	31.6	1.04	0.08

16.6 Discussion

16.6.1 PAH concentrations found in the analyzed matrices

For all matrices analyzed for priority thiaarene, oxaarene, and azaarene compounds, also PAH profiles were determined. The list of PAH contains all Grimmer PAH, the 16 EPA-PAH and thus includes all 8 PAH regulated for tyres according to Commission Regulation (EC) 552/2009 (see Table 43, Table 45 and Table 47 for details on analysed PAH compounds).

As expected, coal tar pitch contains by far the highest PAH concentrations of all analyzed matrices (g/kg range, see Table 45). As the highly aromatic process oil DAE stems from mineral oil, it is not unexpected that PAH concentrations are lower by a factor of 1000. However, still DAE (which has to be labeled for carcinogenicity, IP346 (DMSO extract) >3%) has a PAH content higher by a factor of 20 to 160 compared with modern, labeling-free process oils such as TDAE, RAE and MES (see Table 43). Of these investigated modern process oils, PAH content of MES is lowest (TDAE > RAE > MES).

The analyzed bitumen had a PAH content comparable to modern process oils (see Table 45). It is striking that all PAH but benzo[*ghi*]perylene and coronene are in the sub-mg/kg range. These two PAHs are much

more resistant to hydrotreating (reductive treating with hydrogen) than other PAHs. This makes it highly probable that the analyzed bitumen was hydrotreated in order to reduce the total PAH content.

In furnace black a typical PAH profile is detected (see Table 45) with fluoranthene, pyrene, cyclopenta[*cd*]pyrene, benzo[*ghi*]perylene, and coronene among the PAHs frequently found at the highest levels in carbon black extracts (IARC, 2010).

Among the investigated consumer products the tyre from the DIY market and the corresponding rubber tube contain PAH levels up to g/kg (see Table 47). The toxicological lead compound benzo[*a*]pyrene was found in a concentration of 965 and 670 ppm in the tyre and the tyre tube, respectively. In the flip-flop and in the rubber-boots a PAH profile was found in the lower ppm and higher ppb range, respectively (see Table 47).

16.6.2 Content of Priority Thiaarenes – Relation to PAH Content

Almost all 6 priority thiaarenes (see Table 56 for details on compounds) were detected in all analyzed matrices (see Table 42, Table 44, and Table 46). With the exception of MES and bitumen, concentrations of thiaarenes are in parallel to determined PAH concentrations, i.e. highest thiaarene content for coal tar pitch followed by DAE, TDAE and RAE. The results for MES fit very well to those reported by Null (1999) for MES (see Table 16). He found for for two different MES oils < 0.1 and 0.3 mg/kg benzo[*b*]naphtho[2,1-*d*]thiophene while the sum of 19 PAH for these oils was 6 and 2 mg/kg, respectively.

Generally and as expected, concentrations were highest for coal tar pitch. However, 1-Methylbenzo[b]naphtho[2,1-d]thiophene (1-Methyl-2,1-BNT) was below LOQ for coal tar pitch while present in quantifiable amounts in the mineral oil based matrices including the process oils and bitumen. This fits with observations, that in contrast to petrogenic PAH, where often alkyl PAH are more abundant than the parent compounds, in pyrogenic samples the parent PAH dominate in relation to alkyl PAH and alkylation extent is much more limited (Neff, et al., 2005). As coal tar is a pyrogenic product derived by high-temperature baking of hard coal in a reducing atomosphere and pitch is the distillation residue of coal tar, PAH contained in coal tar pitch are of pyrogenic origin. There seems to be an inverse relationship between abundance of alkyl PAH and the temperature of pyrogenic formation (Neff, et al., 2005). Most probably, these observations are also valid for heterocyclic polyaromatic compounds and could explain the unexpected finding that 1-Methyl-2,1-BNT was below LOQ for coal tar pitch.

MES however, containing only about $1/5^{th}$ of total PAH of RAE has a very similar thiaarene content. This is probably caused by the treatment methodology aimed to reduce IP 346 extract. Similar reasons might be responsible for the strikingly low thiaarene content of bitumen (lowest of all analyzed matrices). As mentioned under section 16.6.1, the analyzed bitumen obviously had been hydrotreated. During this process also concentrations of thiaarenes are reduced and this might be the reason or the unexpectedly low thiaarene content.

Carbon black that is made from high-sulfur feedstocks frequently contains detectable quantities of extractable aromatic compounds including thiaarenes (IARC, 2010). The thiaarene profile, however, appears to be very different from that seen in the mineral oil products. In the investigated furnace black the two alkylated thiaarenes 2-methyldibenzothiophene and 1-methyl-2,1-BNT are below LOQ and all 3 isomeric BNT compounds are present in only very low levels (see Table 44). In contrast, phenanthro[4,5-*bcd*]thiophene is detected in the lower ppm range. This findings support results from previous studies by Lee and Hites (1976) and Nishioka *et al.* (1986a).

In the four consumer products thiaarene profiles are found which are characteristic for process oils. The concentrations of the thiaarenes are in all 4 matrices proportional to the detected PAH level.

Taken together, the results for the 6 priority thiaarenes led to the conclusion that the Grimmer-"PAH" benzo[*b*]naphtho[2,1-*d*]thiophene (2,1-BNT) (Grimmer and Böhnke, 1976) might be a suitable lead compound, as it generally reflects the presence of the other determined thiaarenes in the investigated matrices (except carbon black) in a semi quantitative manner.

16.6.3 Content of Priority Oxaarenes – Relation to PAH Content

As expected the concentrations of the 2 priority oxaarenes (see Table 56 for details on compounds) benzo[*b*]naphtho[1,2-*d*]furan (1,2-BNF) and benzo[*b*]naphtho[2,3-*d*]furan (2,3-BNF) were highest for coal tar pitch (see Table 44). Both BNF isomers occur in the medium ppb range in DAE process oil, whereas in the PAH reduced TDAE, RAE, and MES process oils 1,2-BNF was in the very low ppb range and 2,3-BNF was below LOQ (see Table 42). The latter finding indicates that the two oxaarenes are also reduced in their level along with the removal of PAH in the modern process oils. Also both BNF isomers were below LOQ in bitumen and carbon black (see Table 44).

In all 4 consumer products the two BNF isomers could be determined (see Table 46). In the tyre from the DIY market and the corresponding tyre tube both compounds occur in the range of 60 to 112 ppm, whereas in the flip-flop and rubber-boots only levels of 70 ppb up to 1 ppm were detected. Interestingly, in all products the relative proportion of the two oxaarenes compared to those of the PAH and thiaarenes are higher as expected from their relative occurrence in DAE oil.

16.6.4 Content of Priority Azaarenes – Relation to PAH Content

The 7 priority azaarenes (see Table 56 for details on compounds) and 10-azabenzo[*a*]pyrene are detected in ppm levels in coal tar pitch with large variations (see Table 44). In relation to the PAH level of coal tar pitch the azaarenes occur in similar proportions as the thia- and oxaarenes. In contrast, all investigated azaarenes were close to or below LOQ in all rubber process oils (see Table 42), bitumen, and carbon black (see Table 44).

Among the consumer products benzo[*c*]- and benzo[*a*]acridine as well as dibenzo[*a,h*]acridine and 13H-dibenzo[*a,i*]carbazole are detected in the flip-flop, whereas the other azareness were below LOQ (see Table 46). Similarly in the rubber-boots all azaarenes are close to or below LOQ (see Table 46). Given the low level of azaarenes in rubber process oils it is surprising that in the tyre from the DIY market and the corresponding tyre tube almost all azaarenes could be detected in the ppm range (see Table 46). Taken together the high PAH level and the unexpected occurrence of the azaarenes in these two rubber matrices one could conclude that also other material(s) than high aromatic DAE oil are the source of the detected compounds.

16.7 Conclusions

In summary, selection of the 15 priority semipolar PAC was confirmed based on this analytical work. All six thiaarenes could be quantified in nearly all of the 11 analyzed matrices. Oxaarenes were generally present in coal tar pitch, household products, and to some degree in DAE, while they were detected in only low concentration or below LOQ in modern process oils, furnace black and bitumen. Azaarenes were quite variable in occurrence with high concentrations in coal tar pitch and spare tyre and tube, while concentrations in remaining matrices were rather low or below LOQ.

In a qualitative manner, concentrations of PAH correlated with the concentration of the 15 priority semipolar PAC with the exception of modern process oils (RAE, MES), where higher priority semipolar PAC concentrations are found than would be expected from PAH content, albeit on an overall low level. For more detailed considerations on interdependences of concentrations of priority semipolar PAC among each other and as a group compared to PAH, see section 17.

17 Indicator substances

17.1 Definition

An indicator substance is considered to be a substance, which should be representative for the composition of a certain matrix. That means the concentration of this indicator substance is relatively constant with respect to its content in a certain matrix and also to its ratio to other compounds contained in this matrix. Under this assumption the concentration of the indicator substance allows (in the ideal case) for the extrapolation of the composition of other substances or compound classes in comparable matrices with a low range of variation.

Here we try to identify possible indicator substances in profiles examined within the scope of this project, i.e. profiles of matrices containing semipolar PAC identified in the literature search (see section 3) as well as the results of analysis by BIU (see section 16).

17.2 Representativeness of profiles

The 15 priority semipolar PAC have been not systematically analyzed in the profiles identified in the literature research (see section 3). Mostly only one or two of them have been analyzed per profile, or if more substances were examined, then they had been determined only qualitatively. The limited range of analyzed compounds in these profiles does not allow for a systematic examination of the composition with respect to possible indicator substances. Therefore it is uncertain, how far the single profiles obtained by BIU are representative for those types of matrices.

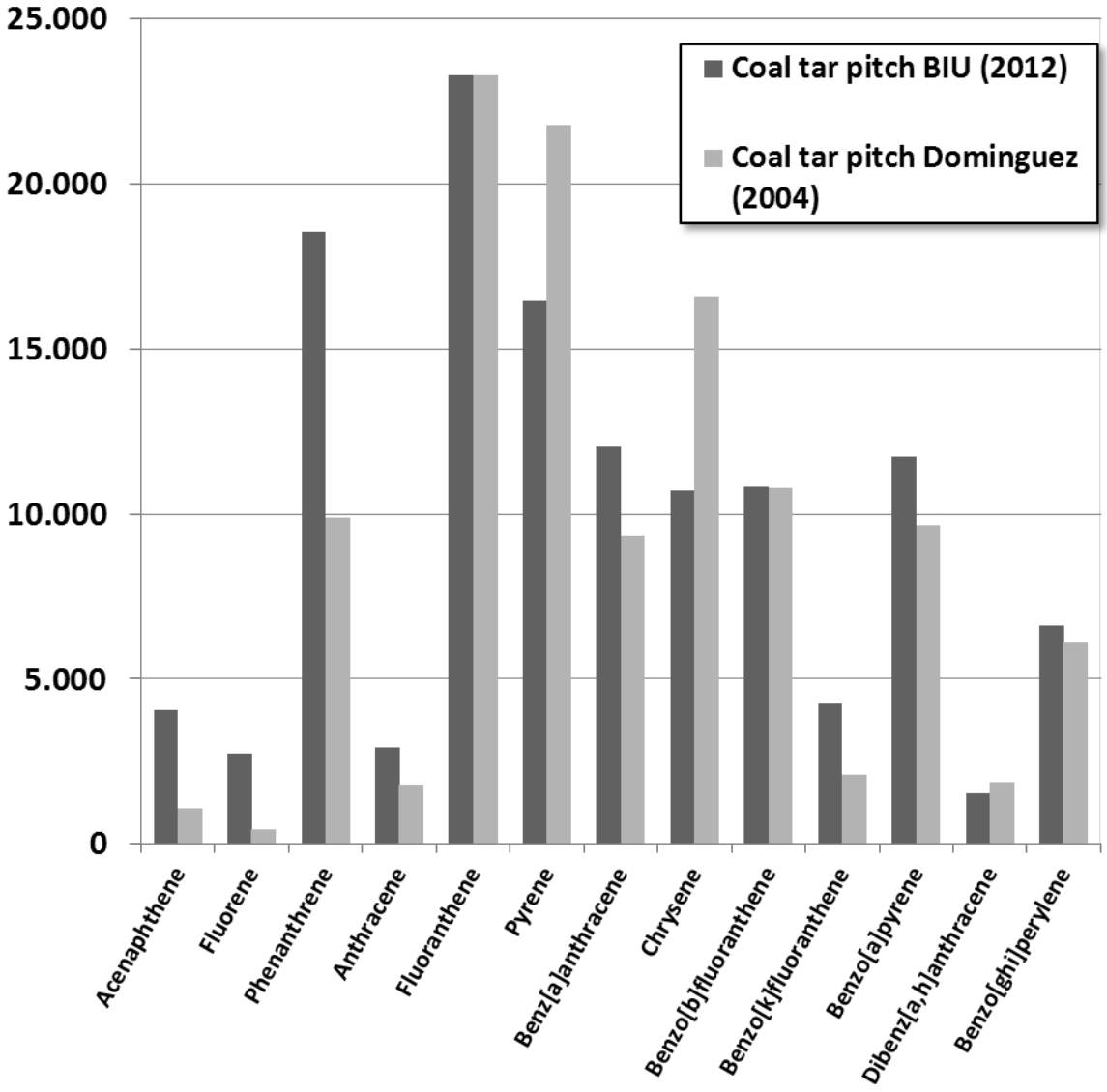


Figure 8: Comparison of composition of pitches [mg/kg] analyzed by BIU and Dominguez et al. (2004) for commonly analyzed PAH

The only possibility of comparison is for pitch: it is a pitch sample with a comparable range of analyzed PAH and semipolar PAC (Domínguez, et al., 2004). The comparison of substances shared by both profiles shows some relative deviations for PAH (Figure 8), however all substances commonly examined were also indeed detected in both profiles and for most substances very similar concentrations were found.

This essentially holds also true for semipolar PAC (Figure 9). 6 of the 15 priority semipolar PAC analyzed by BIU were also analyzed by Domínguez et al. (2004), not including however (beyond others) Benzo[a]acridine and Benzo[b]naphtho[2,3-d]furan analyzed by BIU. However, the respective close isomers (Benzo[a]phenanthridine and Benzo[b]naphtho[1,2-d]furan) are reported and assignment of isomers may have been inaccurate by Dominguez et al. Thus, comparing the sum of both isomers of the respective aza- and oxaarenes again results are very similar.

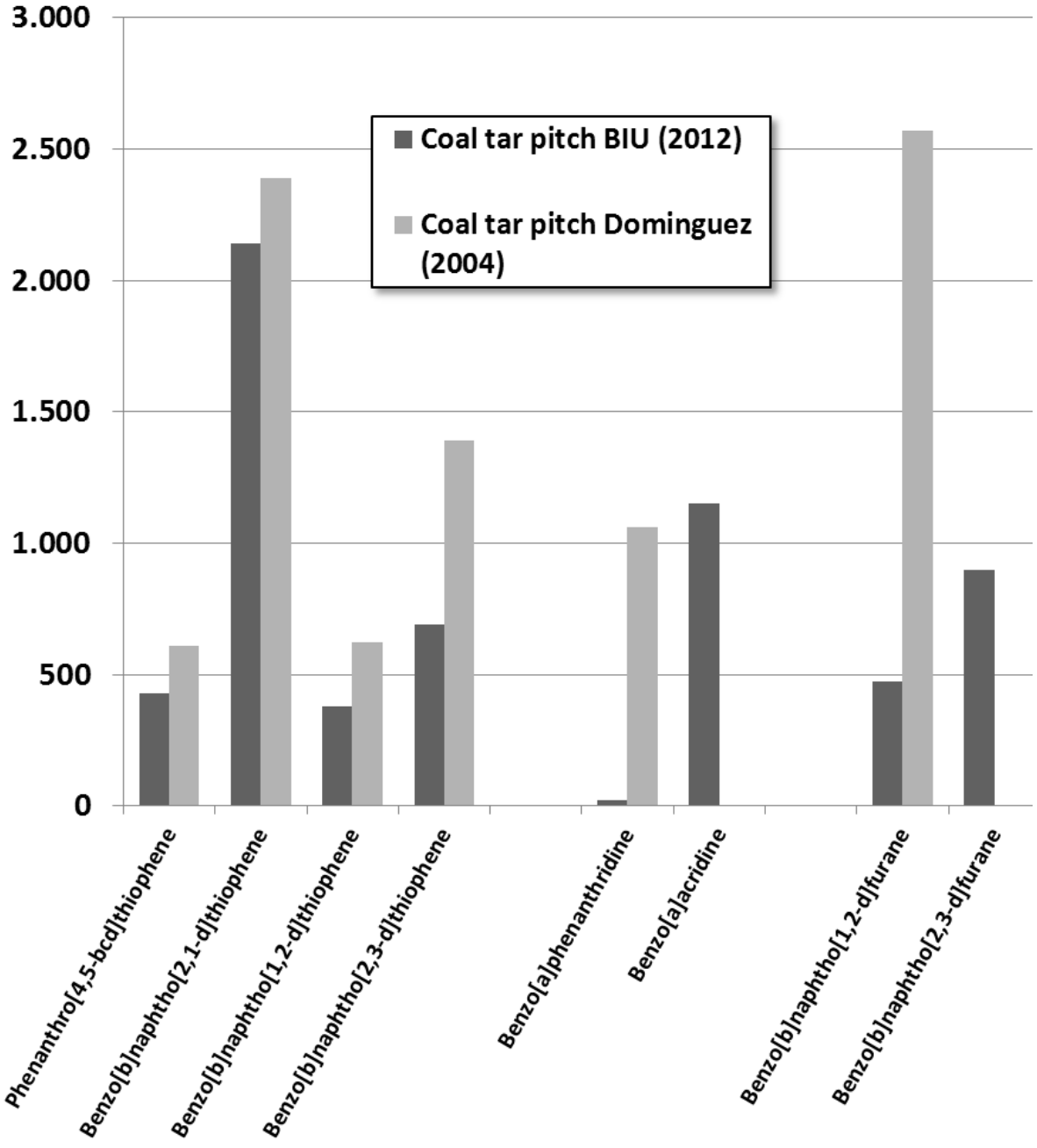


Figure 9: Comparison of composition of pitches [mg/kg] analyzed by BIU and Dominguez et al. (2004) for commonly analyzed priority semipolar PAC: Benzo[a]acridine and Benzo[b]naphtho[2,3-d]furan were not analyzed by Dominguez et al., the respective close isomers (Benzo[a]phenanthridine and Benzo[b]naphtho[1,2-d]furan) however are reported and assignment of isomers may be inaccurate.

Such, this singular profile basically would corroborate the indicator-substance approach. However it can be assumed that the higher processed the analyzed matrices are, the larger the differences in composition will be. This might be due to effects of slightly different technical processing on substance composition of the matrix adding up through the production chain. Therefore, the following steps to identify indicator substances (based on single profiles of different matrices) must be regarded as preliminary. Due to the restrictions regarding the range and quantification of examined semipolar PAC from the profiles identified in the literature search only the results of are discussed in detail in the following section. Additionally, the profile for coal tar

pitch by Domínguez et al. (2004) will be used for comparison to BIU-data and to widen the scope to non-priority semipolar PAC determined therein but not included in the investigation by BIU.

17.3 Results of the analyses of BIU and comparison to coal tar pitch profile from the literature

17.3.1 Overwiew

The 15 semipolar PAC high priority substances and additionally 10-azabenzo[a]pyrene were analyzed in flip-flops, children´s rubber boots, spare tyre and spare tube for garden machinery, furnace black, as well as coal tar pitch, DAE (Distillate Aromatic Extract), TDAE (Treated Distilled Aromatic Extracts), RAE (Residual Aromatic Extract), MES (medium/mildly extracted solvate) and bitumen. The following table summarizes the contents of PAH (25 analyzed, see section 16.1) and semipolar PAC analyzed in the examined matrices.

Table 48: Summary table of the content of PAH and **15 priority** semipolar PAC (all values in mg/kg) in the examined matrices

	Flip-Flop	Children´s rubber boots	Spare tyre for garden machinery	Spare tube for garden machinery	Furnace black	Bitumen
Sum of 16 EPA PAH	394.16	7.42	16953.37	14221.46	946.88	4.99
Sum of 25 PAH	438.82	8.75	19308.61	15802.94	1307.18	8.69
Sum of S-semipolar PAC	12.61	0.32	1150.43	992.07	7.57	0.20
Sum of N-semipolar PAC	1.60	0.06	204.0	129.29	0.08	0.15
Sum of O-semipolar PAC	2.01	0.15	185.56	152.94	<LOQ	< LOQ
Sum of 15 semipolar PAC	16.21	0.52	1536.38	1274.30	7.65	0.35

	Coal tar pitch	Coal tar pitch (Domínguez et al., 2004)	DAE	TDAE	RAE	MES
Sum of 16 EPA PAH	142687.63	120660.00 (14 PAH)	129.86	11.20	3.16	1.28
Sum of 25 PAH	165647.71	Only 14 analyzed	262.44	13.63	5.25	1.63
Sum of S-semipolar PAC	3675.79	5010.00*	11.72	4.09	1.16	1.20
Sum of N-semipolar PAC	3245.57	1060.00 (only Benzo[a]phenanthridine)	0.01	0.01	0.06	0.03
Sum of furans	1370.53	2570.00 (only Benzo[b]naphtho[1,2-d]furan)	0.51	0.04	0.01	0.01
Sum of 15 semipolar PAC	8291.89 6181.03**	8640.00	12.23	4.14	1.22	1.24

LOQ: 0,01 mg/kg ; (*): The two methylated thiaarenes were not determined, these however were either not detected or detected in low concentration only in the analysis of BIU. (**)Sum of only those PAC determined also by Domínguez et al.

As to be expected coal tar pitch revealed the highest contents of polycyclic compounds (PAH as well as semipolar PAC). For comparison, in Table 17 also the data from Domínguez et al. (2004) for coal tar pitch were evaluated, however only 14 of the 16 EPA PAH and not all of the 15 priority semipolar PAC were analyzed

in the publication. To be comparable, for the coal tar pitch analyzed by BIU in parallel to the total sum only those priority semipolar PAC were summed up in the last row of Table 17 which were also analyzed by Domínguez et al. (2004)[64]. Overall and as already obvious from Figure 9 the results are quite close to one another, however relative to PAH and also compared directly the content of priority semipolar PAC is higher for the sample analyzed by Domínguez et al. (2004).

Amongst the household articles, the tyre and tube contained by far the highest amounts of PAH and semipolar PAC (about 20 g/kg and 1.3-1.5 g/kg, respectively), exceeding even the PAH burden of furnace black. Only coal tar pitch contained about 10-times more PAH and about 4-times more semipolar PAC. Furnace black reveals a relatively low content of semipolar PAC compared to the PAH concentration.

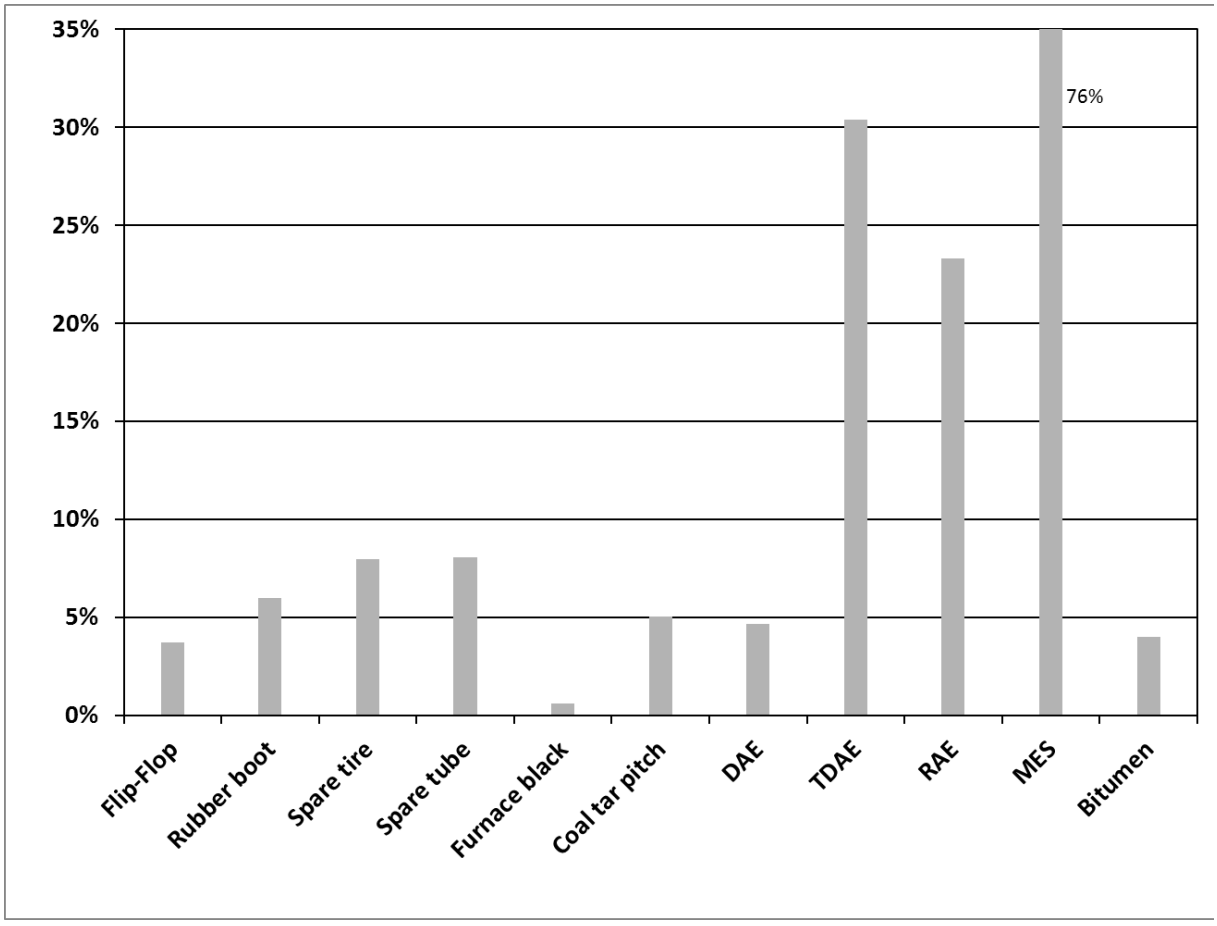


Figure 10: Ratio of total semipolar PAC high priority substances to total PAH

The treatment of process oils (e.g. DAE) reduces the PAH content considerably (from 262 mg/kg to < 15 mg/kg) in TDAE, RAE or MES (lowest PAH-content), but semipolar PAC were reduced only less efficiently (from 12.2 to 1.2-4.1 mg/kg). This clearly increases the ratio of semipolar PAC to PAH which is especially pronounced for MES.

[64] As outlined above, Benzo[a]acridine and Benzo[b]naphtho[2,3-d]furane were not analyzed by Dominguez et al., the respective close isomers (Benzo[a]phenanthridine and Benzo[b]naphtho[1,2-d]furane) however are reported and assignment of isomers may be inaccurate, such that these are treated like sum-values and the corresponding compounds are included in the comparative value of BIU-analysis (6181,03 mg/kg).

The tested bitumen contained few semipolar PAC (0.35 mg/kg) and PAH (8.7 mg/kg). Hydrotreating might have reduced both, PAH and S-heterocyclic PAC (see sections 16.6.1 and 16.6.2). A graphic overview of the ratios of PAH and semipolar PAC (sum values each, results of the BIU-analysis only) is presented in Figure 10. The vertical axis is cut at 35% in order to give also a meaningful overview of the lower concentration values (MES ratio: 76%).

17.3.2 Ratios of priority semipolar PAC substances to 16 EPA-PAH

The content of semipolar PAC (S-, N- and O-containing as well as total) was compared to the sum of PAH based on routine measurements for PAH (sum of 16 PAH according to EPA, in contrast to the 25 PAH determined by BIU, as used in Figure 10 above). These ratios are presented in the following Table 49 and Figure 11 (cut at 40% for a better overview on low values). Values close to 100% indicate approximately equal amounts of semipolar PAC compared to the sum of 16 EPA-PAH.

Table 49: Relative content of priority S-, N-, and O-PAC as well as total priority semipolar PAC (ps-PAC) to PAH (based on 16 EPA-PAH)

	Flip-Flop	Children´s rubber boots	Spare tyre for garden machinery	Spare tube for garden machinery	Furnace black
S-Semipolar PAC/16 EPA PAH	3.20%	4.27%	6.79%	6.98%	0.80%
N-Semipolar PAC/16 EPA PAH	0.41%	0.81%	1.18%	0.91%	0.01%
O-Semipolar PAC/16 EPA PAH	0.51%	1.97%	1.09%	1.08%	0.00%
Total 15 ps-PAC/16 EPA PAH	4.11%	7.04%	9.06%	8.96%	0.81%

	Coal tar pitch	DAE	TDAE	RAE	MES	Bitumen
S-Semipolar PAC/16 EPA PAH	2.58%	9.02%	36.50%	36.78%	94.24%	3.95%
N-Semipolar PAC/16 EPA PAH	2.27%	0.01%	0.09%	1.80%	2.51%	2.99%
O-Semipolar PAC/16 EPA PAH	0.96%	0.39%	0.35%	0.16%	0.63%	0.00%
Total 15 ps-PAC/16 EPA PAH	5.81%	9.42%	36.94%	38.75%	97.37%	6.94%

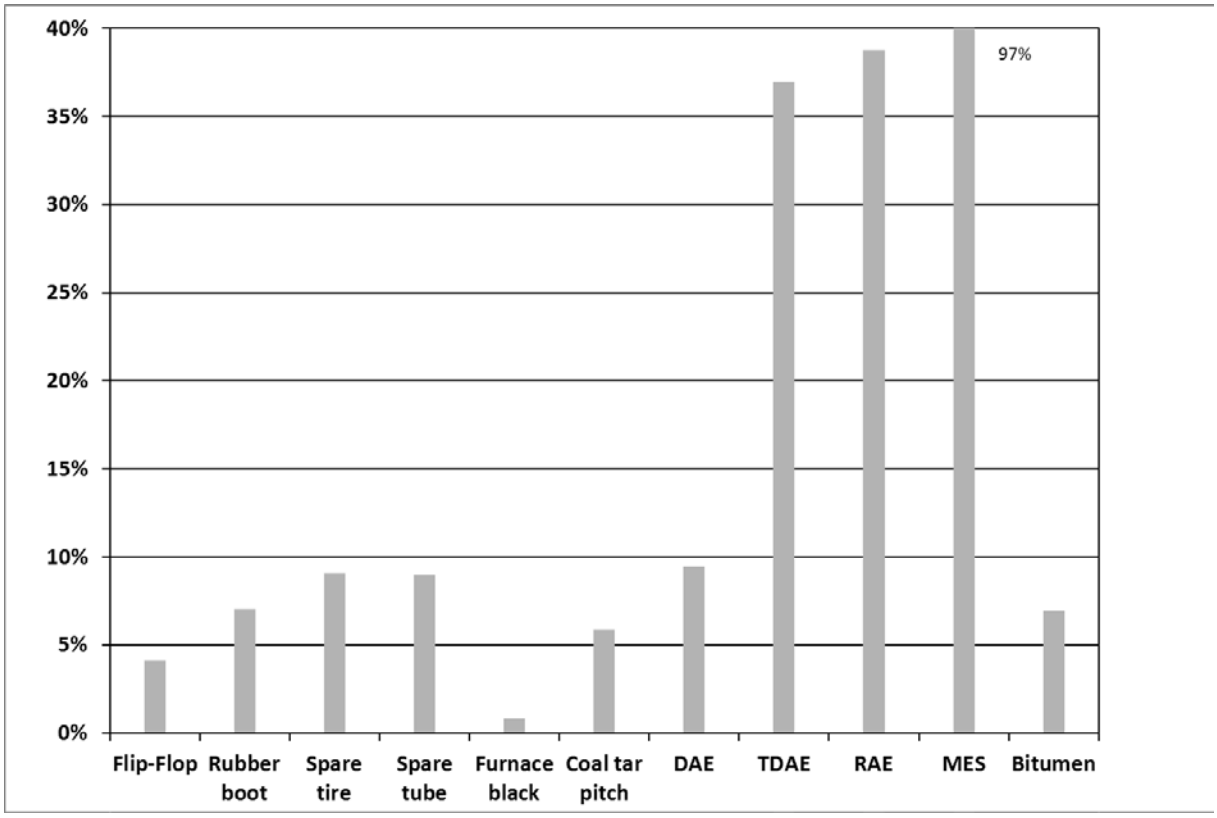


Figure 11: Relative content of total semipolar PAC high priority substances to PAH (based on 16 EPA-PAH)

Whereas the sum of semipolar PAC high priority substances amounts to 4-9% of the sum of 16 EPA-PAH in household articles, coal tar pitch, DAE and bitumen, the ratio varies widely within the other matrices. The depletion of PAH in treated process oils (TDAE, RAE and especially MES) is reflected by ratio values of 37% to 97%.

The ratio of the content of the semipolar PAC high priority substances to the sum of 16 EPA-PAH (either separated with respect to the heteroatoms or in total) was very similar in tube and tyre, indicating the use of process oils with comparable composition. Amongst all household articles, coal tar pitch, DAE and bitumen there was a variation within a factor of roughly 2, whereas a much higher variability was observed in the other matrices. Whether these ratios are typical for the classes of products analyzed would have to be substantiated with additional data from related matrices. In general, the ratio of semipolar PAC high priority substances to the sum of 16 EPA-PAH could be more fluctuating depending on the nature of process oils used.

In conclusion it can be stated that

- the total concentration of priority semipolar PAC is in general always considerably lower than PAH-concentration with the exception of TDAE, RAE and MES (where concentration of semipolar PAC is approximately 1/3 to roughly equal to the sum of 16 EPA-PAH, see Table 49, Figure 11).
- the higher content in TDAE, RAE and MES is due to the targeted reduction of PAH in these oils while semipolar PAC are not reduced to the same amount.
- the ratio of PAH to semipolar PAC varies widely over the different product groups, so the concentration of all determined PAH or the 16 EPA-PAH in particular are not suitable to extrapolate to the total content of the 15 priority semipolar PAC.

17.3.3 S-semipolar PAC high priority substances

The sulphur-containing semipolar PAC (6 compounds tested in total) were present in very high amounts in tyre, tube and coal tar pitch, in moderate amounts (>2 to about 10 mg/kg) in flip-flop, furnace black, DAE and TDAE. The remaining samples revealed concentrations of about 1 mg/kg or less. The results are presented in detail in chapter 16.5.

Benzo[b]naphtho[2,1-d]thiophene was predominant in all household articles, pitch, DAE and bitumen. It amounts to 30 to 60% of the S-semipolar PAC high priority substances in these matrices. While it was not predominant in TDAE, RAE and MES it still accounted for between 25% and 34% of total thiaarenes in these matrices.

2-Methyldibenzothiophene was predominant in TDAE, RAE and MES (53, 31 and 44%, respectively), and phenanthro[4,5-bcd]thiophene in furnace black (98%).

The ratios of the S-semipolar compound with the highest concentration in each sample (S max) compared to the total content of S-semipolar compound (S total) are presented in the left column pairs of the following Figure 12. The right column displays the ratio of benzo[b]naphtho[2,1-d]thiophene to total content of thiaarenes. The values differ only for these matrices, where benzo[b]naphtho[2,1-d]thiophene is not the predominant compound (see above).

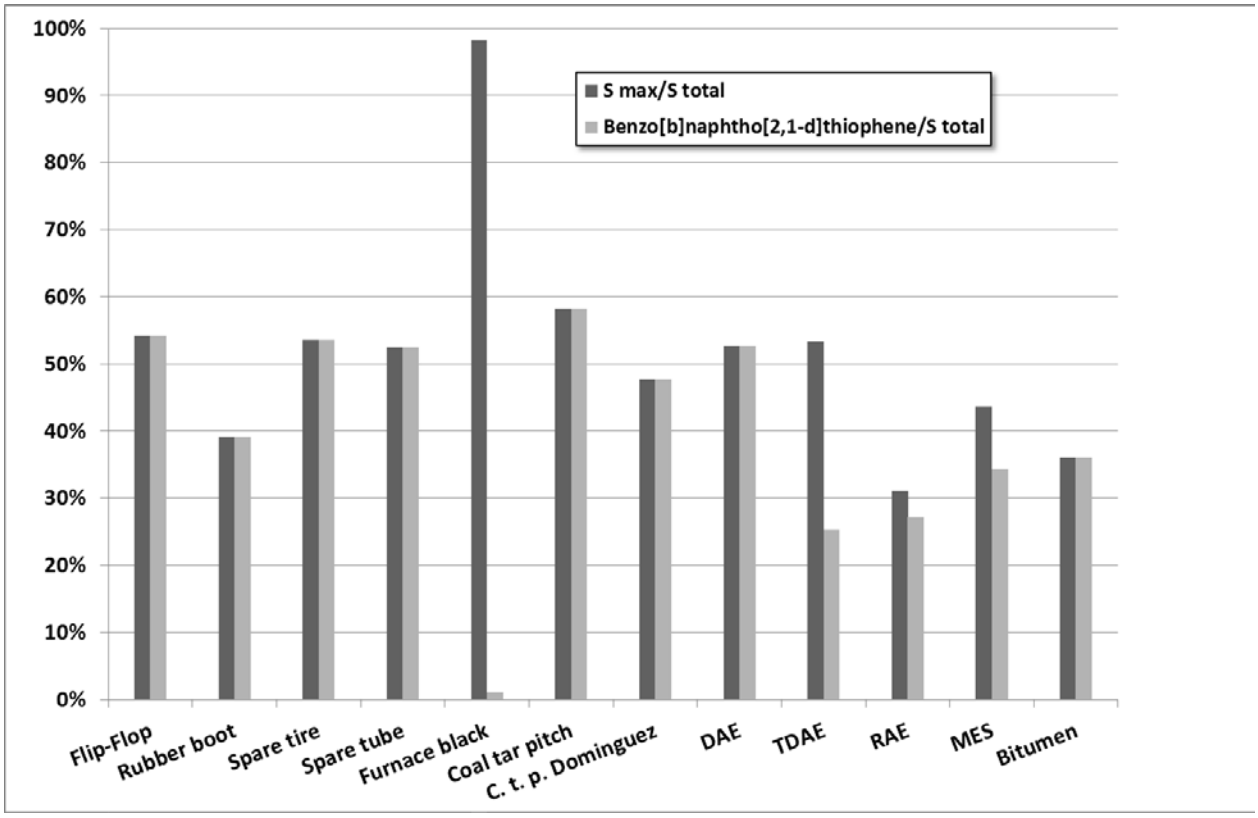


Figure 12: Relative content of the S-semipolar PAC with maximum concentration (left columns) and benzo[b]naphtho[2,1-d]thiophene (right columns) compared to total S-semipolar PAC high priority substances

It is evident from these data that benzo[b]naphtho[2,1-d]thiophene could serve as an appropriate indicator for the total content of sulphur-containing semipolar PAC especially in household articles with a range of 1.4 (minimal 39%, maximal 54%). It could be also a predictor of the S-semipolar PAC in other matrices (except furnace black) with a partial higher uncertainty up to 2.3. The result for coal tar pitch is corroborated by the data reported by Domínguez et al. (2004). However, the limited data base for all other matrices awaits verifi-

cation of this statement. Furthermore, ratios are restricted to the total of six thiaarenes regarded to be of high priority as deduced within the current project. Other thiaarenes most probably will be present to varying extent in the different matrices not assessed here. Extended analysis could corroborate this hypothesis and possibly specify the extrapolation factors for certain subtypes of samples.

17.3.4 N-semipolar PAC high priority substances

The nitrogen-containing semipolar PAC (8 compounds tested in total) were present in low amounts (< 1.6 mg/kg) in most of the samples except tyre, tube and coal tar pitch. The results are presented in detail in chapter 16.5.

The N-semipolar PAC particularly present with the highest concentration in the different matrices analyzed were more heterogeneous compared to the S-containing compounds. Benz[a]acridine was predominant in flip-flops, children´s rubber boots, and coal tar pitch, MES and bitumen, benz[c]acridine in spare tyre and spare tube. Dibenz[a,h]acridine was the predominant priority substance in furnace black, and 13H-dibenzo[a,i]carbazole in RAE. Therefore no clear N-containing indicator substance is identifiable in the examined matrices. If this analysis is restricted to household articles, the extrapolation from benz[a]acridine to the total content of N-semipolar PAC shows a variation with a factor of maximal 2.5 (see Figure 13).

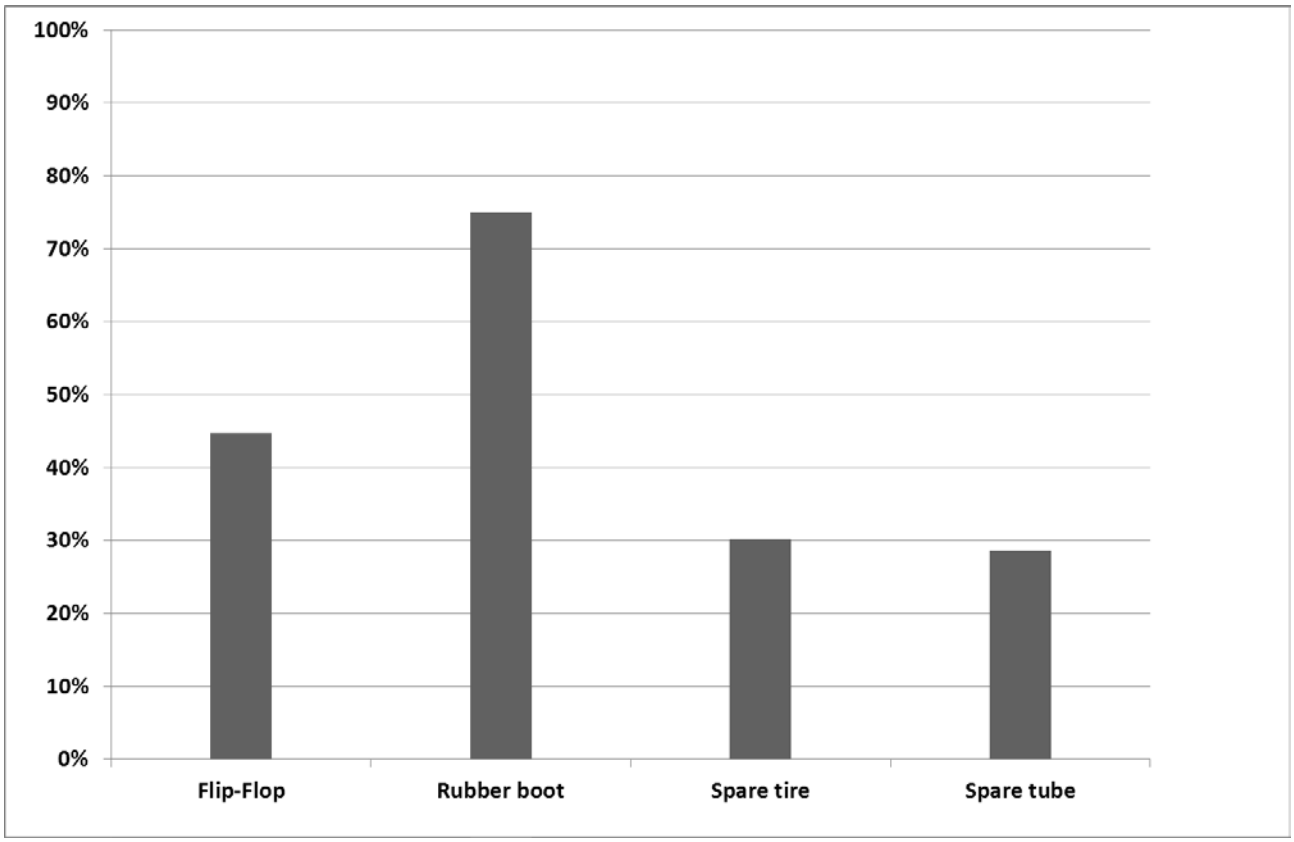


Figure 13: Relative content of benz[a]acridine compared to total the N-semipolar PAC high priority substances in household articles

Interestingly, for the 4 different extender oils tested azaarenes were present only close to or below LOQ. Thus the unexpectedly high azaaren-concentrations detected in household articles tyre and tyre tube and, albeit to a much lesser extent, flip-flop, could be of different origin than extender oils (see chapter 16.6.4).

17.3.5 O-semipolar PAC high priority substances

The oxygen-containing semipolar PAC (2 furans tested in total) were present in low amounts in all samples but tyre, tube and coal tar pitch (with about 7-fold lower contents in tyre and tube compared to pitch). Only low amounts have been detected in bitumen, the various process oils and furnace black. The results are presented in detail in chapter 16.5.

The ratio between these two substances varied between the analyzed matrices, so that no definite conclusions could be drawn.

17.3.6 Relative ratios of priority S-, N-, and O-PAC

In most matrices (except pitch and bitumen) the sulphur-containing semipolar PAC were found in considerably higher relative amounts compared to the nitrogen semipolar PAC and the two furans. This is illustrated in Figure 14. The relative content of the furans varied with respect to the aza-compounds (lower in pitch and bitumen, higher in rubber boots and roughly comparable in the remaining samples). Commercial bitumen is sometimes hydrotreated and analysis of the PAH-pattern indicates that the analyzed bitumen sample was indeed hydrotreated (see section 16.6.1). Hydrotreating not only reduces the PAH-content but also the amount of thiaarenes but not azaarenes and concentrations of thiaarenes found in the analyzed bitumen probe are low (compare section 16.5).

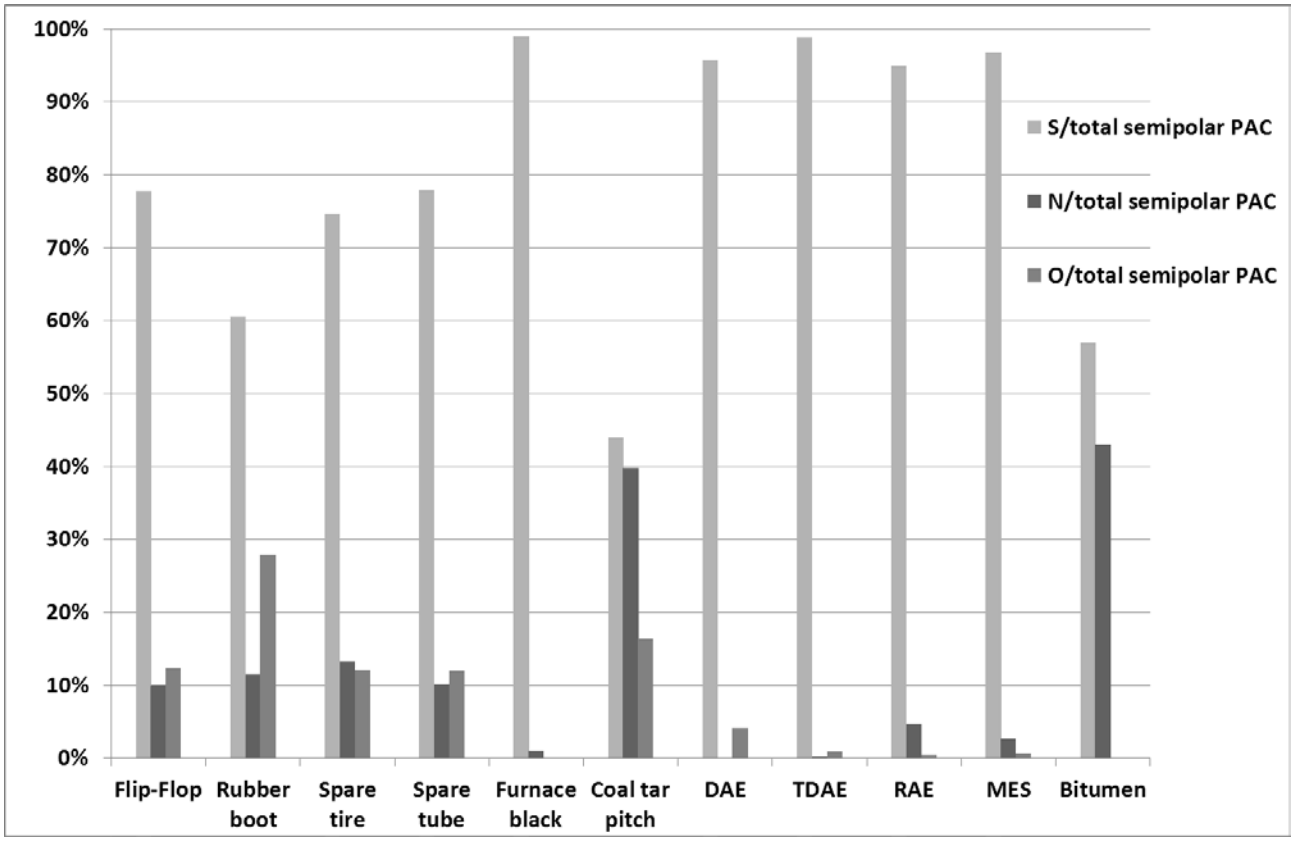


Figure 14: Relative content of S-, N- and O-containing semipolar PAC high priority substances in the examined matrices

It may be deduced from Figure 14, section 17.3.1 and 17.3.3 that

1. Thiaarenes pronouncedly dominate quantitatively compared to aza- and oxaarenes in most matrices and are at least equal to or somewhat higher than azaaarenes in coal tar pitch and bitumen.
2. Thiaarenes show the least variation as regards their relative occurrence to total semipolar PAC in the different matrices
3. Only thiaarenes have one predominant representative over all matrices which is either of the highest or at least second highest concentration of all analyzed thiaarenes and at the same time present in higher concentration than all aza- and oxaarenes analyzed in these matrices. This compound is benzo[b]naphtho[2,1-d]thiophene which due to the same reasons was the sole heterocyclic compound included in the so-called Grimmer-PAC (Grimmer and Böhnke, 1976). One exception is the analyzed bitumen sample, where the azaarene benzo[a]acridine is present in equal amount.

Therefore, in the following sections it will be further evaluated and discussed for what matrices this compound might be used as indicator compound and how the respective quantitative relation to the total semipolar PAC content appears to be.

17.3.7 Ratio of predominant semipolar PAC to total content of semipolar PAC high priority substances

As outlined above, benzo[b]naphtho[2,1-d]thiophene was the common predominant S-semipolar PAC in household products, process oils, pitch and bitumen (however for bitumen benzo[a]acridine in equal concentration). In Figure 15 the relative content of benzo[b]naphtho[2,1-d]thiophene in relation to all analyzed priority semipolar PAC is given.

As may be deduced from the graphical representation of Figure 15, benzo[b]naphtho[2,1-d]thiophene could be used as indicator to estimate the total content of semipolar PAC high priority substances in most matrices.

However, as mentioned earlier, furnace black is an exception, as the vastly predominant semipolar PAC quantified was phenanthro[4,5-bcd]thiophene which might be due to formation during the production process from sulfur and PAH at high temperatures. As this substance was the only semipolar PAC found in three independent carbon black profiles from the literature, it seems to be of special relevance for this matrix but other quantitative data are needed to draw firm conclusions and other types of carbon black might present another pattern of compounds.

Another matrix where benzo[b]naphtho[2,1-d]thiophene seems to be not the best choice as indicator substance is bitumen. Obviously, the bitumen sample analyzed had been hydrotreated as deduced from the PAH profile (see section 16.6.1). As a result besides PAH also S-PAC content is low whereas N-PAC content is not affected by hydrotreating leading to a high N-PAC content in relation to total priority semipolar PAC analyzed and an especially high relative concentration of benzo[a]acridine. Thus we propose to examine whether not both substances, i.e. benzo[b]naphtho[2,1-d]thiophene and benzo[a]acridine and their relation to one another could be used as indicators for priority semipolar PAC-content. Hydrotreated bitumen could possibly be characterized by a ratio near 1:1, whereas benzo[b]naphtho[2,1-d]thiophene would expected to be present in pronouncedly higher concentration for non-hydrotreated bitumen samples. Correspondingly, estimation of total priority semipolar PAC-content could be performed based on ratios specific for hydrotreated and non-hydrotreated bitumen samples, respectively. Further high quality data on priority semipolar PAC in bitumen are needed to validate this suggested approach.

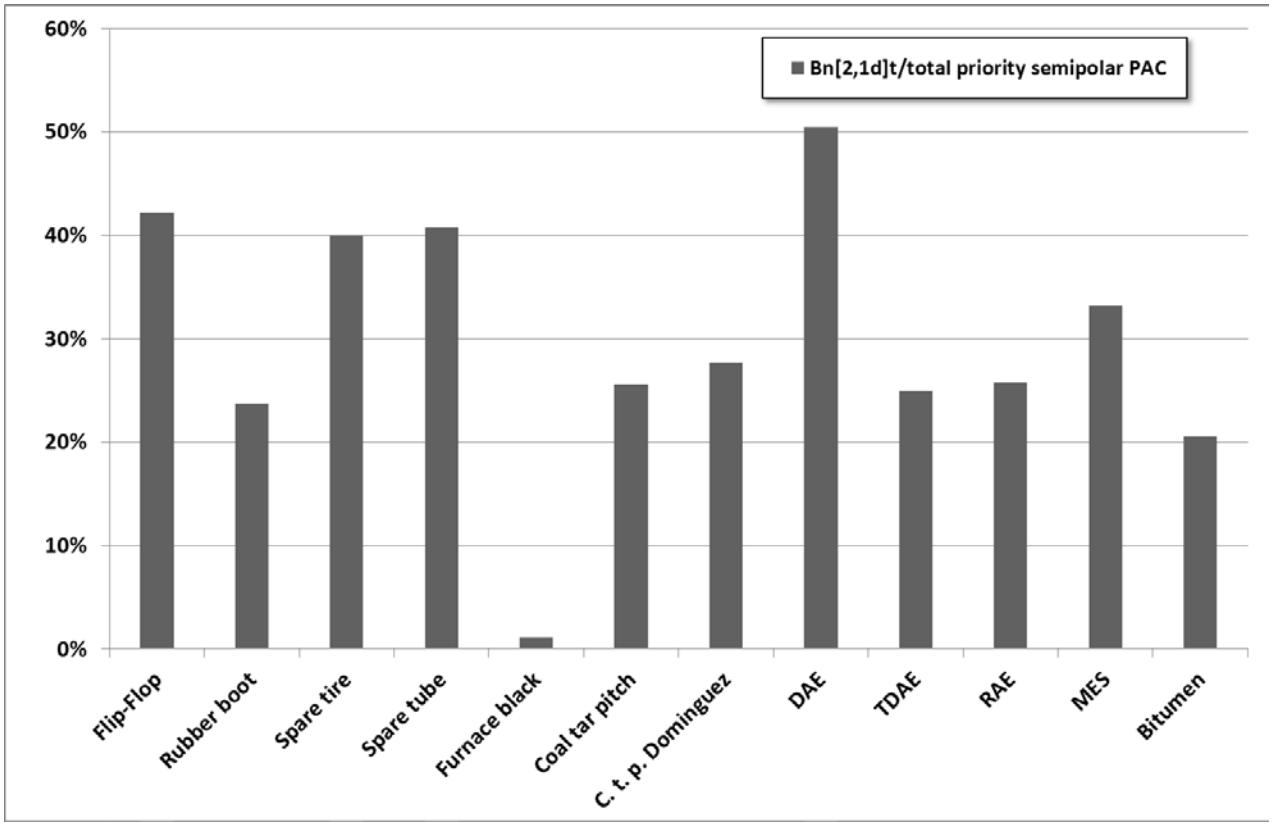


Figure 15: Relative content of benzo[b]naphtho[2,1-d]thiophene compared to total semipolar PAC high priority substances

For the other matrices analyzed, i.e. household products, the different processing oils and coal tar pitch benzo[b]naphtho[2,1-d]thiophene seems to be suited best as indicator substance to judge on total content of priority semipolar PAC analyzed in this project. However this does not hold true in respect to extrapolation on the relative composition of the semipolar PAC-content from S-PAC, N-PAC and O-PAC. The content and exact composition of the latter two is too variable to enable any prediction. Furthermore, the comparably high content of N- and O-PAC found in some household products in relation to the respective contents of these compounds detected in processing oils is not understood. This might be due to another source besides the commonly used processing oils analyzed within this project (see section 16.6.4).

In spite of the uncertainty regarding the origin of N-PAC and O-PAC in household products it is reasonable to assume that in most cases the content of priority semipolar PAC in these products will be due to the processing oils used. Table 50 summarizes available data. In conclusion, we suggest to use for household products as well as processing oils an extrapolation factor of 3.1 (± 1.1, i.e. the relation of highest to lowest single extrapolation factor of only 8 analyzed matrices) on the concentration of benzo[b]naphtho[2,1-d]thiophene [mg/kg] in order to estimate the total content of priority semipolar PAC.

Table 50: Extrapolation from benzo[b]naphtho[2,1-d]thiophene to total priority semipolar PAC (ps-PAC) content in household products and rubber processing oils: Suggested extrapolation factors and range

Household producst	**Flip-Flop**	**Rubber boot**	**Spare tyre**	**Spare tube**	**Mean**	**Range**
2,1-BNT/total 15 ps-PAC	42.2%	23.7%	40.1%	40.8%	36.7%	1.8
Deduced extrapolation Factor on total 15 ps-PAC	2.4	4.2	2.5	2.5	2.9	
Processing oils	**DAE**	**TDAE**	**RAE**	**MES**	**Mean**	**Range**
2,1-BNT/total 15 ps-PAC	50.4%	25.0%	25.7%	33.3%	33.6%	2.0
Deduced extrapolation Factor on total 15 ps-PAC	2.0	4.0	3.9	3.0	3.2	
Mean extrapolation factor on total ps-PAC from all products and processing oils. Range:relation of highest to lowest single extrapolation factor					**3.1**	± 1.1

Further, it is interesting to compare data on coal tar pitch from our investigation (BIU) with data from Domínguez et al. (2004) in regard to extrapolation from benzo[b]naphtho[2,1-d]thiophene to the 15 high priority PAC-concentration and the total PAC-concentration determined by Dominguez et al.(see Table 51).

Table 51: Coal tar pitch: Extrapolation from benzo[b]naphtho[2,1-d]thiophene on total concentration of priority semipolar PAC (ps-PAC) or the total concentration of all semipolar PAC determined by Domínguez et al. (2004) (i.e. including non-priority semipolar PAC not analyzed by BIU).

	Coal tar pitch, BIU-analysis	**Coal tar pitch Dominguez et al (2004), only 6 measured ps-PAC**	**Coal tar pitch Dominguez et al (2004) extrapolated to total ps-PAC with data from BIU analysis**
2,1-BNT/total 15 priority semipolar PAC	25.8%	27.7%	22.2%
Deduced **extrapolation Factor on total ps-PAC**	3.9	3.6	4.5
2,1-BNT/total semipolar PAC		9.2%	8.5%
Deduced **extrapolation Factor on** concentration of **all semipolar PAC** (20 plus several isomers) quantified by Dominguez et al. (2004)		10.9	11.8

Based on BIU-analysis of priority semipolar PAC in coal tar pitch an extrapolation from the concentration of benzo[b]naphtho[2,1-d]thiophene [mg/kg] on the total content of priority semipolar PAC would involve an extrapolation factor of 3.9.

Domínguez et al. (2004) analyzed only 6 from 15 priority semipolar PAC, however probably two close isomers to the reported compounds were implicit in the given concentrations (see section 17.2) which would then add up to 8 determined priority compounds. Table 51 presents both, results based solely on these measured concentrations and results determined by extrapolation to all 15 priority semipolar PAC in that concentrations determined by BIU were taken for the 7 priority compounds not determined by Domínguez et al. (2004).

Based solely on the concentrations of the measured compounds from the data of Domínguez et al. an extrapolation factor of 3.6 results while inclusion of measured data from BIU for the missing compounds leads to a factor 4.5. Both results are very similar to the factor of 3.9 deduced from the coal tar pitch analyzed by BIU within this project.

Interestingly, the analysis of Domínguez et al. was not restricted to the 15 priority semipolar PAC but included other semipolar PAC which we did not assess as high priority compounds and which were not determined within this project. This enables us to derive an extrapolation factor including also these compounds giving an impression of what was left behind focusing on 15 compounds only. Depending on the data base, a factor of rounded 11 or 12 is resulting, compared to a factor around 4 for extrapolation on the sum of 15 priority compounds only. Thus it must be emphasized that our approach to identify an indicator substance (benzo[b]naphtho[2,1-d]thiophene) and derive associated extrapolation factors is based and restricted to the 15 priority semipolar PAC identified and analyzed (BIU) within this project. Most probably a multitude of unidentified compounds is left behind, which is however also true for the generally accepted approach for PAH (see e.g. Lemieux, et al., 2008).

17.4 Qualitative application of the indicator substance approach

Profiles from the literature analyzed within this project often report only qualitative data on semipolar PAC. In addition, target compounds are varying vastly: Some publications are focusing on a certain PAC class with in-detail analysis of substances based on e.g. mass spectra combined with chromatographic signals while others are analyzing only a few commonly known target compounds.

With these data at hand the idea was to select all those profiles containing the identified indicator compound (2,1-BNT, see Table 52: 19 profiles identified) and check whether 2,1-BNT indicates qualitatively the coincidental presence of other semipolar PAC predicted to be of relevance in regard to PBT-properties from our QSAR-screening-approach.

Table 52: Qualitative assessment of literature profiles containing the proposed indicator substance benzo[b]naphtho[2,1-d]thiophene (2,1-BNT): Association of the indicator substance with other semipolar PAC predicted to be relevant in terms of PBT-properties – numerical evaluation.

Profiles containing indicator 2,1-BNT (n=19)		**Total number of semipolar PAC with the following properties predicted from QSAR-Screening**				**Total number of analyzed semipolar PAC**	% relevant **compounds including indicator** 2,1-BNT
	Type of profile	**PBT***	**BT**	**PB**	**Not relevant**		
P2A	bitumen	1	0	0	2	3	33.33%
P2B	bitumen	1	0	0	6	7	14.29%
P2C	bitumen	1	0	0	2	3	33.33%
P2D	bitumen	1	0	0	6	7	14.29%
P2E	bitumen	1	0	0	6	7	14.29%
P2F	bitumen	1	0	0	6	7	14.29%
P2G	bitumen	1	0	0	2	3	33.33%
P13	Coal tar	8	2	0	55	65	15.38%
P15	bitumen	1	0	0	2	3	33.33%

Profiles containing indicator 2,1-BNT (n=19)	**Type of profile**	**Total number of semipolar PAC with the following properties predicted from QSAR-Screening**				**Total number of analyzed semipolar PAC**	% relevant **compounds including indicator** 2,1-BNT
		PBT*	**BT**	**PB**	**Not relevant**		
P19	environment	1	1	0	4	6	33.33%
P21	environment	12	3	0	12	27	55.56%
P37A	Coal tar	7	3	0	19	29	34.48%
P37B	environment	7	3	0	19	29	34.48%
P42	Coal tar fraction	13	2	0	2	17	88.24%
P43	Carbon black	4	2	0	1	7	85.71%
P53C	Coal tar fraction	7	5	0	8	20	60.00%
P62	Coal tar pitch	18	5	0	17	40	57.50%
P69	Coal tar pitch	7	2	0	11	20	45.00%
P70B	Carbon black	3	0	0	1	4	75.00%
Arithmetic mean % weighted by the number of analyzed semipolar PAC							**40.5%**

(*): According to QSAR-screening, the indicator substance benzo[b]naphtho[2,1-d]thiophene (2,1-BNT)is itself classified as PBT: 1 indicates that the indicator substance itself was the only predicted PBT-compound contained in the respective profile.

In the 19 profiles containing 2,1-BNT, 2 to 64 semipolar PAC were analyzed in addition to the indicator compound. Of the analyzed semipolar PAC from single profiles 14.3% to 88.2% were relevant compounds in terms of PBT-properties as defined in this project (including 2,1-BNT). The arithmetic mean % of relevant semipolar PAC present in profiles containing the indicator compound was calculated such that each single percent-number was weighed by the total number of semipolar PAC identified in the respective profile. Overall, from profiles containing the indicator semipolar PAC 2,1-BNT and other semipolar PAC concurrently on average 40.5% of all semipolar PAC contained were relevant in regard to PBT properties.According to the samples tested by BIU benz[a]acridine could be a possible candidate for an indicator substance, but it was analysed only in few profiles (including environmental samples) in the literature.

17.5 Conclusion on Indicator Substance Approach

Concluding from our own analytical data collected within this project (BIU), the concentration of PAH in general or the 16 EPA-PAH are not suitable to extrapolate to the content of the priority semipolar PAC. The so-called Grimmer-PAC benzo[b]naphtho[2,1-d]thiophene (Grimmer and Böhnke, 1976) is suggested as indicator compound to extrapolate on total priority semipolar PAC content in processing oils and consumer products where these oils are used for production (mainly rubber products). Based on the evaluation of four profiles for processing oils and further four profiles for consumer products obviously produced using processing oils an **extrapolation factor of 3.1** (± 1.1, i.e. the relation of highest to lowest single extrapolation factor of only 8 analyzed matrices) on the concentration of benzo[b]naphtho[2,1-d]thiophene [mg/kg] in order to **estimate the total content of priority semipolar PAC** is derived.

In addition, profiles for coal tar pitch from this project (BIU) and the literature (Domínguez, et al., 2004) were analyzed and an extrapolation factor between 3.9 and 4.5 on the concentration of 2,1-BNT was derived to estimate total priority semipolar PAC concentration. Further, by taking into account other semipolar PAC analyzed by Domínguez et al. (2004) not predicted to be of priority in the sense of this project, a factor much higher results, namely approximately 12, on the concentration of 2,1-BNT to extrapolate the concentration of

total semipolar PAC analyzed within this work, yet still being only a fraction of total semipolar PAC compound space. From this it is emphasized that derived extrapolation factors are based and restricted to the 15 priority semipolar PAC identified and analyzed (BIU) within this project.

Further, from literature profiles evaluated in a qualitative manner it was shown that 2,1-BNT could be used also as a qualitative indicator substance in regard to high probability of simultaneous presence of other priority semipolar PAC where this indicator substance had been detected. Thus, we may possibly analyze matrices of interest (including soil samples) solely in regard to the presence of relevant concentrations of the indicator compound 2,1-BNT. However, this tentative approach was to be further examined and confirmed. Depending on the outcome, decisions on detailed chemical analysis including further high priority semipolar PAC could be made.

18 Implications for regulatory consequences

A further central point of the current project was to discuss if semipolar PAC would be a group of compounds with possible special relevance, which, however, is neglected in current chemicals legislation; and thus, if there would be the concurrent need to impose legislative steps for their regulation.

As regards the provisional SVHC-assessment (see 12.1) identifying 4 of the 15 priority compounds as potential PBT and 1 as vPvB compounds, missing data were replaced by QSAR and the available toxicity data are essentially restricted to acute test results for which reliability is often low or not assignable. It must be emphasized that missing experimental data might have also led to not provisionally classifying compounds as PBT or vPvB. Further data are needed for a final assessment of the 15 priority compounds, but available data point to the relevance for most of the 15 priority compounds in terms of hazard.

Therefore, an important question is if current restrictions on PAH may at the same time be regarded to be protective for semipolar PAC. This may only be the case if there would be some correlation between the content of PAH (or rather a selection thereof, e.g. the 16 EPA PAH or the 8 PAH currently regulated under REACH legislation for tyres) and semipolar PAC concentration.

As concluded above (section 17.3.2) correlation is far too weak to allow extrapolation from the sum of 16 EPA PAH on the concentration of the 15 priority semipolar PAC determined within this project. However, as can easily be recognized from Table 53 listing analysed matrices according to their order of sum concentration of 16 EPA-PAH, for most matrices the concentration of priority semipolar PAC decreases with decreasing PAH-concentration. An exception are modern rubber processing oils, where PAH-concentrations are reduced by technical measures and obviously semipolar PAC are depleted less efficiently, leading to a relative enrichment of the latter. This holds also true for the rubber boots probably produced using those oils.

These observations imply the following:

- Where PAH-concentration is high (i.e. sum of 8 PAH above the limit of 10 mg/kg set by REACH annex XVII for extender oils used for production of tyres, grey shaded in Table 53) also benzo[a]pyrene concentration is above the limit of 1 mg/kg specified in the same legal context and also comparably high concentrations of priority semipolar PAC are found, albeit still in much lower concentration than PAH (between 7 and 30% of the sum of 8 PAH).
- Where PAH-concentration is low (i.e. sum of 8 PAH below the limit of 10 mg/kg set by REACH annex XVII for tyres), also benzo[a]pyrene concentration is below the limit of 1 mg/kg specified in same legal context. For these matrices, also low concentrations of priority semipolar PAC are found, although they may be above the sum concentration for the 8 PAH according to REACH (TDAE, MES). Even the sum of 15 priority semipolar PAC *and* 8 PAH regulated under REACH is below the limit of 10 mg/kg set for the sum of 8 PAH for tyres (highest value: 8 mg/kg for TDAE).

Thus, correlation between PAH and semipolar PAC is sufficiently high for matrices where PAH concentration is high.

Current restriction of PAH under REACH is confined to extender oils used for the production of tyres and tyres itself.

Table 53: Concentration dependence of priority semipolar PAC- on concentration of total determined PAH (25), sum of 16 EPA-PAH, sum of 8 PAH according to REACH Annex XVII and Benzo[a]pyrene – the order of matrices is corresponding to their total PAH-concentration.

Matrix, [mg/kg]	Coal tar pitch	Spare tyre	Spare tube	Furnace black	Flip-Flop
Sum 16 EPA PAH	142687.6	16953.4	14221.5	946.9	394.2
Sum 8 PAH according REACH Annex XVII, No. 50	65234.4	5948.5	4324.1	113.6	98.6
Benzo[a]pyrene	**11739.3**	**964.8**	**669.7**	**42.4**	**12.8**
Sum 15 priority semipolar PAC	8291.9	1536.4	1274.3	7.6	16.2
Sum 15 priority PAC/sum of 8 PAH	13%	26%	29%	7%	16%

Matrix, [mg/kg]	DAE	TDAE	Rubber boot	Bitumen	RAE	MES
Sum 16 EPA PAH	129.9	11.2	7.4	5.0	3.2	1.3
Sum 8 PAH according REACH Annex XVII, No. 50	135.1	3.9	0.8	1.3	1.9	0.3
Benzo[a]pyrene	**11.7**	0.8	0.1	0.3	0.1	0.0
Sum 15 priority semipolar PAC	12.2	4.1	0.5	0.3	1.2	1.2
Sum 15 priority PAC/sum of 8 PAH	9%	107%	64%	27%	64%	396%

In regard to extender oils used in tyre production, the sum of 8 PAH including benzo[a]pyrene (10 mg/kg maximum) as well as benzo[a]pyrene itself (1 mg/kg maximum) is restricted. These limits are regarded to be kept if the DMSO extract according to the IP346: 1998 method is below 3% by weight. However, actual concentrations have to be verified in regular intervals to demonstrate correlation with the IP346-based limit. The DMSO-extractable fraction (including PAH and semipolar PAC, see section 7.5) stands for the carcinogenic potency (originating at least in part from the PAH content) of the oils and values < 3% by weight are regarded as safe and avoid their labeling as carcinogenic (Null, 1999; Prince, 2010).

Thus in principle, with a limit of 3% (mass) for the PAC-extract according to IP346 method (DMSO-extract), also priority semipolar PAC are implicitly assessed due to their solubility in DMSO. There is however uncertainty associated with modern process oils: Most probably, the limit of 3% was set based on carcinogenicity data for extracts from high aromatics oils like DAE. But the sum of priority semipolar PAC compared to the sum of 8 PAH is only 9% for DAE, while it amounts between 64% and 396% for modern process oils. As currently available (but incomplete) data on genotoxicity and carcinogenicity do not point to an extraordinarily high toxicity of semipolar PAC but rather a slightly lower or comparable toxicity to PAH, the limit set for PAH should also be protective for priority semipolar PAC considering the interdependencies of PAH and PAC concentrations outlined above: In spite of the relative enrichment of semipolar PAC compared to PAH, absolute concentrations are considerably lower compared to the high aromatic DAE oil.

While, according to REACH, the IP346 method *may* be used as a routine analysis tool to indicate conformity with the set limits for PAH, the *actual* limits are *only* valid for PAH. We do currently not know the background for the height of these limits (1 mg/kg for benzo[a]pyrene and 10 mg/kg for the sum of 8 PAH). While it may be that the underlying toxicological data are based on coal tar derived materials with regulated PAH as quantified indicator compounds, it might also be that specific, compound-related data had been used. For the latter case, semipolar PAC would have been clearly not addressed and it would be important to con-

sider regulatory measures to include this compound group. For the first case, taking a relative content of 13% of priority semipolar PAC compared to the sum of 8 PAH for coal tar pitch, this compound group would have been implicitly included in the assessment.

In regard to tyres itselves, REACH Annex XVII, number 50 addresses explicitly also tyres to enable assessment of conformity for imported final products, too. Limit values for benzo(a)pyrene or the sum of 8 PAH specified for process oils are directly valid also for tyres and concerning these limits the same holds true as outlined for process oils. For tyres, the specified PAH-limits are "regarded as kept, if the vulcanised rubber compounds do not exceed the limit of 0.35% Bay protons as measured and calculated by ISO 21461" intended to determine the aromaticity of oil in vulcanised rubber compounds. This method is based on the structure-activity relationship that bay region PAH are associated with high genotoxic/carcinogenic potency. The NMR-based method determines the ratio of bay protons to total protons (i.e. a relative value). In case of semipolar PAC, hetero-atoms in the aromatic ring system may lead to false negative or false positive results concerning bay protons due to possible alterations in shielding effects. Therefore the ISO 21461 method might not be appropriate to correctly assess bay protons of heterocyclic compounds and consequently, the cut-off value of 0.35% might not be applicable for semipolar PAC. Thus, further work would be required to demonstrate applicability of the method and the associated cut-off value.

In conclusion, regarding PAH restriction for process oils used in tyre production and tyres itself under REACH, there are considerable doubts concerning the implicit coverage of semipolar PAC in the set limit values. This is especially true for the explicit limits on PAH for process oils and tyres and the ISO 21461 method applied for tyres to conclude indirectly on compliance with PAH limits. There is considerable more confidence in the 3% weight limit for the DMSO extract obtained by the IP346 method used to conclude on compliance with PAH limits, as in principle semipolar PAC are implicitly assessed.

Considering these uncertainties and possible limitations of the currently applied method for tyres in regard to semipolar PAC, we would recommend setting in addition to cut-off values for PAH also a regulatory limit value for one or more important representative(s) of semipolar PAC. This could well be the suggested indicator compound benzo[b]naphtho[2,1-d]thiophene. Further work however needs to be done to validate the representativeness of this suggested indicator compound and, even more, to establish a sufficiently conclusive database for (eco)toxicological assessment of the whole compound group to enable one to set a quantitative limit value.

Regulatory implications in regard to aquatic organisms

All the above conclusions are however based on our measured concentrations of PAH and priority semipolar PAC in several matrices and are restricted to human exposure as well as genotoxic/cancerogenic effects, as humans are frequently more or less directly exposed to the matrices analysed. These conclusions are however invalid for aquatic organisms exposed over the water phase.

For assessing the relevance of priority semipolar PAC and thus the possible regulatory gap in regard to organisms exposed predominantly over the water phase (or the sediment), solubility and partitioning behaviour of semipolar PAC compared to PAH are decisive. For semipolar PAC higher water solubility is to be expected compared to their homocyclic PAH-analogues. Thus, upon environmental exposure relative concentrations of semipolar PAC compared to PAH in the water phase may be very different to the concentration ratio in the original matrices (e.g. processing oils or tyres) and thus relevance of semipolar PAC in regard to exposure could be higher than expected from concentration ratios in original media. This important point should be elucidated in a future project by experimental determinations of water solubility for the priority semipolar PAC and comparison with respective PAH analogues. Predictions of water solubility are deviating

vastly from one another applying different QSARs and thus seem to be not sufficiently precise (see Table 37 in section 15). Also their relevance in regard to environmental toxicity is still not clear and may be underestimated, as often data are lacking and generally no chronic toxicity data are available. Further, sediment toxicity was not assessed in this project but sediment could be an important route of exposure depending on the partitioning behaviour of semipolar PAC.

Therefore, in regard to implementation of semipolar PAC in chemicals regulation and – more limited – in regard to assessment of environmental semipolar PAC exposure or analytical considerations encompassing semipolar PAC, the possibility to use benzo[b]naphtho[2,1-d]thiophene as indicator substance might be of high value. This may hold true for the possibility to extrapolate on total priority semipolar PAC concentrations in original matrices as well as the outlined qualitative approach, where a detection of this indicator substance points to the simultaneous presence of other important semipolar PAC and thus reasonable decisions on further analytical determinations may be taken. To consider quantitative regulatory decisions concerning this compound group however, still much work needs to be done to characterise relevant compounds of the group in terms of (eco)toxicological hazard.

19 Combined Actions of Semipolar PAC

From the analyses of the 15 priority semipolar PAC in different matrices performed within this project it became clear that even in consumer products up to all 15 priority semipolar PAC (tyre, also 10-azabenzo[a]pyrene was detected in addition) may simultaneously be present. Therefore, as it is the case for PAH combined toxic action of these compounds has to be taken into consideration.

As semipolar PAC are in relation to their mode of action similar to a comparable degree as are PAH; and with PAH the so-called summation method is commonly applied using toxic equivalency factors (TEF) (KEMI, 2010) to "scale" the toxicity of respective PAH relative to benzo[a]pyrene (see e.g. Muller, et al., 1997); the summation method might be a valid first tier approach also for the group of 15 priority semipolar PAC. The summation method (concentration addition) "is the toxicity-weighted summation of the relevant mixture components and the subsequent analysis whether or not the relative amount of relevant components is above or below a pre-defined threshold" (EC, 2009b).

The suggestion to use the summation method is further supported by recent work (Kortenkamp, et al., 2012) which could demonstrate, that for most cases the summation method, which is applicable in the narrower sense only for chemicals sharing a similar mode of action, does not lead to unduly overestimations of mixture toxicities even when applied to mixtures of chemicals with dissimilar mode of action. Combined toxicity due to dissimilar mode of actions should normally be assessed according to the independent action model (Kortenkamp, et al., 2012).

Therefore, in principle it would be possible to roughly estimate the concentrations of the 15 priority semipolar PAC from the concentration of benzo[b]naphtho[2,1-d]thiophene (valid for consumer products based on materials involving the use of extender oils, extender oils themselves and coal tar pitch, see section 17), weigh them according to their specific toxicities in that e.g. RCRs[65] are formed, and add up their respective RCRs. If the summation result would be ≤ 1, no risk due to combined action would be expected, if it would be >1 adverse effects could not be excluded. However regarding ecotoxicity assessment in the water phase, with the current knowledge no extrapolation to the total concentration of 15 priority semipolar PAC based on the indicator substance benzo[b]naphtho[2,1-d]thiophene is possible as respective water solubilities and partitioning behavior are not known. The same holds true for the sediment (generally not assessed within this project). As such, evaluation of mixture toxicity at the moment would only be possible with measured concentrations for all single substances present in a given medium (e.g. water) and sufficient data regarding aquatic toxicity at hand (which however is momentarily also not the case).

With regard to the regulatory aspect under REACH, implication of combined toxicity of PAH and semipolar PAC could be necessary, if the limits set for benzo(a)pyrene (1 mg/kg) or the sum of 8 PAH (10 mg/kg) exclusively would cover PAH, indeed. Then, assuming additivity in regard to toxic action of PAH and simultaneously present semipolar PAC the limits set by the REACH regulation for single substances may not be sufficiently protective for the UVCB or mixture.

With regard to the note in column 2 stating fulfillment of the respective limits if the IP364-extract is below 3% weight, this might however indicate that carcinogenicity of the extract was related to mass concentrations of total PAC compounds (the DMSO-extract of an aromatic oil contains both, PAH and semipolar PAC) and

[65] RCR: risk characterization ratio, = PEC (predicted environmental concentration) / PNEC (predicted no effect concentration) or = human exposure estimate/DNEL (derived no effect levels, for humane toxicity)

thus, toxic action of semipolar PAC concurrently present in the DMSO-extract would also be included. If this is so, a further consideration of mixture toxicity would not be necessary in this case.

Scientific publications in regard to combined toxicity of semipolar PAC are rare. Meyer and Steinhart (2000) examined the mutual effects of PAH and their heterocyclic analogues on their respective degradation in a soil / compost mixture. The presence of heterocyclic PAC inhibited the degradation of PAH with two to five aromatic rings. On the other hand, degradation of just some heterocyclic PAC was inhibited by the presence of PAH. These observations might be interpreted as the result of combined action of substances from the two compound groups; however, no further conclusions may be drawn.

Lemieux et al. (2008) assessed mutagenic hazards due to complex PAC-mixtures in contaminated soil. Analyzing 10 tar- and creosote-contaminated soils from industrial sites of Sweden, nonpolar neutral aromatic fractions and semipolar aromatic fractions were analyzed for their mutagenic potencies with the Salmonella mutagenicity assay. Additionally, synthetic priority PAH-mixtures were prepared according to compositions found in soils. From the latter, comparison of predicted mutagenicity (implying the TEF-based summation method) with experimentally determined values pointed to an overestimation of toxicity. The authors speculate on saturation effects in regard to activating metabolic enzymes transforming PAHs to active mutagens to be possibly the reason for this observation. However, with the real nonpolar fractions actual mutagenic activities were occasionally underestimated by predicted mutagenicity from known priority compounds involving additivity. This might be due to hitherto unknown compounds included in this fraction. On the other hand, also the semipolar fraction elicited mutagenic response in the Salmonella assay. The authors conclude that traditional risk assessment models generally underestimate the risk involved with real PAC mixtures, especially if the semipolar fraction is included in the assessment. In conclusion, this publication demonstrates the difficulties associated with assessment of real mixtures and the complexity of possible interactions. If e.g. overestimation of mutagenic potency for the synthetic mixtures by summation compared to actual observed mutagenicity for these mixtures would in fact be due to oversaturation of metabolic enzymes, assessment of lower overall concentrations might alleviate this effect and predicted and measured values might be more close to one another.

No more relevant publications regarding combined action of semipolar PAC were identified.

Annex I: Profile list

Table 54: Table of 118 identified and analyzed profiles from the literature

Number	Reference	Matrix	Product
P1A	(Tobias, et al., 1989)	Pechbitumen	
P1B	(Tobias, et al., 1989)		Fugenvergussmasse treibstoffbeständig (Teer)
P1C	(Tobias, et al., 1989)		Fugenvergussmasse nicht treibstoffbeständig (Teer)
P2A	(Arbit, 1997; Knecht, et al., 1999)	Bitumen HB90/100	
P2B	(Arbit, 1997; Knecht, et al., 1999)	Bitumen B45	
P2C	(Arbit, 1997; Knecht, et al., 1999)	Bitumen B80	
P2D	(Arbit, 1997; Knecht, et al., 1999)	Bitumen B200	
P2E	(Knecht, et al., 1999)	Bitumen B65	
P2F	(Knecht, et al., 1999)	Bitumen 85/25	
P2G	(Knecht, et al., 1999)	Bitumen 95/35	
P3A	(Annweiler, et al., 2001)	groundwater, tar oil contaminated	
P3B	(Annweiler, et al., 2001)	groundwater, tar oil contaminated	
P4A	(Schlanges, et al., 2008)	groundwater, tar contaminated	
P4B	(Schlanges, et al., 2008)	groundwater, tar contaminated	
P4C	(Schlanges, et al., 2008)	groundwater, tar contaminated	
P4D	(Schlanges, et al., 2008)	groundwater, tar contaminated	
P5A	(Turney and Goerlitz, 1990)	groundwater, tar contaminated	
P5B	(Turney and Goerlitz, 1990)	soil, gasworks	
P6A	(WHO, 2004b)	Data from Cheremisinoff, 2001	
P7A	(Burchill, et al., 1983d)	Anthracene oil	
P7B	(Burchill, et al., 1983d)	Coke oven pitch	
P7C	(Burchill, et al., 1983d)	Anthracene oil	
P7D	(Burchill, et al., 1983d)	Coke oven pitch	
P7E	(Burchill, et al., 1983d)	Gray King Tar	
P8A	(Cheremisinoff and Rosenfeld, 2010)	coal tar creosote	
P8B	(Cheremisinoff and Rosenfeld, 2010)	coal tar creosote	
P8C	(Cheremisinoff and Rosenfeld, 2010)	coal tar creosote	
P8D	(Cheremisinoff and Rosenfeld, 2010)	coal tar creosote	
P9	(Chuang, et al., 1991)	outdoor air	
P10	(Collin and Höke, 2005)	coke oven coal tar	
P11A	(D'Affonseca, et al., 2008)	coal tar	
P11B	(D'Affonseca, et al., 2008)	well, contaminated by coal tar	
P11C	(D'Affonseca, et al., 2008)	well, contaminated by coal tar	
P11D	(D'Affonseca, et al., 2008)	well, contaminated by coal tar	
P11E	(D'Affonseca, et al., 2008)	soil, contaminated by coal tar	

Number	Reference	Matrix	Product
P12A	(ECHA, 2009)		Impregnating pitch (coal tar, high-temp)
P12B	(ECHA, 2009)		Binding pitch (coal tar, high-temp)
P13	(Fiedler, et al., 1997a)	"Kohlevergasungsteer"	
P14	(Fraser, et al., 1998)	ambient air	
P15	(Rühl, 2006)	Bitumen	
P16	(Hartnik, et al., 2007)	groundwater, creosote contaminated	
P17	Nishioka et al., 1986 (Nishioka, et al., 1986c)	Catalytically cracked petroleum vacuum residue (FCC VR, Exxon Research and Engineering Co., Linden, NJ.)	
P18A	(Nishioka, et al., 1986b)	SRCII heavy distillate coal tar liquid (fuel oil)	
P18B	(Nishioka, et al., 1986b)	coal tar	
P19	(Lemieux, et al., 2009)	gaswork soil	
P20	(Lundstedt, et al., 2006b)	gaswork soil	
P21	(Lundstedt, et al., 2003)	gaswork soil	
P22A	(Lundstedt, et al., 2006a)	soil, contaminated by gas works	
P22B	(Lundstedt, et al., 2006a)	reference soil	
P22C	(Lundstedt, et al., 2006a)	soil, contaminated by wood impregnation	
P22D	(Lundstedt, et al., 2006a)	soil, contaminated by wood impregnation	
P22E	(Lundstedt, et al., 2006a)	soil, contaminated by coke production	
P22F	(Lundstedt, et al., 2006a)	soil, contaminated by wood impregnation	
P22G	(Lundstedt, et al., 2006a)	soil, contaminated by wood impregnation	
P23	(Marynowski, et al., 2004)	urban dust samples	
P24	(Mattsson, et al., 2009)	Data from Lundstedt et al., 2006a	
P25A	(Johansen, et al., 1997)	groundwater, creosote contaminated	
P25B	(Johansen, et al., 1997)	groundwater, creosote contaminated	
P25C	(Johansen, et al., 1997)	groundwater, creosote contaminated	
P26	(Johansen, et al., 1998)	Data from Johansen et al., 1997	
P27	(Kern, et al., 2008)	coal tar	
P28	(King, M. W. G. and Barker, 1999)	creosote	
P29A	(Krone, et al., 1986)	creosote extract	
P29B	(Krone, et al., 1986)	sediment, creosote contaminated	
P30	(Lang and Eigen, 1967)	coal tar	
P31	(Mueller, et al., 1989)	creosote	
P32	(Mundt and Hollender, 2005)	ground water, wood impregnation	
P33	(NRC, 1983)	ambient air	
P34	(Park, et al., 2008)	soil, contaminated by gas works or wood impregnation	
P35	(Reineke, 2008)	groundwater, tar oil contaminated	
P36	(Reineke, et al., 2007)	groundwater, tar oil contaminated	
P37A	(Rostad, et al., 1985)	coal tar	

Number	Reference	Matrix	Product
P37B	(Rostad, et al., 1985)	aqueous eluate from tar	
P38	(Lyapina, et al., 2010)	crude oil	
P39	(Ramdahl and Urdal, 1982)		carbon black
P40	(Peaden, et al., 1980)		carbon black
P41	(Galceran, et al., 1994)	creosote	
P42	(Meyer zu Reckendorf, 2003)	coal tar, pitch, furnace gases (together)	
P43	(Lee and Hites, 1976)		carbon black
P44	(Kozin, et al., 1997)	sediment, no specific source	
P45	(Jacob, et al., 1991)		carbon black
P46A	(Later, et al., 1984)	medium oil	
P46B	(Later, et al., 1984)		carbon black
P47	(Pereira, et al., 1983)	groundwater, coal tar contamina-ted	
P48	(Burchill, et al., 1983c)	Anthracene oil (LC)	
P49A	(Burchill, et al., 1983b)	Anthracene oil	
P49B	(Burchill, et al., 1983b)	Anthracene oil	
P49C	(Burchill, et al., 1983b)	Anthracene oil	
P50	(Burchill, 1982)	Anthracene oil	
P51	(Brack and Schirmer, 2003)	industrial site soil	
P52A	(Later, 1985)	Bituminous coal	
P52B	(Later, 1985)	Anthracene oil	
P52C	(Later, 1985)	Coal tar	
P53A	(Sundström, et al., 1986)	creosote	
P53B	(Sundström, et al., 1986)	refined coal liquid heavy distillate (fuels)	
P53C	(Sundström, et al., 1986)	coal tar distillates	
P53D	(Sundström, et al., 1986)	wood preservation waste water	
P53E	(Sundström, et al., 1986)	water, coal tar contaminated	
P54	(Zamfirescu and Grathwohl, 2001)	groundwater, contaminated by gas works	
P55	(Kriech, et al., 2002)		roofing and paving asphalts (bi-tumen)
P56	(Del Bianco, et al., 1987)	Creosote oil basic fraction	
P57	(Lauer, et al., 1988)	Anthracene oil	
P58A	(Burchill, et al., 1983a)	Anthracene oil	
P58B	(Burchill, et al., 1983a)	Tar	
P59	(Wright, et al., 1985)	Creosote	
P60	(Grimmer, et al., 1981)		Lubricant oil
P61	(Onuska and Terry, 1989)	Sediment	
P62	(Guillén, et al., 1992)	coal tar pitch, volatile fraction (toluene-extract)	
P63A	(Berghof Analytik, 2010)	groundwater, wood preservation	
P63B	(Berghof Analytik, 2010)	groundwater, gaswork	
P64	(Breedveld, et al., 2004)		clay pidgeons
P65	(WHO, 2004a),	asphalt (bitumen) fume	
P66	(Gadd and Kennedy, 2003)		tyres
P67	(IARC, 2010)		carbon black
P68	(EPA, 2009)	creosote	wood preserving

Number	Reference	Matrix	Product
P69	(Domínguez, et al., 2004)	coal tar pitch	
P70A	(Nishioka, et al., 1986a)	coal tar	
P70B	(Nishioka, et al., 1986a)		carbon black

Annex II: Substance list of 443 semipolar polycylclic aromatic compounds

Table 55: *Pool of identified semipolar PAC* (443 compounds)

Running number	Source	CAS yellow field = mixture	Name[1] For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets.	SMILES[2] (incl. Substituents, however not specific for each isomer)	PBT-Screening result	PBT-Screening result enforced	Persistency: Half-life Water (d)[3]	Occurrence in profiles
1	KORA / LAWA	578-95-0	Acridinone	Oc(c(c(nc1cccc2)ccc3)c3)c12	NR	NR	15	6
2	KORA / LAWA	260-94-6	Acridine	n(c(c(ccc1)cc2cccc3)c1)c23	NR	NR	15	34
3	KORA / LAWA	84-65-1	Anthraquinone, 9,10-	O=C(c(c(C(=O)c1cccc2)ccc3)c3)c12	NR	NR	37,5	7
4	KORA / LAWA	225-11-6	Benz(a)acridine	c4ccc3nc2ccc1ccccc1c2cc3c4	PBT	NR	60	5
5	KORA / LAWA	225-51-4	Benz(c)acridine	c4ccc3nc1c(ccc2ccccc12)cc3c4	PBT	NR	60	12
6	KORA / LAWA	243-28-7	Benzo(b)carbazole, 5H-	c12c3cc4ccccc4cc3Nc1cccc2	NR	NR	37,5	26
7	KORA / LAWA	85-02-9	Benzo(f)quinoline	c1ccc2c(c1)ccc3ncccc23	NR	NR	15	20
8	KORA / LAWA	230-27-3	Benzo(h)quinoline	c1ccc2c(c1)ccc3cccnc23	NR	NR	15	23
9	KORA / LAWA	205-43-6	Benzo(b)naphtho(1,2-d)thiophene	c1ccc3c(c1)ccc4sc2ccccc2c34	PBT	PB	60	5
10	KORA / LAWA	239-35-0	Benzo(b)naphtho(2,1-d)thiophene	c1ccc3c(c1)ccc4c2ccccc2sc34	PBT	PB	60	19
11	KORA / LAWA	243-42-5	Benzo(b)naphtho(2,3-d)furan	c1ccc2c(c1)oc1cc3ccccc3cc21	PBT	PB	60	7
12	KORA / LAWA	243-46-9	Benzo(b)naphtho(2,3-d)thiophene	c1ccc2c(c1)sc1cc3ccccc3cc21	PBT	PB	60	6

Semipolar polycyclic aromatic hydrocarbons

Running number	Source	CAS yellow field = mixture	Name[1] For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets.	SMILES[2] (incl. Substituents, however not specific for each isomer)	PBT-Screening result	PBT-Screening result enforced	Persistency: Half-life Water (d)[3]	Occurrence in profiles
13	KORA / LAWA	271-89-6	Benzofuran	c1ccc2ccoc2c1	NR	NR	15	12
14	KORA / LAWA	95-15-8	Benzo(b)thiophene	s(c(c(c1)ccc2)c2)c1	NR	NR	15	34
15	KORA / LAWA	91-64-5	Benzopyran-2-one	c1cc2OC(=O)C=Cc2cc1	NR	NR	15	0
16	KORA / LAWA	119-91-5	Biquinoline, 2,2'-	n(c(c(ccc1)cc2)c1)c2c(nc(c(ccc3)c4)c3)c4	NR	NR	37,5	0
17	KORA / LAWA	86-74-8	Carbazole	n(c(c(c1cccc2)ccc3)c3)c12	NR	NR	15	40
18	KORA / LAWA	86-53-3	Cyanonaphthalene	N#Cc(c(c(ccc1)cc2)c1)c2	NR	NR	15	13
19	KORA / LAWA	224-42-0	Dibenz(a,j)acridine	c1ccc4c(c1)ccc5nc3ccc2ccccc2c3cc45	PBT	PB	60	6
20	KORA / LAWA	215-62-3	Dibenz(a,c)acridine	n5c2c(cc3c5c1c(c4c3cccc4)cccc1)cccc2	PBT	PB	60	2
21	KORA / LAWA	226-36-8	Dibenz(a,h)acridine	c1ccc4c(c1)ccc5nc2c(ccc3ccccc23)cc45	PBT	PB	60	2
22	KORA / LAWA	224-53-3	Dibenz(c,h)acridine	c1ccc2c3nc4c5ccccc5ccc4cc3ccc2c1	PBT	PB	60	2
23	KORA / LAWA	1016-05-3	Dibenzothiophene-S,S-dioxide	c12c3ccccc3S(=O)(=O)c1cccc2	NR	NR	37,5	0
24	KORA / LAWA	132-65-0	Dibenzothiophene	s(c(c(c1cccc2)ccc3)c3)c12	NR	NR	15	42
25	KORA / LAWA	496-15-1	Dihydroindole, 2,3-	N(c(c(ccc1)C2)c1)C2	NR	NR	37,5	0
26	KORA / LAWA	1721-89-7	Dimethylquinoline, 2,3-	c(ccc1cc2C)cc1nc2C	NR	NR	37,5	1

Semipolar polycyclic aromatic hydrocarbons

Running number	Source	CAS yellow field = mixture	Name[1] For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets.	SMILES[2] (incl. Substituents, however not specific for each isomer)	PBT-Screening result	PBT-Screening result enforced	Persistency: Half-life Water (d)[3]	Occurrence in profiles
27	KORA / LAWA	877-43-0	Dimethylquinoline, 2,6-	n(c(c(cc(c1)C)cc2)c1)c2C	NR	NR	37,5	10
28	KORA / LAWA	93-37-8	Dimethylquinoline, 2,7-	n(c(c(ccc1C)cc2)c1)c2C	NR	NR	37,5	4
29	KORA / LAWA	1463-17-8	Dimethylquinoline, 2,8-	Cc2ccc1cccc(C)c1n2	NR	NR	37,5	1
30	KORA / LAWA	826-77-7	Dimethylquinoline, 4,6-	c12nccc(C)c1cc(C)cc2	NR	NR	37,5	0
31	KORA / LAWA	40941-54-6	Dimethylquinoline, 4,7-	c12nccc(C)c1ccc(C)c2	NR	NR	37,5	0
32	KORA / LAWA	2623-50-9	Dimethylquinoline, 5,8-	n(ccc1)c(c(cc2)C)c1c2C	NR	NR	37,5	0
33	KORA / LAWA	2436-93-3	Dimethylquinoline, 6,8-	c12ncccc1cc(C)cc2C	NR	NR	37,5	1
34	KORA / LAWA	486-25-9	Fluorenone, 9-	O=C(c(c(c1cccc2)ccc3)c3)c12	NR	NR	15	9
35	KORA / LAWA	1689-64-1	Fluorenol, 9-	OC(c(c(c1cccc2)ccc3)c3)c12	NR	NR	15	4
36	KORA / LAWA	607-66-9	Methyl-2(1H)-quinolinone, 4-	c(ccc1C(=C2)C)cc1NC2=O	NR	NR	15	1
37	KORA / LAWA	607-67-0	Hydroxy-2-methylquinoline, 4-	c1ccc2c(O)cc(C)nc2c1	NR	NR	15	1
38	KORA / LAWA	826-81-3	Hydroxy-2-methylquinoline, 8-	n(c(c(ccc1)cc2)c1O)c2C	NR	NR	15	0
39	KORA / LAWA	90-43-7	Hydroxybiphenyl, 2-	Oc(c(c(cccc1)c1)ccc2)c2	NR	NR	15	0
40	KORA / LAWA	86-48-6	Hydroxynaphthalene-2-carboxylic acid, 1-	O=C(O)c(c(O)c(c(ccc1)c2)c1)c2	NR	NR	15	0

Semipolar polycyclic aromatic hydrocarbons

Running number	Source	CAS yellow field = mixture	Name[1] For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets.	SMILES[2] (incl. Substituents, however not specific for each isomer)	PBT-Screening result	PBT-Screening result enforced	Persistency: Half-life Water (d)[3]	Occurrence in profiles
41	KORA / LAWA	92-70-6	Hydroxynaphthalene-2-carboxylic acid, 3-	O=C(O)c(c(O)cc(c1ccc2)c2)c1	NR	NR	15	0
42	KORA / LAWA	6351-10-6	Indanol, 1-	c(ccc1C2O)cc1CC2	NR	NR	15	0
43	KORA / LAWA	4254-29-9	Indanol, 2-	c(ccc1C2)cc1CC2O	NR	NR	15	0
44	KORA / LAWA	1641-41-4	Indanol, 4-	c12CCCc1c(O)ccc2	NR	NR	15	0
45	KORA / LAWA	1470-94-6	Indanol, 5-	Oc(ccc(c1CC2)C2)c1	NR	NR	15	0
46	KORA / LAWA	83-33-0	Indanone, 1-	O=C(c(c(ccc1)C2)c1)C2	NR	NR	15	0
47	KORA / LAWA	120-72-9	Indole	c1ccc2ccnc2c1	NR	NR	15	21
48	KORA / LAWA	119-65-3	Isoquinoline	n(ccc(c1ccc2)c2)c1	NR	NR	15	24
49	KORA / LAWA	491-30-5	Isoquinolinone, 1(2H)-	c1ccc2C=CNC(=O)c2c1	NR	NR	15	5
50	KORA / LAWA	2439-04-5	Isoquinolinol, 5-	c12cnccc1c(O)ccc2	NR	NR	15	0
51	KORA / LAWA	7651-82-3	Isoquinolinol, 6-	c12cnccc1cc(O)cc2	NR	NR	15	0
52	KORA / LAWA	4265-25-2	Methylbenzofuran, 2-	Cc2cc1ccccc1o2	NR	NR	15	10
53	KORA / LAWA	21535-97-7	Methylbenzofuran, 3-	c12OC=C(C)c1cccc2	NR	NR	15	4
54	KORA / LAWA	5670-23-5	Methylbenzofuran, 4-	c12OC=Cc1c(C)ccc2	NR	NR	15	0

Semipolar polycyclic aromatic hydrocarbons

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55	KORA / LAWA	18441-43-5	Methylbenzofuran, 5-	c12OC=Cc1cc(C)cc2	NR	NR	15	0
56	KORA / LAWA	17059-51-7	Methylbenzofuran, 6-	c12OC=Cc1ccc(C)c2	NR	NR	15	0
57	KORA / LAWA	17059-52-8	Methylbenzofuran, 7-	O(C=C1)c(c(cc2)C)c1c2	NR	NR	15	0
58	KORA / LAWA	1455-18-1	Methylbenzothiophene, 3-	c12SC=C(C)c1cccc2	NR	NR	15	4
59	KORA / LAWA	14315-14-1	Methylbenzothiophene, 5-	S(C=C1)c(ccc2C)c1c2	NR	NR	15	0
60	KORA / LAWA	6510-65-2	Methylcarbazole, 1-	Cc3cccc2c3nc1c2cccc1	NR	NR	37,5	17
61	KORA / LAWA	3652-91-3	Methylcarbazole, 2-	c(ccc1N2)cc1c(ccc3C)c2c3	NR	NR	37,5	4
62	KORA / LAWA	4630-20-0	Methylcarbazole, 3-	c12c3cc(C)ccc3Nc1cccc2	NR	NR	37,5	5
63	KORA / LAWA	3770-48-7	Methylcarbazole, 4-	Cc3cccc2c3c1c(n2)cccc1	NR	NR	37,5	5
64	KORA / LAWA	1484-12-4	Methylcarbazole, N-	c(ccc1c2cc3)cc1N(c2cc3)C	NR	NR	37,5	0
65	KORA / LAWA	91-63-4	Methylquinoline, 2-	n(c(c(ccc1)cc2)c1)c2C	NR	NR	15	19
66	KORA / LAWA	612-58-8	Methylquinoline, 3-	Cc2cnc1ccccc1c2	NR	NR	15	4
67	KORA / LAWA	491-35-0	Methylquinoline, 4-	n(c(c(c(c1)C)ccc2)c2)c1	NR	NR	15	13
68	KORA / LAWA	7661-55-4	Methylquinoline, 5-	n(ccc1)c(ccc2)c1c2C	NR	NR	15	1

Semipolar polycyclic aromatic hydrocarbons

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69	KORA / LAWA	91-62-3	Methylquinoline, 6-	n(c(c(cc(c1)C)cc2)c1)c2	NR	NR	15	8
70	KORA / LAWA	612-60-2	Methylquinoline, 7-	n(c(c(ccc1C)cc2)c1)c2	NR	NR	15	4
71	KORA / LAWA	611-32-5	Methylquinoline, 8-	n(c(c(ccc1)cc2)c1C)c2	NR	NR	15	2
72	KORA / LAWA	16587-52-3	Methyldibenzothiophene, 3-	Cc1cc2c(c3c(s2)cccc3)cc1	BT	NR	37,5	2
73	KORA / LAWA	7372-88-5	Methyldibenzothiophene, 4-	Cc3cccc2c3sc1c2cccc1	BT	NR	37,5	2
74	KORA / LAWA	603-76-9	Methylindole, 1-	c1ccc2ccn(C)c2c1	NR	NR	15	1
75	KORA / LAWA	95-20-5	Methylindole, 2-	c1ccc2cc(C)nc2c1	NR	NR	15	6
76	KORA / LAWA	83-34-1	Methylindole, 3-	c12c(cccc2)[nH]cc1C	NR	NR	15	5
77	KORA / LAWA	16096-32-5	Methylindole, 4-	c1cc(C)c2ccnc2c1	NR	NR	15	0
78	KORA / LAWA	614-96-0	Methylindole, 5-	Cc2ccc1nccc1c2	NR	NR	15	0
79	KORA / LAWA	3420-02-8	Methylindole, 6-	N(C=C1)c(cc(c2)C)c1c2	NR	NR	15	0
80	KORA / LAWA	933-67-5	Methylindole, 7-	c1ccc2ccnc2c1C	NR	NR	15	1
81	KORA / LAWA	1721-93-3	Methylisoquinoline, 1-	n(ccc1cc2)c(c1cc2)C	NR	NR	15	5
82	KORA / LAWA	1125-80-0	Methylisoquinoline, 3-	c1ccc2cc(C)ncc2c1	NR	NR	15	1

Semipolar polycyclic aromatic hydrocarbons

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83	KORA / LAWA	42398-73-2	Methylisoquinoline, 6-	c12cnccc1cc(C)cc2	NR	NR	15	1
84	KORA / LAWA	232-81-5	Naphtho(1,2-c)thiophene	S1C=C2C=CC3=C(C=CC=C3)C2=C1	NR	NR	15	0
85	KORA / LAWA	261-31-4	Thioxanthene	c1ccc2Sc3ccccc3Cc2c1	NR	NR	37,5	4
86	KORA / LAWA	31317-07-4	Methyldibenzothiophene, 1-	Cc3cccc2c3c1ccccc1s2	BT	NR	37,5	0
87	KORA / LAWA	135-19-3	Naphthol, 2-	Oc(ccc(c1ccc2)c2)c1	NR	NR	15	4
88	KORA / LAWA	86-55-5	Naphthalene-1-carboxylic acid	O=C(O)c(c(c(ccc1)cc2)c1)c2	NR	NR	15	3
89	KORA / LAWA	20928-02-3	Methyldibenzothiophene, 2- (SMILES for 4-methyl-is.)	Cc3cccc2c3sc1c2cccc1	BT	NR	37,5	8
90	KORA / LAWA	234-41-3	Naphtho(1,2-b)thiophene	c12c3SC=Cc3ccc1cccc2	NR	NR	15	4
91	KORA / LAWA	91-22-5	Quinoline	n(c(c(ccc1)cc2)c1)c2	NR	NR	15	35
92	KORA / LAWA	233-02-3	Naphtho(2,1-b)thiophene	c12c3C=CSc3ccc1cccc2	NR	NR	15	0
93	KORA / LAWA	268-77-9	Naphtho(2,3-b)thiophene	c12cc3SC=Cc3cc1cccc2	NR	NR	15	0
94	KORA / LAWA	229-87-8	Phenanthridine	c1ccc2c(c1)cnc3ccccc23	NR	NR	15	25
95	KORA / LAWA	1015-89-0	Phenanthridinone, 6(5H)-	c(ccc1NC2=O)cc1c(ccc3)c2c3	NR	NR	37,5	2
96	KORA / LAWA	635-46-1	Tetrahydroquinoline, 1,2,3,4-	N(c(c(ccc1)CC2)c1)C2	NR	NR	37,5	0

Semipolar polycyclic aromatic hydrocarbons

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97	KORA / LAWA	92-83-1	Xanthene	O(c(c(ccc1)Cc2cccc3)c1)c23	NR	NR	37,5	9
98	KORA / LAWA	2235-15-6	Acenaphthenone, 1-	O=C1c2cccc3cccc(c23)C1	NR	NR	37,5	2
99	KORA / LAWA	239-30-5	Benzo[b]naphtho-[2,1-d]furan	O1C2=C(C=CC=C2)C2=C1C1=C(C=CC=C1)C=C2	BT	NR	37,5	4
100	KORA / LAWA	825-44-5	Benzo(b)thiophene-1,1-dioxid	c1ccc2C=CS(=O)(=O)c2c1	NR	NR	15	0
101	KORA / LAWA	194-59-2	Dibenzocarbazole (7H-Dibenzo(c,g)carbazole)	c12c3c4c(ccc3[nH]c2ccc2c1cccc2)cccc4	PBT	PB	60	5
102	KORA / LAWA	3782-00-1	Dimethylbenzofuran	c1ccc2c(C)c(C)oc2c1	NR	NR	37,5	8
103	KORA / LAWA	1136-77-2	Dimethyldibenzofuran (DMDBF)	c12c3cccc(C)c3Oc1c(C)ccc2	BT	NR	37,5	0
104	KORA / LAWA	1207-12-1	Dimethyldibenzothiophene (DMDBT)	Cc1cccc2c1c3c(s2)ccc(c3)C	BT	NR	37,5	1
105	KORA / LAWA	875-79-6	1,2-Dimethylindole	Cc2cc1ccccc1n2C	NR	NR	15	3
106	KORA / LAWA	875-30-9	1,3-Dimethylindole	c(ccc1C=2C)cc1N(C2)C	NR	NR	15	0
107	KORA / LAWA	91-55-4	2,3-Dimethylindole	CC1=C(C)c2ccccc2N1	NR	NR	37,5	1
108	KORA / LAWA	1196-79-8	2,5-Dimethylindole	c(cc(nc1C)c2c1)c(c2)C	NR	NR	37,5	0
109	KORA / LAWA	1721-94-4	Dimethylisoquinoline	c12c(C)nc(C)cc1cccc2	NR	NR	37,5	0
110	KORA / LAWA	59-31-4	Quinolinone, 2(1H)-	c1ccc2C=CC(=O)Nc2c1	NR	NR	15	7

Semipolar polycyclic aromatic hydrocarbons

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111	KORA / LAWA	70254-42-1	Hydroxyquinoline, 2-	Oc1nc2c(cc1)cccc2	NR	NR	15	0
112	KORA / LAWA	611-36-9	Hydroxyquinoline, 4-	c1ccc2c(O)ccnc2c1	NR	NR	15	0
113	KORA / LAWA	148-24-3	Hydroxyquinoline, 8-	n(c(c(ccc1)cc2)c1O)c2	NR	NR	15	0
114	KORA / LAWA	606-43-9	Methylquinolinone, 1-	c1ccc2C=CC(=O)N(C)c2c1	NR	NR	15	0
115	KORA / LAWA	7320-50-5	1-Methyldibenzofuran	Cc1cccc2oc3ccccc3c12	NR	NR	37,5	6
116	KORA / LAWA	7320-51-6	2-Methyldibenzofuran	c12c3ccc(C)cc3Oc1cccc2	BT	NR	37,5	0
117	KORA / LAWA	7320-52-7	3-Methyldibenzofuran	c12c3cc(C)ccc3Oc1cccc2	BT	NR	37,5	0
118	KORA / LAWA	7320-53-8	4-Methyldibenzofuran	c(ccc1c2cc3)cc1Oc2c(c3)C	BT	NR	37,5	0
119	KORA / LAWA	6072-57-7	Methylindanone	c(ccc1C2C)cc1C(=O)C2	NR	NR	15	0
120	KORA / LAWA	939-23-1	Phenylpyridine, 4-	n(ccc(c1)c(cccc2)c2)c1	NR	NR	37,5	0
121	KORA / LAWA	18123-20-1	Acridinol, 4-	c12nc3c(O)cccc3cc1cccc2	NR	NR	15	0
122	KORA / LAWA	244-99-5	Azafluorene, 4-	c12c3ncccc3Cc1cccc2	NR	NR	37,5	13
123	KORA / LAWA	4790-81-2	Benzofuranol, 7-	c12OC=Cc1cccc2O	NR	NR	15	0
124	KORA / LAWA	132-64-9	Dibenzofuran	o(c(c(c1cccc2)ccc3)c3)c12	NR	NR	15	32

Semipolar polycyclic aromatic hydrocarbons

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125	KORA / LAWA	86-77-1	2-Dibenzofuranol	c(ccc1c2cc3O)cc1Oc2cc3	NR	NR	15	2
126	KORA / LAWA	1013-23-6	Dibenzothiophene-5-oxid	S(=O)(c(ccc1)c-2c1)c(ccc3)c2c3	NR	NR	15	2
127	KORA / LAWA	92-81-9	Dihydroacridine, 9,10-	C2c1ccccc1Nc3ccccc23	NR	NR	37,5	1
128	KORA / LAWA	4565-32-6	Dihydrobenzo(b)-thiophene, 2,3-	S(CC1)c(ccc2)c1c2	NR	NR	15	1
129	KORA / LAWA	86-95-3	Dihydroxyquinoline, 2,4-	Oc(nc(c(c1O)ccc2)c2)c1	NR	NR	15	0
130	KORA / LAWA	2381-40-0	Dimethylbenz(c)acridine	Cc4ccc3c(C)c2ccc1ccccc1c2nc3c4	PBT	PB	180	0
131	KORA / LAWA	30027-44-2	Dimethylbenzo(b)-thiophene	Cc1csc2c1c(ccc2)C	NR	NR	37,5	0
132	KORA / LAWA	54385-63-6	Ethylbenzo(b)thiophene	CCC1=CC2=C(C=CS2)C=C1	NR	NR	15	1
133	KORA / LAWA	1986-00-1	Hydroxy-9-fluorenone, 4-	c12c3c(O)cccc3C(=O)c1cccc2	NR	NR	15	1
134	KORA / LAWA	480-93-3	Hydroxyindole, 3-	Oc1cnc2c1cccc2	NR	NR	15	0
135	KORA / LAWA	2433-56-9	Hydroxyphenanthrene, 1-	c(ccc1c(ccc2)c3c2O)cc1cc3	NR	NR	15	0
136	KORA / LAWA	613-15-0	Methylacridine, 2-	c12nc3cc(C)ccc3cc1cccc2	NR	NR	37,5	7
137	KORA / LAWA	611-64-3	Methylacridine, 9-	c(ccc1)c(nc(ccc2)c3c2)c1c3C	NR	NR	37,5	0
138	KORA / LAWA	84258-62-8	Methylbenzo(b)-naphtho(1,2-d)thiophene	CC1=CC=CC2=C1C1=C(SC3=C1C=CC=C3)C=C2	PBT	PBT	60	0

Semipolar polycyclic aromatic hydrocarbons

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139	KORA / LAWA	4567-41-3	Methylbenzo(b)-naphtho(2,1-d)thiophene	CC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	PBT	PBT	60	6
140	KORA / LAWA	36821-08-6	Methylbenzo(b)-naphtho(2,3-d)thiophene	CC1=CC=CC2=C1SC1=C2C=C2C=CC=CC2=C1	PBT	PBT	60	0
141	KORA / LAWA	85-06-3	Methylbenzoquinoline	n(c(c(c(c(c1)ccc2)c2)cc3)c1)c3C	NR	NR	37,5	13
142	KORA / LAWA	1195-14-8	Methylbenzothiophene, 2-	c12SC(C)=Cc1cccc2	NR	NR	15	3
143	KORA / LAWA	195-52-8	Phenanthrothiophene, 3,4-b	c12c(ccs1)c1c3ccccc3ccc1cc2	PBT	PB	60	1
144	KORA / LAWA	195-68-6	Phenanthrothiophene, 4,3-b	c12c(ccs1)ccc1ccc3ccccc3c21	PBT	PB	60	1
145	KORA / LAWA	224-10-2	Phenanthrothiophene, 3,2-b	c12c(ccs1)cc1ccc3ccccc3c1c2	PBT	PB	60	1
146	KORA / LAWA	3295-64-5	Tetrahydroacridine, 1,2,3,4-	C1CCc2nc3ccccc3cc2C1	NR	NR	37,5	1
147	KORA / LAWA	1196-39-0	Methylisoquinoline, 4-	n(ccc1cc2)cc1cc2C	NR	NR	15	0
148	KORA / LAWA	16587-33-0	Tetrahydrodibenzo(b)-thiophene	S(c(ccc1)c2c1)C(CCC3)=C2C3	BT	NR	37,5	0
149	KORA / LAWA	2437-72-1	Trimethylquinoline	Cc1c2c(nc(c1C)C)cccc2	NR	NR	37,5	1
150	KORA / LAWA	21296-92-4	Trimethylindole, 2,3,5-	c(cc(nc1C)c2c1C)c(c2)C	NR	NR	37,5	0
151	KORA / LAWA	90-47-1	Xanthenone	c1ccc2Oc3ccccc3C(=O)c2c1	NR	NR	37,5	2
152	KORA / LAWA	1198-37-4	2,4-Dimethylquinoline	n(c(c(c(c1)C)ccc2)c2)c1C	NR	NR	37,5	6

Semipolar polycyclic aromatic hydrocarbons

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153	KORA / LAWA	95-14-7	Benzotriazole	c1ccc2nnnc2c1	NR	NR	15	0
154	KORA / LAWA	29385-43-1	Tolyltriazole	Cc1cccc2c1N=NN2	NR	NR	15	0
155	KORA / LAWA	54004-38-5	Methylisoquinoline, 7-	n(ccc1cc2)cc1cc2C	NR	NR	15	0
156	KORA / LAWA	62882-00-2	Methylisoquinoline, 8-	Cc1cccc2ccncc12	NR	NR	15	0
157	KORA / LAWA	93-09-4	Naphthalene-2-carboxylic acid	O=C(O)c(ccc(c1ccc2)c2)c1	NR	NR	15	4
158	KORA / LAWA	62882-01-3	Methylisoquinoline, 5-	Cc1c2ccncc2ccc1	NR	NR	15	0
159	KORA / LAWA	90-15-3	Naphthol, 1-	Oc(c(c(ccc1)cc2)c1)c2	NR	NR	15	5
160	Profiles	18028-55-2	Mixture of other Dimethylcarbazoles (1,4-Dimethylcarbazole, 18028-55-2)	[nH]1c2c(c(C)ccc2C)c2c1cccc2	NR	NR	37,5	2
161	Profiles	18992-67-1	1,2-Dimethylcarbazole	[nH]1c2c(c(C)ccc2C)c2c1cccc2	NR	NR	37,5	5
162	Profiles	18992-68-2	1,3-Dimethylcarbazole	[nH]1c2c(c(C)ccc2C)c2c1cccc2	NR	NR	37,5	2
163	Profiles	54738-93-1	Mixture of Nitroanthracenes	[O-][N+](=O)c1cccc2cc3ccccc3cc12	NR	NR	37,5	1
164	Profiles	602-60-8	9-nitroanthracene	[O-][N+](=O)c1cccc2cc3ccccc3cc12	NR	NR	37,5	4
165	Profiles	313-80-4	1-Azapyrene (313-80-4) and/or 4-Azapyrene	c12c3c4ccc1cccc2ccc3ncc4	NR	NR	60	6
166	Profiles	100663-47-6	Cyclopenta(def)phenanthrone	c1(ccc2cc3cccc4ccc1c2c34)=O	NR	NR	37,5	1
167	Profiles	10299-63-5	Mixture of Trimethyindoles (2,3,4-Trimethylindole, 10299-63-5)	[nH]1c(c(c2c(cccc12)C)C)C	NR	NR	37,5	2
168	Profiles	1196-81-2	2-Ethylbenzo(b)thiophene	S(c(ccc1)c2c1)C(=C2)CC	NR	NR	15	1

Semipolar polycyclic aromatic hydrocarbons

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169	Profiles	1210-12-4	9-Cyanoanthracene	N#Cc(c(c(ccc1)cc2cccc3)c1)c23	NR	NR	37,5	5
170	Profiles	130-15-4	1,4-Naphthoquinone	c1cc2C(=O)C=CC(=O)c2cc1	NR	NR	15	1
171	Profiles	13177-29-2	2-Nitrofluoranthene	O=N(=O)C4=CC2C3C(=CC=CC3=C4)c1c2cccc1	NR	NR	37,5	1
172	Profiles	134-32-7	1-Naphthylamine	c(c(c(N)cc1)ccc2)(c2)c1	NR	NR	37,5	3
173	Profiles	14315-11-8	Mixture of Methylbenzo(b)thiophens (4-Methylbenzo(b)thiophene, 14315-11-8)	s1ccc2c1cccc2C	NR	NR	15	1
174	Profiles	193-98-6	2-Azapyrene = (Naphth(2,1,8-def)isoquinoline)	c12c3c4ccc1cccc2ccc3cnc4	NR	NR	60	10
175	Profiles	195-29-9	Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline = Benzo(a)phenanthridine, 195-29-9)	c12c3c(cccc3)cnc2ccc2c1cccc2	PBT	NR	60	12
176	Profiles	203-65-6	Benzo(def)carbazole	c12c3c4cccc3Nc1cccc2cc4	NR	NR	37,5	14
177	Profiles	205-25-4	Benzo(c)carbazole	c12c3c4ccccc4Nc3ccc1cccc2	NR	NR	37,5	10
178	Profiles	205-39-0	2,3-Benzodiphenylene oxide (3 CAS, Benzo(b)naphtho(1,2-d)furan, 205-39-0;	c1ccc2c(c1)oc1ccc3ccccc3c21	PBT	PB	60	10
179	Profiles	206-49-5	Mixture of Azafluoranthenes (7-Azafluoranthene, 206-49-5)	c12c3c4cccnc4c1cccc2ccc3	NR	NR	60	11
180	Profiles	21064-50-6	Mixture of Methylbenzocarbazoles (6-Methyl-3,4-benzocarbazole = 7H-Benzo(c)carbazole, 10-methyl- ,21064-50-6)	c12c3c(ccc4c3cccc4)[nH]c1ccc(c2)C	PBT	PB	60	5
181	Profiles	2243-89-2	Mixture of Trimethylquinolines and -isoquinolines (2,4,6-Trimethylquinoline, 2243-89-2)	n1c2c(cc(cc2)C)c(cc1C)C	NR	NR	37,5	7
182	Profiles	229-71-0	Benz(h)isoquinoline	c1nccc2ccc3c(c12)cccc3	NR	NR	15	2
183	Profiles	239-01-0	Benzo(a)carbazole	c(ccc1N2)cc1c(ccc3cc4)c2c3cc4	NR	NR	37,5	9

Semipolar polycyclic aromatic hydrocarbons

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184	Profiles	2498-66-0	Benzanthracene-7,12-dione = (7,12-Benz(a)anthraquinone)	O=C(c(c(c(c(c1)ccc2)c2)C(=O)c3cccc4)c1)c34	NR	NR	37,5	8
185	Profiles	2510-55-6	9-Cyanophenanthrene	c12c3ccccc3c(C#N)cc1cccc2	NR	NR	37,5	7
186	Profiles	257-07-8	Dibenz(b,f)(1,4)oxazepine	N1=Cc2ccccc2Oc3c1cccc3	NR	NR	37,5	1
187	Profiles	270-82-6	2-Benzothiophene	c12c(csc1)cccc2	NR	NR	15	1
188	Profiles	2788-23-0	9-Nitroso-9H-carbazole	O=NN(c(c(c1cccc2)ccc3)c3)c12	NR	NR	37,5	2
189	Profiles	29451-76-1	1-Aminodibenzothiophene	NC1=C2C(SC3=C2C=CC=C3)=CC=C1	NR	NR	37,5	1
190	Profiles	3074-00-8	Benzo(cd)pyren-6-one	O=C1C2=C3C(C=CC4=C3C3=C1C=CC=C3C=C4)=CC=C2	PBT	PB	60	4
191	Profiles	36062-93-8	Mixture of Methylcyanonaphthalenes (1-Cyano-4-methylnaphthalene, 36062-93-8)	CC1=CC=C(C#N)C2=C1C=CC=C2	NR	NR	37,5	4
192	Profiles	39327-16-7	Benzoquinoline	C1=CC2=CC3=CC=CC=C3N=C2C=C1	NR	NR	15	2
193	Profiles	4269-15-2	4-Amino-9-fluorenone	c12c3c(N)cccc3C(=O)c1cccc2	NR	NR	37,5	1
194	Profiles	484-17-3	9-Phenanthrol	Oc1cc2c(c3c1cccc3)cccc2	NR	NR	15	2
195	Profiles	493-57-2	Benzothiophene-2,3-dione	C1(Sc2ccccc2C1=O)=O	NR	NR	15	1
196	Profiles	5315-79-7	1-Pyrenol	Oc4ccc2c3c4ccc1cccc(c13)cc2	PBT	NR	60	2
197	Profiles	548-39-0	Phenalenone	c12c3cccc1cccc2C=CC3=O	NR	NR	15	2
198	Profiles	5522-43-0	1-Nitropyrene	O=N(=O)c(c(c(c(c(c1)ccc2)c2cc3)c3c4)c1)c4	PBT	PB	180	5
199	Profiles	56631-57-3	1-Indenol	C=1[C@@H](c2ccccc2C1)O	NR	NR	15	1
200	Profiles	57955-12-1	Mixture of Azafluorenones (1-Azafluorenon = 9H-Indeno(2,1-b)pyridin-9-one, 57955-12-1)	c12c(cccn1)c1ccccc1C2=O	NR	NR	37,5	2
201	Profiles	581-89-5	2-Nitronaphthalene	O=N(=O)c2ccc1ccccc1c2	NR	NR	37,5	2

Semipolar polycyclic aromatic hydrocarbons

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202	Profiles	605-67-4	Mixture of Dimethylbenzoquinolines (Benzo(h)quinoline, 2,4-dimethyl-, 605-67-4)	Cc1nc2c3ccccc3ccc2c(c1)C	NR	NR	37,5	7
203	Profiles	605-88-9	Mixture of other Methylbenzoquinolines (2-Methylbenzo(h)quinoline, 605-88-9)	c12nc(C)ccc2ccc2c1cccc2	NR	NR	37,5	3
204	Profiles	613-46-7	2-Cyanonaphthalene	N#Cc(ccc(c1ccc2)c2)c1	NR	NR	15	6
205	Profiles	6306-07-6	Acenaphthene-1-ol	OC1Cc2cccc3c2c1ccc3	NR	NR	15	7
206	Profiles	6314-28-9	Mixture, including Benzo(b)thiophene-2-carboxylic acid = Thionapthene-2-carboxylic acid (6314-28-9) and Benzo(b)thiophene-5-carboxylic acid and 1 other isomer	c1(sc2ccccc2c1)C(=O)O	NR	NR	15	2
207	Profiles	68967-09-9	Mixture of Pyrene carboxaldehydes	O=Cc1cc2ccc3cccc4ccc(c1)c2c34	PBT	PB	60	1
208	Profiles	7148-92-7	2-Azafluoranthene = (Indeno(1,2,3-de)isoquinoline)	c12c3c4ccccc4c1cncc2ccc3	NR	NR	60	1
209	Profiles	72433-66-0	4-Aminodibenzothiophene	NC1=C2SC3=C(C=CC=C3)C2=CC=C1	NR	NR	37,5	1
210	Profiles	7428-91-3	2-Aminodibenzothiophene	NC1=CC=C2SC3=C(C=CC=C3)C2=C1	NR	NR	37,5	1
211	Profiles	7469-77-4	2-Methyl-1-naphthol	c(ccc1c(O)c2C)cc1cc2	NR	NR	15	4
212	Profiles	76895-43-7	Pyrene-3,4-dicarboxylic acid anhydride = (3H,5H-Pyreno(1,10-cd)pyran-3,5-dione)	c1cc2c3c4c(C(OC(c4cc2)=O)=O)cc2ccc c1c32	PBT	PB	60	1
213	Profiles	78020-40-3	Fluorantheneamine	NC1=CC2=CC=CC3=C2C(=C1)C1=CC =CC=C31	PBT	NR	60	1
214	Profiles	79313-23-8	Propyldibenzothiophene; (Dibenzothiophene, C3-substituted, different isomers)	c12c3c(cccc3)sc1cccc2CCC	BT	BT	37,5	1
215	Profiles	79313-25-0	Butyldibenzothiophene; (Dibenzothiophen, C4-substituted, different isomers)	c12sc3ccccc3c2cccc1C(CCC)	BT	BT	15	1
216	Profiles	81-84-5	1,8-naphthalic anhydride	O=C(OC(=O)c(c1c(ccc2)cc3)c3)c12	NR	NR	15	5
217	Profiles	82-05-3	Benz(de)anthracene-7-one	O=C(c(c(c(c1c(ccc2)cc3)c3)ccc4)c4)c12	BT	NR	37,5	6

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218	Profiles	84909-43-3	4-Methyl-2-Hydroxyquinoline	n1c(cc(C)c2c1cccc2)O	NR	NR	15	1
219	Profiles	86-57-7	1-Nitronaphthalene	N(=O)(=O)c(c(c(ccc1)cc2)c1)c2	NR	NR	37,5	4
220	Profiles	86-79-3	2-Hydroxycarbazole	c12c3ccc(O)cc3Nc1cccc2	NR	NR	37,5	1
221	Profiles	87688-44-6	2-(9H-Xanthene-9-yl)-1H-indene-1,3(2H)-dione	O=C1C(C(=O)C2=C1C=CC=C2)C1=CC=CC2=C1[O]=C1C=CC=CC1=C2	BT	NR	37,5	1
222	Profiles	91-59-8	2-Naphthylamine	c(c(ccc1N)ccc2)(c2)c1	NR	NR	37,5	3
223	Profiles	93-08-3	2-Acetylnaphthalene	O=C(c(ccc(c1ccc2)c2)c1)C	NR	NR	15	3
224	Profiles	94248-40-5	Fluorene carbonitriles	c12c3ccccc3C(C#N)c1cccc2	NR	NR	37,5	1
225	Profiles	95-16-9	Benzothiazole	c1ccc2ncsc2c1	NR	NR	15	3
226	Profiles	954-46-1	9-Nitrophenanthrene	[N+](c1c2c(cccc2)c2c(c1)cccc2)([O-])=O	NR	NR	37,5	1
228	Profiles	4923-91-5	Mixture of C2 Benzo(b)thiophenes (8 Isomere) (Benzo(b)thiophene, 2,3-dimethyl, 4923-91-5)	s1c(c(C)c2c1cccc2)C	NR	NR	37,5	3
229	Profiles	0	Dibenzothiophene, -butylmethyl; (Dibenzothiophen, C5-substituted, different isomers)	CCCCC1=CC=CC2=C1C1=C(S2)C=C(C)C=C1	BT	BT	15	1
230	Profiles	0	Phenanthro(4,5-bcd)thiophene, -ethyl; (Phenanthro(4,5-bcd)thiophene, C2-substituted, different isomers)	CCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	PBT	PBT	60	1
231	Profiles	0	Phenanthro(4,5-bcd)thiophene, -propyl; (Phenanthro(4,5-bcd)thiophene, C3-substituted, different isomers)	CCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	PBT	PBT	60	1
232	Profiles	0	Phenanthro(4,5-bcd)thiophene, -butyl; (Phenanthro(4,5-bcd)thiophene, C4-substituted, different isomers)	CCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	PBT	PBT	60	1

Semipolar polycyclic aromatic hydrocarbons

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233	Profiles	0	Phenanthro(4,5-bcd)thiophene, -pentyl; (Phenanthro(4,5-bcd)thiophene, C5-substituted, different isomers)	CCCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	PBT	PBT	60	1
234	Profiles	0	Phenanthro(4,5-bcd)thiophene, -Hexyl; (Phenanthro(4,5-bcd)thiophene, C6-substituted, different isomers)	CCCCCCC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	PBT	PBT	60	1
235	Profiles	0	Benzo(b)-naphtho(2,1-d)thiophene, -ethyl; (benzo(b)-naphtho(2,1-d)thiophene, C2-substituted, different isomers)	CCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	PBT	PBT	180	3
236	Profiles	0	Benzo(b)-naphtho(2,1-d)thiophene, -propyl; (benzo(b)-naphtho(2,1-d)thiophene, C3-substituted, different isomers)	CCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	PBT	PBT	180	1
237	Profiles	0	Benzo(b)-naphtho(2,1-d)thiophene, -butyl; (benzo(b)-naphtho(2,1-d)thiophene, C4-substituted, different isomers)	CCCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	PBT	PBT	60	1
238	Profiles	0	Benzo(b)-naphtho(2,1-d)thiophene, -pentyl; (benzo(b)-naphtho(2,1-d)thiophene, C5-substituted)	CCCCCC1=CC=CC2=C1C1=C(C=C2)C2=C(S1)C=CC=C2	PBT	PBT	60	1
239	Profiles	0	(1)Benzothieno(3,2-b)(1)benzothiophene, C3-substituted, different isomers	CCCC1=CC2=C(C=C1)C1=C(S2)C2=CC=CC=C2S1	BT	BT	37,5	1
240	Profiles	0	(1)Benzothieno(3,2-b)(1)benzothiophene, C4-substituted	CCCCC1=CC2=C(C=C1)C1=C(S2)C2=CC=CC=C2S1	BT	BT	15	1
241	Profiles	0	Naphthobenzothiophane, C3-substituted	CCCC1=CC2=C(C=C1)C1C=CC3=C(C=CC=C3)C1S2	BT	BT	37,5	1
242	Profiles	0	Chryseno(4,5-bcd)thiophene, C1-substituted	CC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	PBT	PBT	180	1
243	Profiles	0	Chryseno(4,5-bcd)thiophene, C2-substituted, different isomers	CCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	PBT	PBT	180	1

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244	Profiles	0	Chryseno(4,5-bcd)thiophene, C3-substituted, different isomers	CCCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	PBT	PBT	180	1
245	Profiles	0	Chryseno(4,5-bcd)thiophene, C4-substituted, different isomers	CCCCC1=C2SC3=CC=CC4=C3C2=C(C=C4)C2=CC=CC=C12	PBT	PBT	60	1
246	Profiles	0	Dinaphthothiophene, C2-substituted	CCC1=C2SC3=C(C=CC4=C3C=CC=C4)C2=CC2=C1C=CC=C2	BT	BT	37,5	1
247	Profiles	0	3-Aminodibenzothiophene	NC1=CC=C2C(SC3=C2C=CC=C3)=C1	NR	NR	37,5	1
248	Profiles	0	4-Azadibenzothiophene	S1C2=C(C=CC=C2)C2=C1N=CC=C2	NR	NR	15	2
V3-001	Profiles	147-47-7	Dihydrotrimethylquinoline (1,2-Dihydro-2,2,4-trimethylquinoline)	N(c(c(C(=C1)C)ccc2)c2)C1(C)C	NR	NR	37,5	1
V3-002	Profiles	72072-20-9	Benzophenanthrothiophene (Benzo(2,3)phenanthro(4,5-bcd)thiophene)	C1=CC=C2C(=C1)C=C3C=CC4=C5C3=C2SC5=CC=C4	PBT	PBT	60	3
V3-003	Profiles	100441-36-9	Methylhydroxyanthracenes or -phenanthrenes (2-Methylanthracene-1-ol)	CC1=C(C2=CC3=CC=CC=C3C=C2C=C1)O	NR	NR	37,5	1
V3-004	Profiles	10240-08-1	Methylnaphthols (4-Methyl-1-naphthol, 10240-08-1)	c(ccc1c(c2)C)cc1c(c2)O	NR	NR	15	1
V3-005	Profiles	102859-52-9	Nitrocyclopenta(cd)pyrene (4-Nitrocyclopenta(cd)pyrene, 102859-52-9)	C1=CC2=C3C(=C1)C=C4C(=CC5=C4C3=C(C=C2)C=C5)[N+](=O)[O-]	PBT	PB	60	1
V3-006	Profiles	104313-09-9	Dibenzo(b,def)carbazole	N1C2=C3C4=C1C=C1C=CC=CC1=C4C=CC3=CC=C2	PBT	PB	60	1
V3-007	Profiles	1090-13-7	Naphthacene-5,12-dione	O=C(c(c(C(=O)c1cccc2)cc(c3ccc4)c4)c3)c12	BT	NR	37,5	3
V3-008	Profiles	12041-95-1	Benzacridine (12041-95-1) and/or Azachrysene	C1=CC=C2C(=C1)C=CC3=NC4=CC=CC=C4C=C32	PBT	NR	60	2
V3-009	Profiles	128-66-5	Dibenzo(b,def)chrysene-7,14-quinone	O=C(c(c(c(c1c(c(c(c(C2=O)ccc3)c3)cc4)c2c5)c5)ccc6)c6)c14	BT	BT	37,5	1

Semipolar polycyclic aromatic hydrocarbons

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V3-010	Profiles	135-67-1	Phenoxazine	c1ccc2Oc3ccccc3Nc2c1	NR	NR	37,5	1
V3-011	Profiles	14461-85-9	C4-Naphtholes (1,6-Diethyl-2-naphthol, 14461-85-9)	CCC1=CC2=C(C=C1)C(=C(C=C2)O)CC	BT	NR	37,5	1
V3-012	Profiles	149-30-4	Mercaptobenzothiazole	N(c(c(S1)ccc2)c2)=C1S	NR	NR	15	1
V3-013	Profiles	17580-20-0	Dimethylisoquinolines(1,3-Dimethylisoquinoline, 17580-20-0)	CC1=CC2=CC=CC=C2C(=N1)C	NR	NR	37,5	1
V3-014	Profiles	18028-56-3	C3-Carbazoles or benzoindoles (9H-Carbazole, 1,4,6-trimethyl- RN: 18028-56-3	CC1=CC2=C(C=C1)NC3=C(C=CC(=C23)C)C	BT	NR	37,5	1
V3-015	Profiles	189-90-2	Azabenzopyrenes (2-Azabenzo(b)pyrene, 189-90-2)	c4c5ccccc5c2c3c4ccc1cncc(c13)cc2	PBT	PB	60	2
V3-016	Profiles	194-03-6	Benzo(lmn)phenanthridine = 4-Azapyrene = Dibenzo(c,d,e)quinoline	c12c3c4cccc3ncc1cccc2C=C4	NR	NR	60	5
V3-017	Profiles	194-65-0	Dinaphtho(2,1-b:1',2'-d)thiophene	S(c(ccc1cc2)c-3c1cc2)c(ccc4cc5)c3c4cc5	BT	BT	37,5	4
V3-018	Profiles	19694-02-1	Pyrene carboxylic acid (1-Pyrenecarboxylic acid, 19694-02-1)	OC(=O)C1=C2C=CC3=CC=CC4=CC=C(C=C1)C2=C34	PB	PB	60	1
V3-019	Profiles	200-23-7	Benzo(kl)xanthene	O(c(c(c(c1c(ccc2)cc3)c3)ccc4)c4)c12	BT	NR	37,5	4
V3-020	Profiles	206-49-5	7-Azafluoranthene	n(c(c(c(c1c(cc2)ccc3)c2)cc4)c13)c4	NR	NR	60	11
V3-021	Profiles	206-56-4	Indeno(1,2,3-ij)isoquinoline = 1-Azafluoranthene	n(c(c(c(c1)ccc2)c2c3cccc4)c34)c1	NR	NR	60	4
V3-022	Profiles	20668-33-1	6,7-Dimethylquinoline	Cc1cc2c(cc1C)nccc2	NR	NR	37,5	1

Semipolar polycyclic aromatic hydrocarbons

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V3-023	Profiles	218-08-6	Azachrysenes (Naphtho(2,1-f)quinoline = 1-Azachrysene, 218-08-6)	c4cc2c(c3c4c1c(cc3)nccc1)cccc2	PBT	NR	60	3
V3-024	Profiles	218-34-8	Diazabenz(a)anthracene or -chrysene (4,10-Diazachrysene, 218-34-8	C1=CC2=C(C3=C(C=C2)C4=C(C=CC=N4)C=C3)N=C1	NR	NR	60	1
V3-025	Profiles	219-25-0	Phenanthro(2,1-b)thiophene	c1ccc2ccc3c4C=CSc4ccc3c2c1	PBT	PB	60	1
V3-026	Profiles	224-07-7	Phenanthro(2,3-b)thiophene	c12c(ccs1)cc1c3ccccc3ccc1c2	PBT	PB	60	1
V3-027	Profiles	2243-89-2	2,4,6-Trimethylquinoline	c12nc(C)cc(C)c1cc(C)cc2	NR	NR	37,5	7
V3-028	Profiles	227-86-1	Anthra(1,2-b)thiophene	C1=CC=C2C=C3C(=CC2=C1)C=CC4=C3SC=C4	PBT	PB	60	1
V3-029	Profiles	229-67-4	Benzo(f)isoquinoline	c12c3c(cncc3)ccc1cccc2	NR	NR	15	1
V3-030	Profiles	230-17-1	Benzo(c)cinnoline	c1ccc2c(c1)nnc3ccccc23	NR	NR	15	1
V3-031	Profiles	233-34-1	Naphthopyrrole (1H-Benz(g)indole, 233-34-1)	C1=CC=C2C(=C1)C=CC3=C2NC=C3	NR	NR	15	1
V3-032	Profiles	236-01-1	Phenanthro(9,10-b)thiophene	c32c(ccs3)c4ccccc4c1ccccc21	PBT	PB	60	1
V3-033	Profiles	239-64-5	13H-Dibenzo(a,i)carbazole	c12c(c3ccc4c(c3[nH]2)cccc4)ccc2c1cccc2	PBT	PB	60	1
V3-034	Profiles	243-51-6	11H-Indeno(1,2-b)quinoline	c12c3nc4ccccc4cc3Cc1cccc2	NR	NR	37,5	2
V3-035	Profiles	2443-58-5	2-Hydroxyfluorene (9H-Fluoren-2-ol)	c12c3ccc(O)cc3Cc1cccc2	NR	NR	15	1

Semipolar polycyclic aromatic hydrocarbons

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V3-036	Profiles	24496-65-9	Dibenzophenanthridine or isomer (Dibenzo(i,lmn)phenanthridine = 12-Azabenzo(a)pyrene)	c12c3c4ncc1c1c(cccc1)cc2ccc3ccc4	PBT	PB	60	1
V3-037	Profiles	2523-48-0	9H-Fluorene-2-carbonitrile = 2-Cyanofluorene	c(ccc1C2)cc1c(ccc3C#N)c2c3	NR	NR	37,5	3
V3-038	Profiles	2840-51-9	Methyl-9-fluorenones (9H-Fluoren-9-one, 2-methyl, 2840-51-9)	O=c1c2c(c3c1cccc3)ccc(c2)C	NR	NR	37,5	2
V3-039	Profiles	29062-95-1	C2-Dibenzofurans (Dimethyldibenzofurans, 29062-95-1)	CC1=C(C2=C(C=C1)OC3=CC=CC=C32)C	BT	NR	37,5	1
V3-040	Profiles	3067-13-8	Benzo(a)pyrene-1,6-quinone	O=C1C=CC2=C3C1=CC=C1C4=C(C=CC=C4)C(=O)C(C=C2)=C31	BT	NR	37,5	1
V3-041	Profiles	3067-14-9	Benzo(a)pyrene-3,6-quinone	O=C1C=CC2=CC=C3C4=C(C=CC=C4)C(=O)C4=C3C2=C1C=C4	BT	NR	37,5	1
V3-042	Profiles	3074-03-1	11H-Benzo(b)fluoren-11-one	O=C1C2=C(C=CC=C2)C2=C1C=C1C=CC=CC1=C2	BT	NR	37,5	1
V3-043	Profiles	30796-92-0	Phenanthro(4,5-bcd)thiophene	c1cc2sc3cccc4ccc(c1)c2c34	PBT	PB	60	11
V3-044	Profiles	31096-91-0	Phenyl-1H-indoles	C1=CC=C(C=C1)N2C=CC3=CC=CC=C32	NR	NR	15	1
V3-045	Profiles	313-80-4	1-Azapyrene	n4ccc2c3c4ccc1c3c(ccc1)cc2	NR	NR	60	6
V3-046	Profiles	31473-75-3	Perylo(1,12-bcd)thiophene (4 isomers)	S1C2=CC=C3C=CC=C4C5=CC=CC6=CC=C1C(C2=C34)=C56	BT	BT	37,5	3
V3-047	Profiles	3254-91-9	Methylbenzo(def)carbazoles or indenoindoles (5,10-Dihydroindeno(1,2-b)indole, 3254-91-9	C1C2=CC=CC=C2C3=C1C4=CC=CC=C4N3	NR	NR	37,5	1
V3-048	Profiles	33583-02-7	Trimethyl- or methylethylnaphtholes (2,5,8-Trimethyl-1-naphthol, 33583-02-7)	c(cc(C)c1c(O)c2C)c(C)c1cc2	NR	NR	37,5	1

Semipolar polycyclic aromatic hydrocarbons

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V3-049	Profiles	3558-24-5	Methylphenylindoles (1-methyl-2-phenyl-1H-Indole, 3558-24-5)	c1ccc2cc(c3ccccc3)n(C)c2c1	NR	NR	15	1
V3-050	Profiles	3634-16-0	Methylbenzoacridine (5-Methylbenz(a)acridine, 3634-16-0)	CC1=CC2=NC3=CC=CC=C3C=C2C4=CC=CC=C14	PBT	PB	60	2
V3-051	Profiles	37069-37-7	2,3-Dimethylbenzo(f)quinoline	CC1=NC2=C(C(C)=C1)C1=C(C=CC=C1)C=C2	NR	NR	37,5	1
V3-052	Profiles	3752-42-9	(1-Anthracene nitrile, 3752-42-9)	C1=CC=C2C=C3C(=CC2=C1)C=CC=C3C#N	NR	NR	37,5	1
V3-053	Profiles	386-77-6	Aza-benzofluoranthenes (Indeno(1,2,3-kl)acridine, 386-77-6 or azabenzopyrenes	c1cccc2nc3cccc4c3c(c12)c1ccccc41	PBT	PB	60	1
V3-054	Profiles	39258-30-5	2-Methylbenzo(f)quinoline	c12c3cc(C)cnc3ccc1cccc2	NR	NR	37,5	1
V3-055	Profiles	40174-37-6	Methylbenzoquinolines (4-Methylbenzo(h)quinoline, 40174-37-6)	c12c(ccc3c1cccc3)c(C)ccn2	NR	NR	37,5	1
V3-056	Profiles	4053-35-4	Methylquinolone (7-Methyl-2-quinolone, 4053-35-4)	c12NC(=O)C=Cc1ccc(C)c2	NR	NR	37,5	1
V3-057	Profiles	4107-64-6	Cyanopyrenes or -fluoranthenes (1-Cyanopyrene, 4107-64-6)	C1=CC2=C3C(=C1)C=CC4=C(C=CC(=C43)C=C2)C#N	PBT	NR	60	2
V3-058	Profiles	42135-22-8	3-Nitro-9-fluorenone	O=N(=O)c1cc2c3ccccc3C(=O)c2cc1	NR	NR	37,5	1
V3-059	Profiles	42397-64-8	1,6-Dinitropyrene	O=N(=O)c1ccc2ccc3c(ccc4ccc1c2c34)N(=O)=O	PB	PB	180	2
V3-060	Profiles	42397-65-9	1,8-Dinitropyrene	O=N(=O)c1ccc2ccc3ccc(N(=O)=O)c4ccc1c2c34	PB	PB	180	2
V3-061	Profiles	4657-93-6	5-Acenaphthenamine, 4657-93-6	C1CC2=CC=CC3=C(C=CC1=C23)N	NR	NR	37,5	1

Semipolar polycyclic aromatic hydrocarbons

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V3-062	Profiles	4709-20-0	Dimethylnaphtholes (2,4-Dimethyl-1-naphthol, 4709-20-0)	c12c(c(C)cc(c1cccc2)C)O	NR	NR	37,5	1
V3-063	Profiles	518-85-4	Perinaphthanone	O=C1CCC2=CC=CC3=C2C1=CC=C3	NR	NR	37,5	1
V3-064	Profiles	51913-96-3	Biquinoline, different isomers	n3cccc4c3c(ccc4)c1c2ncccc2ccc1	NR	NR	37,5	1
V3-065	Profiles	529-86-2	Hydroxyanthracenes or -phenanthrenes (Anthranol = anthracene-9-ol, 529-86-2)	c1ccc2cc3ccccc3c(O)c2c1	NR	NR	15	1
V3-066	Profiles	53123-73-2	Ethylquinolines	CCc1cc2ncccc2cc1	NR	NR	15	1
V3-067	Profiles	5737-13-3	4H-Cyclopenta(def)phenanthren-4-one	O=C1c4cccc3c4c2c(cc3)cccc12	NR	NR	37,5	5
V3-068	Profiles	58426-99-6	Phenanthro(1,2-b)thiophene	C1=CC=C2C(=C1)C=CC3=C2C=CC4=C3SC=C4	PBT	PB	60	1
V3-069	Profiles	602-87-9	5-Nitroacenaphthene	O=N(=O)c(c(c(c(cc1)CC2)c2c3)c1)c3	NR	NR	37,5	1
V3-070	Profiles	604-49-9	Methylbenzoquinolines (1-Methylbenzo(f)quinoline, 604-49-9)	CC1=C2C(=NC=C1)C=CC3=CC=CC=C32	NR	NR	37,5	1
V3-071	Profiles	6051-98-5	7H-Benzo(b)fluoren-7-one	O=C1C2=C(C=CC=C2)C2=C1C=CC1=C2C=CC=C1	BT	NR	37,5	1
V3-072	Profiles	607-42-1	Anthracene carboxylic acid (Anthracene-1-carboxylic acid, 607-42-1)	C1=CC=C2C=C3C(=CC2=C1)C=CC=C3C(=O)O	NR	NR	15	1
V3-073	Profiles	610-49-1	1-Aminoanthracene, 610-49-1	Nc1c2cc3ccccc3cc2ccc1	NR	NR	37,5	1
V3-074	Profiles	612-96-4	2-Phenylquinoline	c1ccc2ccc(c3ccccc3)nc2c1	NR	NR	15	1

Semipolar polycyclic aromatic hydrocarbons

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V3-075	Profiles	615-22-5	Methylthiobenzothiazole (2-(Methylthio)benzothiazole)	N(c(c(S1)ccc2)c2)=C1SC	NR	NR	15	1
V3-076	Profiles	63041-90-7	6-Nitrobenzo(a)pyrene	O=N(=O)c2c1ccccc1c3ccc4cccc5ccc2c3c45	PBT	PBT	180	2
V3-077	Profiles	6344-63-4	Fluoren-1-amine, 6344-63-4	Nc1c2Cc3ccccc3c2ccc1	NR	NR	37,5	1
V3-078	Profiles	65777-07-3	Dibenzacridines	C1=CC=C2C(=C1)C=C3C4=CC=CC=C4C5=CC=CC=C5C3=N2	PBT	PB	60	1
V3-079	Profiles	68518-20-7	Dinaphthofurans	c1ccc2cc3c(cc2c1)oc1cc2ccccc2cc31	PBT	PBT	60	2
V3-080	Profiles	69716-03-6	Cyanoacenaphthylenes (5-Cyanoacenaphthylene, 69716-03-6)	C1=Cc2ccc(c3cccc1c23)C#N	NR	NR	37,5	1
V3-081	Profiles	70021-48-6	C3-Dibenzothiophenes (Trimethyldibenzothiophenes)	Cc3ccc(c2c3sc1c2cc(cc1)C)C	BT	BT	37,5	1
V3-082	Profiles	72076-98-3	Benzo(def)naphthobenzothiophene (= Chryseno(4,5-bcd)thiophene)	c15c3c4ccc5c2c(cc1sc3ccc4)cccc2	PBT	PBT	60	2
V3-083	Profiles	7470-14-6	Phenanthrene carboxylic acid (3-Phenanthrenecarboxylic acid, 7470-14-6)	c12c3cc(C(=O)O)ccc3ccc1cccc2	NR	NR	15	1
V3-084	Profiles	75321-19-6	1,3,6-Trinitropyrene	O=N(=O)c4cc(c2c3c4ccc1c3c(c(cc1)N(=O)=O)cc2)N(=O)=O	PBT	NR	180	2
V3-085	Profiles	75321-20-9	1,3-Dinitropyrene	O=N(=O)c4c2c3c(c(c4)N(=O)=O)ccc1c3c(ccc1)cc2	PBT	PB	180	2
V3-086	Profiles	7651-86-7	Phenanthrenols (4-Hydroxyphenanthrene, 7651-86-7)	c12c3c(O)cccc3ccc1cccc2	NR	NR	15	1
V3-087	Profiles	7661-60-1	Ethylisoquinolines (1-Ethylisoquinoline, 7661-60-1)	n(ccc1cc2)c(c1cc2)CC	NR	NR	15	1

Semipolar polycyclic aromatic hydrocarbons

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V3-088	Profiles	76723-60-9	Benzofluorenones (Benzofluorenone, 76723-60-9; 11H-Benzo(b)fluoren-11-one, 3074-03-1)	C1(c4c3c(ccc4c2ccccc12)cccc3)=O	BT	NR	37,5	4
V3-089	Profiles	78210-35-2	Hydroxyphenylnaphthalenes (o-(2-Naphthyl)phenol, 78210-35-2)	OC1=CC=CC=C1C1=CC=C2C=CC=CC2=C1	NR	NR	15	1
V3-090	Profiles	218-19-9	Naphthoquinoline (Naphtho(1,2-h)quinoline 218-19-9)	C1=CC2=C(C=C1)C1=C(C=C2)C2=C(C=CC=N2)C=C1	PBT	NR	60	1
V3-091	Profiles	79313-22-7	5-Ethyldibenzothiophene (CAS for unspecified isomer)	c12c(c3c(s1)cccc3)c(ccc2)CC	BT	NR	37,5	1
V3-092	Profiles	80440-44-4	Dibenzanthracenone (7H-Dibenz(de,j)anthracene-7-one 80440-44-4)	O=C1C2=C3C(C=CC=C3C3=C1C1=C(C=CC=C1)C=C3)=CC=C2	BT	BT	37,5	2
V3-093	Profiles	81503-62-0	2,4,6-Trimethylbenzo(h)quinoline	n1c(cc(c2cc(c3c(c12)cccc3)C)C)C	BT	NR	37,5	1
V3-094	Profiles	84540-55-6	Methylnaphthobenzofurans (Methylbenzo(b)naphtho(2,3-d)furan)	CC1=C2C(OC3=C2C=CC=C3)=CC2=C1C=CC=C2	BT	BT	37,5	3
V3-095	Profiles	84-54-8	2-Methylanthracenedione	O=C(c(c(c(C(=O)c1cccc2)ccc3C)c3)c12	NR	NR	37,5	2
V3-096	Profiles	84-56-0	Azabenz(a)anthracenes (Naphtho(2,3-h)quinoline, 84-56-0)	c3c4ccccc4cc2c3c1c(cccn1)cc2	PBT	NR	60	1
V3-097	Profiles	892-21-7	3-Nitrofluoranthene	O=N(=O)c2ccc3c1ccccc1c4cccc2c34	PBT	PB	180	1
V3-098	Profiles	89816-99-9	C2-Dibenzothiophenes (4-Ethyldibenzothiophene 89816-99-9)	c12c(c3c(s1)cccc3)cccc2CC	BT	NR	37,5	3
V3-099	Profiles	90-44-8	Anthrone = 9(10H)-Anthracenone	O=C(c(c(ccc1)Cc2cccc3)c1)c23	NR	NR	37,5	1
V3-100	Profiles	934-34-9	2(3H)-Benzothiazolone	O=C(Nc(c1ccc2)c2)S1	NR	NR	15	1

Semipolar polycyclic aromatic hydrocarbons

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V3-101	Profiles	954-07-4	Methylanthracene-9,10-dione (1-Methylanthraquinone, 954-07-4)	c(ccc1C(=O)c2c(c3)C)cc1C(=O)c2cc3	NR	NR	37,5	2
V3-102	Profiles	99339-80-7	C2-Acridines (1,5-Dimethyl acridine, 99339-80-7)	Cc1cccc2nc3c(cccc3C)cc12	NR	NR	37,5	1
V3-103	Profiles	0	Azabenzofluoranthenes (SMILES for 2-Aza-isomer)	C1=CC2=C3C(=C1)C1=CN=CC4=C1C3=C(C=C2)C=C4	PBT	NR	60	3
V3-104	Profiles	0	Dibenzoisoquinolines (SMILES for Dibenzo(c,f)isoquinoline)	C1=CC2=NC=C3C=CC4=CC=CC=C4C3=C2C=C1	PBT	NR	60	1
V3-105	Profiles	0	6,7-Ethylene-benzo(h)quinoline	C1CC2=CC3=C(N=CC=C3)C3=C2C1=CC=C3	NR	NR	37,5	1
V3-106	Profiles	0	Aza-acenaphthene (SMILES for 4-Aza-isomer) or phenylpyridine	C1CC2=CN=CC3=C2C1=CC=C3	NR	NR	37,5	5
V3-107	Profiles	0	9,10-Dihydro-2-azapyrene	C1CC2=CN=CC3=C2C2=C(C=CC=C12)C=C3	NR	NR	37,5	1
V3-108	Profiles	0	4H-Naphtho(1,2,3,4-def)carbazole or isomer	N1C2=CC=CC3=C4C=CC=CC4=C4C=CC=C1C4=C23	PBT	PB	180	1
V3-109	Profiles	0	Dibenzonaphthofuran or isomers (SMILES forAnthra(2,1-b)benzo(d)furan)	O1C2=CC=CC=C2C2=C1C=CC1=C2C=C2C=CC=CC2=C1	BT	BT	37,5	1
V3-110	Profiles	0	Azabenzonaphthotiophene isomers	S1C2=CC=CC=C2C2=CC3=C(C=CC=C3)N=C12	NR	NR	37,5	2
V3-111	Profiles	0	Dibenzophenanthridine or isomer (Dibenzophenanthridine)	C1=CC2=C(C=C1)C1=C(C=C2)N=CC2=CC3=CC=CC=C3C=C12	PBT	PB	60	1
V3-112	Profiles	0	3-Azaphenanthro(4,3-b)thiophene	S1C=C[N+]2=C1C1=C(C=CC3=C1C=CC=C3)C=C2	PBT	PB	60	1
V3-113	Profiles	0	Dimethyl- or ethylhydroxypyrenes or -fluoranthenes (SMILES for 2,3-Dimethylpyren-1-ol)	CC1=C(C)C2=CC=C3C=CC=C4C=CC(=C1O)C2=C34	BT	NR	15	1

Semipolar polycyclic aromatic hydrocarbons

Running number	Source	CAS yellow field = mixture	Name[1] For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets.	SMILES[2] (incl. Substituents, however not specific for each isomer)	PBT-Screening result	PBT-Screening result enforced	Persistency: Half-life Water (d)[3]	Occurrence in profiles
V3-114	Profiles	0	5,6-Dimethylquinoline	CC1=C(C)C2=CC=CN=C2C=C1	NR	NR	37,5	1
V3-115	Profiles	0	C3-Hydroxyanthracenes or -phenanthrenes (SMILES for 1,9,10-Trimethylanthracene-2-ol)	CC1=C2C=CC=CC2=C(C)C2=C(C)C(O)=CC=C12	BT	BT	37,5	1
V3-116	Profiles	0	Dimethyl- or ethylydroxyanthracenes or -phenanthrenes (SMILES for 9,10-Methylanthracene-2-ol)	CC1=C2C=CC=CC2=C(C)C2=C1C=CC(O)=C2	BT	NR	37,5	1
V3-117	Profiles	0	Dimethylbenzo(def)carbazole = Dimethylphenanthro(bcd)pyrrole (SMILES for 1,7-dimethylphenanthro(bcd)pyrrole)	CC1=C2C=CC3=C(C)C=CC4=C3C2=C(N4)C=C1	PBT	PB	60	3
V3-118	Profiles	0	Methylphenanthro(bcd)pyrrole (SMILES for 1-Methyl-isomer)	CC1=C2C=CC3=CC=CC4=C3C2=C(N4)C=C1	NR	NR	60	2
V3-119	Profiles	0	Methylphenanthro(4,5-bcd)thiophenes (SMILES for 1-Methyl isomer)	CC1=C2C=CC3=CC=CC4=C3C2=C(S4)C=C1	PBT	PB	60	2
V3-120	Profiles	0	Methyl-2,3-Dihydrobenzo(b)thiophenes (SMILES for 4-Methyl-isomer)	CC1=C2CCSC2=CC=C1	NR	NR	37,5	1
V3-121	Profiles	0	Methylazabenzothiophenes (SMILES for 7-Methyl-5-azabenzothiophene = 7-Methyl-thieno(3,2-c)pyridine)	CC1=C2SC=CC2=CN=C1	NR	NR	15	1
V3-122	Profiles	0	C5-Acenaphthenols (3,4,5,6,8-Pentamethylacenaphthene-1-ol)	CC1=CC(C)=C2C(O)CC3=C(C)C(C)=C(C)C1=C23	BT	BT	37,5	1
V3-123	Profiles	0	C4-Acenaphthenols (SMILES for 3,5,6,8-Tetramethylacenaphthene-1-ol)	CC1=CC(C)=C2CC(O)C3=C(C)C=C(C)C1=C23	BT	BT	37,5	1
V3-124	Profiles	0	Dimethyl- or ethylacenaphthenols (SMILES for 3,5-Dimethylacenaphthene-1-ol)	CC1=CC(C)=C2CC(O)C3=CC=CC1=C23	NR	NR	37,5	1
V3-125	Profiles	0	C2-Azaacenaphthenes (SMILES for 6,8-Dimethyl-4-aza-acenaphthene)	CC1=CC(C)=C2CCC3=CN=CC1=C23	NR	NR	37,5	2

Semipolar polycyclic aromatic hydrocarbons

Running number	Source	CAS yellow field = mixture	Name[1] For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets.	SMILES[2] (incl. Substituents, however not specific for each isomer)	PBT-Screening result	PBT-Screening result enforced	Persistency: Half-life Water (d)[3]	Occurrence in profiles
V3-126	Profiles	0	Dimethyl-2,3-Dihydrobenzo(b)thiophenes (SMILES for 4,6-Dimethyl-isomer)	CC1=CC(C)=C2CCSC2=C1	NR	NR	37,5	1
V3-127	Profiles	0	Methylhydroxypyrenes or -fluoranthenes (SMILES for 3-Methylpyren-1-ol)	CC1=CC(O)=C2C=CC3=CC=CC4=CC=C1C2=C34	PBT	PB	60	1
V3-128	Profiles	0	Methylhydroxyphenylnaphthalenes (SMILES for 5-Methyl-2(naphthalen-2-yl)-phenol)	CC1=CC=C(C(O)=C1)C1=CC=C2C=CC=CC2=C1	BT	NR	37,5	1
V3-129	Profiles	0	3,4,5,8-Tetramethylquinoline	CC1=CC=C(C)C2=C(C)C(C)=CN=C12	NR	NR	37,5	1
V3-130	Profiles	0	C3-Acenaphthenols (SMILES for 3,5,6-Trimethylacenaphthene-1-ol)	CC1=CC=C2C(O)CC3=C(C)C=C(C)C1=C23	NR	NR	37,5	1
V3-131	Profiles	0	Methylhydroxyacenaphthenes (SMILES for 6-Methyl-1,2-dihydroacenaphthylen-1-ol)	CC1=CC=C2C(O)CC3=CC=CC1=C23	NR	NR	15	1
V3-132	Profiles	0	2,6,7-Trimethylquinoline	CC1=CC=C2C=C(C)C(C)=CC2=N1	NR	NR	37,5	1
V3-133	Profiles	0	Methyldibenzoquinolines (SMILES for Methyldibenzo(c,f)quinoline)	CC1=CC=C2C=CC3=NC=C4C=CC=CC4=C3C2=C1	PBT	PB	60	3
V3-134	Profiles	0	Methylaminoacenaphthenes (SMILES for 5-Methylacenaphthene-1 amine)	CC1=CC=C2CC(N)C3=CC=CC1=C23	NR	NR	37,5	1
V3-135	Profiles	0	Methylacenaphthenols (SMILES for 5-Methylacenaphthene-1-ol)	CC1=CC=C2CC(O)C3=CC=CC1=C23	NR	NR	15	1
V3-136	Profiles	0	Methylazaacenaphthenes (SMILES for 6-Methyl-4-aza-acenaphthene)	CC1=CC=C2CCC3=CN=CC1=C23	NR	NR	37,5	2
V3-137	Profiles	0	Dimethyl- or ethyl-azafluoranthenes/pyrenes or azabenzofluorenes (SMILES for 1,6-Dimethyl-4-azapyrene)	CC1=CC=C2N=CC3=C(C)C=CC4=CC=C1C2=C34	PBT	PB	180	1
V3-138	Profiles	0	Methylcyanophenanthrene or-anthracene (SMILES for 4-Methylanthracene-9-carbonitrile)	CC1=CC=CC2=C1C=C1C=CC=CC1=C2C#N	NR	NR	37,5	1

Semipolar polycyclic aromatic hydrocarbons

Running number	Source	CAS yellow field = mixture	Name[1] For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets.	SMILES[2] (incl. Substituents, however not specific for each isomer)	PBT-Screening result	PBT-Screening result enforced	Persistency: Half-life Water (d)[3]	Occurrence in profiles
V3-139	Profiles	0	Methylphenanthridines (SMILES for 7-Methylphenanthridine)	CC1=CC=CC2=C1C=NC1=C2C=CC=C1	NR	NR	37,5	1
V3-140	Profiles	0	Methylazafluorenes (SMILES for 8-Methyl-4-azafluorene, SMILES)	CC1=CC=CC2=C1CC1=C2N=CC=C1	NR	NR	37,5	2
V3-141	Profiles	0	Methylazadibenzothiophene (SMILES for 4-Methyl-1-azadibenzothiophene)	CC1=CC=NC2=C1SC1=CC=CC=C21	NR	NR	37,5	1
V3-142	Profiles	0	C4-Quinolines (SMILES for 2,4,6, 8-Tetramethylquinoline)	CC1=CC2=C(C)C=C(C)N=C2C(C)=C1	NR	NR	37,5	1
V3-143	Profiles	0	Trimethyl- or methylethylbenzo(def)carbazoles or dimethylindenoindoles (Trimethylbenzo(def)carbazole = Trimethylphenanthro(bcd)pyrrole (SMILES for 1,7,8--trimethylphenanthro(bcd)pyrrole)	CC1=CC2=C(C)C=CC3=C2C2=C(N3)C=CC(C)=C12	PBT	PB	180	1
V3-144	Profiles	0	Dimethylbenzonaphthofuran (SMILES for 9,11-dimethylbenzo(b)naphtho(2,3-d)furan)	CC1=CC2=C(C=C1)C=C1OC3=C(C=CC=C3)C1=C2C	BT	BT	37,5	1
V3-145	Profiles	0	Methylfluorenols (SMILES for 7-Methylfluoren-2-ol)	CC1=CC2=C(C=C1)C1=C(C2)C=C(O)C=C1	NR	NR	37,5	1
V3-146	Profiles	0	Methyl-Azadibenzofurans (SMILES for 8-Methyl-1-Azadibenzofuran)	CC1=CC2=C(OC3=CC=CN=C23)C=C1	NR	NR	37,5	4
V3-147	Profiles	0	Methylnaphthobenzothiophenes (SMILES for 5-Methylnaphtho(1,2-b)thiophene)	CC1=CC2=C(SC=C2)C2=C1C=CC=C2	BT	NR	37,5	1
V3-148	Profiles	0	Methylazadihydropyrene or C2-azacyclopenta(def)phenanthrene (SMILES for 7-Methyl-5-aza-2,3dimethylpyrene)	CC1=CC2=C3C(=C1)N=CC1=C3C(C=C2)=CCC1	BT	NR	37,5	1
V3-149	Profiles	0	10-Methyl-1-aza-pyrene	CC1=CC2=C3C(C=CC4=CC=NC1=C34)=CC=C2	PBT	NR	60	3
V3-150	Profiles	0	Methylbenzophenanthro(4,5-bcd)thiophene (SMILES for 2-Methyl isomer)	CC1=CC2=C3C(SC4=C5C=CC=CC5=CC(C=C2)=C34)=C1	PBT	PBT	180	2

Semipolar polycyclic aromatic hydrocarbons

Running number	Source	CAS yellow field = mixture	Name[1] For mixtures of isomers, a close representative is given in brackets, for which a CAS-number is specified in Column CAS. This however does not mean, that this compound was indeed identified, if not otherwise stated outside the brackets.	SMILES[2] (incl. Substituents, however not specific for each isomer)	PBT-Screening result	PBT-Screening result enforced	Persistency: Half-life Water (d)[3]	Occurrence in profiles
V3-151	Profiles	0	Methylphenanthro(4,5-bcd)thiophene (SMILES for 10-Methyl-isomer)	CC1=CC2=CC=CC3=C2C2=C(S3)C=CC=C12	PBT	PB	60	0
V3-152	Profiles	0	5-Methyl-1aza-fluoranthene	CC1=CC2=CC=NC3=C2C(=C1)C1=CC=CC=C31	PBT	NR	60	3
V3-153	Profiles	0	2,4,7-Trimethylquinoline	CC1=CC2=NC(C)=CC(C)=C2C=C1	NR	NR	37,5	1
V3-154	Profiles	0	3,4,7-Trimethylquinoline	CC1=CC2=NC=C(C)C(C)=C2C=C1	NR	NR	37,5	1
V3-155	Profiles	0	3,5,8-Trimethylquinoline	CC1=CN=C2C(C)=CC=C(C)C2=C1	NR	NR	37,5	1
V3-156	Profiles	0	Dimethyl- or ethyl-Azadibenzofurans (SMILES for 3,8-Dimethyl-1-Azadibenzofuran)	CC1=CN=C2C(OC3=C2C=C(C)C=C3)=C1	NR	NR	37,5	1
V3-157	Profiles	0	C3-Alkylisoquinolines (SMILES for 1,3,4-Trimethylisoquinoline)	CC1=NC(C)=C(C)C2=C1C=CC=C2	NR	NR	37,5	1
V3-158	Profiles	0	Dimethyl- or ethylfluorenols (SMILES for 7,9-Dimethylfluoren-2-ol)	CC1C2=C(C=CC(C)=C2)C2=C1C=C(O)C=C2	NR	NR	37,5	1
V3-159	Profiles	0	Dimethyldibenzonaphthofurans or isomers (SMILES for 3,7-Dimethylanthra(2,1-b)benzo(d)furan)	CC1=CC=C2C(OC3=C2C2=C(C=C4C=CC=CC4=C2)C(C)=C3)=C1	BT	BT	37,5	1
V3-160	Profiles	0	Methylbenzophenanthridine or isomer (SMILES for 10-methylbenzo(a)phenanthridine)	CC1=CC=C2C(C=CC3=C2C2=C(C=CC=C2)C=N3)=C1	PBT	PB	60	1
V3-161	Profiles	0	Aminodibenzothiophene, C1-substituted (SMILES for 8-methyl-1-Aminodibenzothiophene)	CC1=CC=C2SC3=C(C2=C1)C(N)=CC=C3	NR	NR	37,5	1
V3-162	Profiles	0	Azabenzonaphthothiophene isomers, C1-substituted	CC1=CC=CC2=C1C=C1C(SC3=C1C=CC=C3)=N2	BT	NR	37,5	1

Semipolar polycyclic aromatic hydrocarbons

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V3-163	Profiles	0	4-Ethyl-3,6-dimethylquinoline	CCC1=C2C=C(C)C=CC2=NC=C1C	NR	NR	37,5	1
V3-164	Profiles	0	C5-Naphtholes (SMILES for 4-Methyl-1,6-diethyl-2-naphthol)	CCC1=CC2=C(C)C=C(O)C(CC)=C2C=C1	BT	NR	37,5	1
V3-165	Profiles	0	C3-Fluorenols (SMILES for 7-Ethyl-9-methylfluoren-2-ol)	CCC1=CC2=C(C=C1)C1=C(C=C(O)C=C1)C2C	BT	NR	37,5	1
V3-166	Profiles	0	C7-Naphtholes (SMILES for 8-Methyl-1,4,6-triethyl-2-naphthol)	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C(C)=C1	BT	BT	37,5	1
V3-167	Profiles	0	C8-Naphtholes (SMILES for 1,4,6,8-Tetraethyl-2-naphthol)	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C(CC)=C1	BT	BT	37,5	1
V3-168	Profiles	0	C6-Naphtholes (SMILES for 1,4,6-Triethyl-2-naphthol)	CCC1=CC2=C(CC)C=C(O)C(CC)=C2C=C1	BT	BT	37,5	1
V3-169	Profiles	0	Aminodibenzothiophene, C2-substituted (SMILES for 8-ethyl-1-Aminodibenzothiophene)	CCC1=CC=C2SC3=C(C2=C1)C(N)=CC=C3	NR	NR	37,5	1
V3-170	Profiles	0	Cyanofluoranthene or -pyrene or azabenzo(ghi)fluoranthene (SMILES for Fluoranthene-1-carbonitrile)	N#CC1=C2C3=CC=CC=C3C3=C2C(C=C1)=CC=C3	PBT	NR	60	1
V3-171	Profiles	0	Cyanopentapyrenes or -benzo(ghi)fluoranthenes (SMILES for Benzo(ghi)fluoranthene carbonitrile)	N#CC1=CC2=C3C(=C1)C1=CC=CC4=C1C3=C(C=C4)C=C2	PBT	PB	60	1
V3-172	Profiles	0	Chrysenenitriles (SMILES for Chrysene-6-carbonitrile)	N#CC1=CC2=C3C=CC=CC3=CC=C2C2=CC=CC=C12	PBT	PB	60	1
V3-173	Profiles	0	Cyanoacenaphthenes (SMILES for 1,2-Dihydroacenaphthylene-1-carbonitrile)	N#CC1CC2=CC=CC3=C2C1=CC=C3	NR	NR	37,5	1
V3-174	Profiles	0	Naphthoindoles (SMILES for 1H-Naphtho(1,2-g)indole	N1C=CC2=C1C1=C(C=C2)C2=C(C=CC=C2)C=C1	PBT	TP	60	1

Semipolar polycyclic aromatic hydrocarbons

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V3-175	Profiles	0	Benzophenanthro(bcd)pyrrole	N1C2=C3C4=C1C1=C(C=CC=C1)C=C4C=CC3=CC=C2	PBT	PB	60	1
V3-176	Profiles	0	4,5-Dihydropyrene-1-amine	NC1=CC=C2CCC3=CC=CC4=CC=C1C2=C34	NR	NR	37,5	1
V3-177	Profiles	0	4-Oxapyrene-5-one	O=C1OC2=CC=CC3=C2C2=C1C=CC=C2C=C3	NR	NR	15	2
V3-178	Profiles	0	Azabenzonaphthofurans (SMILES for 4-Azabenzo(b)naphtho(1,2-d)furan)	O1C2=C(C=CC=C2)C2=CC=C3N=CC=CC3=C12	NR	NR	37,5	1
V3-179	Profiles	0	Azaphenanthro(bcd)furan isomers (SMILES for 10-Aza-isomer) or cyanodibenzofurans	O1C2=CC=CC3=C2C2=C(C=CC=C12)N=C3	NR	NR	37,5	1
V3-180	Profiles	0	Azadibenzofurans (SMILES for 1-Azadibenzofuran)	O1C2=CC=CN=C2C2=C1C=CC=C2	NR	NR	37,5	3
V3-181	Profiles	0	4-Azabenzothiophene	S1C=CC2=C1C=CC=N2	NR	NR	15	1
V3-182	Profiles	0	6-Azabenzothiophene	S1C=CC2=C1C=NC=C2	NR	NR	15	1
V3-183	Profiles	0	3-Azaphenanthro(9,10-b)thiophene	S1C=CC2=C1C1=C(C=CC=C1)C1=C2C=CN=C1	NR	NR	60	1
V3-184	Profiles	0	7-Azabenzothiophene	S1C=CC2=C1N=CC=C2	NR	NR	15	1
V3-185	Profiles	0	Dibenzo(c,g)phenanthro(12,9-bcd)thiophene = Benzo(6,7)perylo(1,12-bcd)thiophene (only defined by structure,isomers exisiting)	S1C2=C3C4=C1C=CC1=C4C4=C(C=C1)C=CC1=C4C3=C(C=C2)C=C1	PBT	PBT	180	1
V3-186	Profiles	0	Phenanthro(7,6,5,4b,4a,4:12,12a,12b,1,2,3)peryleno(6,7-bcd)thiophene	S1C2=C3C4=C1C=CC1=C4C4=C5C(C=CC(C=C2)=C35)=CC2=C4C3=C(C=CC=C13)C=C2	PBT	PBT	180	1

Semipolar polycyclic aromatic hydrocarbons

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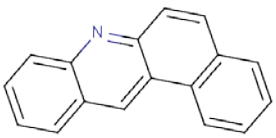
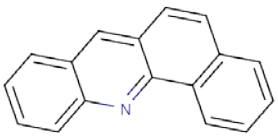
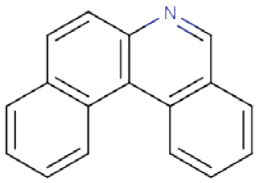
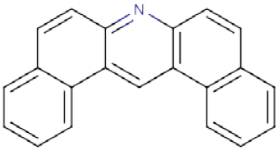
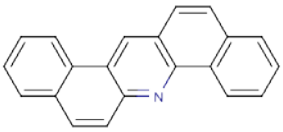
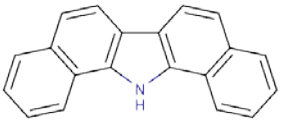
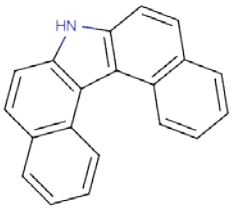
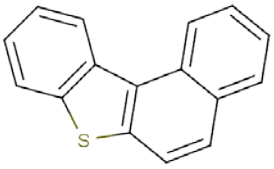
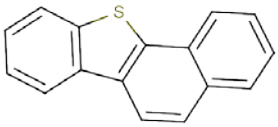
V3-187	Profiles	0	Tribenzo(2,1,4,5,6,7)chryseno(10,11,bcd)thiophene	S1C2=C3C4=C1C1=C5C(C=CC6=C5C4=C(C=C6)C4=C3C(C=C2)=CC=C4)=CC=C1	PBT	PBT	180	1
V3-188	Profiles	0	Benzo(9,10)pyreno(3,4,5,6-jklma)dibenzothiophene	S1C2=CC=C3C=CC4=C5C=CC=CC5=C5C=CC6=C7C(=C1C=C6)C2=C3C4=C57	PBT	PBT	180	1
V3-189	Profiles	0	Only defined by structure (isomers existing, SMILES for Cyclopenta(kl)phenanthro(4,5-bcd)thiophene)	S1C2=CC=C3C=CC4=CC5=CC=CC1=C5C2=C34	BT	NR	37,5	1
V3-190	Profiles	0	Benzo(1,2)pyreno(3,4,5,6-jklma)dibenzothiophene	S1C2=CC=C3C4=CC=CC=C4C4=CC=C5C=CC6=C7C(=C1C=C6)C2=C3C4=C57	PBT	PBT	180	1
V3-191	Profiles	0	1-Azadibenzothiophene	S1C2=CC=CC=C2C2=C1C=CC=N2	NR	NR	37,5	2
V3-192	Profiles	0	2-Azadibenzothiophene	S1C2=CC=CC=C2C2=C1C=CN=C2	NR	NR	37,5	1
V3-193	Profiles	0	3-Azadibenzothiophene	S1C2=CC=CC=C2C2=C1C=NC=C2	NR	NR	37,5	1
V3-194	Profiles	0	Azaphenanthro(4,5-bcd)thiophene isomers (SMILES for 9-Aza-isomer)	S1C2=CC=CC3=C2C2=C(C=CC=C12)N=C3	NR	NR	37,5	1
V3-195	Profiles	0	Coroneno(2,3,4-bcd)benzothiophene	S1C2=CC=CC3=C2C2=C4C5=C(C=CC6=C5C5=C(C=C6)C=CC6=C5C4=C3C=C6)C=C12	PBT	PBT	180	1
V3-196	Profiles	68558-73-6	Triphenyleno(4,5-bcd)thiophene	S1C2=CC=CC3=C4C=CC=CC4=C4C=CC=C1C4=C23	PBT	PBT	60	1

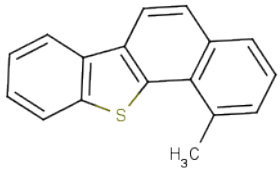
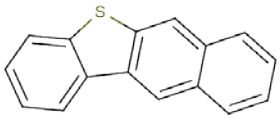
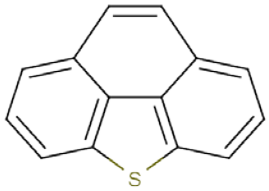
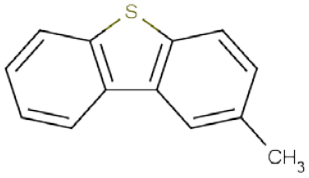
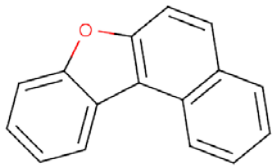
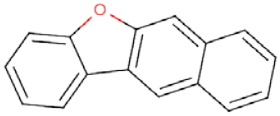
(1) For mixtures of isomers, a close representative is given in brackets for which a CAS-number is specified in Column CAS. This however does not mean that this compound was indeed identified if not otherwise stated outside the brackets.

(2) SMILES codes including substituents; for mixtures or groups of isomers detected together (e.g. C2-substituted) they are however not always specific for each isomer regarding the position of substituents on the ring system, the position of a ring-heteroatom within the ring system or the relative position of aromatic rings within the ring system (see sections 9.4.1 and 14).
(3) Persistency assessment according to US-EPAs PBT Profiler methodology (http://www.pbtprofiler.net/) based on BioWin3 textual output, resulting in half-life Water (d): According to enforced screening criteria, substances rated PB had to fulfil a half-life of > 60d (180d) additionally (vPvB-candidates).

Annex III: Substance list of 15 Priority Semipolar PAC

Table 56: NSO-heterocyclic polyaromatic target compounds for analytical determinations in matrices of UVCBs and products

CAS-No.	Chemical name	Structure	Literature reports: prevalence in profiles
225-11-6	Benz(a)acridine		environment (2); tar (1); tar fraction (2)
225-51-4	Benz(c)acridine		environment (2); pitch (2); tar (1); tar fraction (7)
195-29-9	Mixture of Dibenzoquinolines (Dibenzo(c,f)quinoline = Benzo(a)phenanthridine, 195-29-9)		environment (1); pitch (4); tar (1); tar fraction (6)
224-42-0	Dibenz(a,j)acridine		environment (3); pitch (1); tar fraction (2)
226-36-8	Dibenz(a,h)acridine		environment (2)
239-64-5	13H-Dibenzo(a,i)carbazole		tar fraction (1)
194-59-2	7H-Dibenzo(c,g)carbazole and other dibenzocarbazoles		environment (2); pitch (1); tar fraction (2)
205-43-6	Benzo(b)naphtho(1,2-d)thiophene		environment (1); pitch (2); tar (1); tar fraction (1)
239-35-0	Benzo(b)naphtho(2,1-d)thiophene		bitumen (8); carbon black (2); environment (3); pitch (2); tar (2); tar fraction (2)

CAS-No.	Chemical name	Structure	Literature reports: prevalence in profiles
4567-41-3	1-Methylbenzo(b)-naphtho(2,1-d)thiophene, several methylation isomers		environment (1); pitch (1); tar (2); tar fraction (1); oil fraction hydrotreated (1)
243-46-9	Benzo(b)naphtho(2,3-d)thiophene		environment (2); pitch (2); tar (1); tar fraction (1)
30796-92-0	Phenanthro(4,5-bcd)thiophene		carbon black (3); environment (3); pitch (2); tar (2); tar fraction (1)
20928-02-3	Methyldibenzothiophene, 2- (several isomers)		environment (3); tar (2); tar fraction (2); oil fraction hydrotreated (1)
205-39-0	Benzo(b)naphtho(1,2-d)furan, several Benzonaphthofurans		carbon black (1); environment (3); pitch (2); tar (2); tar fraction (2)
243-42-5	Benzo(b)naphtho (2,3-d)furan and other naphthobenzofurans		environment (2); tar (3); tar fraction (2)

Annex IV: Groups of heterocyclic PAC differing only in their heteroatom (O, S, N)

Table 57: Groups of heterocyclic PAC differing only in their heteroatom (O, S, N): Given are calculated values from US EPA´s EPI Suite programs, the total C-number and number of heteroatoms present in the compounds. For persistency assessment using BIOWIN-modules see section 5.2.1.

CAS	Name	ECOSAR L(E)C50 [mg/L]	ECOSAR class	Log Kow (preference for measured)	Persistency	PBT-Screening result	Number of C	Number of O	Number of S	Number of N
271-89-6	Benzofuran	11.20	Neutral Organics	2.67	not P	NR	8	1	0	0
95-15-8	Benzo(b)thiophene	6.64	Neutral Organics	3.12	not P	NR	8	0	1	0
120-72-9	Indole	0.75	Pyrazoles/Pyrroles	2.14	not P	NR	8	0	0	1
132-64-9	Dibenzofuran	2.92	Neutral Organics	4.12	not P	NR	12	1	0	0
132-65-0	Dibenzothiophene	1.37	Neutral Organics	4.38	not P	NR	12	0	1	0
86-74-8	Carbazole	5.83	Neutral Organics	3.72	not P	NR	12	0	0	1
92-83-1	Xanthene	1.04	Neutral Organics	4.23	not P	NR	13	1	0	0
261-31-4	Thioxanthene	0.72	Neutral Organics	3.86	not P	NR	13	0	1	0
92-81-9	Dihydroacridine, 9,10-	3.99	Neutral Organics	3.55	not P	NR	13	0	0	1
239-30-5	Benzo(b)naphtho-(2,1-d)furan	0.42	Neutral Organics	4.89	not P	BT	16	1	0	0
239-35-0	Benzo(b)naphtho(2,1-d)thiophene	0.19	Neutral Organics	5.19	P1P2	PBT	16	0	1	0
239-01-0	Benzo(a)carbazole	0.92	Neutral Organics	4.47	not P	NR	16	0	0	1
72076-98-3	Benzo(def)naphthobenzothiophene (= Chryseno(4,5-bcd)thiophene)	0.07	Neutral Organics	5.93	P1P2	PBT	18	0	1	0
104313-09-9	Dibenzo(b,def)carbazole	0.38	Neutral Organics	4.99	P1P2	PBT	18	0	0	1
68518-20-7	Dinaphthofurans	0.06	Neutral Organics	6.07	P1P2	PBT	20	1	0	0
194-65-0	Dinaphtho(2,1-b:1',2'-d)thiophene	0.02	Neutral Organics	6.64	not P	BT	20	0	1	0

Semipolar polycyclic aromatic hydrocarbons

CAS	Name	ECOSAR L(E)C50 [mg/L]	ECOSAR class	Log Kow (preference for measured)	Persistency	PBT-Screening result	Number of C	Number of O	Number of S	Number of N
194-59-2	Dibenzocarbazole (7H-Dibenzo(c,g)carbazole)	0.14	Neutral Organics	6.4	P1P2	PBT	20	0	0	1

Annex V: List of companies inquired in the telephone survey

List of manufacturers and confederations inquired in the telephone survey

Tar-based products (general)

- Rütgers Chemicals AG Castrop-Rauxel (Products based on tar: wash oils, creosote, furnace oils; also: fluxbitumen and heavy fuel oils)

-AVENARIUS-AGRO GmbH Wels, Austria (Products based on tar and naphtha, anthracene oil (corrosion prevention products, sealings), bitumen-based coatings)

Carbon electrodes

-SGL Carbon Wiesbaden

-HTW Hochtemperatur-Werkstoffe Thierhaupten

-Schunk GmbH Hodenhagen

-Rheinfelden Carbon, Rheinfelden

Coatings on coal tar basis

-Remmers Baustofftechnik Löningen (Wood preservation, floor coatings)

-Uferkamp Korrosionsschutz GmbH Oberhausen (Protective coatings)

-Jotun GmbH Hamburg (Protective coatings, especially for marine purposes)

- Sigma Marine & Protective Coatings, Wolverhampton UK (Protective coatings, especially for marine purposes)

Bitumen/Asphalt

-Nynas AB Stockholm, Sweden (Bitumen products)

-Polysafe Bautenschutztechnik GmbH Augsburg (Bitumen-based silo coatings)

-Deutscher Asphaltverband (DAV) e.V. Bonn (Confederation)

-Unipetrol Deutschland GmbH Langen (Hessen) (Road asphalt)

-DEUTAG Asphalttechnik GmbH Soest (Road asphalt)

-AGH - Aktionsgemeinschaft Gussasphalt im Hochbau Sankt Augustin (Confederation)

-bga, Beratungsstelle für Gussasphaltverwendung Bonn (Confederation)

-gisbau (Employer's Liability Insurance Association)

-Bayerische Asphalt-Mischwerke GmbH & Co. KG Hofolding (Road asphalt)

-Südhessische Asphalt-Mischwerke GmbH & Co. KG Hanau (Road asphalt)

-ESSO Deutschland GmbH Abteilung Bitumen Hamburg (Bitumen manufacturer)

-ARBIT, Arbeitsgemeinschaft Bitumen Hamburg (Confederation)

-BP Europa SE Bitumen/Coke Bochum (Bitumen manufacturer)

-RASCO Bitumentechnik GmbH Augustdorf (Bitumen-based coatings)

-Shell Bitumen Hamburg (Bitumen manufacturer)

-TOTAL (und ELF) Bitumen Deutschland GmbH, Brunsbüttel (Bitumen manufacturer)

-ENI (Agip) Deutschland, München (Bitumen manufacturer)

-ARAL Deutschland (Bitumen manufacturer)

-MiRO Mineraloelraffinerie Karlsruhe (Bitumen manufacturer)

-Conoco Phillips Germany GmbH Hamburg (Bitumen manufacturer)

Roofing cardboards and sealing or coating materials

-Dachpappe Portal (online) (Confederation)

-Icopal GmbH Werne (Roofing cardboard)

- vdd Industrieverband Bitumen-Dach-und Dichtungsbahnen e.V. Frankfurt am Main (Confederation)

- Watco GmbH Viersen (Bitumen-based sealings)

-ARDEX GmbH Witten-Annen (Bitumen-based sealings)

-PHOENIX Dichtungstechnik GmbH Hamburg (Roofing cardboard)

-Binné & Sohn GmbH & Co.KG Dachbaustoffwerk Pinneberg (Roofing cardboard)

-Kebulin-Gesellschaft Kettler GmbH & Co KG Herten

(Roofing cardboard)

-Süddeutsche Teerindustrie GmbH & Co. KG Malsch (Roofing cardboard and bituminous coatings)

Coal briquettes

-Grolman GmbH & Co. KG, Gustav Neuss (also binders and soot)

-SAAR COAL INTERNATIONAL GmbH Saarbrücken

-JUSTEN KARL HEINZ GmbH Ettringen

Clay pigeons

-CCI-International (United Kingdom)

-CORSIVIA S.A.; Vivaz (Spain)

-Emiliana Piattelli; Eurotarget; FAB; ROSSINI; Mattarelli (Italy)

-Nasta (Finland)

-SUPER STAR (Sweden)

Tyres

-Stiftung Warentest (Test organisation for tyres)

-wdk Wirtschaftsverband der deutschen Kautschukindustrie e.V. Frankfurt am Main (Confederation)

-Bundesverband Reifenhandel und Vulkaniseur-Handwerk e.V. Bonn (Confederation)

-European Tyre and Rubber Manufacurer's Association (Confederation)

-Nynas, Stockholm, Sweden (process oils for tyres)

Extender and Process oils

-Total, Düsseldorf /Osnabrück (Extender and process oils)

-Evonik Essen (Extender and process oils)

-Nynas, Stockholm, Sweden (Extender and process oils)

-Sunoco, Philadelphia, USA (Extender and process oils)

- Esso Hamburg (Extender and process oils)

-Shell Hamburg (Extender and process oils)

-Normenausschuss Kautschuktechnik Frankfurt (Extender and process oils)

-DKI, Deutsches Kunststoff-Institut Darmstadt (Extender and process oils)

-ENI (Agip) München (Extender and process oils)

Organisations examining extender oils

-German Federal Environment Agency Berlin/Dessau

-TÜV Rheinland Produkt und Umwelt GmbH Köln

Carbon black/carbon black oils

-Deutsches Institut für Kautschuktechnologie (DIK)

-Degussa-Hüls Hürth

-Rütgers Chemicals AG Castrop-Rauxel

Annex VI: Priority Compounds (including isomers) of LAWA AG subcommittee

Table 58: 92 Priority Compounds (including isomers) of LAWA AG subcommittee for GFS-derivation (LAWA AG, 2010)

Name (German)	CAS-number	Name (German)	CAS-number
Acridinon	578-95-0	Methyldibenzo-thiophen, 4-	7372-88-5
Acridin	260-94-6	Methylisochinolin, 1-	1721-93-3
Benzofuran	271-89-6	Methylisochinolin, 3-	1125-80-0
Benzo(b)thiophen	95-15-8	Methylisochinolin, 4-	1196-39-0
Benzopyran-2-on (Cumarin)	91-64-5	Methylisochinolin, 5-	62882-01-3
Carbazol	86-74-8	Methylisochinolin, 6-	42398-73-2
Chinolin	91-22-5	Methylisochinolin, 7-	54004-38-5
Dibenzothiophen	132-65-0	Methylisochinolin, 8-	62882-00-2
Dihydroxypyridin, 2,3-	16867-04-2	Methylpyrrol, 1-	96-54-8
Dihydroxypyridin, 2,4-	626-03-9	Methylthiophen, 2-	554-14-3
Dihydroxypyridin, 2,5-	5154-01-8	Methylthiophen, 3-	616-44-4
Dihydroxypyridin, 2,6-	626-06-2	Phenanthridin	229-87-8
Dihydroxypyridin, 3,4-	10182-48-6	Phenanthridinon, 6(5H)-	1015-89-0
Dimethylchinolin, 2,3-	1721-89-7	Pyridin	110-86-1
2,4-Dimethylchinolin	1198-37-4	Pyrrol	109-97-7
Dimethylchinolin, 2,6-	877-43-0	Thiophen	110-02-1
Dimethylchinolin, 2,7-	93-37-8	Xanthen	92-83-1
Dimethylchinolin, 2,8-	1463-17-8	Benzo(b)thiophen-1,1-dioxid	825-44-5
Dimethylchinolin, 4,6-	826-77-7	Dimethylbenzofurane, 2,3-	3782-00-1
Dimethylchinolin, 4,7-	40941-54-6	Dimethylthiophen, 2,3-	632-16-6
Dimethylchinolin, 5,8-	2623-50-9	Dimethylthiophen, 2,4-	638-00-6
Dimethylchinolin, 6,8-	2436-93-3	Dimethylthiophen, 2,5-	638-02-8
Furan	110-00-9	Dimethylthiophen, 3,4-	632-15-5
Methyl-2(1H)-chinolinon, 4-	607-66-9	Chinolinon, 2(1H)-	59-31-4
Hydroxybiphenyl, 2-	90-43-7	Methylchinolinon, 1-	606-43-9
Indol	120-72-9	Methyldibenzofurane, 1-	7320-50-5
Isochinolin	119-65-3	Methyldibenzofurane, 2-	7320-51-6
Isochinolinon, 1(2H)-	491-30-5	Methyldibenzofurane, 3-	7320-52-7
Methylbenzofuran, 2-	4265-25-2	Methyldibenzofurane, 4-	7320-53-8
Methylbenzofuran, 3-	21535-97-7	Piperazin	110-85-0
Methylbenzofuran, 4-	5670-23-5	Trimethylthiophene, 2,3,4-	1795-04-6
Methylbenzofuran, 5-	18441-43-5	Trimethylthiophene, 2,3,5-	1795-05-7
Methylbenzofuran, 6-	17059-51-7	Acridinol, 4-	18123-20-1
Methylbenzofuran, 7-	17059-52-8	Dibenzofuran	132-64-9

Name (German)	CAS-number	Name (German)	CAS-number
Methylbenzothiophen, 3-	1455-18-1	Dimethylbenzo(b)-thiophen	30027-44-2
Methylbenzothiophen, 5-	14315-14-1	Dimethylfuran, 2,4-	3710-43-8
Methylchinolin, 2-	91-63-4	Dimethylfuran, 2,5-	625-86-5
Methylchinolin, 3-	612-58-8	Ethylthiophen, 2-	872-55-9
Methylchinolin, 4-	491-35-0	Hydroxyindol, 3-(1H-Indol-3-ol)	480-93-3
Methylchinolin, 5-	7661-55-4	Methylbenzothiophen, 2-	1195-14-8
Methylchinolin, 6-	91-62-3	Propylpyridin, 2-	622-39-9
Methylchinolin, 7-	612-60-2	Propylpyridin, 3-	4673-31-8
Methylchinolin, 8-	611-32-5	Propylpyridin, 4-	1122-81-2
Methyldibenzo-thiophen, 1-	31317-07-4	Xanthenon	90-47-1
Methyldibenzo-thiophen, 2-	20928-02-3	Benzotriazol	95-14-7
Methyldibenzo-thiophen, 3-	16587-52-3	Methylbenzotriazol, 6- or 7-	29385-43-1

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