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Effect-directed analysis and mixture effects of AhR-active PAHs in crude oil and coastal sediments contaminated by the Hebei Spirit oil spill



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ABSTRACT

The major AhR-active PAHs were identified in crude oil and oil-contaminated sediments by use of effect-directed analysis. As part of the study, an enhanced potency balance analysis was conducted by establishing the novel relative potency values of (alkyl)-PAHs from the H4IIE-*luc* bioassay. Silica gel column fractionation of crude oil resulted in greater AhR-mediated potencies in fractions of aromatics (F2) and resins (F3), and such trend was also observed for field collected sediment samples. AhR-mediated potencies of six F2 sub-fractions from HPLC indicated that the majority of F2 responses were attributable to 3–4 ring aromatics. Target PAHs including C4-phenanthrene, C1-chrysene, and C3-chrysene in sediments explained ~18% of the bioassay-derived TCDD-EQs, however, the unknown AhR agonists and potential mixture effects remain in question. Overall, the AhR-potency and antagonistic potential of residual oil in sediment tended to decrease over time, thus monitoring of weathering process would be key for the post management of oil-contaminated sites.

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1. Introduction

Over the last century, spills of petroleum hydrocarbons into the marine environment have become a global problem, representing a major threat to marine ecosystems and associated organisms because of the oil-derived toxic chemicals being inevitably produced (Di Toro et al., 2007; Incardona et al., 2005; Peterson et al., 2003). Many studies have been conducted on the toxic effects and various modes of actions associated with crude oil, for

example, analyses of aryl hydrocarbon receptor (AhR)- or estrogen receptor (ER)-mediated activities and genotoxicity (Di Toro et al., 2007; Hong et al., 2012a; Ji et al., 2011; Lee et al., 2011). AhR-mediated dioxin-like effects can often be the critical mechanism of toxicity caused by exposure to polycyclic aromatic hydrocarbons (PAHs) (Hong et al., 2013; Lee et al., 2013b). Many studies relating to petroleum chemistry have reported the chemical properties and compositions of crude oil (Page et al., 2002; Yim et al., 2011). However, only few studies have addressed linkages between hydrocarbon chemistry and environmental toxicology in terms of a potency balance analysis (Rowland et al., 2001). In particular, field studies are lacking in addressing the potential toxic effects of residual oils in environmental media including sediments. Further, toxicological characteristics of sediments contaminated with crude oil, which experience natural weathering followed by

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compositional changes of major components (PAHs and alkyl-PAHs), remain largely unknown (Hong et al., 2012a; Incardona et al., 2005; Jonker et al., 2006).

On December 7, 2007, the worst oil spill occurred about 10 km off the Taean coast, in Korea, when the tanker *Hebei Spirit* foundered and released approximately 10,900 tons of crude oil (Hong et al., 2012a, 2014; Yim et al., 2012). Several previous studies indicated that the water quality, bioaccumulation, and benthic community of the Taean coast appeared to be fairly (or almost fully) recovered in five years after the *Hebei Spirit* oil spill (HSOS) (Hong et al., 2012a; Ji et al., 2011; Jung et al., 2011; Kim et al., 2010; Lee et al., 2011, 2013a; Yu et al., 2013). However, coastal sediments in the vicinity of an oil spill site are subject to a relatively long history of contamination, particularly in semi-closed sheltered areas. For instance, residual oils are still found in the inner regions of small bays and muddy bottom layers of several sites along the Taean area, which might cause adverse effects on marine organisms (Hong et al., 2012a, 2014; Yu et al., 2013).

It was previously found that AhR-mediated potencies of sediment samples about two years after the HSOS generally reflected the degree of oil contamination, viz., concentrations of PAHs and alkyl-PAHs homologues (Hong et al., 2012a). The well-known AhR-active PAHs (several parent PAHs including chrysene (Chr)) only explained a small proportion (<1.5%, on average) of total AhR-mediated activity in a potency balance analysis (Hong et al., 2012a; Villeneuve et al., 2002). However, the previous work seemed to be incomplete in characterizing alkyl-PAHs which might be more potent AhR-agonists after the weathering in situ (Hong et al., 2012a). For an accurate potency balance analysis between bioassay and instrumental analysis data, assay-specific relative potency values (RePs) of more individual PAHs should be employed. For example, alkyl-PAHs are the major components of residual oils, but their collective contribution to the total AhR-mediated potencies have not been well addressed due to limited reports of the ReP values (Machala et al., 2008; Marvanova et al., 2008; Trilecova et al., 2011; Vondracek et al., 2007). Also, considering the timely weathering in sedimentary compositions of the residual oils, the analysis of a time series samples should be of great benefit.

Effect-directed analysis (EDA) has become a powerful tool for identification of major toxicant(s) that occur in complex mixtures in the environment encompassing composites of sediments, soil, and effluents (Brack et al., 2002; Regueiro et al., 2013; Vrabie et al., 2012). The separated fractions contain successively fewer groups or individual organic chemicals of similar functionality or polarity, allowing an easier identification of toxic causative chemicals when combined with bioassays. There are used to determine the fractional potency of the mixture and are read out in potency equivalents. Recently, by use of such an EDA approach, Vrabie et al. (2012) suggested that the predominant AhR agonists in crude and refined oils were aromatics and resins with log K_{ow} of 5–8, such as alkyl-benzenes, alkyl-PAHs, and/or aromatic chemicals containing nitrogen, sulfur, or oxygen atoms (including heterocyclic compounds). However, more than a thousand such chemicals would be expected in the complex mixtures of oil contaminated sediment. Thus, it is extremely challenging to determine certain AhR agonist(s) in oil contaminated sediments and how the relative proportions might change during weathering.

In the present study, the EDA approach combining the H4IIE-*luc* bioassay with GC/MSD analysis was performed to isolate and identify the causative chemicals in sediments affected by the HSOS. The specific aims were to: i) identify the major AhR agonist(s) and/or antagonist(s) in crude oil (Iranian Heavy Crude (IHC)), and oil contaminated sediments, ii) establish AhR-mediated RePs of individual (alkyl)-PAHs ($n = 30$) for enhancing potency balance analysis, iii) evaluate contributions of (alkyl)-PAHs (expressed as

TEQ_{PAHs}) to the total induced AhR-mediated potencies (expressed as TCDD-EQ) of sediments, and finally iv) determine the relationship between oil weathering and AhR-mediated potency by use of a time-series analysis of oil contaminated sediments. To the best of our knowledge, this is the first study that used EDA to identify major AhR agonists of sedimentary residual oils affected by an oil spill and that reports the H4IIE-*luc* ReP values for (alkyl)-PAHs.

2. Materials and methods

2.1. Sampling sites and sample collection

Sediments were collected from heavily oil contaminated areas of the Taean coast over a 5 year period; specifically within ~1 month (December 2007 and January 2008), after ~3 years (December 2010), and after ~4 years (January 2012) of the HSOS (Table S1 of Supplemental Materials (S)). A total of 11 surface sediments were collected from the Sinduri dune, Sinduri mudflat, and Sogeu-nri mudflat.

2.2. Silica gel fractionation

A 10 g of wet sediment was mixed with anhydrous sodium sulfate (Sigma Aldrich, Saint Louis, MI), and was extracted with 300 mL dichloromethane (DCM, Burdick and Jackson, Muskegon, MI) in a Soxhlet extractor for 24 h. The isotopically-labeled internal standards were not added in the extraction and fractionation procedures, because the chemicals would contribute to the AhR-mediated potency. Elemental sulfur was removed by use of activated copper powder (Merck, Darmstadt, Germany) and the extracts were concentrated to 5 mL under a gentle stream of nitrogen (raw extract (RE), 2 g sediment mL⁻¹). One milliliter of the RE or 0.1 g of crude oil (IHC) was passed through 10 g of activated silica gel (70–230 mesh, Merck) in a packed glass column for fractionation (Khim et al., 1999a, 1999b). The first fraction (F1) was eluted with 40 mL of hexane (Burdick and Jackson) containing saturate hydrocarbons. The aromatic fraction (F2) was collected by the elution of 50 mL of 20% DCM in hexane (v/v). The third fraction (F3) contained resins and polar compounds was eluted in 50 mL of 60% DCM in acetone (Burdick and Jackson). All eluents were concentrated to 1 mL for use in the bioassay, instrumental analysis, and further fractionation using a rotary evaporator and nitrogen concentrator. Column residues, such as asphaltene, were excluded as AhR agonists in the present study.

2.3. HPLC fractionation

The F2 fraction contained PAHs and alkyl-PAHs being further fractionated into six sub-fractions (F2.1–F2.6) based on the number of aromatic rings by use of a normal-phase HPLC column (Nucleosil 100-5 NO₂, 250 mm length, 5 μm particle size, 4.6 mm i.d., Macherey–Nagel, Düren, Germany) (Brack and Schirmer, 2003; Brack et al., 2002). Separation and fraction collection were performed in an Agilent 1260 HPLC System (Agilent technologies, Avondale, PA) with UV detection at 254 nm. The hexane:DCM mobile phase (95:5, v/v) was delivered isocratically at a flow rate of 0.7 mL min⁻¹ (details provided in Table S2). Separation conditions were optimized through several tests with GC/MSD confirmation using 16 EPA priority PAHs (Table S3). Standards of the PAHs and crude oil were successfully fractionated and identified according to the ring number of aromatics (Fig. 1a and Fig. S1). Unfortunately, HPLC fractionation was only conducted on crude oil, because insufficient amounts of sediment extracts were available. Detailed information for the F2 sub-fractions is present in Table S4.

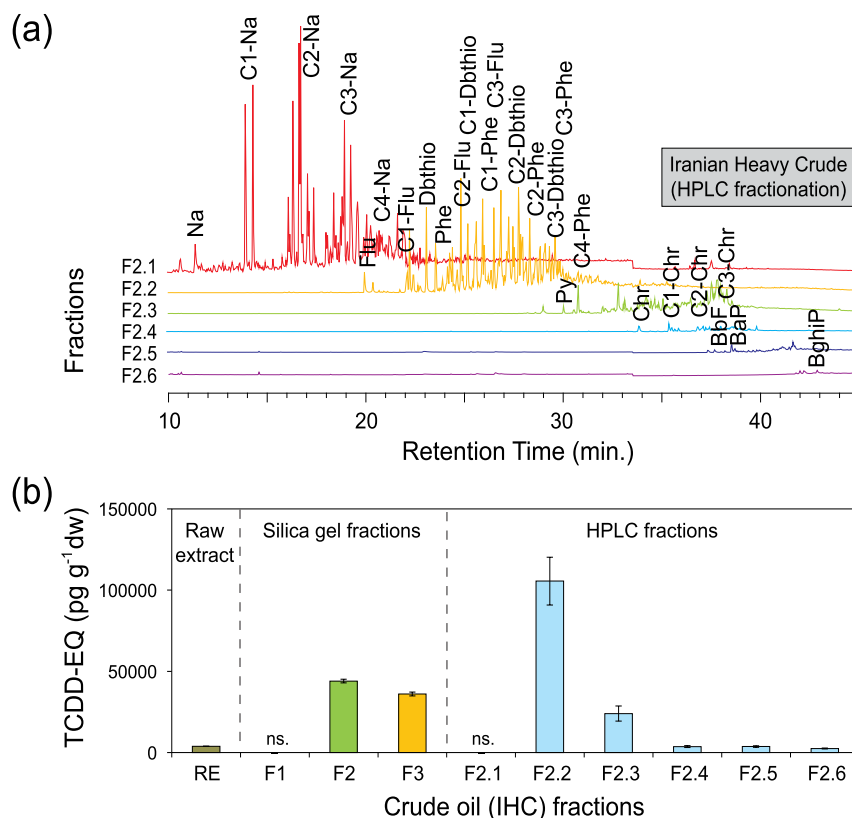


Fig. 1. (a) GC-MSD chromatograms for the sub-fractions of the F2 (aromatics) of crude oil (Iranian Heavy Crude). (b) AhR-mediated potencies (TCDD-EQs) in the fractions of crude oil (RE: raw extracts; error bar: mean \pm SD ($n = 3$)).

2.4. H4IIE-*luc* bioassay

The H4IIE-*luc* bioassay was performed according to previously reported methods (Hong et al., 2012a, 2012b). Viability of cells and cytotoxicity of all samples were determined using the MTT assay (Yoo et al., 2006) and the dilution factors of the samples were determined. Trypsinized cells (density of $\sim 8.0 \times 10^4$ cells mL⁻¹) were seeded into the 60 interior wells of 96 micro well plates at a volume of 250 μ L per well. After 24 h, test and control wells were each dosed with 0.25 μ L (0.1% dose) of the appropriate standards (2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD)), sample extracts (all fractions), and solvent controls (0.1% of DMSO) (Hutchinson et al., 2006). This concentration of DMSO had no significant effect on the activity of the H4IIE-*luc* bioassay. Luciferase assays were conducted after 72 h of exposure using an ML3000 microplate reading luminometer (Tecan, Infinite 200, Mannedorf, Switzerland). Responses of the H4IIE-*luc* bioassay (expressed as average relative luminescence units) were converted to percentages of the maximum response (%TCDD_{max}) observed for a 300 pM (=100 % TCDD_{max}) of 2,3,7,8-TCDD. Finally, the AhR-mediated potency was expressed as a TCDD standard equivalent concentration (pg TCDD-EQ g⁻¹ dry weight sediment), which was determined directly from sample dose–response relationships generated by testing samples at multiple dilutions (1.2, 3.7, 11, 33, 100, and 300 pM TCDD) (Hong et al., 2012a; Villeneuve et al., 2000).

2.5. Identification and quantification of PAHs and alkyl-PAHs

Concentrations of PAHs and alkyl-PAHs were identified and quantified by use of a previously described method (Hong et al., 2012a). A total of 45 PAHs and alkyl-PAHs were measured using

an Agilent 7890 gas chromatograph (GC) coupled to a model 5975C mass-selective detector (MSD, Agilent Technologies). Since various alkyl-PAHs isomers with the same molar mass have very similar fragmentation patterns in oil residues, total concentrations of groups of alkyl-PAHs were quantified rather than identifying several hundreds of alkyl PAH isomers. The target chemicals were mostly detected in the F2 fraction of the crude oil and sediment samples, with no significant detections in the F1 and F3 fractions. Detailed instrumental conditions are provided in Table S3. As QA/QC purpose, the matrix spike test was performed for five sediment samples in a separate experiment using 16 EPA PAHs, and recoveries were generally acceptable with average of 85%. The method detection limits (MDL) for individual PAHs ranged from 0.1 to 0.5 ng g⁻¹ dw. The degree of weathering of residual oil in sediments was estimated by use of alkyl-PAHs double ratios (Hong et al., 2012a; Sauer et al., 1998).

2.6. Relative potency values of PAHs and alkyl-PAHs

The RePs for AhR-mediated potencies were developed by use of the optimized H4IIE-*luc* bioassay based on the EC-50 of each PAH and alkyl-PAH isomer compared to that of 2,3,7,8-TCDD (Hori et al., 2009; Lee et al., 2013b; Villeneuve et al., 2002). A total of 30 individual parent and alkyl-PAHs were chosen as the model compounds to evaluate novel RePs (not described previously) for AhR-mediated potency (Table S5). The chemicals were prepared at six concentrations using 3-fold serial dilution (e.g., 1000, 333, 111, 37, 12, and 4 ng mL⁻¹), and were tested as described above. The ReP values were estimated by fitting the curves of the dose–response relationship (EC-50) according to the method reported previously (Villeneuve et al., 2000).

2.7. Potency balance analysis

Concentrations of TCDD-EQs determined by use of the H4IIE-*luc* bioassay were compared to instrument-derived TEQs. The TEQ values were calculated as the sum of TEQs by multiplying the concentration of individual PAHs and alkyl-PAHs using assay-specific RePs obtained from this study (Table S5) and reported previously (Hong et al., 2013; Villeneuve et al., 2002) using Equation (1).

$$TEQ_{PAHs} = \sum_{i=1}^n (C_i \times ReP_i) \quad (1)$$

where: C_i is the concentrations of individual PAH isomers and ReP_i is the assay-specific ReP value of individual PAHs.

3. Results and discussion

3.1. AhR-mediated potency of crude oil and oil contaminated sediments

Among silica gel fractions of crude oil, greater AhR-mediated potencies were observed in the fractions of F2 (aromatics) and F3 (resins and polar compounds), whereas F1 (saturates) showed no significant response (Fig. 1b). Most AhR-mediated responses appeared to be caused by the aromatic compounds associated with crude oil. To examine the unknown AhR agonist(s) of the F2, an HPLC column was used to separate F2 of crude oil (IHC) into six sub-fractions (F2.1 to F2.6) based on the number of aromatic rings. Among the six sub-fractions of F2, significantly greater AhR-mediated potencies were found in the F2.2 and F2.3 containing 3 to 4 rings of aromatics and/or aromatics of similar molar mass (166–234) in crude oil (Table S4). The further identification of specific AhR agonist(s), targeting sub-fractions of F2.2 and F2.3 in crude oil and/or sedimentary residual oils, would be necessary to narrow down possible causative chemicals to individual

compound(s) (Vrabie et al., 2012).

All sediments collected from the Taean coast from 2007 to 2012 exhibited significant AhR-mediated potencies (Table 1 and Fig. 2). Of the three major fractions (F1–F3), relatively high concentrations of TCDD-EQs were found in F2 compared to F1 and F3, in general. Total concentrations of PAHs showed a similar temporal trend to AhR-mediated potencies in the F2 of the sediment extracts (Fig. 2 and Table S6). The result was consistent with the previous finding that samples from the oil contaminated areas of Taean coast elicited relatively great AhR-mediated potencies, with a strong correlation ($r^2 = 0.85$, $p < 0.001$) between concentration and potency (Hong et al., 2012a). However, known AhR-active parent PAHs might explain a relatively small proportion of the overall AhR-mediated potency (Table 1 and Table S7), i.e., unknown AhR agonists and possible mixture effects between them remain in question (Hong et al., 2012a).

Concentrations of TCDD-EQs in F2 and F3 of the IHC were found to be remarkably greater (~9 times) compared to that of the RE, which indicated that AhR antagonists are present in crude oil (Fig. 1b) (Vrabie et al., 2009, 2012). In addition, the AhR-mediated potency associated with the F2.2 was about 2-fold greater than its parent fraction (F2). Such antagonistic effects after fractionation were also found in extracts of sediments. For example, TCDD-EQ in the F2 of the Sinduri dune sediment (in 2007) increased ~11 times compared to that of the RE (Fig. 2). AhR-mediated activities of various refined oils and crude oils mixed with well-known AhR-active PAHs (benzo[a]pyrene) were significantly less than expected (1.2–4.3 times), which suggested antagonism (Vrabie et al., 2009). In addition, Vrabie et al. (2012) found that AhR activities in fractions of crude oil much greater than those of the initial activity of the unfractionated sample after HPLC fractionation. They suggested that this phenomenon can only be explained by antagonistic effects in the whole oil mixture.

To further verify such mixture effects cross the parent and sub-fractions of the IHC or sedimentary residual oils, the AhR potencies between given fractions and their combined ones were further analyzed (Fig. 3). F2 and F3 both appeared to contain the AhR

Table 1
Bioassay-derived TCDD-EQs and instrument-derived TEQs of crude oil and sediments collected from the Taean coast after the Hebei Spirit oil spill.

Samples	Sampling year	H4IIE- <i>luc</i> responses (TCDD-EQ, pg g ⁻¹ dw)				Chemical analysis (TEQ, pg g ⁻¹ dw)			% Identified (%) ^d	% Major AhR agonists (%) ^e	Weathering stage ^f
		Raw extracts (RE)	Saturates (F1)	Aromatics (F2)	Resins (F3)	Parent-PAHs ^a	Alkyl-PAHs ^b	Total-PAHs ^c			
<i>Crude oil</i>											
Iranian heavy crude		3.8×10^3	<DL ^g	4.4×10^4	3.6×10^4	3.3×10^2	3.4×10^3	3.7×10^3	8.4	91	Not weathered
<i>Taeon sediments</i>											
Sinduri dune	Dec. 2007	95	2.2	1.0×10^3	7.2×10^2	4.4	28	32	3.1	86	I
	Dec. 2010	54	5.5	74	39	0.92	11	12	16	92	II
	Jan. 2012-1	50	7.1	2.8×10^2	18	3.4	50	53	19	94	II
Sinduri mudflat	Jan. 2012-2	30	< DL	1.1×10^2	5.2	3.1	47	50	44	94	II
	Jan. 2008	89	3.7	35	5.8	1.0	7.3	8.3	23	87	III
	Dec. 2010	98	55	1.2×10^3	2.3×10^2	7.5	1.0×10^2	1.1×10^2	9.2	93	I
Sogeenri mudflat	Jan. 2012	91	3.6	2.4×10^2	1.1×10^2	6.5	71	77	32	91	II
	Dec. 2010	27	< DL	1.1×10^2	23	1.5	19	21	19	92	I
	Jan. 2012-1	61	2.7	1.1×10^3	37	14	1.5×10^2	1.6×10^2	15	91	II
	Jan. 2012-2	52	2.9	3.1×10^2	13	2.9	22	25	8.1	88	II
Jan. 2012-3		1.2×10^2	2.8	1.2×10^2	39	2.3	4.5	6.8	5.6	65	III

^a TEQ values of parent-PAHs were summed by use of the chemical concentrations of Dbthio, BaA, Chr, BbF, BkF, BaP, IcdP, and DbahA multiplied by the ReP values obtained in this study and reported previously (Villeneuve et al., 2002).

^b TEQ values of alkyl-PAHs were summed by use of the chemical concentrations of C4-Phe, C1-Chr, C2-Chr, and C3-Chr multiplied by the ReP values obtained in this study (Table S5).

^c Total TEQ values were summed as $TEQ_{parent-PAHs}$ and $TEQ_{alkyl-PAHs}$.

^d % Identified: The percentage of total-TEQs to the bioassay-derived TCDD-EQs of F2 fraction.

^e % Major AhR agonist: The percentage of TEQs of three major AhR agonists such as C4-Phe, C1-Chr, and C3-Chr to the total TEQs of PAHs and alkyl-PAHs.

^f The weathering stages of residual oil in sediments were estimated by from C2-Dbthio/C3-Dbthio and C2-Phe/C3-Phe double ratios. I: Slightly weathered; II: Moderately weathered; and III: Severely weathered (see Fig. 7a).

^g <DL: below detection limit (2.0 pg g⁻¹ dw).

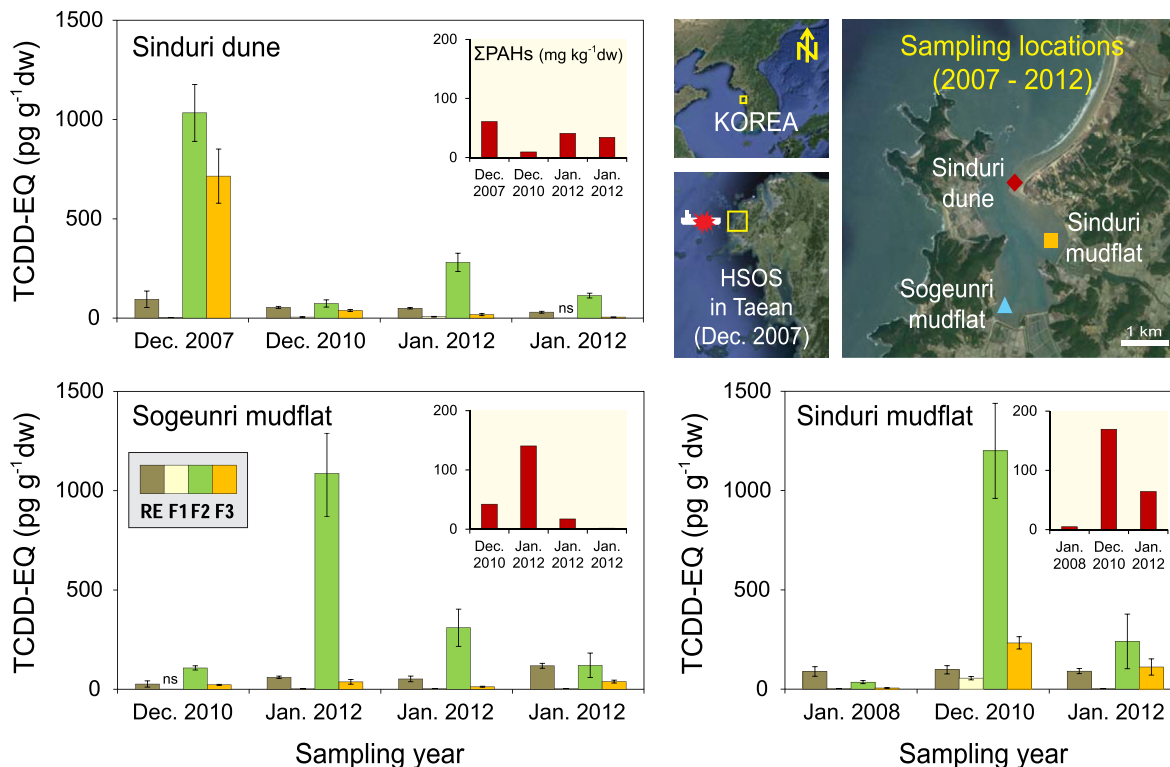


Fig. 2. AhR-mediated potencies (TCDD-EQs) in fractions of organic extracts and total PAHs concentrations in the sediments collected from the Taean Coast (Sinduri dune, Sinduri mudflat, and Sogeuuri mudflat) from 2007 to 2012 after the *Hebei Spirit* oil spill (RE: raw extract; ns: not significant; error bar: mean \pm SD (n = 3)).

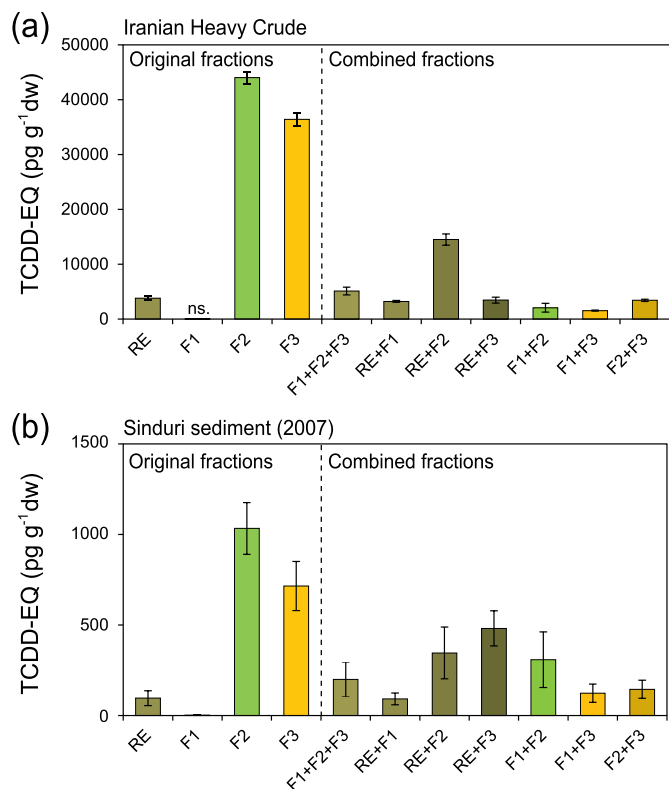


Fig. 3. AhR-mediated potencies of raw extract (RE), fractions (F1, F2, and F3) and re-combined fractions to predict the AhR agonists and antagonists in (a) crude oil and (b) oil contaminated sediment (ns: not significant; error bar: mean \pm SD (n = 3)).

antagonist(s) for the respective fraction, because the AhR-mediated potencies drastically decreased after combined F2+F3 samples, compared to those in either of F2 or F3. F1 did not appear to contain the AhR antagonists, because the AhR-mediated potencies were comparable between RE and RE+F1 and between F2+F3 and F1+F2+F3, respectively (Fig. 3). Another possible explanation for this phenomenon is that competition between the chemicals of oil component cross the F2 and F3 fractions for AhR binding sites might exist (Vrabie et al., 2009, 2012). In addition, such antagonistic effects of AhR-mediated potency of sedimentary residual oils decreased over time, which indicated that the compositional changes during the weathering process might be the key for such mixture effects in the natural environment.

The AhR-mediated potency of F3 in the IHC was comparable to that of F2 (Fig. 1b). However, in general, the AhR-mediated potencies of F3 in extracts of sediments showed a decreasing trend from 2007 to 2012 (Table 1 and Fig. 2). For example, great AhR-mediated potency was found in the F3 of the Sinduri dune sediment from December 2007, whereas TCDD-EQs in the F3 of the sediment extracts in December 2010 and January 2012 were relatively small. Thus, the AhR agonists in F3 appeared to be eliminated and/or transformed to other less toxic chemicals during the natural oil weathering process, whereas AhR agonists in F2 were relatively persistent. Results of previous studies have suggested that possible AhR-active compounds in the polar fraction (F3) were keto-, hydroxyl-, amino-, and nitro-compounds in sediments contaminated by industrial activities (Lübcke-von Varel et al., 2011). Additional follow up studies focusing on major toxicant(s) in polar fraction of crude oil and oil contaminated sediments are necessary.

Meanwhile, some of the F2 fractions collected in 2012 (in Sogeuuri) showed great TCDD-EQ concentrations compared to those from 2007 (in Sinduri dune) and 2010 (in Sinduri mudflat). This result appeared to be caused by the inhomogeneous patch

distribution of residual oils in the corresponding sediments, emphasizing a site-specific distribution and/or variations in the degree of oil weathering. Altogether, the results of the present study improved the understanding of fractional responses and their combined mixture effects of unknown AhR agonists and antagonists in crude oil and oil contaminated sediments, if any.

3.2. Composition of (alkyl)-PAHs and oil weathering

Concentrations of 45 PAHs and alkyl-PAHs were measured in crude oil (IHC) and oil contaminated sediments, supporting hydrocarbon chemistry data and also compound-specific potency balance analysis (Table S6). Concentrations of crude-derived PAHs in the sediments collected from a heavily oil contaminated area (Hong et al., 2012a, 2014) exhibited site-specific distributions, and their compositional patterns reflected the temporal progress of weathering, with increase of alkylation of parent PAHs (Fig. 4). Effects of such weathering were also investigated by comparison of the compositional profiles in crude oil and time series of oil contaminated sediments to find out the degree of alkylation of several PAHs. Among the 3 sets of time-series sediment samples, the sample collected at initial stage of the HSOS (collected within 1 month) showed a similar composition of alkyl homologues, supporting lesser weathering at the beginning stage of oil exposure in the environment. However, the degree of alkylation much increased after 3 and 4 years of the HSOS, with great proportion of C3 and C4 to the total alkyl-PAHs (Hong et al., 2012a; Yim et al.,

2011).

Based on analysis of alkyl-PAHs double ratios, samples from the Sinduri dune in 2007, Sinduri mudflat in 2010, and Sogeuinri mudflat in 2010 were categorized into slightly weathered (I), while samples from the Sinduri mudflat in 2008 and Sogeuinri in 2012 were found to be severely weathered (III), and the others were grouped into moderately weathered (II). These results further indicated that the weathering degree of PAHs in Taean sediments was a good agreement with their exposure duration in general, but not always. In addition, weathering of residual oils in sediments could be affected by other environmental factors including geographical and physical conditions of the sites and variations of microbial activity in given site and time (Barakat et al., 2001; Hong et al., 2012a; Page et al., 2002).

Meanwhile, several previous reports suggested that the sediment quality and epi-benthic faunal community in the Taean area began to apparently recover after four years of the HSOS (Hong et al., 2014; Lee et al., 2013a; Yu et al., 2013). However, another set of data in turn indicated that sedimentary residual oils remain in certain inner regions, particularly the locations with low tidal energy conditions accompanying a lack of flushing into bottom layers of sediment and low biological activity such as bottom-dwelling organisms (Hong et al., 2012a, 2014). Overall, the PAHs and alkyl-PAHs concentrations in coastal sediments and their homologue distributions varied among studies likely due to physiographic, ecological, and methodological differences.

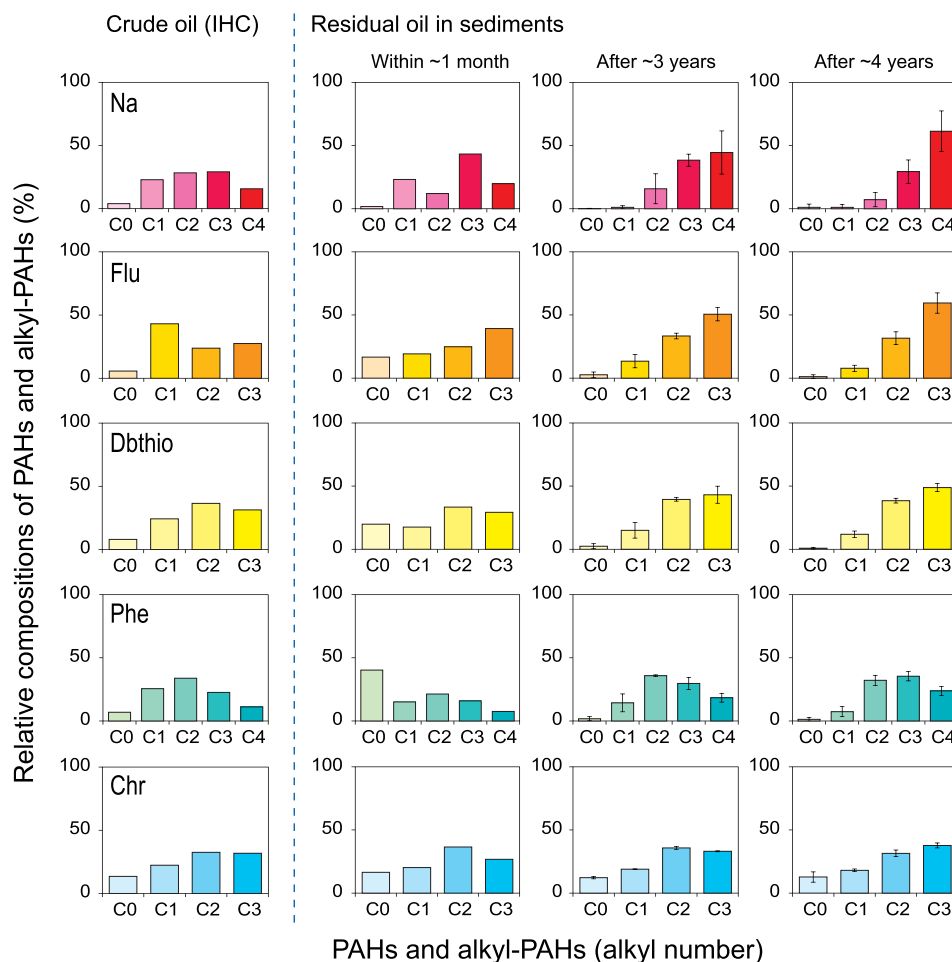


Fig. 4. Relative compositions of PAHs and alkyl-PAHs in crude oil (Iranian Heavy Crude) and sediments contaminated with oil collected from Taean coasts within ~1 year ($n = 2$), after ~3 years ($n = 3$), and after ~4 years ($n = 6$) of the Hebei Spirit oil spill (error bar: mean \pm SD).

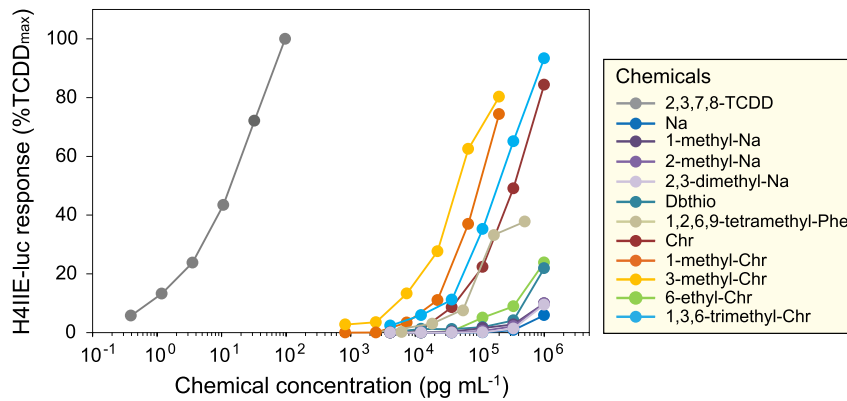


Fig. 5. Dose–response curves of individual PAHs and alkyl-PAHs determined by H4IIE-*luc* bioassay.

3.3. Potency balance analysis

One effective usage of the H4IIE-*luc* bioassays is to determine the potential risks posed by both known and unknown chemicals in environmental mixtures. For accurate potency balance analysis, bioassay-specific RePs for target compounds are needed, including those for parent and alkyl-PAHs. The ReP values of parent and mono-methyl PAHs for AhR-mediated activity were suggested in the previous studies (Machala et al., 2008; Marvanova et al., 2008; Trilecova et al., 2011; Vondracek et al., 2007). However, the ReP values of dominant alkyl-PAHs in crude oil (C1–C4 PAHs) were less reported. Thus, we have newly developed H4IIE-*luc* assay-specific RePs of alkyl-PAHs for those yet unknown.

Out of 30 chemical tested, 11 chemicals exhibited significant AhR-mediated responses at the test concentration levels and of these, 7 chemicals elicited strong enough responses to enable calculation of corresponding RePs (Fig. 5 and Table S5). The existing ReP for Chr was updated and RePs were calculated for the first time for six alkyl-PAHs, including dibenzothiophene (Dbthio), 1,2,6,9-phenanthrene (C4-Phe), 1-Chr (C1-Chr), 3-Chr (C1-Chr), 6-ethyl-Chr (C2-Chr), and 1,3,6-Chr (C3-Chr). The potencies of these 7 AhR-active PAHs (see Table S5) relative to the potency of 2,3,7,8-TCDD were found to be comparable to those for other known AhR-active PAHs including benzo[k]fluoranthene (1.4×10^{-4}) and benzo[a]pyrene (1.6×10^{-4}) (Hong et al., 2013; Villeneuve et al., 2002).

The potency balance analysis between bioassay-derived TCDD-EQs and instrument-derived TEQs in Taaen sediments was conducted to evaluate their individual contribution to the total AhR-mediated potencies (Table 1). Instrument-derived TEQs were calculated based on the reported RePs determined previously (Hong et al., 2012b; Villeneuve et al., 2002) and newly obtained RePs for 7 target PAHs detected in the oil contaminated sediments. The identified contribution of AhR-active PAHs in sediments, viz. proportion of total TEQ_{PAHs} to TCDD-EQs in F2, varied greatly but was fairly significant with an average of ca. 20% ($n = 11$), with a maximum contribution of 44% to the total potency of sediments collected from the Sinduri dune in January 2012 (Table 1 and Fig. 6). Alkyl-PAHs, such as C1-Chr, C3-Chr, and C4-Phe were the most potent AhR agonists, accounting for the majority of F2 TCDD-EQs. The parent PAHs explained only a small portion of the F2 TCDD-EQs, ranging from 0.42 to 2.9% (average = 1.6%).

The proportion of AhR potency identified that was accounted for by these 3 alkyl-PAHs were approximately the same for crude oil (~91%) and samples of weathering stage I (~91%) and II (~92%), but fairly weakened for samples of weathering stage III (~76%). This result indicated that other AhR-active PAHs contributed more to the overall AhR-mediated potency as weathering progressed (Table 1). In anyhow, the relatively lesser contribution of parent PAHs to F2 TCDD-EQs found in the present study was consistent with the previous studies (mostly < 10%) (Hong et al., 2012a). Overall, the power of explanation for TCDD-EQs by measured AhR-active

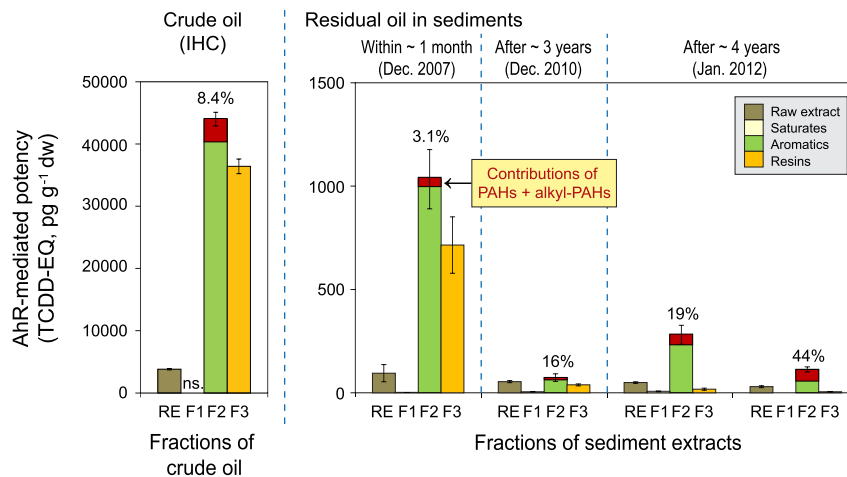


Fig. 6. AhR-mediated potencies (TCDD-EQs) in the fractions of organic extracts and contributions of PAHs and alkyl-PAHs in the crude oil (IHC) and sediments collected from the Taaen coast (Sinduri dune, 2007–2012) after the *Hebei Spirit* oil spill (RE: raw extract; ns: not significant; error bar: mean \pm SD ($n = 3$)).

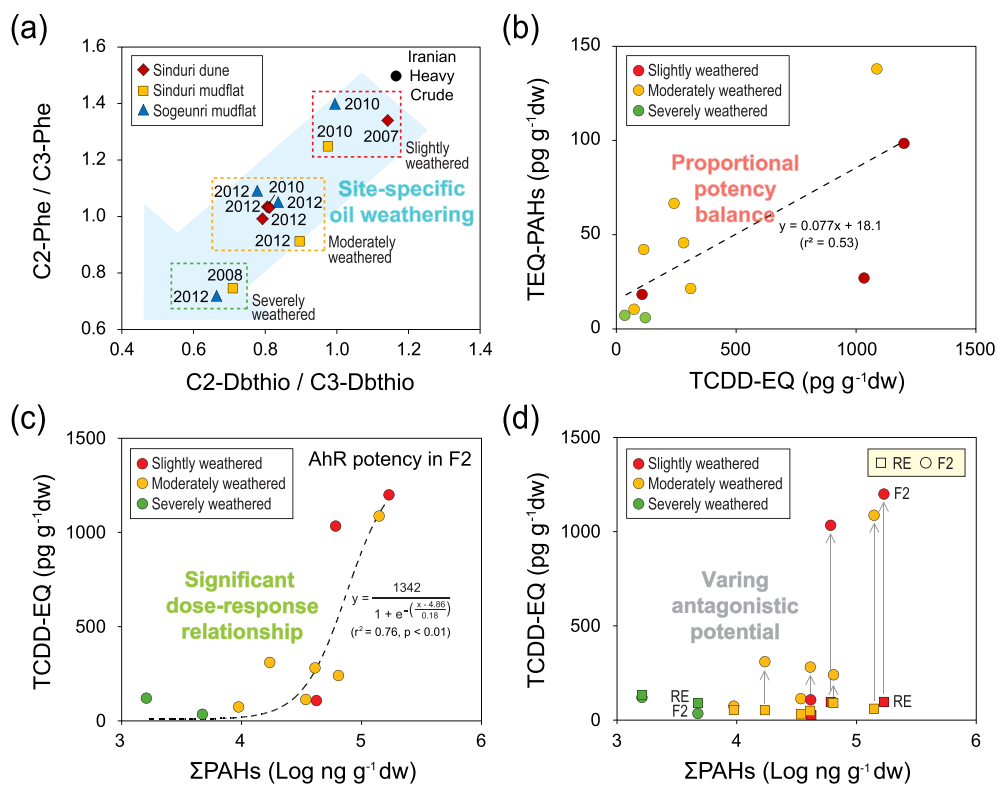


Fig. 7. Degree of relationship between weathering and AhR-mediated activities of the residual oil in the Hebei Spirit oil spill contaminated sediments. (a) Degree of weathering estimated by alkyl-PAHs double ratios, (b) Relationship between bioassay-derived TCDD-EQs and instrument-derived TEQs in the F2 fraction, (c) Dose–response relationships between TCDD-EQs and total PAHs, and (d) Antagonistic potentials for AhR-mediated potencies.

compounds in environmental mixtures, oil contaminated sediment at this time, was significantly improved by the analysis of several alkyl-PAHs in terms of potency balance analysis.

3.4. Significance of weathering characteristics

To further characterize the AhR-mediated potencies of sedimentary residual oils associated with the degree of weathering, alkyl-PAHs double ratios were calculated for all the samples (Fig. 7). The scatter plot of double ratios also supported the site-specific characteristics of sedimentary residual oils regardless of time-dependent sampling effect (Fig. 7a). By examining the relationship between TCDD-EQs and TEQs by scatter plot of sample data set (Fig. 7b), a proportion increase of TEQs to the TCDD-EQs were found with a ratio of approximately 0.077. For severely or slightly weathered samples, the slopes of the TEQ to TCDD-EQ plots were relatively low or comparable to the average (viz., slope = 0.077), while the slopes for the moderately weathered samples were higher. This phenomenon indicated that the more AhR-active compounds could be produced in the middle of the weathering process or certain mixture effects possibly accelerated synergistic effects in given samples.

Next, the simple summation of chemical concentrations of PAHs and alkyl-PAHs was significantly correlated with bioassay F2 TCDD-EQs ($r^2 = 0.76$, $p < 0.01$) (Fig. 7c). The result generally supported that the majority of AhR-active compounds in sedimentary residual oils are PAHs and their derived compounds. Further a comparison of F2 TCDD-EQs to RE response suggested varying differences in the magnitude of bioassay responses between samples. The result clearly suggested that some antagonistic compounds masking AhR-mediated responses were present in residual oil in sediments (Fig. 7d). At first glance, such antagonistic effects seemed to be

much strong for the more highly contaminated sediments with PAHs. Meantime, the antagonistic effects apparently decreased as the natural weathering progressed, indicating AhR antagonists seemed to be degraded and/or transformed during the weathering process.

4. Conclusions

Overall, the present study provided valuable addition into the current understandings of hydrocarbon chemistry and oil toxicology in terms of i) identification of major AhR agonists among PAHs and alkyl-PAHs in oil contaminated sediments and their fractional and/or combined effects, ii) compositional changes of alkylation in sedimentary environment over time, iii) report of novel RePs for yet unknown set of 7 individual (alkyl)-PAHs allowing the improvement of potency balance analysis, and finally iv) evidence for antagonistic or synergistic effects of sedimentary residual oils associated with weathering. At present, most studies of the oil spill are focused on the known toxic chemicals such as PAHs and alkyl-PAHs. However, the results of present study indicated that known toxic PAHs explained only a small portion of the overall toxicity in crude oil and oil contaminated sediments. Unknown toxic chemicals and more long-term weathering process of sedimentary residual oils and their potential toxic effects on benthic organisms remain in question. Current findings would warrant adaptive management scheme for environmental monitoring and risk assessment in the oil contaminated sites in timely fashion.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2015.01.009>.

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<Supplemental Materials>

**Effect-directed analysis and mixture effects of AhR-active PAHs in crude oil
and coastal sediments contaminated by the *Hebei Spirit* oil spill**

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Supplemental Tables

Table S1. Locations of sampling sites and sample information for sediments in the Taean area after the *Hebei Spirit* oil spill.

Location	Sampling period	Latitude (N)			Longitude (E)			Sediment type	Water contents (%)
		°	'	''	°	'	''		
Sinduri dune	Dec. 2007	36	50	59	126	11	40	Sand	4.1
	Dec. 2010	36	50	4	126	10	45	Sand	22.4
	Jan. 2012-1	36	50	6	126	10	48	Sand	22.9
	Jan. 2012-2	36	50	5	126	10	46	Sand	21.0
Sinduri mudflat	Jan. 2008	36	49	42	126	10	58	Mud	37.8
	Dec. 2010	36	49	18	126	11	12	Mud	37.8
	Jan. 2012-2	36	49	22	126	11	14	Mud	27.3
Sogunri mudflat	Dec. 2010	36	49	9	126	10	17	Mud	27.9
	Jan. 2012-1	36	49	9	126	10	17	Mud	32.9
	Jan. 2012-2	36	49	8	126	10	15	Mud	30.8
	Jan. 2012-3	36	49	6	126	10	14	Mud	32.8

Table S2. HPLC instrumental conditions for the fractionation of aromatics (F2).

HPLC system	Agilent 1200 Series
Column	Nucleosil 100-5 NO ₂ (25 cm, 5 μm, 4.6 mm), Normal phase column
Mobile phase	Hexane:DCM (95:5. v/v), Isocratic elution
Flow rate	0.7 mL/min
Delay time	0.3 min
Injection volume	100 μL
Test standard	16 PAHs mixture (10 ppm)
Fraction collect	0 – 4 min: discard 4 – 6.6 min: F2.1 (containing naphthalene) 6.6 – 9.2 min: F2.2 (containing acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene) 9.2 – 11.8 min: F2.3 (containing fluoranthene, pyrene) 11.8 – 14.4 min: F2.4 (containing benzo[<i>a</i>]anthracene, chrysene) 14.4 – 19.6 min: F2.5 (containing benzo[<i>b</i>]fluoranthene, benzo[<i>k</i>]fluoranthene, benzo[<i>a</i>]pyrene) 19.6 – 25 min: F2.6 (containing indeno[<i>1,2,3-cd</i>]pyrene, dibenz[<i>a,h</i>]anthracene, benzo[<i>g,h,i</i>]perylene)

Table S3. GC/MSD instrumental conditions for the determination of PAHs and alkyl-PAHs.

GC/MSD system	Agilent 7890A GC and 5975C MSD
Column	DB-5MS (30 m, 0.25 mm, 0.25 μ m)
Gas flow	1 mL/min He
Injection mode	Split 1 mL/min (ratio 1:1)
Injection volume	2 μ L
MS temperature	175 $^{\circ}$ C
Detector temperature	280 $^{\circ}$ C
Oven temperature	60 $^{\circ}$ C hold 2 min Increase 6 $^{\circ}$ C/min to 300 $^{\circ}$ C 300 $^{\circ}$ C hold 13 min

Table S4. HPLC fractionation results and GC/MSD confirmation of 45 PAHs and alkyl-PAHs in the F2 fractions of Iranian Heavy Crude oil.

Compounds	MW	F2.1	F2.2	F2.3	F2.4	F2.5	F2.6	Benzene (and total) rings
Naphthalene	128	•						2
2-Methylnaphthalene	142	•						2
1-Methylnaphthalene	142	•						2
2,6-Dimethylnaphthalene	156	•						2
C2-Naphthalene	156	•						2
2,3,5-Trimethylnaphthalene	170	•						2
C3-Naphthalene	170	•						2
C4-Naphthalene	184	•						2
Acenaphthylene	152		•					2
Acenaphthene	154		•					2
Fluorene	166		•					2 (3)
C1-Fluorene	180		•					2 (3)
C2-Fluorene	194		•					2 (3)
C3-Fluorene	208		•					2 (3)
Phenanthrene	178		•					3
Anthracene	178		•					3
3-Methylphenanthrene	192		•					3
2-Methylphenanthrene	192		•					3
4/9-Methylphenanthrene	192		•					3
1-Methylphenanthrene	192		•					3
C2-Phenanthrene	206		•					3
C3-Phenanthrene	220		•					3
C4-Phenanthrene	234		•					3
Dibenzothiophene	184		•					3
4-Methyldibenzothiophene	198		•					3
2-/3-Methyldibenzothiophene	198		•					3
1-Methyldibenzothiophene	198		•					3
C2-Dibenzothiophene	212		•					3
C3-Dibenzothiophene	226		•					3
Fluoranthene	202			•				3 (4)
Pyrene	202			•				4
Benz[<i>a</i>]anthracene	228				•			4
Chrysene	228				•			4
C1-Chrysene	242				•			4
C2-Chrysene	256				•			4
C3-Chrysene	270				•			4
Benzo[<i>b</i>]fluoranthene	252					•		4 (5)
Benzo[<i>k</i>]fluoranthene	252					•		4 (5)
Benzo[<i>e</i>]pyrene	252					•		5
Benzo[<i>a</i>]pyrene	252					•		5
Indeno[1,2,3- <i>cd</i>]pyrene	276						•	5 (6)
Dibenz[<i>a,h</i>]anthracene	278						•	5
Benzo[<i>g,h,i</i>]perylene	276						•	6

Table S5. List of PAHs and alkyl-PAHs and the relative potency values of AhR-mediated activity.

Compounds	Company	Test concentration range (ng mL ⁻¹) ^a	%TCDD _{max} ^b	ReP values ^c
Naphthalene	Fluka	4 - 1000	5.9	-
1-Methylnaphthalene	Fluka	4 - 1000	10	-
2-Methylnaphthalene	Fluka	4 - 1000	9.9	-
1,3-Dimethylnaphthalene	Chiron	4 - 1000	ns	-
2,3-Dimethylnaphthalene	Aldrich	4 - 1000	9.5	-
1,4,5-Trimethylnaphthalene	Chiron	2 - 500	ns	-
2,3,5-Trimethylnaphthalene	Chiron	2 - 500	ns	-
1,2,5,6-Tetramethylnaphthalene	Chiron	2 - 500	2.1	-
Fluorene	Aldrich	4 - 1000	2.4	-
1-Methylfluorene	Chiron	4 - 1000	1.0	-
9-Methylfluorene	Chiron	4 - 1000	1.8	-
1,7-Dimethylfluorene	Chiron	2 - 500	ns	-
9-n-Propylfluorene	Chiron	4 - 1000	1.7	-
Phenanthrene	Aldrich	4 - 1000	3.6	-
2-Methylphenanthrene	Chiron	4 - 1000	ns	-
3-Methylphenanthrene	Chiron	4 - 1000	ns	-
1,2-Dimethylphenanthrene	Chiron	2 - 500	ns	-
1,6-Dimethylphenanthrene	Chiron	2 - 500	ns	-
1,2,6-Trimethylphenanthrene	Chiron	2 - 500	1.1	-
1,2,9-Trimethylphenanthrene	Chiron	2 - 500	1.0	-
1,2,6,9-Tetramethylphenanthrene	Chiron	2 - 500	38	1.0 x 10 ⁻⁵
Dibenzothiophene	Aldrich	4 - 1000	22	2.5 x 10 ⁻⁷
2-Methyldibenzothiophene	Chiron	2 - 500	1.7	-
2,4-Dimethyldibenzothiophene	Chiron	2 - 500	ns	-
2,4,7-Trimethyldibenzothiophene	Chiron	2 - 500	ns	-
Chrysene	Supelco	4 - 1000	76	2.0 x 10 ⁻⁵
1-Methylchrysene	Chiron	1 - 200	62	1.0 x 10 ⁻⁴
3-Methylchrysene	Chiron	1 - 200	74	2.0 x 10 ⁻⁴
6-Ethylchrysene	Chiron	4 - 1000	31	3.5 x 10 ⁻⁷
1,3,6-Trimethylchrysene	Chiron	4 - 1000	91	5.0 x 10 ⁻⁵

^a Chemicals were prepared at six concentrations using 3-fold serial dilution and were tested.

^b %TCDD_{max}: a percentage of maximum response observed for a TCDD standard containing 30 nM (0.1% dosing).

^c ReP values were calculated based on EC 50 concentrations which were relative to that of TCDD estimated.

Observed maximum response for the chemical was < %TCDD_{max}, ReP values could not be calculated.

Table S6. Concentrations of PAHs and alkyl-PAHs in Iranian Heavy Crude ($\mu\text{g g}^{-1}$) and Taaen sediments (ng g^{-1} dw).

Compounds	Iranian Heavy Crude	Sediments										
		Sinduri dune		Sinduri mudflat				Sogeenri mudflat				
		2007	2010	2012-1	2012-2	2008	2010	2012	2010	2012-1	2012-2	2012-3
Naphthalene	172	766	4.72	1.37	2.39	3.99	40.1	4.45	53.4	48.1	38.2	46.7
2-Methylnaphthalene	-	5390	4.10	1.36	0.843	4.45	57.1	3.53	444	70.5	41.5	21.5
1-Methylnaphthalene	-	5170	3.91	3.37	4.31	3.30	110	9.28	70.6	35.3	44.7	19.7
C1-Naphthalene	991	10600	8.01	4.73	5.15	7.75	167	12.8	515	106	86.2	41.2
2,6-Dimethylnaphthalene	-	5380	6.16	3.06	3.28	5.08	1560	127	1050	166	114	14.6
C2-Naphthalene	1230	87.9	55.1	124	133	21.3	8990	817	4200	978	765	91.2
2,3,5-Trimethylnaphthalene	-	6250	158	220	235	21.6	4630	738	1400	1200	485	30.8
C3-Naphthalene	1270	19800	719	1470	1380	112	24000	4140	8140	9790	2850	181.6
C4-Naphthalene	682	9040	1390	5570	4420	286	22600	6850	6140	20600	3470	205.2
Acenaphthylene	0.280	3.71	0.865	3.55	1.62	0.45	87.1	2.35	30.1	6.13	1.47	1.63
Acenaphthene	4.64	107	1.80	5.51	5.95	0.38	74.1	38.3	26.9	114	20.2	3.53
Fluorene	27.3	644	1.31	4.87	4.26	1.71	383	45.9	111	64.7	46.5	7.81
C1-Fluorene	207	747	58.8	132	94.6	12.3	1770	393	440	990	211	17.0
C2-Fluorene	114	964	279	573	487	71.1	3790	1510	793	4370	657	61.2
C3-Fluorene	132	1530	437	1660	1420	201	5880	2670	1140	6780	785	92.2
Phenanthrene	50.9	1360	8.47	30.8	30.6	8.92	278	66.1	184	141	77.6	17.8
Anthracene	3.38	160.9	3.22	17.0	15.5	1.41	33.7	20.4	9.36	307	28.4	3.36
3-Methylphenanthrene	-	119	27.8	41.6	32.6	14.9	722	106	221	326	84.4	5.91
2-Methylphenanthrene	-	87.1	8.54	87.8	72.4	9.30	443	60.7	196	156	73.1	6.20
4/9-Methylphenanthrene	-	183	58.0	365	294	24.3	1340	195	434	648	176	8.35
1-Methylphenanthrene	-	121	32.9	93.8	97.6	12.5	796	105	256	383	102	7.95
C1-phenanthrene	189	510	127	588	497	61.1	3300	466	1100	1510	436	28.4
C2-Phenanthrene	250	720	612	2390	1980	282	8600	3080	1800	7100	1060	89.2
C3-Phenanthrene	167	537	592	2320	1990	379	6900	3380	1290	6760	975	124
C4-Phenanthrene	84.4	254	368	1520	1260	223	4490	2080	740	4250	600	108
Dibenzothiophene	122	1380	6.70	126	93.0	8.92	2380	360	582	634	105	2.21
4-Methyldibenzothiophene	-	525	30.1	1440	1020	74.6	5730	1520	1270	2430	258	8.17
2-/3-Methyldibenzothiophene	-	412	183	1080	745	55.0	4510	1490	956	3230	162	6.25
1-Methyldibenzothiophene	-	280	145	1010	798	36.4	3220	1610	650	3160	136	6.51
C1-Dibenzothiophene	370	1220	359	3530	2560	166	13500	4620	2880	8810	555	20.9
C2-Dibenzothiophene	556	2320	1870	8890	7460	1100	29800	15200	5720	29000	1670	66.2

C3-Dibenzothiophene	477	2030	2320	11000	9400	1550	30600	16900	5750	34700	2150	99.5
Fluoranthene	0.560	24.1	1.80	11.2	13.2	7.29	33.7	22.8	6.52	85.0	28.7	28.9
Pyrene	3.64	89.2	10.2	44.0	35.9	10.6	12.5	68.9	23.7	166	36.5	22.5
Benz[<i>a</i>]anthracene	1.99	29.7	6.68	20.5	21.3	6.17	48.8	47.5	10.5	107	21.5	15.3
Chrysene	8.85	122	26.8	97.9	94.1	22.3	192	164	36.7	329	53.7	22.7
C1-Chrysene	14.7	150	38.8	164	158	27.6	300	250	60.4	530	79.8	16.5
C2-Chrysene	21.4	270	71.0	283	288	51.0	557	443	120	847	122	24.9
C3-Chrysene	20.9	198	66.5	355	355	43.9	527	488	108	974	157	33.5
Benzo[<i>b</i>]fluoranthene	1.17	17.7	4.61	12.7	11.7	10.7	41.4	34.8	8.57	96.6	27.5	29.9
Benzo[<i>k</i>]fluoranthene	0.150	1.70	0.506	1.59	1.29	1.65	4.68	7.43	1.08	15.0	6.16	8.75
Benzo[<i>e</i>]pyrene	-	40.1	7.41	33.5	33.6	9.16	68.3	54.7	13.3	116	23.7	16.8
Benzo[<i>a</i>]pyrene	1.05	13.5	1.13	11.2	12.9	4.34	28.5	18.3	6.80	53.6	12.7	12.6
Indeno[1,2,3- <i>cd</i>]pyrene	0.22	1.50	1.07	4.71	< DL	4.39	9.31	9.25	3.44	51.0	12.2	15.4
Dibenz[<i>a,h</i>]anthracene	0.49	1.40	1.32	5.38	< DL	1.57	9.42	8.38	2.00	20.0	5.53	2.51
Benzo[<i>g,h,i</i>]perylene	0.64	5.30	2.27	10.8	11.6	4.42	22.8	21.8	5.81	54.6	14.7	17.2
C2-Phe/C3-Phe	1.50	1.34	1.03	1.03	0.99	0.75	1.25	0.91	1.40	1.05	1.09	0.72
C2-Dbthio/C2-Dbthio	1.17	1.14	0.81	0.81	0.79	0.71	0.98	0.90	1.00	0.84	0.78	0.66
Weathering Stage ^a	NW	I	II	II	II	III	I	II	I	II	II	III
Σ PAHs	7160	67300	9610	41200	34500	4700	176000	65100	44500	142000	17800	1620

^a Weathering stage. I: Slightly weathered, II: Moderately weathered, III: Severely weathered (Hong et al., 2012; Yim et al., 2011).

-: not analyzed; NW: not weathered; < DL: below detection limits^b.

^b Detection limits for individual PAHs and alkyl-PAHs were from 0.1 to 0.5 ng g⁻¹ dw.

Table S7. TEQs concentrations of PAHs and alkyl-PAHs in Iranian Heavy Crude (pg g^{-1}) and Taaen sediments (pg g^{-1} dw).

Compounds	Iranian Heavy Crude	Sediments										
		Sinduri dune		Sinduri mudflat				Sogeenri mudflat				
		2007	2010	2012-1	2012-2	2008	2010	2012	2010	2012-1	2012-2	2012-3
C4-Phenanthrene	846	2.61	3.78	15.7	13.0	2.29	46.1	21.3	7.70	43.7	6.16	1.11
Dibenzothiophene	30.4	0.344	0.002	0.031	0.023	0.002	0.592	0.090	0.145	0.158	0.026	0.001
Benz[<i>a</i>]anthracene	3.78	0.057	0.013	0.039	0.041	0.012	0.093	0.090	0.020	0.204	0.041	0.029
Chrysene	261	3.59	0.791	2.89	2.78	0.657	5.67	4.85	1.08	9.71	1.58	0.671
C1-Chrysene	1470	15.0	3.88	16.4	15.8	2.76	30.0	25.0	6.05	53.1	8.00	1.66
C2-Chrysene	7.44	0.093	0.025	0.099	0.100	0.018	0.194	0.154	0.042	0.295	0.042	0.009
C3-Chrysene	1050	9.91	3.33	17.8	17.8	2.20	26.4	24.5	5.40	48.9	7.85	1.68
Benzo[<i>b</i>]fluoranthene	5.97	0.090	0.024	0.065	0.060	0.055	0.211	0.177	0.044	0.493	0.140	0.152
Benzo[<i>k</i>]fluoranthene	21.0	0.233	0.071	0.222	0.180	0.231	0.655	1.04	0.150	2.10	0.863	1.23
Benzo[<i>a</i>]pyrene	1.68	0.022	0.002	0.018	0.021	0.007	0.046	0.029	0.011	0.086	0.020	0.020
Indeno[1,2,3- <i>cd</i>]pyrene	3.30	0.022	0.016	0.071	< DL	0.066	0.140	0.137	0.052	0.765	0.184	0.231
Dibenz[<i>a,h</i>]anthracene	2.25	0.007	0.006	0.025	< DL	0.007	0.043	0.039	0.009	0.092	0.025	0.012
TEQs-PAHs	3700	32.0	11.9	53.4	49.8	8.31	110	77.4	20.7	159	24.9	6.79

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Supplemental Figure

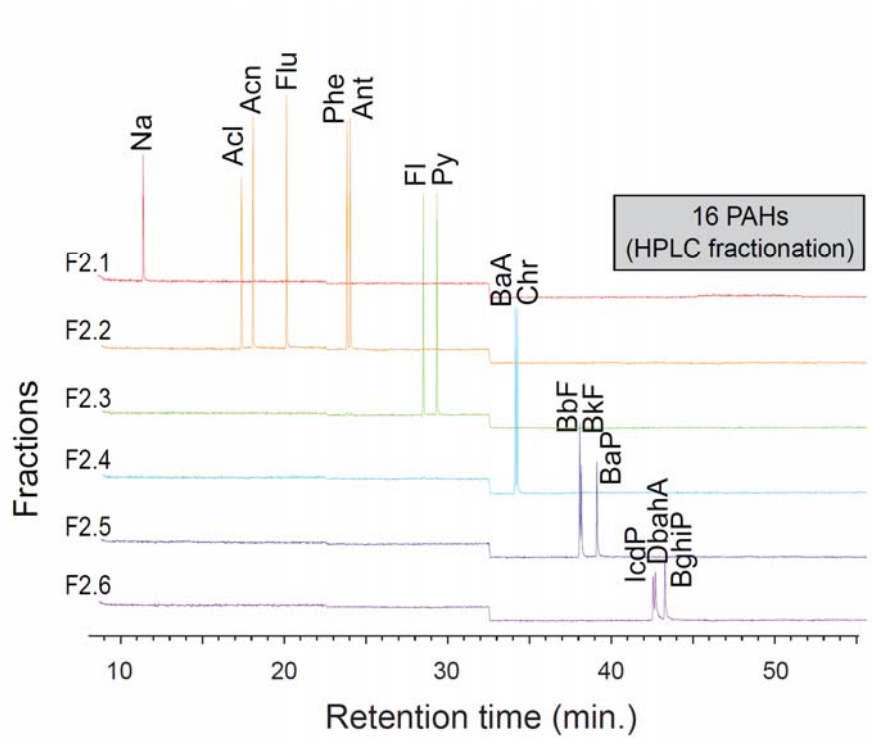


Fig. S1. GC/MSD chromatograms of HPLC fractions (F2.1-F2.6) of 16 EPA priority PAHs.