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Occurrence and leachability of polycyclic aromatic compounds in contaminated soils: Chemical and bioanalytical characterization

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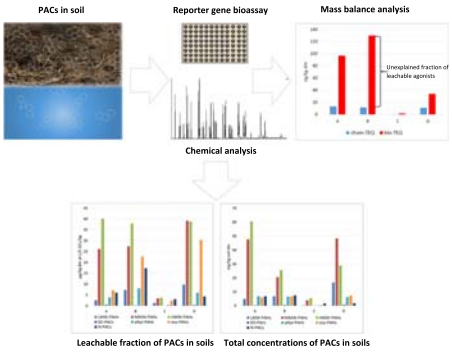
HIGHLIGHTS

- Bioassay and chemical analysis combined with column leaching tests
- PAHs, oxy-PAHs, alkyl-PAHs and NSO-PACs are present in soils and leachates of soils.
- Presence of non-analyzed or unknown AhR agonists in soils and leachates
- Low leachability of PACs and AhR agonists from soils
- Polar PACs more leachable than parent PAHs

GRAPHICAL ABSTRACT



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ABSTRACT

An important concern regarding sites contaminated with polycyclic aromatic compounds (PACs) is the risk of groundwater contamination by release of the compounds from soils. The goal of this study was to investigate the occurrence and leachability of 77 PACs including polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic compounds (NSO-PACs) among total aryl hydrocarbon receptor (AhR) agonists in soils from historical contaminated sites. A novel approach combining chemical and bioanalytical methods in combination with characterization of leachability by use of a column leaching test was used. Similar profiles of relative concentrations of PACs were observed in all soils, with parent PAHs accounting for 71 to 90% of total concentrations in soils. Contribution of oxy-PAHs, alkyl-PAHs and N-PACs ranged from 2 to 9%, 3 to 9% and 1 to 14%, respectively. Although the contributions of groups of PACs were small, some compounds were found in similar or greater concentrations than parent PAHs. Leachable fractions of 77 PACs from soils were small and ranged from 0.002 to 0.54%. Polar PACs were shown to be more leachable than parent PAHs. The contribution of analyzed PACS to overall AhR-mediated activities in soils and leachates suggests presence of other AhR agonists in soils, and a potential risk. Only a small fraction of AhR agonists was available in soils, indicating an overestimation of the risk if only total initial concentrations in soils would be considered in risk assessment. The results of the study strongly support that focus on 16US EPA PAHs may result in inadequate assessment of risk and hazard of PACs in complex environmental samples.

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1. Introduction

Due to widespread sources and persistence, polycyclic aromatic compounds (PACs) are ubiquitous contaminants in soils worldwide. PACs are natural components in most fossil fuels, such as petroleum and coal, and are formed and released into the environment through burning of organic material. Soil and groundwater at industrial areas, especially abandoned gasworks sites, gas stations and former wood preservation facilities where creosote was used, can be contaminated with a variety of PACs, such as polycyclic aromatic hydrocarbons (PAHs), oxy-, and/or alkyl-substituted PAHs and heterocyclic compounds (NSO-PACs), among others (Arp et al., 2014; Bergknut, Frech, Andersson, Haglund, and Tysklind, 2006; Boll, Nejrup, Jensen, and Christensen, 2015). The composition of PACs varies, depending on sources of contamination and weathering processes in the environment.

Despite the complexities of areas contaminated with PACs, current analyses are commonly based on quantification of 16, priority PAHs, originally selected by the U.S. EPA. Over the past 40 years, these 16 PAHs have become widely accepted as representatives for all PACs and routinely analyzed in monitoring programs and used for assessing hazards and risks. Consequently, hundreds of previous environmental and toxicological studies have focused on these "priority PAHs" or only benzo[*a*]pyrene, and less attention has been paid to frequency of occurrence, environmental fate and toxicological effects of other PACs. Over the last few years, more attention has been directed to substituted PAHs and heterocyclic compounds, such as oxy-PAHs and N-heterocyclic compounds (azaarenes) (Andersson and Achten, 2015; Lundstedt et al., 2014; Wilcke et al., 2014). So far, these compounds have mainly been analyzed during research investigations and are seldom included in environmental monitoring, probably due to lack of regulations. Some compounds have been reported to be potentially more toxic and mutagenic to living organisms than parent PAHs (Trilecová et al., 2011).

Because it gives little information about the actual release of PACs from soil to surrounding areas, groundwater or availability of the compounds to organisms, measurement of total concentrations of 16 US EPA PAHs in soil is a poor indicator of either hazards or risks posed to the environment or humans at contaminated sites. Due to their hydrophobic character, PACs tend to bind strongly to soil organic matter, thus reducing mobilities and bioavailabilities of contaminants (Alexander, 2000). Assessment of hazards or risks posed by PACs should therefore be based on the bioaccessible or mobile fraction rather than total concentrations in soil. In addition, some more polar PACs, like oxy-PAHs and N-PACs might, besides being toxic, have a greater tendency to leach than less polar PACs (Lundstedt et al., 2007). So far, research has focused on distribution and leachability of 16 US EPA PAHs in soil (Dalgren, Düker, Arwidsson, von Kronhelm, and van Hees, 2011; Enell, Reichenberg, Warfvinge, and Ewald, 2004; Kim and Osako, 2003; Revitt, Balogh, and Jones, 2014; Zand, Grathwohl, Nabibidhendi, and Mehrdadi, 2010) and only a few studies have examined the distribution and leachability of other PACs, like oxy-PAHs in soil (Enell et al., 2016; Musa Bandowe, Sobocka, and Wilcke, 2011).

Because they enable an estimation of the total toxicological potential of all compounds present in a sample acting through the same mechanism of action, mechanism-specific bioassays are a good complement to instrumental identification and quantification of individual contaminants (Behnisch, Hosoe, and Sakai, 2001; Larsson, Hagberg, Rotander, van Bavel, and Engwall, 2013). Similar to unsubstituted PAHs, some oxy-PAHs, alkyl-PAHs and NSO-heterocyclic compounds are agonists of the aryl hydrocarbon receptor (AhR) and therefore AhR-based bioassays, like the H4IIE-*luc,* can be used to screen for PACs in the environment (Lam et al., Unpublished results; Larsson, Hagberg, Giesy, and Engwall, 2014; Larsson, Orbe, and Engwall, 2012; Lee et al., 2015; Sun, Miller 3rd, Wiese, and Blake, 2014). Previous studies have shown that the observed biological response in the H4IIE-*luc* to extracts of samples from sites contaminated with PACs is considerably underestimated by equivalents calculated as the sum of products of relative potency factors and concentrations of the 16 US EPA PAHs (Andersson et al., 2009; Keiter et al., 2008; Larsson et al., 2013). However, a majority of studies have focused on bioassay analysis of total concentrations of compounds in exhaustive extracts of soils with strong organic solvents, rather than leachable or available fraction.

The present study was conducted to provide deeper insight into occurrence and leachability of PACs in soils at contaminated sites. A novel approach combining chemical and bioanalytical measures combined with characterization of leachability by use of a column leaching test was used. Aims of the study were to: i) examine total concentrations and profiles of relative concentrations of 77 PACs in soils from various industrial areas contaminated with PACs; ii) investigate leachabilities of 77 PACs in soils; iii) determine leachable fractions of AhR-activating compounds in soils and iv) by use of potency (mass) balance estimations, in which predicted AhR-mediated responses of samples based on concentrations of equivalents calculated as the sum of the product of concentrations of individual compounds and their relative potency values (REPs) are compared to concentrations of equivalents measured by use of the H4IIE-*luc* trans-activation assay, determine relative contributions of quantified PACs to total AhR-mediated responses in the H4IIE-*luc* assay. This approach allowed screening of potentially toxic metabolites of PACs in soils and leachates.

2. Materials and methods

2.1. Chemicals

Anhydrous sodium sulfate (99% purity) and n-hexane (≥98%) were purchased from VWR (Stockholm, Sweden). Silica gel 60, dichloromethane (99.8%) and dimethyl sulfoxide (DMSO) (99.9%) were purchased from Sigma Aldrich (Stockholm, Sweden). Steady Lite was purchased from Perkin Elmer (Hägersten, Sweden).

An internal standard (IS) solution, PAH-mix 9, containing 16 deuterated PAHs was purchased from Labor Dr. Ehrenstrofer-Schäfers (Augsburg, Germany). IS solutions of 1-methylnaphthalene-d10, 9-methylanthracene-d12, dibenzothiophene-d8, anthraquinone-d8, acridine-d9 and carbazole-d8 were purchased from Chiron AS (Trondheim, Norway), and the recovery standard (RS) perylene-d12 was purchased from Sigma-Aldrich (Stockholm, Sweden). A standard mixture of alkylated PAHs and dibenzothiophenes (S-4406-200-2 T) containing 20 analytes was purchased from Chiron AS. A PAH standard mixture containing 36 native PAHs (SRM 2260a) from National Institute of Standards and Technology (NIST), was purchased from Sigma-Aldrich. Standards of 1-methylchrysene (99.1%), 2-methylchrysene (99.3%), 3-methylchrysene (99.3%), 7-methylbenzo[*a*]pyrene (98%) and 7-methylbenzo[*a*]anthracene (n/a) were purchased from Sigma-Aldrich, 9-methylacridine (99%) and 11H-benzo[*a*]carbazole (99.8%) were purchased from Chiron AS, and benzo[*a*]fluorene (98%) was purchased from Analytical Solutions (North Kingstown, USA). Dibenzo[*a,h*]acridine (99.6%) was purchased from LGC standards (Wesel, Germany). 1,4-chrysenequinone (93%) was purchased from Tokyo Chemicals. 2-methylanthracene (97%), 9-fluorenone (98%), naphthacene-5,12-dione (97%), 9,10-dihydrobenzo[*a*]pyren-7(8H)-one (97%), quinoline (98%), carbazole (99.3%), anthracene-9,10-dione (99.8%), 4Hcyclopenta[*d,e,f*]phenanthrenone (BCR-338; 99.5%), benzo[*a*]fluorenone (BCR-342; 99.8%), benzo[*b*]naphtho[2,1-*d*]furan (BCR-341; 99.6%), and 6H-benzo[*cd*]pyren-6-one (BCR-339; 98.8%) were purchased from Sigma-Aldrich, 1-indanone (>99%), 2-methylanthracene-9,10-dione (97%), benzo[*a*]anthracene-7,12-dione (>98%), 7H-benzo[*de*]anthracene-7-one (99%), benzo[*h*]quinoline (98%), and acridine (> 98%) were purchased from Alfa Aesar (Karlsruhe, Germany), and dibenzo[*ah*]acridine (99.6%) was purchased from LGC standards. Naphtho[2,3-*a*]pyrene (99% purity) was purchased from Ultra Scientific Analytical Solutions. The 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD)

standard, with a purity of 99.1%, was from AccuStandard Inc. (New Haven, USA).

2.2. Sample collection, pre-treatment and characterization

Nine soils used in the experiment were sampled at four sites in Sweden contaminated with PACs. Three different samples were collected at the site of an abandoned gasworks plant in Norrköping (G1-G3), three different samples were collected in an area close to a railway station in Mjölby (R1-R3), two different samples were collected at a former wood preservation facility contaminated with creosote in Nässjö (C1-C2) and one sample was collected at a housing area in Örebro containing filling material (H1). Soil samples were sieved (≤2 mm) and homogenized thoroughly by stirring for at least 5 min by use of a stainless-steel spoon, and stored below 7 °C in the dark until use. Water content (heating at 105 °C in 24 h), organic matter (continued heating at 550 °C in 3 h), clay content according to ISO method 11277 (ISO, 2009) and pH according to ISO method 10390 (ISO, 2005) were determined for all soils. The major characteristics of the soils are given (Table 1).

2.3. Analytical methods

2.3.1. Column leaching test

An up-flow percolation test was performed according to the procedure described in EN 14405 (2017). Briefly, wet soil samples were packed in glass columns with capacities of approximately 600 cm^3 (i.d. = 5 cm; L = 30 cm) at saturated conditions. Compounds were eluted with a continuous vertical up-flow of deionized water at the rate given in the standard (12 ml/h). Leachates were collected at liquid-solid ratios (L/S) 0.1, 2 and 10 L/kg in amber glass containers and sodium azide was added (0.98 g/L) to prevent biodegradation. A volume of 10 ml of each leachate was collected for measurement of pH and conductivity. Leachates were stored at 7 °C prior to extraction. Triple liquid-liquid extractions were performed to extract PACs from the eluates, with two times n-hexane, 5 min each, followed by a single extraction with dichloromethane for 5 min. The volumes extracted were 50 ml, 150 ml and 400 ml for L/S 0.1, 2 and 10 l/kg, respectively, and the eluate/organic solvent ratio was 4:1 (v/v) in all extractions. Extracts were passed through a column with anhydrous sodium sulfate to remove remaining water, before solvents were evaporated to 1 ml by use of a rotary evaporator. Extracts were split into two fractions used for chemical and biological analysis. IS solutions (50 ng) was added to extracts intended for chemical analysis, and the extracts were then concentrated under a gentle stream of nitrogen and solvent changed into 100 μl in toluene, after addition of RS (50 ng). Extracts to be used in the H4IIE-*luc* were evaporated under a gentle stream of nitrogen into 30 μl DMSO. All extracts were stored at −18 °C until analysis.

2.3.2. Pressurized liquid extraction of soils

Total concentrations of PACs in soils were determined by use of pressurized liquid extraction (PLE™, fluid management systems, Inc.) with in-cell cleanup, as described previously in Larsson et al. (2013) with minor modifications. Extraction cells made of stainless steel (44 ml) were packed with 4 g of basic silica at the bottom, followed by a thin layer of anhydrous sodium sulfate, soil homogenate (2 to 4 g of soil + anhydrous sodium sulfate, in a ratio 1:5) and finally a layer of sodium sulfate at the top. Extractions were performed in two static cycles at 120 °C and 12 MPa for 10 min, with n-hexane/dichloromethane (9:1 v/v) as extraction solvent. After extraction, extracts were evaporated and split into two aliquots. Aliquots aimed for chemical analysis were solvent exchanged into 500 μl toluene after addition of IS solutions (50 to 500 ng) and RS (500 ng), and aliquots aimed for bioassay analysis were solvent exchanged into 50 μl DMSO.

Table 1
Physical and chemical characteristics of the investigated soils.

Samples	Water content	Organic matter	Clay content	pH
	(%)	(%)	(%)	
H1	11	3	12	5.9
R1	8	6	4	6.2
R2	19	19	12	7.6
R3	8	5	2	8.2
G1	14	13	8	5.3
G2	15	5	13	4.5
G3	11	6	<2	8.4
C1	8	2	<2	6.6
C2	9	3	2	6.8

2.3.3. H4IIE-luc bioassay

Concentrations of AhR-mediated equivalents in extracts of soil or leachates were measured by use of the H4IIE-*luc* assay. This assay is based on a rat hepatoma cell line stably transfected with a luciferase reporter gene under control of the AhR (Murk et al., 1996). The H4IIE-*luc* assay was performed as previously described in Larsson et al. (2013), with minor modifications described here. Prior to the H4IIE-*luc* analysis, a four-fold dilution series was prepared for each extract in culture medium. To each 96 well plate, two extracts were added at six concentrations (three replicates per concentration), and a standard curve of TCDD (0–300 pM) in triplicate wells. The final DMSO concentration in all wells was 0.4%. After 24 h of exposure, medium was removed from plates, and cells were washed with twice with phosphate buffered saline solution (PBS). PBS (25 μl) and Steady Lite substrate mix (25 μl) were added to cells and plates were stored in darkness at room temperature in 15 to 20 min for cell lysis and enzymatic reaction to take place. Cell lysates were transferred to white, 96-well, microtiter plates, and the luciferase activity in each well was measured in a luminometer (Fluostar Omega). Concentration-response curves were performed for extracts and TCDD by use of a sigmoidal (variable slope) curve fitting equation (GraphPad Prism® 5.01). A number of extracts of soils were further diluted (10 or 100 fold in DMSO) and reanalyzed to achieve adequate concentration-response curves. TCDD equivalents determined by use of the H4LLE-*luc* bioassay (Bio-TEQs), were calculated from concentration-response curves by relating the luciferase induction potency of the extracts to that of the TCDD standard as described in Larsson et al. (2013).

2.3.4. GC/MS analysis

Identification and quantification of 77 PACs were performed by use of an Agilent 7890A gas chromatograph coupled to a 5975C low-resolution mass spectrometer (GC/LRMS). Analytes (1 μL) were injected into the gas chromatograph in the splitless mode. Separation of compounds was achieved on a capillary column (30 m × 0.25 mm, 0.15 μm film thickness) (Select PAH; Agilent Technologies).The initial oven temperature was 70 °C (0 min), 8 °C/min to 205 °C (2 min), 8 °C/min to 250 °C, 3 °C/min to 270 °C (2 min), 9 °C/min to 279 °C, 1 °C/min to 280 °C (3 min), and then 5 °C/min to 325 °C where it was held for 5 min. All measurements were performed in the selected ion monitoring mode. Identification and quantification of the PACs in the extracts were done by use of quantification mixtures including all 77 PACs in addition to IS and RS.

Chemically derived TCDD equivalents (chem-TEQs) were calculated as the sum of the product of concencentrations of individual concentrations of 61 PACs multiplied by relative potency factors (REPs) specific to the H4IIE-*luc* assay (Lam et al., Unpublished results; Larsson et al., 2014; Larsson et al., 2012) as previously described in Larsson et al. (2013).

2.3.5. Quality assurance/quality control (QA/QC)

The accuracy of PAH measurements of total initial concentrations in soils was checked by including a certified reference material ERM®-CC013a (BAM, Berlin, Germany) into our analysis. Reference soil was tested in triplicates and spiked with IS solutions before extraction by use of PLE™ and spiked RS before GC/MS-analysis. The average results

agreed well with the certified values available for a number of PAHs. The confidence limits were overlapping in all but one occasion (i.e., for naphthalene). Relative standard deviation (RSD) of the triplicates were 1 to 15% for all compounds except for quinoline and benzo[*h*]quinoline (60 and 35%, respectively). Procedure blanks were included in all batches.

Target compounds were quantified by use of five to six point calibration curves. RSD of the relative response factor (RRF) values was <15% for PAHs and <25% for alkylated PAHs, oxy-PAHs and azaarenes. Quantification standards were analyzed after every tenth sample. Concentrations of PACs were calculated by use of the internal standard method. In lack of internal standards, relative response factor (RRF) values for the compounds were calculated using the compound nearest in retention time. The limit of detection (LOD) was defined as mean concentration in blanks + 3 times the standard deviations. Samples which had concentrations exceeding the range of the calibration curve were diluted and reanalyzed.

Only plates with a standard deviation of ≤14% within triplicates, a TCDD EC50 value between 8 and 18 pM, and a TCDD maximal induction factor >6 were used for quantification of AhR-mediated response (bio-TEQ). Limit of detection (LOD) was calculated as the mean luciferase activity of DMSO control triplicates + 3 times standard deviation (SD).

3. Result and discussion

In this study, both chemical and bioanalytical approaches were combined with a column leaching test used to measure the occurrence and leachability of PACs and overall AhR-activating compounds in soils from historical contaminated sites.

3.1. Characterization of PACs in soils and leachates of soils

3.1.1. Concentrations of PACs in soils

Total concentrations of PACs varied among soils. Sum of 77 PACs ranged from 3.6 to 290 mg/kg dry mass (dm) (Table 2). The 16 US EPA PAHs were among the most abundant PACs in all soils, with concentrations of 16 PAHs ∑16PAHs ranging from 2.3 to 195 mg/kg. Fluoranthene and pyrene were found to be the most abundant compounds in seven soils, with concentrations from 0.3 to 52 mg/kg, and 0.3 to 40 mg/kg, respectively (Supplementary information, Table S2). However, other non-16 US EPA PAHs were also found in relatively great concentrations; sum of 19 parent PAHs ranged from 0.8 to 44 mg/kg. Concentrations of ∑17alkyl-PAHs, ∑12oxy-PAHs and ∑8 N-PACs (azaarenes) in soils were in similar range (0.13 to 7.6, 0.22 to 7.3 and 0.27 to 7.5 mg/kg, respectively), except for one soil sampled close to a railway (R2) containing ∑N-PAC of 41 mg/kg. Sulfur and oxygen containing heterocyclic compounds (SO-PACs) were detected in lesser concentrations (∑5SO-PACs <1.9 mg/kg). Concentrations of 16 US EPA PAHs, alkyl-PAHs, oxy-PAHs and N-PACs were in the range found at other historically PAC contaminated sites (Arp et al., 2014; Lundstedt et al., 2003).

Similar profiles of relative concentrations were observed for all soils (Fig. 1), with 35 parent PAHs accounting for 71 to 90% of total concentrations of PACs in soils. Parent PAHs were dominated by compounds with four to six fused rings (molecular weights from 228 to 302) in all soils regardless of source of contamination. Soils (C1 and C2) contained a greater portion of PAHs with four rings, and also a greater portion of three ring PAHs than the other soils, which is characteristic for weathered soils from a former wood preservation facility with creosote (Murphy and Brown, 2005), while soils (G1, G2 and G3) from a former gasworks site contained a greater portion of PAHs with five and six rings. This can be explained by volatilization, degradation and mobility of low molecular weight PAHs in soils over time, which lead to an enhancement in the relative concentration of high molecular weight PAHs in most contaminated sites (Thomas and Lester, 1993).

Contribution of substituted PAHs was smaller, oxy-PAHs and alkyl-PAHs accounted for 2 to 9%, and 3 to 9% of ∑77PACs, respectively. Although the contribution of 12 oxy-PACs to total sum of PACs in soil was relatively low, some oxy-PAHs were found in similar or greater concentrations than their parent PAHs (Supplementary information, Table S3). For example, concentrations of 9-fluorenone were similar to or greater than fluorene in five soils (R2, R3, G1, G2 and G3), and concentrations of benzo[*a*]fluorenone were similar to or greater than benzo[*a*]fluorene in three soils (R1, G2 and G3). The oxy-PAHs may have entered the soils from sources similar to those of parent PAHs, but may also have been formed through oxidation of PAHs in the soils. Similar concentrations of oxy-PAHs and parent compounds have been reported in previous studies of historical contaminated soils, and might be due to the accumulation potential of many oxy-PAHs during degradation of PAHs in soils (Lundstedt et al., 2003; Lundstedt et al., 2007). The composition of the oxy-PAHs in the soils are similar to previous studies, which reported 9-fluorenone, anthracene-9,10-dione, benzo[*a*]anthracene-7,12-dione and benzo[*a*]fluorenone as major contributors to the sum of oxy-PAHs in soils from urban and industrial areas (Bandowe et al., 2014; Lundstedt et al., 2007). Alkylated PAHs were less abundant than parent PAHs in tested soils, except for alkylated isomers of naphthalenes, which were present in similar concentration as those of naphthalene. Nitrogen containing heterocyclic compounds

Table 2
Total concentrations (mg/g soil dm) of polycyclic aromatic compound (PACs) groups in soils.

Samples	LMW-PAHs[a]	MMW-PAHs[b]	HMW-PAHs[c]	16PAHs[d]	19PAHs[e]	alkyl-PAHs[f]	oxy-PAHs[g]	N-PACs[h]	SO-PACs[i]	PACs[j]
H1	0.16	1.2	1.6	2.3	0.8	0.13	0.22	0.27	0.016	3.6
R1	0.27	3.9	5.6	7	3.1	0.31	0.63	1.7	0.023	12
R2	10	130	89	195	44	7.6	7.2	41	1.2	290
R3	1.9	15	7.9	21	4.6	1.2	1.3	2.6	0.18	29
G1	4.9	48	61	83	34	6.9	6.1	6.9	0.48	130
G2	2.2	15	26	34	13	2.1	3.1	3.9	0.23	53
G3	6.9	21	26	40	16	6.6	6.7	7.5	0.64	76
C1	10	106	16	110	29	5.9	5.4	0.97	1.9	150
C2	17	48	29	73	33	6.2	7.3	1.9	0.79	110

[a] Total concentrations of 7 parent PAHs with molecular weight 128–178.
[b] 9 parent PAHs with molecular weight 190–228.
[c] 19 parent PAHs with molecular weight 252–302.
[d] Total concentrations of 16 priority PAHs.
[e] Total concentrations of 19 parent "non-priority" PAHs.
[f] Total concentrations of 17 alkyl-PAHs.
[g] Total concentrations of 12 oxy-PAHs.
[h] Total concentrations of 8 N-PACs (azaarenes) including 9-methylacridine and acridone.
[i] Total concentrations of measured 3 alkylated dibenzothiophenes, dibenzothiophene and benzo[*b*]naphtho[2,1-*d*]furan.
[j] Total concentrations of all 77 PACs.

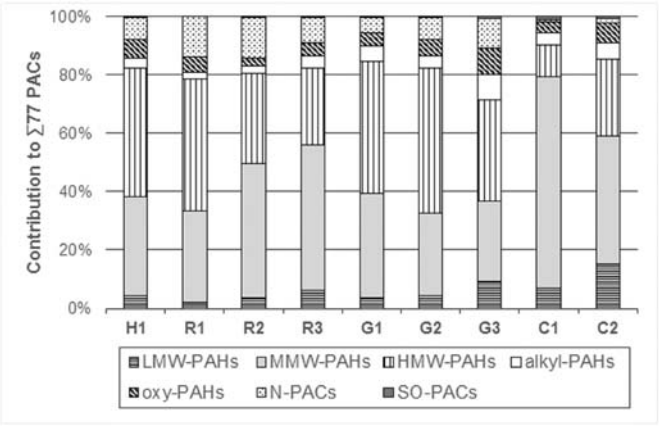

Fig. 1. Relative contributions (%) of groups of PACs to the sum of 77 PACs in soils. Groups of PACS: 7 parent PAHs with molecular weight 128–178 (LMW-PAHs), 9 parent PAHs with molecular weight 190–228 (MMW-PAHs), 19 parent PAHs with molecular weight 252–302 (HMW-PAHs), 17 alkyl-PAHs, 12 oxy-PAHs, 8 N-PACs and 3 alkylated dibenzothiophenes, dibenzothiophene and benzo[*b*]naphtho[2,1-*d*]furan (SO-PACs).

(N-PACs) accounted for 1 to 14% of the total concentrations of PACs in soil, with lesser relative concentrations in soils (C1 and C2) from a former wood preservation facility contaminated with creosote. However, comparisons of concentrations of individual N-PACs with their homocyclic PAH analogous in soils showed that carbazole was present in concentrations up to five times greater than fluorene, and dibenzo[*ah*]acridine in concentrations up to half the concentrations of dibenzo[*ah*]antracene, while other N-PACs were present at lesser concentrations than their homocyclic PAH analogues. For example, acridine was present in concentrations 1 to 14% of anthracene. Surface soils from urban areas were dominated by carbazole (0 to 86% of ∑4N-PACs) (Bandowe et al., 2014). Carbazole was also the second most abundant N-PAC in soils from this study (3.6 to 28% of ∑8N-PACs). 11H-benzo[*a*]carbazole was the most abundant azaarene in all soils (46 to 96% of ∑8N-PACs), with concentrations ranging from 0.25 to 38 mg/kg. Even though concentrations of N-PACs were relatively low in many soils, toxicity and the generally greater water solubility of these compounds, may imply a greater environmental impact (Bleeker et al., 2002).

Contribution of SO-PACs was <1% in all soils, but only five SO-PACs were included in the study.

3.1.2. Concentrations of PACs in leachates of soils

Concentrations of the sum of PACs in leachates ranged from 0.2 to 50 μg/L in first fractions (L/S 0.1 L/kg), from 0.1 to 129 μg/L in second fractions (L/S 2 L/kg) and from 0.1 to 68 μg/L in last fractions (L/S 10 L/kg) (Supplementary information, Table S4). Leached accumulated amounts of PACs per kg soil at L/S 10 L/kg were calculated by multiplying the concentrations of PACs by the L/S ratio and subsequently summing them up. Sum of PACs ranged from 1.5 to 790 μg/kg dm soil (Table 3). Similar to initial concentrations in soils, 16 US EPA PAHs were among the most abundant compounds in leachates and leached amounts of 16 US EPA PAHs (∑16PAHs) ranged between 0.89 and 560 μg/kg dm soil.

Profiles of relative concentrations in leachates differed slightly compared to profiles in soils. Similar to soils parent PAHs were dominant compounds in leachates (Fig. 2), but with lesser relative concentrations to ∑PACs (60 to 81%), while the contribution of alkyl-PAHs was slightly greater than in soils (3 to 14%).

Greater relative concentrations of more hydrophilic PACs were found in leachates compared to relative concentrations in soils; sum of oxy-PAHs and N-PACs accounted for 0.5 to 23% and 3 to 21% of the sum of leachable PACs, respectively. Comparison of individual concentrations of oxy-PAHs and parent PAHs showed that oxy-PAHs were relatively abundant in the leachates. 9-Fluorenone was present in concentrations up to seven times greater than fluorene, and benzo[*a*]fluorenone in concentrations up to four times greater than benzo[*a*]fluorene. Greater concentrations of N-PACs compared to their PAH analogues were also shown for a number of samples, for example carbazole was present in concentrations up to 11 times greater than those of flourene, and quinoline up to two times greater than naphthacene, while other N-PACs were present in lesser concentrations than analogues PAHs, for example acridine and dibenzo[*ah*]acridine.

3.2. Characterization of leachable fractions of PACs and AhR agonists in soils

3.2.1. Leachability of PACs from soils

Leachable concentrations of ∑77PACs were considerably less than initial concentrations of ∑77PACs in soils, and leached percentages of initial amounts of ∑77PACs in soils ranged from 0.002 to 0.54% (Supplementary information, S5). Leachable fractions of ∑17alkyl-PAHs in soils ranged from 0.03 to 0.38%. The leachable fraction in soils was generally greater for more hydrophilic PACs, which is illustrated in Fig. 3 by the negatively correlation between leached accumulated fractions of initial amounts in soils and log K_{OW} values for the studied PAHs ($r = -0.25$, $P < 0.0001$), oxy-PAHs (-0.53, < 0.0001), alkyl-PAHs (-0.30, < 0.05) and N-PACs (-0.68, < 0.0001). This is in agreement with results

Table 3
Leached accumulated amount in μg/kg dm soil of groups of polycyclic aromatic compounds (PACs) at L/S-ratio 10.

Samples	LMW-PAHs[a]	MMW-PAHs[b]	HMW-PAHs[c]	16PAHs[d]	19PAHs[e]	alkyl-PAHs[f]	oxy-PAHs[g]	N-PACs[h]	SO-PACs[i]	PACs[j]
H1	0.24	0.44	0.51	0.89	0.30	0.07	0.17	0.10	0.01	1.5
R1	1.4	3.5	3.8	6.6	2.0	0.46	2.5	3.1	0.05	15.0
R2	0.47	1.5	1.3	2.5	0.72	0.23	0.58	0.55	0.03	4.7
R3	0.31	0.51	0.46	1.0	0.28	0.10	0.084	0.18	0.01	1.7
G1	2.7	26	40	52	17	4.0	7.3	6.1	0.60	87
G2	7.4	27	38	54	19	8.1	23	17	0.28	120
G3	1.0	3.6	5.6	7.3	2.9	2.3	2.3	1.1	0.10	16
C1	18	595	30	560	89	21	110	5.2	7.70	790
C2	10	39	39	66	22	6.1	30	4.4	0.52	130

[a] Concentrations of 7 parent PAHs with molecular weight 128–178.
[b] 9 parent PAHs with molecular weight 190–228.
[c] 19 parent PAHs with molecular weight 252–302.
[d] Concentrations of 16 priority PAHs.
[e] Concentrations of 19 parent "non-priority" PAHs.
[f] Concentrations of 17 alkyl-PAHs.
[g] Concentrations of 12 oxy-PAHs.
[h] Concentrations of 8 N-PACs (azaarenes) including 9-methylacridine and acridone.
[i] Concentrations of measured 3 alkylated dibenzothiophenes, dibenzothiophene and benzo[*b*]naphtho[2,1-*d*]furan.
[j] Concentrations of all 77 PACs.

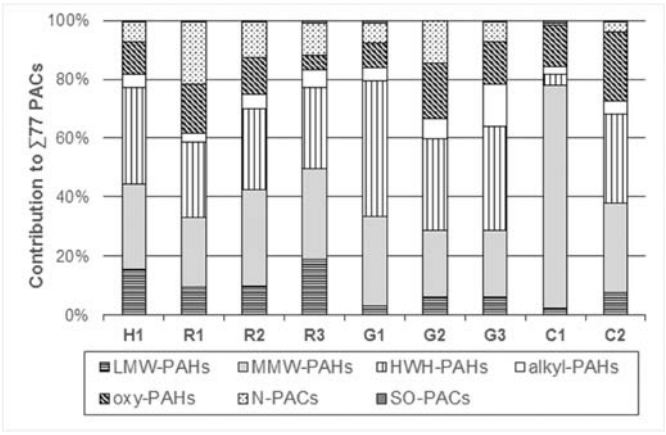

Fig. 2. Relative contributions (%) of 7 parent PAHs with molecular weight 128–178 (LMW-PAHs), 9 parent PAHs with molecular weight 190–228 (MMW-PAHs), 19 parent PAHs with molecular weight 252–302 (HMW-PAHs), 17 alkyl-PAHs, 12 oxy-PAHs, 8 N-PACs and 3 alkylated dibenzothiophenes, dibenzothiophene and benzo[*b*]naphtho[2,1-*d*]furan (SO-PACs) to the leached accumulated amounts of 77 PACs (L/S 10 L/kg).

of previous studies of PACs (Enell et al., 2016; Enell et al., 2004). Percentages of initial amounts of ∑LMW-PAHs, ∑8N-PACs and ∑12Oxy-PAHs that were leached from soils ranged from 0.005 to 0.5%, 0.001 to 0.5% and 0.01 to 2%, respectively. In most soils, greater leachability was observed for polar PACs composed of two or three rings, that is, 1-indanone, quinoline and benzo[*h*]quinoline, than for other PACs.

Besides polarities and aqueous solubilities of compounds, organic matter content in soil is an important factor that influences the leachability of compounds from soils (Wilcke, 2000). The soil organic matter is the main sorbent for organic compounds in soil. No significant correlation between organic matter content and leachability of ∑77PACs in the nine soils was observed in this study (<0.05, $p = 0.0769$). However, greatest leachability of most PAC groups was observed for soil C1, which also had the smallest amount of organic matter content, and lesser leachability of PACs was observed for soil (R2), which had the greatest amount of organic matter (Table 1). N-PACs (azaarenes) are in comparison to PAHs weak bases and ionizable in the pH range corresponding to their pKa values. Leachability of N-PACs in soils are thereby affected by pH and studies of sorption to soils and minerals have shown that the overall sorption of N-PACs is dominated by cation exchange (Burgos et al., 2002; Bi et al., 2006). No significant correlation was observed between leachable fraction of N-PACs and pH values in soils (<0.05, $p = 0.5044$).

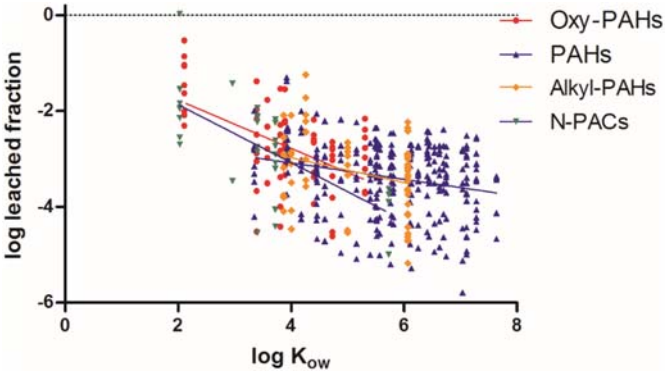

Fig. 3. Relationship between leached accumulated fractions of initial amounts of 56 PACs in soils and their octanol–water partitioning coefficients (K_{ow}). K_{ow}-values for PACs are obtained from Achten and Andersson (2015).

3.2.2. Leachability of AhR agonists from soils

To get a more comprehensive understanding of potential risks of leachable fractions in soils, all soils and leachates were also tested in the AhR-based H4IIE-*luc* bioassay. Bio-TEQs in soils ranged from 840 to 67,470 ng/kg dm soil (Table 4). Cumulative leaching of bio-TEQs from soils at L/S 10 L/kg was calculated by multiplying the bio-TEQ by the L/S ratio and subsequently summing the products. The low leachability of PACs in soils is also reflected by the bio-TEQ values. Bio-TEQs of leached fractions ranged from 0.54 to 130 ng/kg dm soil, and percentages of the initial bio-TEQs in soils, that could be leached, were 0.005% to 0.54%. This result indicates that even if soils contain a greater amount of AhR agonists, only a small portion of AhR agonists was leachable with water. Similar to concentrations of PACs that could be leached from soils, no significant correlation was observed between concentrations of bio-TEQs in soils and concentrations of leachable AhR agonists (bio-TEQs) (<0.05, $p = 0.3455$), or between content of organic material in soils and leachable fraction of AhR agonists (bio-TEQs) (<0.05, $p = 0.7224$). This indicates that the leachable fraction of compounds is influenced not only by soil properties like organic matter content but also by residence time of compounds in soils, their properties and history of contamination of soils.

3.2.3. Contribution of PACs to AhR-mediated responses of leachates and soils

Combining quantification of individual PACs with measurement of AhR agonists by use of the H4IIE-*luc* bioassay allows an integrated measure of all AhR-activating compounds in soils including potential toxic metabolites, but can also provide information on contributions of individual compounds to overall AhR-mediated potency observed in the bioassay (Larsson et al., 2013; Machala et al., 2001b). To assess the fraction of AhR-mediated response explained by PACs, concentrations of chem-TEQs, calculated by use of 61 REPs for PACs that have been measured in the H4IIE-*luc* assay (Lam et al., Unpublished results; Larsson et al., 2014; Larsson et al., 2012), were compared with concentrations of bio-TEQs measured in leachates and extracts of soils directly, by use of the H4IIE-*luc* bioassay (Fig. 4). The contribution of PACs could explain 5 to 72% of concentrations of bio-TEQs in soils. The 16 US EPA PAHs were predominant contributors to the chem-TEQs in all soils, and explained 4 to 55% of the AhR-mediated activities. Also alkyl-PAHs had a greater contribution to the bio-TEQs in soils (1 to 10%).

Higher molecular weight PAHs with a greater affinity for the AhR were predominant contributors to the chem-TEQs in soils. Priority PAHs benzo(b)fluoranthene and dibenzo[*ah*]anthracene and nonpriority PAHs dibenzo[*aj*]anthracene and dibenzo[*ac*]anthracene were the most dominant contributors to the chem-TEQs along with alkyl-PAHs 7-methylbenzo(a)anthracene, 1-methylchrysene, 2-methylchrysene, and 3-methylchrysene.

Both chemical and bioassay results revealed a relatively small leachability of PACs from contaminated soils. Chem-TEQs based on 61 PACs could explain 3 to 49% of concentrations of bio-TEQs in leachable fractions of soils (Fig. 5). Similar to soils, the 16 US EPA PAHs were

Table 4
Bioassay derived TEQs (bio-TEQs) in soils (ng/kg dm soils) and in leachates of soils (ng/kg dm soil) at L/S 10 L/kg).

Samples	bio-TEQ in soil	bio-TEQ in leachate	%[a]
H1	840	0.54	0.06
R1	31,200	3.6	0.01
R2	35,600	1.8	0.005
R3	8000	4.4	0.06
G1	61,500	97	0.16
G2	14,760	16	0.11
G3	24,250	130	0.54
C1	67,470	31	0.05
C2	23,960	34	0.14

[a] Leached percentage of initial bio-TEQS in soil.

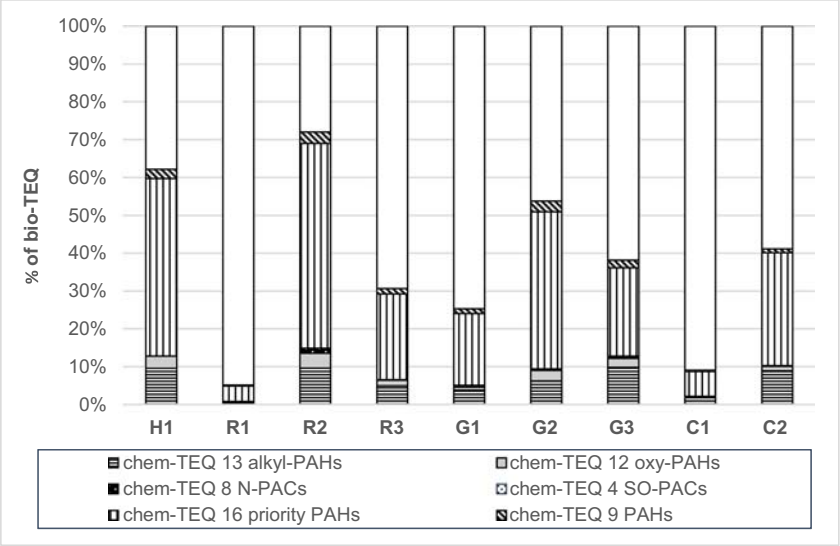

Fig. 4. Relative contributions (%) of PACs in chem-TEQs to bio-TEQs in soils from PAH-contaminated sites determined by use of the H4IIE-*luc* assay.

predominant contributors to the chem-TEQs in leachates, and explained 2 to 30% of the AhR-mediated response. Also non-priority PAHs and alkyl-PAHs had a greater contribution to the bio-TEQs in some leachates, 2 to 19% and 0.3 to 16%, respectively. Benzo(b)fluoranthene, 1-methylchrysene and 2-methylchrysene were among the most dominant contributors to the chem-TEQs in leachates.

Groups of oxy-PAHs and heterocyclic aromatic compounds (NSO-PACs) contributed little to the overall AhR-mediated responses detected in soils and leachates. This result can be explained by small concentrations of more potent compounds, like dibenzo[*ah*]acridine and naphthacene-5,12-dione, and/or lesser AhR-mediated potencies of lesser molecular weight oxy-PAHs, N-PACs and S-PACs (Larsson et al., 2014; Machala et al., 2001a). O-PACs have been reported to be potential contributors to the AhR-mediated responses in environmental samples (Brack and Schirmer, 2003). However, because only benzo[*b*]naphtho[2,1-*d*]furan, a weak AhR agonist, was included in the chemical analysis, that could not be demonstrated during the present study.

There was no correlation between contribution of known AhR agonists in soils and contributions of known AhR agonists in corresponding leachates, for example, contribution of chem-TEQs to bio-TEQs for samples C1 and C2 was 49 and 32% in leachates compared to 9 and 41% in soils. This indicates that soil C1 contained a great portion of unquantified or/and unknown AhR agonists with little leachability from soils.

Several studies have shown that chem-TEQs based on the 16 US EPA PAHs only account for a portion of the AhR-mediated activities in environmental samples contaminated with PACs (Andersson et al., 2009; Keiter et al., 2008; Larsson, et al., 2013). Discrepancies between chem-TEQs and bio-TEQs can be due to mixture interactions (antagonistic or synergistic effects) and/or additional known or unknown AhR agonists in the samples not target by the chemical analysis, and lack of REPs for target compounds.

Even though, in the present study, chem-TEQs were based on REPs of 61 PACs, specific for the H4IIE-*luc* bioassay, only 5 to 72% of the AhR-mediated responses in soils could be explained by the chemical

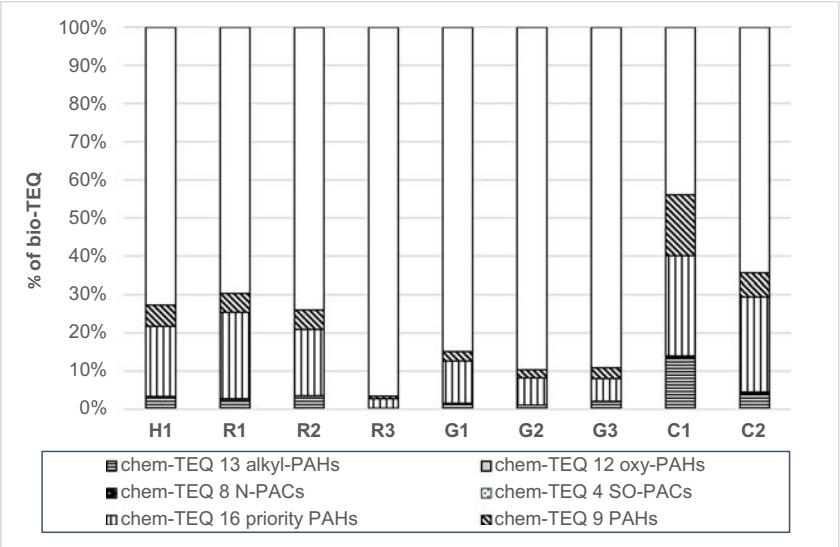

Fig. 5. Relative contributions (%) of PACs in chem-TEQs to bio-TEQs in water leachates of soils from PAC-contaminated soils determined by use of the H4IIE-*luc* assay.

analyzed PACs. Several studies have shown that some organic pollutants, for example, polychlorinated naphthalenes (PCNs), polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs), and polychlorinated biphenyls (PCBs) have the potential to induce AhR-mediated activities in the H4IIE-*luc* bioassay (Behnisch et al., 2003; Villeneuve et al., 2000). At sites heavily contaminated with PACs, for example abandoned gasworks plants and former wood preservation facilities contaminated with creosote, complex mixtures of hundreds or even thousands of PACs can be present (Bergknut et al., 2006; Lundstedt et al., 2003; Stout et al., 2015). This is the most likely explanation for discrepancies between chem-TEQs and bio-TEQs in the studied soils and leachates.

3.3. Environmental relevance

Soils contaminated with PACs are a worldwide problem. Due to the toxic, mutagenic, and carcinogenic properties of many PACs and their persistence in soil, sites contaminated with PACs are of greatest environmental concern. The present study combined chemical and bioanalytical measures with a column leaching test for the characterization of PACs and other AhR-active compounds and their leachability in soils. Results of this study show that chemical analysis of 16 US EPA PAHs to determine the degree of contamination of PACs in soils greatly overlooks toxicologically relevant PACs and AhR agonists in soils. Even though the 16 US EPA PAHs were among the most abundant of 77 analyzed PACs in soils, other toxicological relevant PACs were present in all soils. For example, higher molecular weight PAHs, such as benzo[*a*]fluoranthene, benzo[*b*]chrysene dibenzo[*b*,*k*]fluoranthene, dibenzo[*a*,*e*]pyrene, and isomers of methylchrysene along with more polar PACs, like 11H-benzo[*a*]carbazole, dibenzo[*ah*]acridine, and benzo[*a*]fluorenone (Bleeker et al., 2002; Machala et al., 2008). Chemical analysis of 77 PACs could explain only 5 to 72% of the activity observed in the bioassay, and confirm that other AhR agonists were present in soils.

The results of this work provide knowledge about presence of PACs in contaminated soils and leachabilities of those compounds from soils. Although PACs are strongly sorbed in soil they are found at greater soil depth, indicating release of the compounds from surface soil (Musa Bandowe et al., 2011). Leachable concentrations of PACs were considerably lesser than total initial concentrations of PACs in all soils, which indicated limited leachability of compounds from soils. Only a smaller portion of the AhR-inducing compounds in the studied soils was available for release to ground or surface water. Leachability in soils was generally greater for more hydrophilic PACs, like 1-indanone, quinoline and benzo[*h*]quinoline, than for other PACs. Even though the leachable concentrations of polar PACs were relatively small, toxicity and the generally greater water solubility of the compounds may imply a greater environmental impact (Lemieux et al., 2008; Lundstedt et al., 2007). Contribution of low molecular PACs, like quinoline, to the observed activity in bioassays was low due to the weak AhR-inducing potency of low molecular weight PACs. However, the bioassay analysis of leachates revealed that even though the leachable fraction of compounds in soils was small, there was a great portion of unknown AhR agonists released from the soils. Our results also indicate that the leachability of groups of PACs might not reflect the leachable characteristics of individual PACs.

4. Conclusions

An important concern at most sites contaminated with PACs is the risk of groundwater contamination by release of the PACs from soils. Results of the study reported here demonstrated that leachable fractions of 77 PACs from nine historically contaminated soils were small. Furthermore, polar PACs, like oxy-PAHs and N-PACs were more leachable than parent PAHs containing the same number of fused rings. Therefore, analysis of only PAHs would give misleading information about the actual risk of the soil, especially because some of the polar compounds are known to be toxic. Also other important PACs were quantified in soils, like a number of high molecular weight PAHs and alkyl-PAHs. Results of this study support the suggestion that additional PACs should be included among the 16 US EPA PAHs in environmental monitoring and assessment of risk of contaminated soils. At present it is difficult to suggest which specific PACs should be included in the target list and more research is needed to find out which PACs are frequently present at contaminated sites and to improve the existing knowledge about their toxic mechanisms and fate in environment. Reporter gene cell bioassays, like the AhR-based H4IIE-luc bioassay are valuable tools to measure the integrated potencies of all chemicals in an environmental sample based on an established mechanism of action. Contribution of the analyzed PACs to the overall AhR-mediated activities detected in soils and leachates was small and confirms the presence of several other AhR agonists in soils. A small fraction of AhR agonists was available in soils, indicating an overestimation of the risk if only total initial concentrations in soils would be considered in risk assessment. However, the results show that leaching studies based on only 16 US EPA PAHs have the potential to underestimate the risk of the soils. A broad range of PACs and bioassay active compounds have been related to leachable fractions of soils, which reflect the risk more than initial amounts of compounds in soils, and provides a more comprehensive picture of the chemical risks of sites contaminated with PACs. The results of the study presented here strongly support that focus on 16 US EPA PAHs may result in inadequate assessment of risk and hazard of PACs in complex environmental samples.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.12.015.

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Supplementary data of

Occurrence and leachability of polycyclic aromatic compounds in contaminated soils: Chemical and bioanalytical characterization

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Table S1 Characteristics of leachates.

	pH			temperature			conductivity		
samples	L/S 0.1 L/kg	L/S 2 L/Kg	L/S 10 L/kg	L/S 0.1 L/kg	L/S 2 L/Kg	L/S 10 L/kg	L/S 0.1 L/kg	L/S 2 L/Kg	L/S 10 L/kg
H1	6.9	7.6	7.2	22.9	21.2	21.5	230	270	130
R1	7.8	8.4	7.7	25.1	24.1	22.6	84	74	170
R2	7.9	8	7.6	25.1	23.9	22.6	68	100	200
R3	7.7	7.9	7.5	25	17.3	22.6	77	57	140
G1	6.2	6.7	6.7	23.3	23.8	23.9	210	210	16
G2	6.5	7.0	7.0	23.4	23	23.9	150	220	170
G3	7.7	8.1	7.6	23.2	23.3	24	240	210	180
C1	7.1	7.8	7.5	22.4	21.7	19.4	120	89	170
C2	7.7	7.7	7.5	22.6	21.5	20.4	73	180	160

Table S2 Concentrations (µg/kg dm) of polycyclic aromatic hydrocarbons (PAHs) in soils.

	samples								
compounds	H1	R1	R2	R3	G1	G2	G3	C1	C2
Naphthalene	6.2	21	148	68	143	95	309	167	170
Biphenyl	1.4	2.8	44	24	26	15	92	58	110
Acenaphthylene	32	28	12	61	560	252	523	330	390
Acenaphthene	1.2	4.6	198	57	70	37	192	1260	990
Fluorene	4.3	3.5	246	100	152	74	513	1660	1580
Phenanthrene	85	146	7800	1280	3190	1360	4280	2760	3620
Anthracene	27	66.3	1780	296	750	411	949	3810	9800
4H-Cyclopenta[def]phenanthrene	21	30	1200	190	960	320	870	9700	1880
Fluoranthene	370	653	51640	9470	13010	3670	5530	51990	16130
Pyrene	330	578	40130	1840	16880	3690	5710	26000	12490
Benzo(a)fluorene	52	109	4640	404	1523	537	1024	4750	2530
Benzo[ghi]fluoranthene	17	28	535	79	460	212	255	530	510
Benzo[c]phenanthrene	24	49	1380	166	767	391	591	1220	855
Benzo(a)anthracene	208	573	25970	1308	7560	3790	3720	6450	6700
Chrysene	148	1690	4440	1077	5206	1791	2102	4090	5160
Triphenylene	41	142	2660	279	1374	657	800	1520	2130
Benzo(b)fluoranthene	213	785	13850	1195	7360	3979	3228	3470	4870
Benzo[k,j]fluoranthene	207	723	12250	1170	7370	4340	4080	3300	4740
Benzo[a]fluoranthene	56	168	2860	272	7360	1003	1252	705	5170
Benzo[e]pyrene	167	612	8420	861	6170	3030	2800	1970	1650
Benzo(a)pyrene	254	252	21100	1222	6970	3330	3390	2260	3020
Perylene	84	241	2930	272	2600	756	776	655	4430
Indeno(1,2,3-cd)pyrene	201	746	7520	884	7120	3690	2800	1100	970
Benzo(g,h,i)perylene	173	651	7300	738	6140	2750	2340	720	1670
Dibenzo(a,h)anthracene	20	105	1570	132	711	272	533	177	1146
Dibenzo[a,c]anthracene	13	67	984	86	530	370	446	119	158
Dibenzo[a,j]anthracene	15	51	844	85	672	288	331	122	175
Picene	46	452	2970	303	1500	621	821	328	184
Anthanthrene	67	229	2720	216	2740	484	423	159	303
Benzo[b]chrysene	29	227	2080	178	1310	584	689	227	247
Coronene	17	49	371	48	512	236	187	28	65
Dibenzo[b,k]fluoranthene	21	116	317	111	694	336	1120	957	81
Dibenzo[a,e]pyrene	<14	82	859	74	457	209	263	51	78
naphtho(2,3-a)pyrene	<130	50	173	59	401	150	139	<240	<230

Table S3 Concentrations (µg/kg dm) of alky-PAHs, oxy-PAHs and NSO-PACs in soils.

	samples								
compounds	H1	R1	R2	R3	G1	G2	G3	C1	C2
2-Methylnaphthalene	2.3	6.0	91	74	88	<0.23	314	97	246
1-Methylnaphthalene	<0.14	4.0	58	49	64	<0.23	219	78	144
1,6-Dimethylnaphthalene	2.8	2.4	56	63	149	24	331	82	128
2,3,5-Trimethylnaphthalene	<0.14	1.5	32	31	90	11	239	186	42
Dibenzothiophene	4.9	7.3	547	88.3	174	79.4	304	424	330
2-Methyldibenzothiophene	0.8	1.2	80.9	14.3	19	13.7	99.7	100	37
2-Methylphenanthrene	18	31	1260	239	936	243	1580	903	483
2-Methylanthracene	13	21	643	116	301	111	537	1800	777
2,8-Dimethyldibenzothiophene	<20	0.36	14	2.51	5.43	3.08	24	45	10
2,4-Dimethylphenanthrene	4.5	6.4	189	45	216	52.1	<20	393	178
2,4,7-Trimethyldibenzothiophene	0.3	0.4	11	2.7	48	3.0	19.0	3	20
2,3-Dimethylanthracene	3.5	5.14	113	26.7	194	29	149	165	131
1-Methylfluoranthene	22	67	1700	177	1220	379	792	1090	1280
1,2,8-Trimethylphenanthrene	2.4	2.8	67	19	260	23	138	88	63
1,2,6-Trimethylphenanthrene	2.2	3.3	42	17	207	17	78	51	43
7-Methylbenzo(a)anthracene	8.1	21	736	56	1600	684	419	149	1390
3-Methylchrysene	17	37	861	100	516	208	584	325	546
2-Methylchrysene	25	58	1180	136	658	243	849	431	515
1-Methylchrysene	10	47	579	67	438	110	372	128	265
6-Ethylchrysene	<0.14	<0.1	4.8	0.4	<0.08	0.1	<0.08	0.9	<0.08
7-Methylbenzo(a)pyrene	<0.14	0.1	0.1	0.1	0.1	<0.08	<0.08	<0.08	<0.08
Quinoline	0.76	0.3	9.4	4.4	5.7	3.3	<0.1	5.3	8.0
1-Indanone	0.29	1.2	4.3	3.4	50	13	46	23	39
Carbazole	9.65	70	2000	208	300	147	339	269	377
Benzo[h]quinoline	<3.7	2.6	146	25	46	17	59	67	70
Acridine	2.2	7.8	253	41	36	17	19	130	137
9-Fluorenone	<35	<36	249	108	165	101	380	98	234
9-methylacridine	<8.4	<8.4	<14.3	<4.3	33	<8.6	17	10	20
Acridone	<0.90	<0.90	<1.6	<0.70	<0.90	<0.92	<1.0	78	54
4H-Cyclopenta[def]phenanthrenone	16	40	441	98	452	260	422	2360	3260
Anthracene-9,10-dione	19	51	631	216	477	262	594	362	558
11H-Benzo[a]carbazole	252	1620	37930	2260	6370	3590	6930	452	1240
2-Methylanthracene-9,10-dione	5.4	13	142	53	318	75	355	106	103
Benzo[a]fluorenone	26	122	2134	276	873	672	2380	421	613
7H-Benzo[de]anthracen-7-dione	57	28	222	83	1263	412	919	121	166
6-HBenzo[cd]pyren-6-one	65	25	112	65	1483	483	668	45	95
Benzo[a]anthracene-7,12-dione	11	49	414	106	384	335	479	274	401
Naphthacene-5,12-dione	21	301	2810	282	637	450	465	1554	1850
9,10-dihydrobenzo[a]pyren-(8H)-none	<3.8	<2.4	6.4	2.9	3.9	<3.9	<4.2	<4.3	<4.1
Benzo[b]naphtho[2,1-d]furan	10	14	511	67	231	131	193	1286	393
dibenzo[ah]acridine	6.4	20	373	32	132	113	158	36	68

Table S4 Concentrations (μg/L) of groups of PACs in leachates at different L/S ratios.

samples	L/S ratio	16PAHs	19PAHs	alkyl-PAHs	oxy-PAHs	N-PACs	SO-PACs	PACs
H1	L/S 0.1 L/Kg	0.4	0.1	0.03	0.03	0.03	0.004	0.6
H1	L/S 2 L/Kg	0.1	0.1	0.01	0.02	0.02	0.001	0.3
H1	L/S 10 L/Kg	0.1	0.02	0.01	0.02	0.01	<0.001	0.1
R1	L/S 0.1 L/Kg	0.2	0.2	0.03	0.1	0.1	0.005	0.6
R1	L/S 2 L/Kg	0.2	0.05	0.02	0.03	0.05	0.001	0.3
R1	L/S 10 L/Kg	0.8	0.2	0.1	0.3	0.4	0.01	1.8
R2	L/S 0.1 L/Kg	0.1	0.1	0.02	0.03	0.01	<0.005	0.2
R2	L/S 2 L/Kg	0.2	0.1	0.02	0.04	0.1	0.004	0.4
R2	L/S 10 L/Kg	0.3	0.1	0.02	0.1	0.1	0.002	0.5
R3	L/S 0.1 L/Kg	0.1	0.1	0.03	0.05	0.1	<0.005	0.3
R3	L/S 2 L/Kg	0.1	0.03	0.01	0.01	0.02	<0.002	0.1
R3	L/S 10 L/Kg	0.1	0.03	0.01	0.01	0.02	0.001	0.2
G1	L/S 0.1 L/Kg	13	4.3	1.2	1.7	1.1	0.05	21
G1	L/S 2 L/Kg	5.9	2.0	0.4	0.8	0.7	0.2	10
G1	L/S 10 L/Kg	4.9	1.6	0.4	0.7	0.6	0.02	8.2
G2	L/S 0.1 L/Kg	2.6	1.2	0.7	0.5	0.3	0.02	50
G2	L/S 2 L/Kg	1.1	0.5	0.4	0.4	0.2	0.01	14
G2	L/S 10 L/Kg	0.6	0.2	0.2	0.2	0.1	0.01	11
G3	L/S 0.1 L/Kg	25	9.4	4.0	6.1	5.4	0.1	5.3
G3	L/S 2 L/Kg	6.2	2.2	1.0	2.6	2.0	0.04	2.6
G3	L/S 10 L/Kg	4.9	1.7	0.7	2.2	1.6	0.02	1.3
C1	L/S 0.1 L/Kg	2.2	0.6	0.1	3.0	0.1	0.04	6.1
C1	L/S 2 L/Kg	95	13	2.1	15	0.6	1.2	127
C1	L/S 10 L/Kg	47	7.9	2.2	10	0.5	0.7	68
C2	L/S 0.1 L/Kg	1.0	0.4	0.1	0.4	0.04	<0.001	1.9
C2	L/S 2 L/Kg	5.9	2.2	0.6	2.8	0.4	0.04	12
C2	L/S 10 L/Kg	6.8	2.2	0.6	3.1	0.5	0.1	13

Table S5 Leached percentages of initial amounts in soils*.

samples	LMW-PAHs[a]	MMW-PAHs[b]	HMW-PAHs[c]	16PAHs[d]	alkyl-PAHs	oxy-PAHs	N-PACs[e]	SO-PACs[f]	PACs[g]
H1	0.15	0.04	0.03	0.04	0.05	0.08	0.04	0.04	0.04
R1	0.51	0.09	0.07	0.10	0.15	0.39	0.18	0.22	0.12
R2	0.005	0.001	0.001	0.001	0.003	0.01	0.001	0.002	0.002
R3	0.02	0.003	0.01	0.005	0.01	0.01	0.01	0.01	0.01
G1	0.05	0.05	0.07	0.06	0.06	0.12	0.09	0.12	0.07
G2	0.33	0.18	0.14	0.16	0.38	0.74	0.45	0.12	0.23
G3	0.01	0.02	0.02	0.02	0.03	0.03	0.01	0.01	0.02
C1	0.17	0.56	0.18	0.53	0.36	2.05	0.53	0.41	0.54
C2	0.06	0.08	0.13	0.10	0.10	0.41	0.23	0.07	0.12

*Ratio of leached accumulated amount/kg soil at L/S 10 L/kg and initial concentrations in soils.

[a]7 parent PAHs with molecular weight 128-178, [b]9 parent PAHs with molecular weight 190-228. [c] 9 parent PAHs with molecular weight 252-302. [d]16 priority PAHs. [e]8 N-PACs (azaarenes) including 9-methylacridine and acridone. [f]3 alkylated dibenzothiophenes, dibenzothiophene and benzo[b]naphtho[2,1-d]furan..[g]∑77 PACs.