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Newly Identified AhR-Active Compounds in the Sediments of an Industrial Area Using Effect-Directed Analysis

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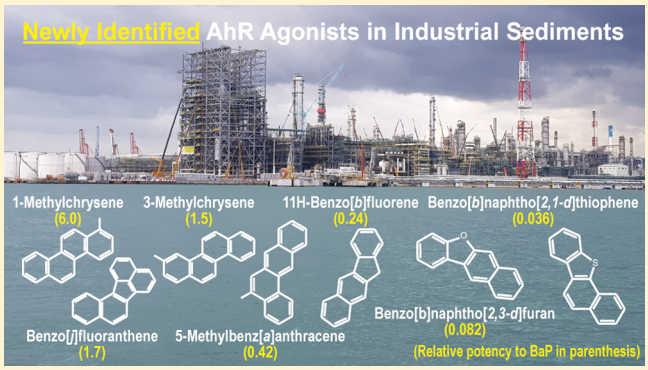
Supporting Information

ABSTRACT: Effect-directed analysis was used to identify previously unidentified aryl hydrocarbon receptor (AhR) agonists in sediments collected from a highly industrialized area of Ulsan Bay, Korea. The specific objectives were to (i) investigate potent fractions of sediment extracts using the H4IIE-*luc* bioassay, (ii) determine the concentrations of known AhR agonists (polycyclic aromatic hydrocarbons (PAHs) and styrene oligomers (SOs)), (iii) identify previously unreported AhR agonists in fractions by use of GC-QTOFMS, and (iv) evaluate contributions of individual compounds to overall AhR-mediated potencies, found primarily in fractions containing aromatics with log K_{ow} 5−8. Greater concentrations of PAHs and SOs were also found in those fractions. On the basis of GC-QTOFMS and GC-MSD analyses, 16 candidates for AhR agonists were identified in extracts of sediments. Of these, seven compounds, including 1-methylchrysene, benzo[*j*]fluoranthene, 3-methylchrysene, 5-methylbenz[*a*]anthracene, 11*H*-benzo[*b*]fluorene, benzo[*b*]-naphtho[2,3-*d*]furan, and benzo[*b*]naphtho[2,1-*d*]thiophene, exhibited significant AhR activity. Relative potency values of newly identified AhR agonists were found to be greater than or comparable to that of benzo[*a*]pyrene (BaP). The potency balance analysis showed that newly identified AhR agonists explained 0.07−16% of bioassay-derived BaP-EQs. These chemicals were widely distributed in industrial sediments; thus, it is of immediate importance to conduct studies on sources and potential effects of those chemicals.



INTRODUCTION

There are various hydrophobic toxic chemicals in sediments of coastal environments, where sediments act as both a final sink and occasionally sources of potentially toxic chemicals to marine ecosystems. In particular, areas surrounding industrial complexes are polluted by polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers, and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs).[1,2] Because diverse compounds are present in environmental samples, including sediments, water, sewage sludge, and biota, it is often difficult to assess the potential toxic effects of known and unknown chemicals.[3,4]

Ulsan City, which is one of the largest cities in Korea, is located in the southeastern part of the country. Many industries operate adjacent to Ulsan Bay, including automobile manufacturing, ship building, petrochemical, and rubber industries.[5] PAHs, PCBs, and butyltins in sediments of Ulsan Bay primarily originate from surrounding industrial complexes.[6] Concentrations of persistent substances, such as PAHs, PCBs, nonylphenols, and octylphenols in sediments, are

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greater in Ulsan Bay compared to other coastal areas of Korea.[7,8] In 2000, to protect water and sediment quality of this area, the South Korean Government designated Ulsan Bay as a "Special Management Coastal Zone". The government initiative led to a reduction in concentrations of well-known AhR agonists; however, AhR-mediated potency is still relatively great.

Effect-directed analysis (EDA) has been widely applied to identify key toxicant(s) in environmental samples.[3] The EDA approach includes toxicity testing, fractionations, and instrumental analyses. If significant responses of raw extracts (REs) of environmental samples are observed in the bioassay, fractionation is performed to reduce complexity and separate more potent fractions that contain potentially active compounds.[3] After fractionation, instrumental analyses are conducted to identify and quantify specific compounds. Several studies that applied EDA have been successfully performed to identify key toxicant(s) in a variety of environmental samples, including freshwater,[9] river sediments,[10] and oil-contaminated sediments.[11] A previous study using EDA identified mainly PAHs and nitrogen/oxygen-containing polyaromatic compounds (N/O-PAC) from marine sediment as aryl hydrocarbon receptor (AhR) agonists in mouse hepatoma (H1L6.1c3) cell line (CALUX assay).[12] Another study tentatively identified 11*H*-indeno[2,1,7-*cde*]pyrene, methylbenzo[*e*]pyrene, and methylperylene in creek sediment as AhR agonists determined by use of a rainbow trout liver cell line (EROD assay).[13] In addition, AhR agonists including 1,3-diphenylproane (SD1), 2,4-diphenyl-1-butene (SD3), and 1e-phenyl-4e-(1-phenylethyl)-tetralin (ST2) were identified in sediments adjacent to an industrial complex.[14] However, target compounds detected in environmental samples could not fully explain bioassay results.[15]

More recently, the EDA approach has been used in combination with full-scan screening analysis (FSA) to detect unknown and potentially toxic substances in environmental samples, such as sediment and water.[16] FSA is a powerful tool that is used to identify unknown toxicants in environmental samples based on accurate molecular mass by use of high-resolution mass spectrometry (HRMS), such as time-of-flight mass spectrometry (TOFMS).[17−19] A previous study using FSA successfully identified 2,3- and 2,8-phenazinediamine from river water, which was subsequently confirmed by use of authentic standards and shown to be a potent mutagenicity in the Ames test.[20] Through combining EDA with FSA, 4-methyl-7-diethylaminocoumarin was confirmed as a potent androgenic receptor agonist in the surface water of rivers with the relative potency (ReP) value of the reference material flutamide exceeding 5.2.[21] By using RePs, it is possible to determine contributions of chemicals to total potencies of mixtures of chemicals as determined by use of end point-specific transactivation in vitro bioassays. However, few studies have investigated contributions of novel chemicals identified from EDA by use of ReP values to total potencies as determined by bioassays.[14]

In this study, EDA was combined with FSA to identify previously unidentified AhR-active compounds in sediments. Specific objectives were to (i) investigate major AhR-active fractions of organic extracts of sediments by use of the H4IIE-*luc* bioassay, (ii) measure concentrations and relative compositions of known AhR agonists (PAHs and styrene oligomers (SOs)) in sediments using GC-MSD, (iii) identify previously unidentified AhR agonists in more potent fractions by use of GC-QTOFMS, and (iv) evaluate relative contributions of AhR agonists to total potencies as determined by use of H4IIE-*luc* bioassay.

MATERIALS AND METHODS

Sample Collection and Preparation. Surface sediments were collected from the inland creeks (C1−C4) connected to Ulsan Bay by use of a grab sampler in June 2017 (Figure S1 of the Supporting Information). Samples were transferred to precleaned glass jars and immediately stored at −20 °C until analysis. Sample preparation methods for biological and chemical analyses were conducted according to previous studies with some modifications.[14,22,23] In brief, a 40 g of freeze-dried sediment was extracted with 350 mL of dichloromethane (DCM; J.T. Baker, Phillipsburg, NJ) for 24 h on a Soxhlet extractor. Activated copper was used to remove elemental sulfur from sediment organic extracts (Sigma-Aldrich, Saint Louis, MO). Organic extracts were concentrated to 4 mL (10 g sediment equivalents (SEq) mL^{-1}). Extracts were divided into two aliquots for use in the in vitro bioassay or for instrumental analysis and further fractionations. To avoid contribution to the AhR-mediated potency, isotopically labeled surrogate standards were not added during extraction and fractionation.

Silica Gel and RP-HPLC Fractionations. A column packed with 8 g of activated silica gel (70−230 mesh, Sigma-Aldrich) and hexane (Honeywell, Charlotte, NC) was used for fractionation of 1 mL of sediment REs.[11] First, the nonpolar fraction (F1) was eluted with 30 mL of hexane. The second fraction (F2), containing mainly aromatic compounds, was collected by elution with 60 mL of 20% DCM in hexane (v/v). The polar fraction (F3) was obtained by using 50 mL of 60% DCM in acetone (J.T. Baker). Before use in the H4IIE-*luc* bioassay, instrumental analysis and further fractionation elutriates were concentrated to 1 mL by rotary evaporation followed by gentle nitrogen gas. The F2 fraction was further separated into 10 subfractions, according to the log K_{ow} values of chemicals by use of reverse-phase (RP)-HPLC (Agilent 1260 HPLC; Agilent Technologies, Santa Clara, CA) with a multiple-wavelength detector.[14] A C18 column was used for fractionation (PrepHT XBD-C18, 21.2 × 250 mm, 7 μm, Agilent Technologies). By using the fractionation method developed in a previous study, 10 subfractions were collected at 1 log K_{ow} intervals.[14] The injection volume of each fraction was 1 mL of 20% water in MeOH (v/v), and the flow rate of the mobile phase was 10 mL min^{-1}. Subfractions were collected and exchanged into hexane or dimethyl sulfoxide (DMSO, Sigma-Aldrich) for further use in instrumental analyses or the H4IIE-*luc* bioassay, respectively.

In Vitro Transactivation Bioassay. To assess AhR-mediated activity, an in vitro bioassay was conducted using H4IIE-*luc* cells by the method previously established with slight modifications.[14,22] In brief, sediment REs, fractions (silica gel column and RP-HPLC) and individual AhR-active compounds were assayed. To minimize the influence of cytotoxicity, noncytotoxic doses were determined by use of WST-1 assay (Roche Applied Science, Mannheim, Germany) prior to performing the AhR-mediated activity assay (i.e., >80% viability vs control). The results of the WST-1 assays confirmed that no cytotoxicity occurred at the doses tested (maximum concentration 10 g SEq mL^{-1}, 0.1% dose). For AhR assay, trypsinized cells (~7.0×10^4 cells mL^{-1}) were seeded into 96 microwell plates. To avoid effects of edges of

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microwell plates, the interior 60 wells were used at a volume of 250 μL per well. After 24 h incubation, wells were dosed with the appropriate standards (benzo[*a*]pyrene (BaP) for 4 h; 0.1% dose), samples (REs, fractions, or compounds; 0.1% dose), and solvent controls (0.1% DMSO). After 4 h of exposure, the results were expressed as relative luminescence units that were quantified using a Victor X3 multilabel plate reader (PerkinElmer, Waltham, MA).

Responses of the H4IIE-*luc* bioassay were converted to percentages of the maximum response (% BaP_{max}) observed for a 50 nM BaP (=100% BaP_{max}). The magnitude-based % BaP_{max} is given for screening purposes and thus not normalized for diluted samples. Significant responses (5% BaP_{max}) were defined as those produced responses that were three times as great as the standard deviation of the mean solvent controls. Potencies of samples expressed as BaP equivalent concentrations (potency-based BaP-EQ, ng BaP-EQ g^{-1} dm) were determined directly from the full sample dose−response relationships generated by testing samples at multiple (at least 3 points) dilutions. All bioassays were conducted in triplicate.

According to the results of previous studies, strong AhR agonists, such as PCDD/Fs and coplanar-PCBs, tend to take longer to form stable bonds to the AhR than do PAHs.[24,25] The relatively luminescence unit (RLU) of PCDD/Fs tended to increase with duration of exposure and showed a maximum at 72 h. Alternatively, PAHs showed the maximum responses at shorter durations of exposure (∼4−6 h), and RLU values gradually decreased in the following time due to metabolism. Considering that concentrations of PCDD/Fs in sediments are generally about 1000-fold less than those of PAHs,[6] contributions of PCDD/Fs to the overall AhR-mediated potencies would not be expected as great as those of PAHs during a 4 h exposure in the H4IIE-*luc* bioassay.

Identification and Quantification of Target Compounds. Standard materials for target PAHs were obtained from ChemService (West Chester, PA) and included acenaphthylene (Acl), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fl), pyrene (Py), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), BaP, indeno[1,2,3-*c*,*d*]pyrene (IcdP), dibenz[*a*,*h*]anthracene (DahA), and benzo[*g*,*h*,*i*]perylene (BghiP). Authentic standards for SOs were obtained from Wako Pure Chemical Ind. (Osaka, Japan) and Hayashi Pure Chemical Industries (Osaka, Japan) and included SD1, *cis*-1,2-diphenylcyclobutane (SD2), SD3, *trans*-1,2-diphenylcyclobutane (SD4), 2,4,6-triphenyl-1-hexene (ST1), ST2, 1a-phenyl-4e-(1-phenylethyl)-tetralin (ST3), 1a-phenyl-4a-(1-phenylethyl)-tetralin (ST4), 1e-phenyl-4a-(1-phenylethyl)-tetralin (ST5), and 1,3,5-triphenylcyclohexane (isomer mix) (ST6). Isotopically labeled surrogate standards (Ace-d_{10}, Phe-d_{10}, Chr-d_{12}, and perylene-d_{12}) were added in aliquots after extraction, and instrumental internal standard (2-fluorobiphenyl) was added before GC injection. Both standards were obtained from ChemService. Target compounds in organic extracts of sediments were quantified by use of an Agilent 7890B gas chromatograph equipped with a 5977B mass-selective detector (GC-MSD). Instrumental conditions used to detect target compounds are provided in Figure S2. Recoveries of surrogate standards ranged from 59% to 68% (n = 4, mean = 64%) for Ace-d_{10}, from 67% to 90% (n = 4, mean = 80%) for Phe-d10, from 73% to 111% (n = 4, mean = 90%) for Chr-d_{12}, and from 76% to 111% (n = 4, mean = 92%) for perylene-d_{12}. Method detection limits (MDL) for individual SOs and PAHs ranged from 0.031 to 0.20 and from 0.11 to 0.89 ng g^{-1} dm, respectively.

Full-Scan Screening Analysis. Fractions F2.6 and F2.7 of organic extracts of sediment from C2 (Yeocheon) that showed greater AhR-mediated potencies were subjected to FSA (see Results and Discussion). The gas chromatograph Agilent 7890B equipped with a quadrupole TOFMS (QTOFMS) (Agilent 7200, Agilent Technologies) was used for FSA. A DB-5MS UI (30 m × 0.25 mm i.d. × 0.25 μm film) column was used, and the carrier gas was He with 1.2 mL min^{-1} flow rate. Details on instrumental conditions for FSA are present in Table S1. The selection criteria of candidates for AhR agonists from the results of GC-QTOFMS analysis consisted of four steps.[26] First, compounds that were registered in the NIST library were chosen.[27] Second, considering the reliability of data, the minimum match factor score of the spectral library was set to 70.[28] Third, because target AhR agonists of bioassay at 4 h exposure time were mainly aromatics, aromatic compounds were screened.[29,30] Finally, compounds with more than 3 benzene rings were selected as tentative candidates for AhR agonists.[31] Among the compounds, commercially available compounds for standard materials were chosen for chemical and biological confirmation.

According to the selection process, 11*H*-benzo[*b*]fluorene (11BF), benzo[*b*]naphtho[2,1-*d*]thiophene (BBNT), benzo[*b*]naphtho[2,3-*d*]furan (BBNF), benzo[*e*]pyrene (BEP), 1,12-dimethylbenzo[*c*]phenanthrene (BCP), 2-methylanthracene (2MA), 5-methylbenz[*a*]anthracene (5MBA), 2-methylphenanthrene (2MP), and triphenylene (TRI) were selected as candidate AhR-active compounds by use of GC-QTOFMS. In addition, benzo[*j*]fluoranthene (BjF), 1,2-dimethylphenanthrene (12DMP), 1,6-dimethylphenanthrene (16DMP), 9-ethylphenanthrene (9EP), 1-methylchrysene (1MC), 3-methylchrysene (3MC), and 3-methylphenanthrene (3MP) were identified in fractions by use of GC-MSD (50−550 m/z). Finally, 16 compounds were purchased for use in confirmation of the structures derived and to characterize their potencies for AhR-mediated responses. 11BF, BBNT, BBNF, BEP, BCP, 2MA, 5MBA, TRI, BjF, 9EP, 1MC, 3MC, and 3MP were purchased from Sigma-Aldrich. 2MP and 16DMP were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX). 12DMP was obtained from AHH chemical Co., Ltd. (ChangZhou, China). Properties of the candidate AhR-active compounds as well as GC-MSD retention times and mass fragment ions are listed in Table S2.

Relative Potency Values of AhR Agonists. RePs for the AhR-mediated effects of individual compounds including traditional and newly identified AhR-active compounds (see Tables S2 and S3) were determined by use of the H4IIE-*luc* bioassay based on the effective concentrations at 50% (EC50) of maximum BaP concentration.[31] Compounds with eight concentrations using 3-fold serial dilution (viz., 10, 3.3, 1.1, 0.37, 0.12, 0.046, 0.014, and 0.0041 ng mL^{-1}) were prepared and tested as described above. If the magnitude of induction was sufficient to allow a reasonable estimate, RePs were calculated. The linear portion of each dose response (%BaP_{max} plotted as a function of log dose) was defined until an $R^2 \geq 0.98$ by dropping points from the tails was obtained, and a linear regression model was fitted to the remaining points.[32] At least three points were used in all cases. The linear regression equations for the samples and corresponding BaP standard were used. Estimation of the ReP values of compounds from

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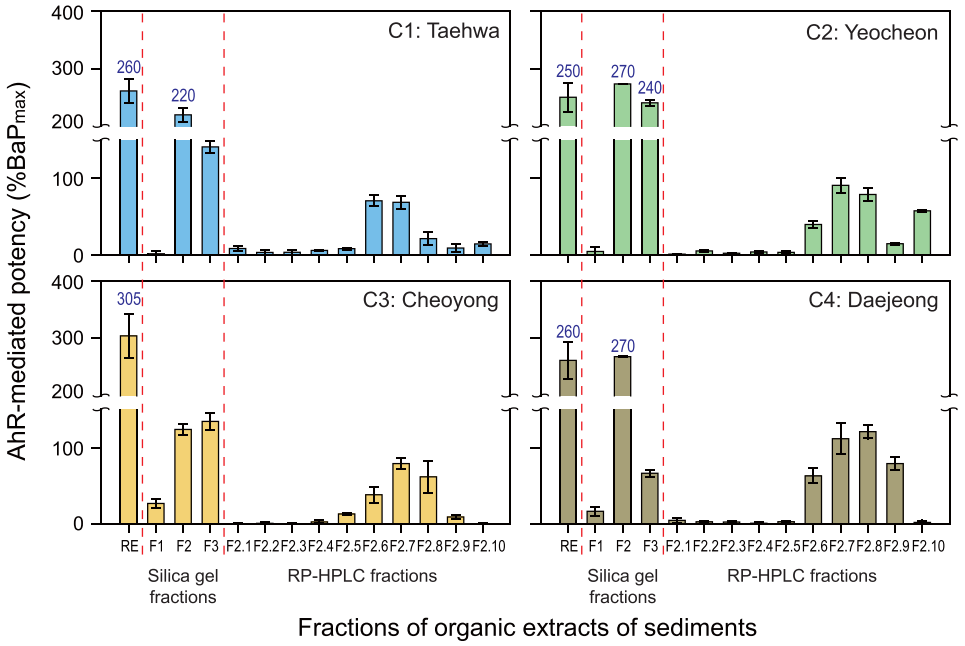

Figure 1. AhR-mediated potencies of raw extracts (RE) and fractions (silica gel and RP-HPLC) of inland creek sediments of Ulsan Bay, Korea (Error bar: mean ± SD; n = 3).

the dose−response relationship was basically assumed to equal efficacy, and parallelism between reference compound (BaP) and unknown were met.[33] ReP20, ReP50, and ReP80 values were determined at the doses of a given chemical of which AhR responses are equivalent to 20%, 50%, and 80% response levels of the maximum BaP concentration in standard curves, respectively.[25,33,34]

Potency Balance Analysis. Potency balance analyses between bioassay-derived BaP-EQ and instrument-derived BaP equivalent concentrations (BEQs) were performed to determine relative contributions of individual chemicals to total induced AhR-mediated potencies. Instrument-derived BEQs were calculated by multiplying the concentrations of individual compounds and their ReP values (eq 1)

$$\mathrm{BEQ} = \sum [(\mathrm{AhR\ agonist}_i) \times \mathrm{ReP}_i] \quad (1)$$

where [AhR agonist$_i$] is the measured concentration in the sample and ReP$_i$ is the ReP for a given compound. ReP values for some SOs were previously reported,[14] while others were newly obtained for seven traditional PAHs and seven newly identified AhR agonists in this study (Table S3).

RESULTS AND DISCUSSION

Screening of AhR-Mediated Potencies in Sediments. All sediment REs samples showed significant AhR-mediated potencies (Figure 1). Of the three silica gel fractions, relatively greater AhR-mediated potencies were observed in F2 (aromatics) and F3 (polar) compared to that of F1 (nonpolar). This result was not surprising, with strong AhR responses in the aromatic fraction being reported many times previously.[14] This phenomenon occurs because AhR agonists including PAHs occur in the F2.[35,36] Fraction F3 also contained significant AhR-mediated potency. Results of previous studies have shown that AhR-mediated potencies in polar fractions were significant with (hydroxy-)quinones, keto-, dinitro-, hydroxyl-PAHs, and N-heterocycles considered as AhR-active compounds.[37−39] However, due to its great complexity and polar characteristics it is very challenging to identify the key toxicants in polar fraction of sediment REs. FSA of polar contaminants in environmental samples has not been widely conducted and has been successfully performed in only a few studies.[16,20] Thus, polar AhR agonists in environmental samples remain unclarified with further study being required. In this study, the focus was on aromatic AhR agonists in F2 fractions of sediment organic extracts.

Among 10 RP-HPLC subfractions of F2, considerable AhR-mediated potencies were found in F2.6−F2.8, which contained aromatics with 5−8 log K_{ow} values (Figure 1). In these fractions, well-known AhR-active PAHs with 4−6 aromatic rings (such as Chr, BaA, Fl, Py, BkF, BbF, BaP, IcdP, and DahA) were included. Patterns showing strong AhR potencies found in F2.6−F2.8 of sediment organic extracts were also found during a previous study conducted at Lake Sihwa, Korea.[14] Novel AhR agonists were also found in fractions of sediment organic extracts, including SD1, SD3, and ST2 (in F2.6 and F2.7).[14] Although concentrations of SOs in sediments were comparable to PAHs, due to their lesser ReP values, SOs did not explain the large proportion of overall induced AhR-mediated potencies. This result was probably due to insufficient aromatic rings. Typically, AhR-active compounds mainly consist of 3−5 benzene rings.[31] Compounds binding to the AhR tend to be planar in configuration and approximately 3 × 10 Å.[40] A similar tendency was reported for strong AhR-mediated potencies in F2.6−F2.8 of sediments on the west coast of South Korea.[41] However, it remains unclear whether this phenomenon is due to the same AhR agonists or similar chemical properties.

After a screening for all of the REs and fractions to identify more potent fractions, dose−response tests, to determine potency-based BaP-EQ concentrations (based on EC50), were performed on selected fractions, such as F2.6 and F2.7 (Figure S3). Concentrations of BaP-EQ were directly compared to instrument-derived BEQ in a potency balance analysis. However, in the case of F2.6 of C1, AhR response was

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Table 1. Summary of the Results for Improved Potency Balance Analysis between Bioassay-Derived BaP-EQs and Instrument-Derived BEQs in the Fractions (F2.6 and F2.7) of Sediment Organic Extracts from Ulsan Bay, South Korea

	C1 (Taehwa)		C2 (Yeocheon)		C3 (Cheoyong)		C4 (Daejeong)	
target compounds	F2.6	F2.7	F2.6	F2.7	F2.6	F2.7	F2.6	F2.7
bioassay-derived BaP-EQ (ng BaP-EQ g^{-1} dm)								
magnitude-based BaP_{max}-EQ[a]	7.3	3.5×10^2	4.6×10	1.5×10^4	8.3×10	2.0×10^3	3.3×10	3.1×10^2
magnitude-based BaP_{max} (%)	16	100	56	180	65	150	48	99
potency-based BaP-EQ[b]	ne[c]	6.1×10^2	1.9×10^2	5.7×10^3	4.2×10^2	2.5×10^3	9.9×10	1.2×10^4
instrument-derived BEQ (ng BEQ g^{-1} dm)								
PAHs and SOs								
benzo[*a*]anthracene	1.1		100		2.1		21	
chrysene	4.6		320		10		34	
1,3-diphenylproane	0.0037		0.0081		0.0011		0.020	
2,4-diphenyl-1-butene	0.0046		0.017		0.023		1.1	
benzo[*b*]fluoranthene		2.8		180		43		4.3
benzo[*k*]fluoranthene		<D.L.[d]		71		88		5.0
benzo[*a*]pyrene		1.6		120		3.0		36
indeno[1,2,3-*cd*]pyrene		2.8		110		5.3		23
dibenz[*a*,*h*]anthracene		0.48		44		1.5		13
1e-phenyl-4e-(1-phenylethyl)tetralin		0.003		0.008		0.018		0.025
sum of PAHs and SOs (ng BEQ g^{-1} dm)	5.7	7.7	420	530	12	140	56	81
contribution of PAHs and SOs (%)	79	1.3	218	9.3	2.9	5.6	57.0	0.68
newly identified AhR agonists								
benzo[*b*]naphtho[2,3-d]furan	0.20		14		0.29		0.22	
11*H*-benzo[*b*]fluorene		1.3		30		0.58		0.50
benzo[*b*]naphtho[2,1-*d*]thiophene		0.082		4.3		0.039		2.7
1-methylchrysene		<D.L.		480		<D.L.		28
3-methylchrysene		3.3		6.8		3.0		79
5-methylbenz[*a*]anthracene		0.35		6.6		<D.L.		1.9
benzo[*j*]fluoranthene		14		420		7.3		140
sum of newly identified AhR agonists (ng BEQ g^{-1} dm)	0.20	19	14	950	0.29	11	0.22	250
contributions of newly identified AhR agonists (%)	2.7	3.2	7.3	17	0.069	0.44	0.22	2.1
total contributions (potency balance, %)	82	4.4	225	26	3.1	6.0	57	2.8

[a]Magnitude-based BaP-EQ concentrations were calculated from percentage of the maximum response observed for a 50 nM BaP standard (set to 100%-BaP_{max}) elicited by 100% sediment raw extracts (10 g SEq mL^{-1}). [b]Potency-based BaP-EQ (BaP-EQ 50) were obtained from sample dose−response relationships generated by testing samples at multiple levels of dilution (see Figure S3). [c]Not enough responses. [d]<D.L.: below detection limits.

insufficient; thus, the magnitude-based BaP-EQ was used. Concentrations of BaP-EQs ranged from 7.3 to 4.2×10^2 ng BaP-EQ g^{-1} dm and from 6.1×10^2 to 1.2×10^4 ng BaP-EQ g^{-1} dm in F2.6 and F2.7 of sediment extracts, respectively (Table 1). F2.8 fractions also showed relatively great AhR-mediated potencies in the H4IIE-*luc* bioassay (Figure 1). This is presumably due to the 7−9 ring PAHs (≥C24-PAH).[42] However, they are very large in molecular mass and occurred with low concentrations in the sediments which resulted in difficulty for the instrumental analysis with low-resolution GC-MSD.[42] Thus, this study did not cover it, and further research on the distributions and potential biological effects of ≥C24-PAH would be necessary.

Concentrations of PAHs and SOs in Sediments. Known AhR agonists, such as PAHs and SOs, were detected in all sediments (Tables S4 and S5). The greatest concentrations of sedimentary PAHs were detected at site C2, followed by sites C4, C3, and C1. Concentrations of PAHs in sediments from C2 and C4 exceeded interim sediment quality guidelines (ISQGs) suggested by the Canadian Council of Ministers of the Environment (CCME).[43] For example, in sediment from C2, concentrations of Na, Ace, Acl, Flu, Phe, Ant, Fl, Py, BaA, Chr, BaP, and DahA exceeded the ISQGs of CCME. Concentrations of Ace, Phe, and DahA in the C4 sediment were greater than the ISQGs of CCME (Table S4). Concentrations of SOs in sediments ranged from 36 to 3700 ng g^{-1} dm (mean = 990 ng g^{-1} dm). The greatest concentrations of SOs were observed in site C4, followed by sites C3, C2, and C1. Spatial distributions of PAHs and SOs in sediments from Ulsan Bay were distinguished by their sources. PAHs mainly originate from incomplete combustion or pyrolysis of coal, oil, wood, and petroleum products.[44] In comparison, SOs originate primarily from degradation of polystyrene plastics.[14]

Relative compositions of PAHs in sediments indicated that larger molecular mass PAHs with 4−6 rings (such as Fl, Py, BaA, and Chr) were dominant (77−83%, mean = 80%) (Table S4). To predict sources of PAHs, the diagnostic ratios were used by comparing the relative contributions of individual PAHs, including Ant/(Ant + Phe), Fl/(Fl + Py), BaA/(BaA + Chr), and IcdP/(IcdP + BghiP) (details in Figure S4). For example, according to results of previous studies, the ratio of Ant/(Ant + Phe) > 0.1 indicates a dominance of combustion source, and a Fl/(Fl + Py) ratio > 0.4 indicates coal, wood, or grass combustion source.[45−47] Results of the diagnostic ratios indicated that most PAHs were of pyrogenic origin. Results of

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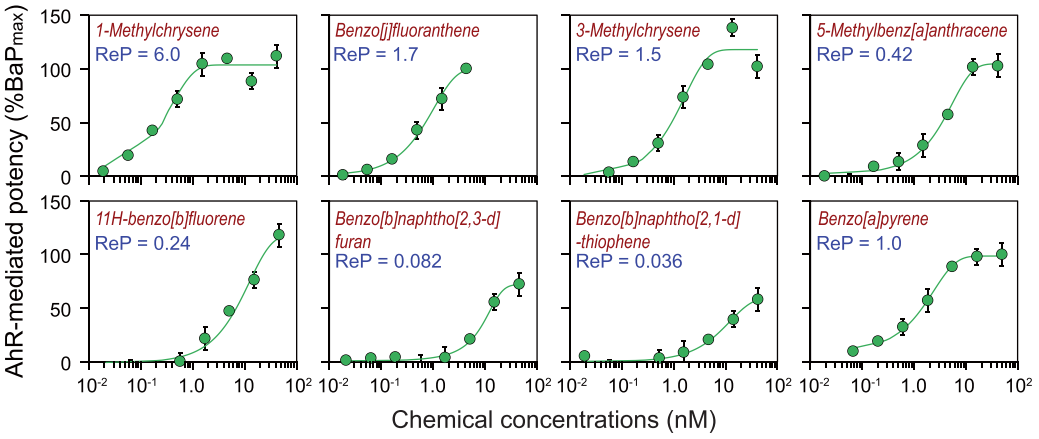

Figure 2. Dose−response relationships for AhR-mediated potencies of newly identified AhR-active compounds and benzo[*a*]pyrene in the H4IIE-*luc* bioassay (error bar: mean ± SD. n = 3. ReP: relative potency value).

a previous study, conducted in 1999, indicated that sources of PAHs in sediments from Ulsan Bay, especially near the harbor and ship repairing dock, were mainly of petrogenic origin.[8] Current concentrations of sedimentary PAHs in Ulsan Bay were comparable to those reported 18 years ago; however, the main sources have changed from petrogenic to pyrogenic. Despite efforts to improve the environment of Ulsan Bay, the results of the present study indicate that the pollution of persistent toxic substances in the sediments of inland creeks remains serious.

To confirm portions of AhR-mediated potencies in fractions of sediment organic extracts (bioassay-derived BaP-EQs) that could be explained by known AhR agonists, such as PAHs and SOs (instrument-derived BEQs), potency balance analysis was performed.[48,49] The subfractions that exhibited greatest potencies, such as F2.6 and F2.7, were targeted. RePs of 7 PAHs and 3 SOs were used to calculate instrument-derived BEQs. Results indicated that concentrations of target AhR agonists in F2.6 (i.e., BaA, Chr, SD1, and SD3) and F2.7 (i.e., BbF, BaP, BkF, IcdP, DahA, and ST2) explained only a small portion of bioassay-derived BaP-EQs (mean = 22%) except for C2 (Table 1). Instrument-derived BEQs in F2.6 of the C2 sediment extract was approximately a factor of 2 greater compared to bioassay-derived BaP-EQ. This phenomenon could be attributed to mixture effects among chemicals (i.e., antagonism).[50,51] The main cause of this mixture effect was not elucidated, but the compound group was less or more toxic when present as an individual compound.[11]

Full-Scan Screening Analysis and Chemical and Biological Confirmation. Greatest AhR-mediated potencies were found in F2.6 and F2.7 of organic extract of sediment from site C2. Thus, these fractions were subjected to FSA using GC-QTOFMS and GC-MSD. Selection for candidates of AhR agonists from the results of GC-QTOFMS analyses consisted of four steps (Figure S5). First, formula derived from accurate mass were compared to those in the NIST library, although two-step fractionations of sediment organic extracts, 405 and 463 compounds were detected in F2.6 and F2.7, respectively. Overall, 477 compounds (222 for F2.6 and 255 for F2.7) in the fractions had matching factor scores greater than 70. Next, 75 and 145 compounds were selected as aromatics in F2.6 and F2.7, respectively. Finally, 13 and 57 compounds with 3−6 benzene rings were detected in F2.6 and F2.7, respectively. These chemicals were selected as tentative AhR-active compounds based on FSA (Table S6).

Chemical properties of a total of 16 candidate AhR-active compounds, including GC retention times and mass fragment ion patterns, were determined using GC-MSD (Table S2). Concentrations of these chemicals in sediments were then determined (Table S7). During biological characterization of the 16 candidate compounds, 7 compounds, such as 1MC, BjF, 3MC, 5MBA, 11BF, BBNF, and BBNT (Figure S6) showed significant AhR responses in the H4IIE-*luc* bioassay (significant level = 5% BaP_{max}) (Figure 2). ReP values of seven compounds for the AhR-mediated potency compared to that of BaP were obtained by use of dose−response relationships. There is an uncertainty in the ReP estimations for compounds with lesser AhR-mediated potencies.[25,34] However, the AhR-mediated potencies of 7 newly identified AhR agonists are sufficiently great (58−118% BaP_{max}), and the variations among ReP20, ReP50, and ReP80 were generally small for most estimates (Table S8). Thus, the use of ReP50 as a parameter for indicating AhR-mediated potency is considered as reliable.[33,34]

Assay-Specific Relative Potency Values. Assay-specific ReP values for traditional PAHs such as BaA, Chr, BbF, BkF, BaP, IcdP, and DahA were newly obtained by use of H4IIE-*luc* for 4 h exposure as part of our study. ReP values for traditional PAHs were generally similar when compared to the previously reported values using PLHC-1 cell line.[31] However, the ReP values of BkF and DahA were different, seemingly due to differences in species origin between the cell lines. We used assay-specific ReP values for more accurate comparison between instrumental analysis and bioassay results in this study (Tables S3 and S7). Meanwhile, among the 7 newly identified compounds, 1MC (6.0), BjF (1.7), and 3MC (1.5) exhibited great RePs values for AhR-mediated activity compared to BaP. Although the ReP values of other compounds were not greater than that of BaP, they were comparable to well-known AhR-active compounds, such as BaA, Chr, and BbF (Table S3). Some of these chemicals, such as 11BF, 1MC, 3MC, and BjF, had been previously reported to act as AhR agonists.[12,13,52,53] In this study, we confirmed that the previously untargeted and poorly monitored chemicals (e.g., 7 newly identified compounds) have strong AhR-mediated potency and occurred in sediments of industrial area. To the best of our knowledge, the remaining compounds, BBNT, BBNF, and 5MBA, were confirmed as novel AhR

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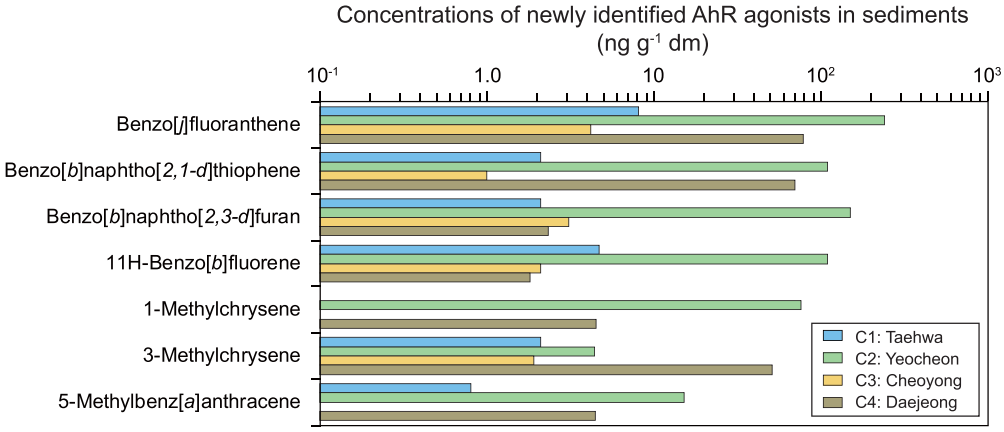

Figure 3. Distributions and concentrations of newly identified AhR-active compounds in organic extracts of sediments from the inland creeks of Ulsan Bay, Korea.

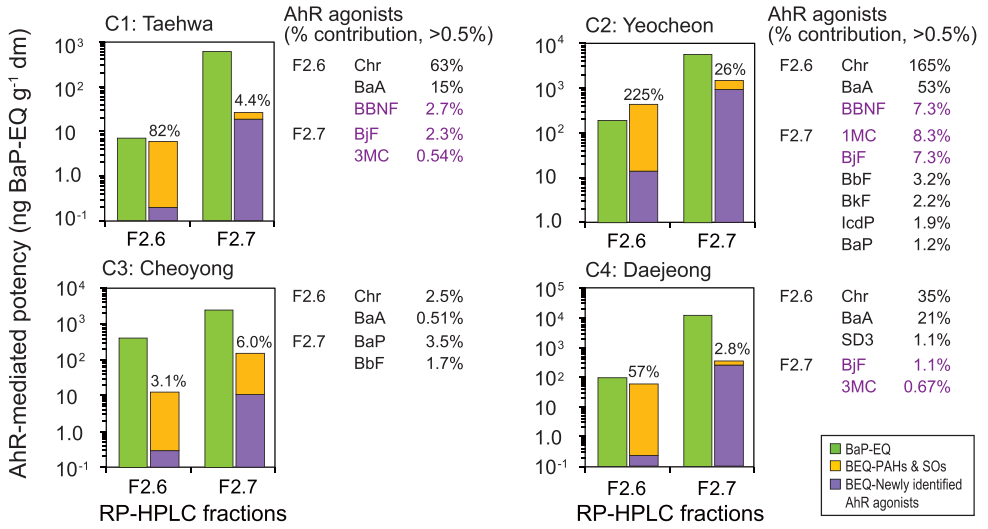

Figure 4. Potency balance analysis between bioassay-derived BaP-EQs and instrument-derived BEQs in the RP-HPLC fractions (F2.6 and F2.7) of organic extracts of sediments from inland creeks connected to Ulsan Bay, Korea (percentage numbers in the figure indicate total contributions of traditional and newly identified AhR agonists to BaP-EQs).

agonists occurring in sediments, with ReP values being reported for the first time in the present study.

Previous studies using the H4IIE-*luc* bioassay have shown that the AhR-mediated potency is additive in a mixture of PAHs.[51,54] Additive activities of PAHs mixtures along with the insignificant effect of the soil matrix support the use of concentration addition in potency balance calculations.[54] The parallelism of the dose−response curves of AhR agonists means that the mode of toxic action is likely the same; thus, the response would be additive. In the present study, equal efficacy and parallelism between BaP and newly identified AhR agonists were met (Table S7). Thus, in this study, contributions of traditional and newly identified AhR agonists to the total AhR potencies were evaluated by use of potency balance analysis assuming that it acts as additive. However, environmental samples are very complex in composition, which sometimes causes mixture effects to increase or decrease the AhR-mediated potency.[55] There is a case that the explanatory power exceeds 100% even in our sample (Table 1), and it can be guessed that the mixture effect occurred. Future studies will need to be conducted on the mixture toxic effects of environmental samples.

Distribution of Newly Identified AhR Agonists in Sediments. Newly identified AhR agonists were widely distributed in sediments from Ulsan Bay (Figure 3 and Table S7). Great concentrations of newly identified AhR agonists were detected in the sediments from sites C4 and C2. BjF had the greatest concentrations of the novel AhR-active compounds in organic extracts of sediments, followed by BBNF and BBNF. Newly identified AhR-active PAHs were detected greatly in sediments of industrial areas and seemed to originate from the surrounding industrial area. Previous studies have shown the sources of these AhR-active compounds.[56−61] For instance, BBNT primarily originates from coal tar, crude oil, shale oil, engine oil, diesel exhaust, tobacco, and aluminum reduction plant.[56] 5MBA is thought to originate from crude oil, urban dust, sedimentary rock, and tobacco smoke.[57] 11BF originates from coal tar and fossil fuel combustion emission.[58,59] BjF seems to originate from pyrolysis and fuel-rich combustion of bituminous coal primary tar.[60] In addition, 1MC and 3MC originate from the processes of wood combustion.[61] The results of this study provide useful information about novel AhR-active compounds for future research work.

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Improved Potency Balance Analysis. Potency-based BaP-EQs derived by use of the H4IIE-*luc* bioassay could not be fully explained by known AhR agonists in sediment extracts, except for the F2.6 of C2 (Table 1). An improved potency balance analysis was performed to determine to what extent the newly identified compounds could explain unexplained portions of concentrations of BaP-EQs (Figure 4). The potency balance showed power to explain concentrations of BaP-EQ increased by including newly identified AhR agonists (mean = 4.1%) (Table 1). In F2.6, BBNF contributed significantly to BaP-EQs in C1 (2.7%) and C2 (7.3%) due to the relatively great concentrations, although with a small ReP value (0.082). BjF, 1MC, and 3MC significantly contributed to the BaP-EQs of F2.7 in C2, C1, and C4 sediments. 1MC was the most potent AhR agonist (ReP = 6.0); this compound was a major contributor in F2.7 of C2 sediment. However, the explanatory powers of 1MC in sites C1 and C3 were not significant because it occurred at lower concentrations in the samples (<D.L.). Among the traditional AhR agonists, Chr and BaA were found to be the major contributors in F2.6, and BaP, BbF, and BkF were contributed significantly in F2.7. Overall, the results of potency balance analysis indicated that the major AhR agonists in sediments of Ulsan Bay were site specific.

Powers to explain AhR-mediated potencies in sediment organic extracts were significantly improved by including the newly identified AhR agonists determined by using EDA combined with FSA; however, a large quantity of unexplained portions remained. Thus, other unknown AhR agonists might exist in sediments. A previous study indicated that several untargeted PAHs and/or oxygenated-, methylated-, and N-containing derivatives (e.g., benzo[*a*]fluorene, naphthacene, 9,10-dihydrobenzo[*a*]pyrene-7(8*H*)-none, dibenzo[*a*,*h*]-acridine, 7*H*-benz[*d*,*e*]anthracnene, and 2-methylanthracene-9,10-dione) are AhR-active chemicals.[56] However, these chemicals were not detected during the study, results of which are presented here. Of the final 70 compounds identified through the selection criteria, due to the lack of authentic standards, only 9 compounds could be considered. Thus, additional toxicological confirmation for the remaining compounds is required. Meanwhile, some of AhR agonists identified in this study have also other potential toxicities. For instance, 1MC showed great estrogen receptor-mediated potency using the MVLN bioassay.[62] BjF has been reported to cause significant tumorigenic reactions.[63] In addition, 11BF causes developmental toxicity to the zebrafish *Danio rerio*.[64] Therefore, to assess the ecotoxicological effects of AhR-active compounds in coastal environments, effect-based management with various end points should be considered.

In the future, studies identifying mechanisms that lead to the production of different ReP values among compounds as well as sources and fate of novel AhR agonists in the environments and ecotoxicological effects are strongly needed. In particular, PAHs are a group of compounds that are unwanted byproducts during the combustion process and can generate many unknown toxic substances simultaneously; thus, we suggest that more studies on unknown toxic PAHs are needed. The present study is a much more complete outcome than previous EDA studies that did not perform biological confirmation using toxicity testing and/or has been limited to suggest candidates for toxic substances based on quantitative structure−activity relationship (QSAR) model. Overall, the EDA approach combined with FSA used in the present study is expected to prove very useful for selecting effect-based chemicals of concern in terms of ecological risk assessment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b02166.

Additional details of instrumental conditions, raw data of chemical concentrations and bioassay, FSA results, sampling locations, and other helpful materials (PDF)

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Notes

The authors declare no competing financial interest.

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<Supporting Information>

Newly Identified AhR-Active Compounds in the Sediments of An Industrial Area Using Effect-Directed Analysis

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Supplementary Tables

Supplementary Figures

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Supplementary Tables

Table S1. Instrumental conditions of GC-QTOFMS for full-scan screening analysis.

Instrument	GC : Agilent 7890B
	QTOFMS : Agilent 7200
Samples	C2 (Yeocheon Creek)
	F2.6 and F2.7 RP-HPLC fractions
Column	DB-5MS UI (30 m × 0.25 mm i.d. × 0.25 μm film)
Carrier gas	He
Flow rate	1.2 mL min^{-1}
Injection volume	2 μL
Mass range	50-600 *m*/*z*
Ion source temperature	230°C
Ionization mode	EI mode (70 eV)
Software	Qualitative analysis B.07.01
	MassHunter Quantitative analysis
	Unknown analysis
	NIST Library (ver. 2014)

Table S2. Chemical properties and GC-MSD retention times and mass fragment ions of candidate AhR-active compounds.

Compounds	**Abb.** [a]	**Molecular formula**	**CAS number**	**Molecular weight**	**GC RT** [b] **(min.)**	**Mass fragment ions (*m/z*)**
3-Methylphenanthrene	3MP	$C_{15}H_{12}$	832-71-3	192.256	25.797	***192*** [c], 191, 89
2-Methylphenanthrene	2MP	$C_{15}H_{12}$	2531-84-2	192.256	25.891	***192***, 191, 89
2-Methylanthracene	2MA	$C_{15}H_{12}$	613-12-7	192.256	26.041	***192***, 191, 189
9-Ethylphenanthrene	9EP	$C_{16}H_{14}$	3674-75-7	206.282	27.597	***191***, 206, 189
1,6-Dimethylphenanthrene	16DMP	$C_{16}H_{14}$	20291-74-1	206.282	28.137	***206***, 191, 189
1,2-Dimethylphenanthrene	12DMP	$C_{16}H_{14}$	20291-72-9	206.282	28.896	***206***, 191, 189
Benzo[*b*]naphtho[*2,3-d*]furan	BBNF	$C_{16}H_{10}O$	243-42-5	218.255	29.881	***218***, 189, 219
11H-Benzo[*b*]fluorene	11BF	$C_{17}H_{12}$	243-17-4	216.277	30.991	***216***, 215, 213
Benzo[*b*]naphtho [*2,1*-d]thiophene	BBNT	$C_{16}H_{10}S$	239-35-0	234.316	33.098	***234***, 235, 232
Triphenylene	TRI	$C_{18}H_{12}$	228.2879	228.294	34.156	***228***, 226, 229
3-Methylchrysene	3MC	$C_{19}H_{14}$	3351-31-3	242.315	35.718	***242***, 241, 293
5-Methylbenz[*a*]anthracene	5MBA	$C_{19}H_{14}$	2319-96-2	242.315	35.998	***242***, 241, 239
Benzo[*c*]phenanthrene, 1,12-Dimethyl	BCP	$C_{20}H_{16}$	4076-43-1	256.341	36.107	***256***, 241, 239
1-Methylchrysene	1MC	$C_{19}H_{14}$	3351-28-8	242.315	36.180	***242***, 241, 293
Benzo[*j*]fluoranthene	BjF	$C_{20}H_{12}$	205-82-3	252.309	38.032	***252***, 253, 250
Benzo[*e*]pyrene	BEP	$C_{20}H_{12}$	192-97-2	252.309	38.868	***252***, 250, 253

[a] Abb.: Abbreviations.

[b] GC RT: Gas chromatography retention time.

[c] Quantification ion.

Table S3. Relative potency values for AhR-mediated activity of PAHs and SOs obtained from this study and previously reported values.

Compounds	**Abb.** [a]	**Relative potency values (RePs)**		
		H4IIE-*luc*, 4h exposure (This study)	PLHC-1, 4h exposure (Louiz et al., 2008)	H4IIE-*luc*, 4h exposure (Hong et al., 2016)
Acenaphthylene	Acl	ns [b]	5.56×10^{-3}	
Fluoranthene	Fl	ns	1.44×10^{-2}	
Pyrene	Py	ns	3.58×10^{-3}	
Benz[*a*]anthracene	BaA	3.2×10^{-1}	2.58×10^{-1}	
Chrysene	Chr	8.5×10^{-1}	2.92×10^{-1}	
Benzo[*b*]fluoranthene	BbF	5.0×10^{-1}	6.94×10^{-1}	
Benzo[*k*]fluoranthene	BkF	4.8×10^{-1}	2.94	
Benzo[*a*]pyrene	BaP	1.0	1.0	1.0
Indeno[*1,2,3-c,d*]pyrene	IcdP	5.8×10^{-1}	8.43×10^{-1}	
Dibenz[*a,h*]anthracene	DahA	6.6×10^{-1}	3.66	
1,3-Diphenylpropane	SD1			2.3×10^{-3}
2,4-Diphenyl-1-butene	SD3			3.0×10^{-4}
2,4,6-Triphenyl-1-hexene	ST1			2.7×10^{-3}

[a] Abb.: Abbreviations.
[b] ns: Not significant.

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Table S4. Concentrations of PAHs in the sediments of inland creeks of Ulsan Bay.

Compounds	**Abb.**[a]	**ISQG**[b]	**C1**	**C2**	**C3**	**C4**
(ng g^{-1} dry mass)						
Naphthalene	Na	34.6	2.6	77 [c]	6.1	34
Acenaphthene	Ace	6.71	< DL[d]	7.8	0.7	< DL
Acenaphthylene	Acl	5.87	< DL	58	< DL	41
Fluorene	Flu	21.2	1.1	85	1.2	13
Phenanthrene	Phe	86.7	5.6	680	11	99
Antracene	Ant	46.9	1.0	120	1.4	3.0
Fluoranthene	Fl	113	8.2	520	15	86
Pyrene	Py	153	9.4	500	19	100
Benzo[a]anthracene	BaA	74.8	3.2	290	6.0	59
Chrysene	Chr	108	4.9	340	11	37
Benzo[b]fluoranthene	BbF		5.5	360	86	8.6
Benzo[k]fluoranthene	BkF		< DL	70	88	5.0
Benzo[a]pyrene	BaP	88.8	3.3	260	6.3	75
Indeno[1,2,3-cd]pyrene	IcdP		5.2	210	10	42
Dibenz[a,h]anthracene	DahA	6.22	0.8	74	2.6	22
Benzo[g,h,i]perylene	BghiP		8.4	380	14	110
Sum of PAHs			60	4000	280	740

[a] Abb.: Abbreviations.

[b] ISQG: Interim sediment quality guidelines (ISQGs) recommended by the Canadian Council of Ministers of the Environment (CCME, 2002).

[c] Shade indicates concentrations that exceed ISQG values.

[d] < DL: Below detection limits.

Canadian Council of Ministers of the Environment (CCME), Canadian sediment quality guidelines for the protection of aquatic life summary tables. CCME: Winnipeg, MB, **2002**.

Table S5. Concentrations of SOs in the sediments of inland creeks of Ulsan Bay.

Compounds (ng g^{-1} dry mass)	**Abb.[a]**	**C1**	**C2**	**C3**	**C4**
1,3-Diphenylproane	SD1	1.2	2.7	< DL	6.9
cis-1,2-Diphenylcyclobutane	SD2	< DL[b]	< DL	1.0	11
2,4-Diphenyl-1-butene	SD3	13	47	64	2900
trans-1,2-Diphenylcyclobutane	SD4	1.0	2.9	5.9	72
4,6-Triphenyl-1-hexene	ST1	4.3	7.6	7.2	20
1e-Phenyl-4e-(1-phenylethyl)-tetralin	ST2	1.6	3.6	8.4	11
1a-Phenyl-4e-(1-phenylethyl)-tetralin	ST3	3.2	5.9	10	11
1a-Phenyl-4a-(1-phenylethyl)-tetralin	ST4	3.1	1.7	5.0	1.8
1e-Phenyl-4a-(1-phenylethyl)-tetralin	ST5	8	29	36	720
1,3,5-Triphenylcyclohexane (isomer mix)	ST6	0.62	< DL	3.3	5.5
Sum of SOs		36	100	140	3700

[a] Abb.: Abbreviations.
[b] < DL: Below detection limits.

Table S6. List of candidates for AhR agonists in fractions samples (F2.6 and F2.7) of organic extracts of sediments from C2 based on the GC-QTOFMS and GC-MSD.

Fraction	Compounds	CAS#	MW[a]	Formula	RT[b]	Matching Factor	AhR agonists
GC-QTOFMS							
F2.6	Benzo[*b*]naphtho[*2,3-d*]furan	243-42-5	218	$C_{16}H_{10}O$	29.368	74	+
	Benzo[*kl*]xanthene	200-23-7	218	$C_{16}H_{10}O$	17.951	80	
	Benzo[*b*]naphtho[*1,2-d*]thiophene	205-43-6	234	$C_{16}H_{10}S$	21.659	81	
	Phenanthrene, 4,5-dimethyl-	3674-69-9	206	$C_{16}H_{14}$	15.790	84	
	Naphthacene, 5,12-dihydro-	959-02-4	230	$C_{18}H_{14}$	20.157	86	
	Naphtho[*2,1-b*]benzofuran	205-39-0	218	$C_{16}H_{10}O$	17.513	86	
	Benzo[*c*]phenanthrene	195-19-7	228	$C_{18}H_{12}$	22.157	87	
	2-Methylphenanthrene	2531-84-2	192	$C_{15}H_{12}$	25.517	87	ns[c]
	Phenanthro[*9,10-b*]furan	235-98-3	218	$C_{16}H_{10}O$	17.745	93	
	1(2H)-phenanthrenone, 3,4,9,10-tetrahydro-	62264-34-0	198	$C_{14}H_{14}O$	14.695	93	
	1,1':4',1"-Terphenyl	92-94-4	230	$C_{18}H_{14}$	18.373	93	
	o-Terphenyl	84-15-1	230	$C_{18}H_{14}$	14.555	94	
	2-Methylanthracene	613-12-7	192	$C_{15}H_{12}$	14.656	94	
F2.7	2-(3-Chlorophenyl)-4-methylquinazoline	2000377-83-6	254	$C_{15}H_{11}ClN_2$	25.906	71	
	Anthra[*2,3-b*]benzo[*d*]thiophene	249-05-8	284	$C_{20}H_{12}S$	31.139	72	
	Methylbis(phenylmethyl)benzene	2000440-25-8	272	$C_{21}H_{20}$	20.702	72	
	2-Phenylphenalen-1-one	73873-15-1	256	$C_{19}H_{12}O$	24.449	72	
	Benzo[*b*]thiophene, 2-(2-naphthalenyl)-	17164-77-1	260	$C_{18}H_{12}S$	24.759	72	
	Triphenylene, 1,2,3,4-tetrahydro-	Not available	232	$C_{18}H_{16}$	21.773	73	
	2H-phenanthro[*9,10-b*]pyran	217-67-4	232	$C_{17}H_{12}O$	19.206	73	
	Pyrene, 1,9-dimethyl-	74298-70-7	230	$C_{18}H_{14}$	20.990	74	
	Benzo[*b*]naphtho[*2,1-d*]thiophene	239-35-0	234	$C_{16}H_{10}S$	32.611	74	+[d]
	1,1':2',1"-Terphenyl, 4'-ethyl-	61875-99-8	258	$C_{20}H_{18}$	16.901	76	
	1,1':4',1":4",1'"-Quaterphenyl	135-70-6	306	$C_{24}H_{18}$	30.506	76	
	Phenanthrene, 2,7-dimethyl-	1576-69-8	206	$C_{16}H_{14}$	16.185	76	
	Phenanthrene, 1,7-dimethyl-	483-87-4	206	$C_{16}H_{14}$	16.129	76	
	2-Methyl-9,10-dihydroanthracene	948-67-4	194	$C_{15}H_{14}$	14.048	76	
	4-Benzylbiphenyl	613-42-3	244	$C_{19}H_{16}$	19.532	77	
	11H-Indeno[*2,1-a*]phenanthrene	220-97-3	266	$C_{21}H_{14}$	28.095	77	
	5-Methoxy(5H)dibenzo[a,b]cycloheptene	55789-73-6	222	$C_{16}H_{14}O$	15.998	77	
	o-Terphenyl	84-15-1	230	$C_{18}H_{14}$	20.640	77	
	9H-Fluorene, 9-phenyl-	789-24-2	242	$C_{19}H_{14}$	21.451	78	
	Benzo[*b*]naphtho[*2,3-d*]thiophene, 7-methyl-	24964-09-8	248	$C_{17}H_{12}S$	23.466	78	
	p-Terphenyl	92-94-4	230	$C_{18}H_{14}$	17.826	78	

	1,2,3,4-Tetramethylanthracene	2000312-95-5	234	$C_{18}H_{18}$	18.654	78	
	Fluorantheno[*1,2-b*]thiophene	129527-38-4	258	$C_{18}H_{10}S$	26.958	80	
	Phenanthro[*9,10-b*]furan, 2-methyl-	36000-02-9	232	$C_{17}H_{12}O$	20.275	80	
	1,1':3',1"-Terphenyl, 5'-phenyl-	612-71-5	306	$C_{24}H_{18}$	28.520	80	
	4-Phenyldibenzofuran	2000345-36-0	244	$C_{18}H_{12}O$	20.614	81	
	2,2'-Binaphthalene	612-78-2	254	$C_{20}H_{14}$	24.290	81	
	5-Methylbenz[*a*]anthracene	2319-96-2	242	$C_{19}H_{14}$	35.644	81	+
	Phenanthrene, 4,5-dimethyl-	3674-69-9	206	$C_{16}H_{14}$	16.005	82	
	Phenanthrene, 1-methyl-7-(1-methylethyl)- retene	483-65-8	234	$C_{18}H_{18}$	20.160	83	
	2-(4-Methylphenyl)naphthalene	2000258-92-1	218	$C_{17}H_{14}$	16.973	83	
	m-Terphenyl	92-06-8	230	$C_{18}H_{14}$	18.377	84	
	7-Methylbenz[*a*]anthracene	2541-69-7	242	$C_{19}H_{14}$	23.947	84	
	9H-Cyclopenta[*a*]pyrene	50861-05-7	240	$C_{19}H_{12}$	24.121	85	
	10-Methylbenzo(a)pyrene	63104-32-5	266	$C_{21}H_{14}$	28.886	85	
	Benzo[*b*]naphtho[*2,3-d*]thiophene, 8-methyl-	24964-07-6	248	$C_{17}H_{12}S$	22.827	86	
	8H-Indeno[*2,1-b*]phenanthrene	241-28-1	266	$C_{21}H_{14}$	28.263	86	
	1,1':2',1":3",1"'-Quaterphenyl	1165-57-7	306	$C_{24}H_{18}$	21.336	86	
	Pyrene, 4,5,9,10-tetrahydro-	781-17-9	206	$C_{16}H_{14}$	16.618	86	
	Phenanthrene, 2,3,5-trimethyl-	3674-73-5	220	$C_{17}H_{16}$	17.932	87	
	Benzo[*b*]naphtho[*1,2-d*]thiophene	205-43-6	234	$C_{16}H_{10}S$	21.406	87	
	1,12-DimethylBenzo[*c*]phenanthrene	4076-43-1	256	$C_{20}H_{16}$	33.553	87	ns
	7H-Benzanthrene	199-94-0	216	$C_{17}H_{12}$	18.729	87	
	Benzo[*a*]pyrene, 4,5-dihydro-	57652-66-1	254	$C_{20}H_{14}$	22.656	88	
	1-Methylphenanthro[*4,5-bcd*]thiophene	88114-01-6	222	$C_{15}H_{10}S$	18.490	88	
	Benzo[*b*]chrysene	214-17-5	278	$C_{22}H_{14}$	31.242	89	
	Dibenz[*a,j*]anthracene	224-41-9	278	$C_{22}H_{14}$	31.650	90	
	Pyrene, 1-methyl-	2381-21-7	216	$C_{17}H_{12}$	19.406	90	
	Cyclopenta[*cd*]pyrene	27208-37-3	226	$C_{18}H_{10}$	21.213	91	
	1,1':2',1"-Terphenyl, 4'-phenyl-	1165-53-3	306	$C_{24}H_{18}$	25.355	93	
	11H-Benzo[*b*]fluorine	243-17-4	216	$C_{17}H_{12}$	30.449	93	+
	1,1':2',1":4",1"'-Quaterphenyl	1165-58-8	306	$C_{24}H_{18}$	25.230	94	+
	Phenanthrene, 2,5-dimethyl-	3674-66-6	206	$C_{16}H_{14}$	16.371	94	
	Perylene	198-55-0	252	$C_{20}H_{12}$	27.841	94	
	Benzo[*b*]fluoranthene	205-99-2	252	$C_{20}H_{12}$	27.520	95	
	Benzo[*e*]pyrene	192-97-2	252	$C_{20}H_{12}$	38.296	96	ns
	Triphenylene	217-59-4	228	$C_{18}H_{12}$	33.587	96	ns
GC/MSD							
F2.6	9-Ethylphenanthrene	3674-75-7	206	$C_{16}H_{14}$	27.071		ns
	3-Methylphenanthrene	832-71-3	192	$C_{15}H_{12}$	25.382		ns

	1,2-Dimethylphenanthrene	29062-98-4	206	$C_{16}H_{14}$	28.355	ns
	1,6-Dimethylphenanthrene	20291-74-1	206	$C_{16}H_{14}$	27.611	ns
F2.7	Benzo[*j*]fluoranthene	205-82-3	252	$C_{20}H_{12}$	37.473	+
	1-Methylchrysene	3351-22-8	242	$C_{19}H_{14}$	35.644	+
	3-Methylchrysene	3351-31-3	242	$C_{19}H_{14}$	35.461	+

[a] MW: Molecular weight.
[b] RT: Retention time.
[c] ns: bioassayed but not significant response.
[d] +: significant AhR activity found.

Table S7. Relative potency values for newly identified AhR agonists relative to the potency of BaP in the H4IIE-*luc* bioassay.

Compounds	**%BaP$_{max}$[a]**	**Slope**	**ReP20-50-80[b]**		
			ReP20	**ReP50**	**ReP80**
Benzo[*a*]pyrene	100	49	1.0	1.0	1.0
1-Methylchrysene	111	53	5.4	6.0	6.8
Benzo[*j*]fluoranthene	100	59	1.4	1.7	2.2
3-Methylchrysene	102	66	1.0	1.5	2.2
5-Methylbenz[*a*]anthracene	102	61	0.31	0.42	0.56
11H-benzo[*b*]fluorene	118	61	0.18	0.24	0.31
Benzo[*b*]naphtho[*2,3-d*]furan	72	54	0.070	0.082	NQ[c]
Benzo[*b*]naphtho[*2,1-d*]thiophene	58	29	0.091	0.036	NQ

[a] %BaP$_{max}$: Max-efficacy found to be over 50%BaP$_{max}$, appropriate to report ReP20-50 values for those compounds.
[b] ReP20-50-80: RePs reported as the range of ReP values generated from multiple point values over a response range from 20 to 50 to 80%BaP$_{max}$.
[c] NQ: not quantifiable for ReP calculation, dose–response relationship insufficient for estimation.

Table S8. Concentrations of candidates for AhR agonists and newly identified AhR agonists in the sediments of inland creeks of Ulsan Bay.

Compounds (ng g^{-1} dry mass)	**AhR agonists**	**C1**	**C2**	**C3**	**C4**
3-Methylphenanthrene	ns[a]	2.1	87	4.1	7.0
2-Methylphenanthrene	ns	3.4	58	6.3	13
2-Methylanthracene	ns	1.1	150	< DL	< DL
9-Ethylphenanthrene	ns	< DL[c]	100	0.72	< DL
1,6-Dimethylphenanthrene	ns	3.4	100	2.4	240
1,2-Dimethylphenanthrene	ns	0.6	28	< DL	4.0
Benzo[*b*]naphtho[*2,3-d*]furan	+[b]	2.1	150	3.1	2.3
11H-Benzo[*b*]fluorene	+	4.7	110	2.1	1.8
Benzo[*b*]naphtho[*2,1-d*]thiophene	+	2.1	110	1.0	70
Triphenylene	ns	12	290	13	57
3-Methylchrysene	+	2.1	4.4	1.9	51
5-Methylbenz[*a*]anthracene	+	0.8	15	< DL	4.4
Benzo[*c*]phenanthrene, 1,12-dimethyl	ns	< DL	< DL	< DL	< DL
1-Methylchrysene	+	< DL	6.8	< DL	16
Benzo[*j*]fluoranthene	+	< DL	76	< DL	4.5
Benzo[*e*]pyrene	ns	8.1	240	4.2	79
Sum of compounds	ns	40	1500	39	550

[a] ns: bioassayed but not shown significant response.
[b] +: significant AhR activity found.
[c] < DL: Below detection limits.

Supplementary Figures

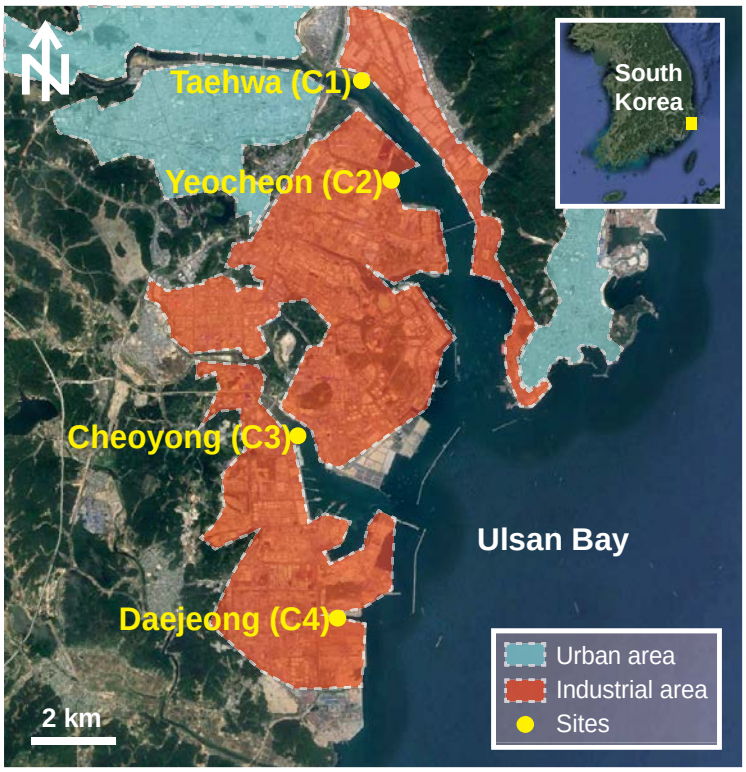

Figure S1. Sampling sites of sediments from inland creeks of Ulsan Bay, South Korea (June, 2017).

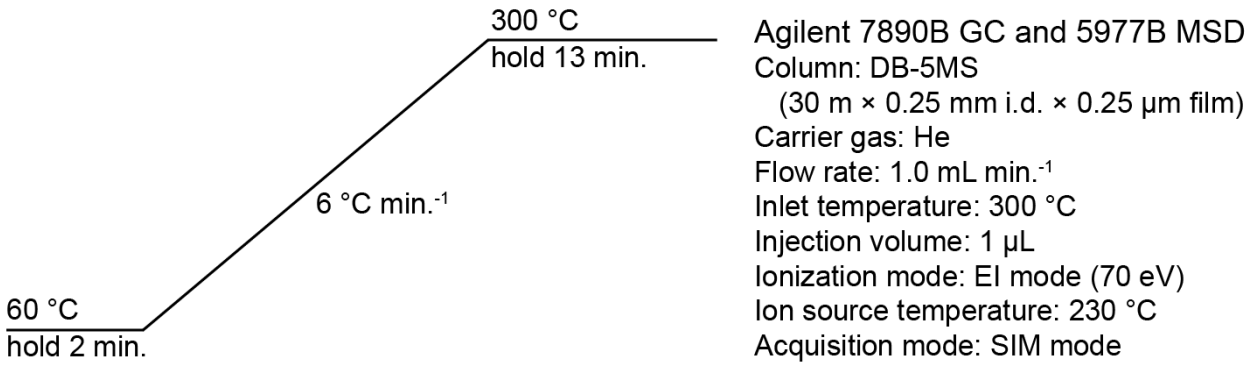

Figure S2. Instrumental conditions of GC-MSD for target analyses of PAHs and styrene oligomers.

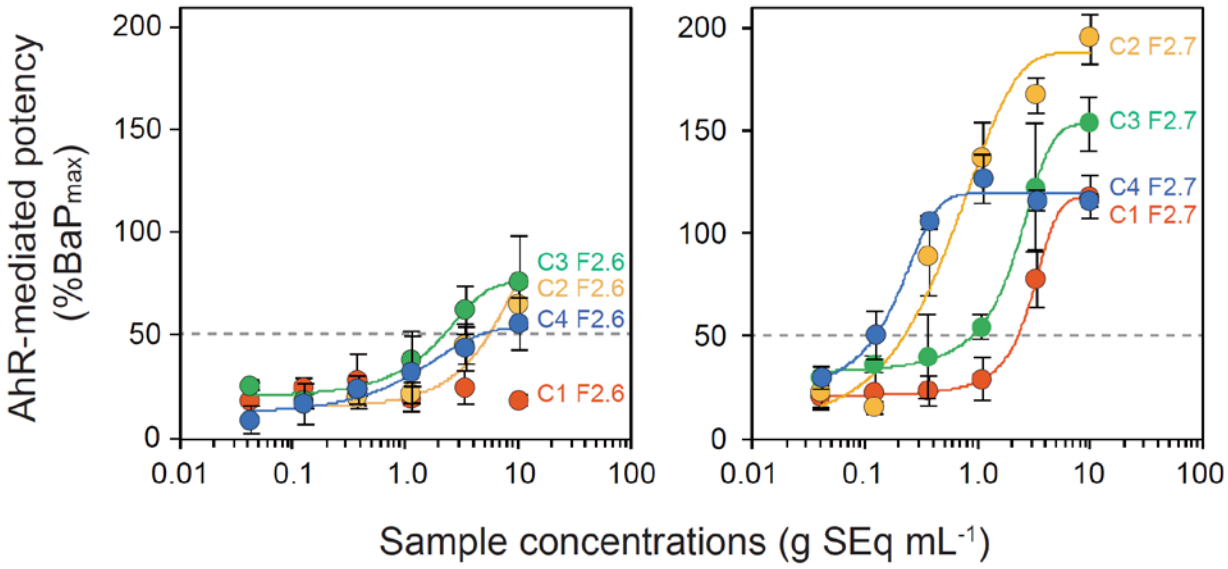

Figure S3. Dose-response curves for the AhR-mediated potency of the RP-HPLC fractions (F2.6 and F2.7 of C1–C4 sediment extracts) from inland creeks of Ulsan Bay, South Korea (SEq: sediment equivalents; Error bar: mean ± SD; n = 3).

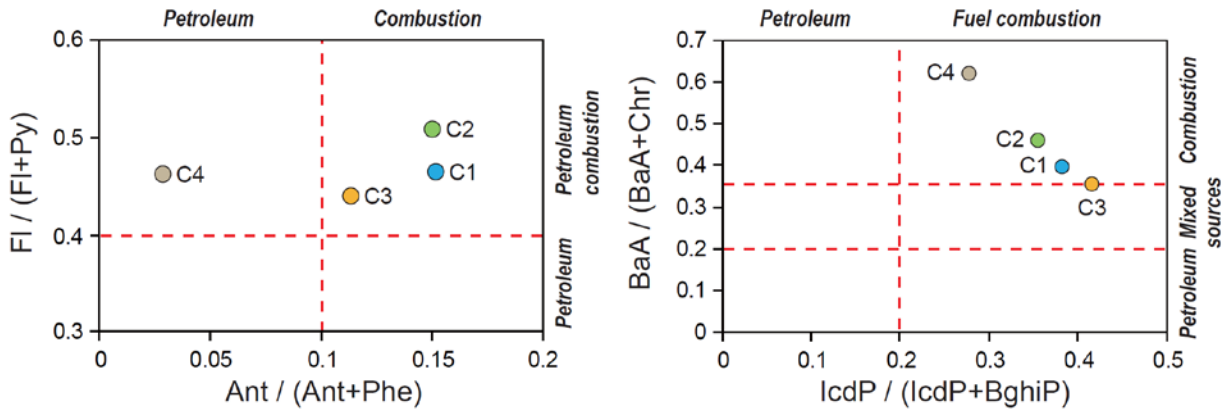

Figure S4. Diagnostic ratios for source identification of PAHs in sediments from inland creeks of Ulsan Bay, South Korea (Fl: fluoranthene; Py: pyrene; Ant: anthracene; Phe: phenanthrene; BaA: benzo[*a*]anthracene; Chr: chrysene; IcdP: indeno[*1,2,3-cd*]pyrene; BghiP: benzo[*g,h,i*]perylene; modified from Bucheli et al. (2004), Wang et al. (2017), and Yunker et al. (2002)).

Bucheli, T. D.; Blum. F.; Desaules, A.; Gustafsson, Ö. Polycyclic aromatic hydrocarbons, black carbon, and molecular markers in soils of Switzerland. *Chemosphere*. **2004**, 56, 1061-1076.

Wang, C.; Wu, S.; Zhou, S.; Shi, Y.; Song, J. Characteristics and source identification of polycyclic aromatic hydrocarbons (PAHs) in urban soils: A Review. *Pedosphere* **2017**, 27, (1), 17-26.

Yunker, M. B.; Macdonald, R. W.; Vingarzan, R.; Mitchell, R. H.; Goyette, D.; Sylvestre, S. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Org. Geochem*. **2002**, 33, (4), 489-515.

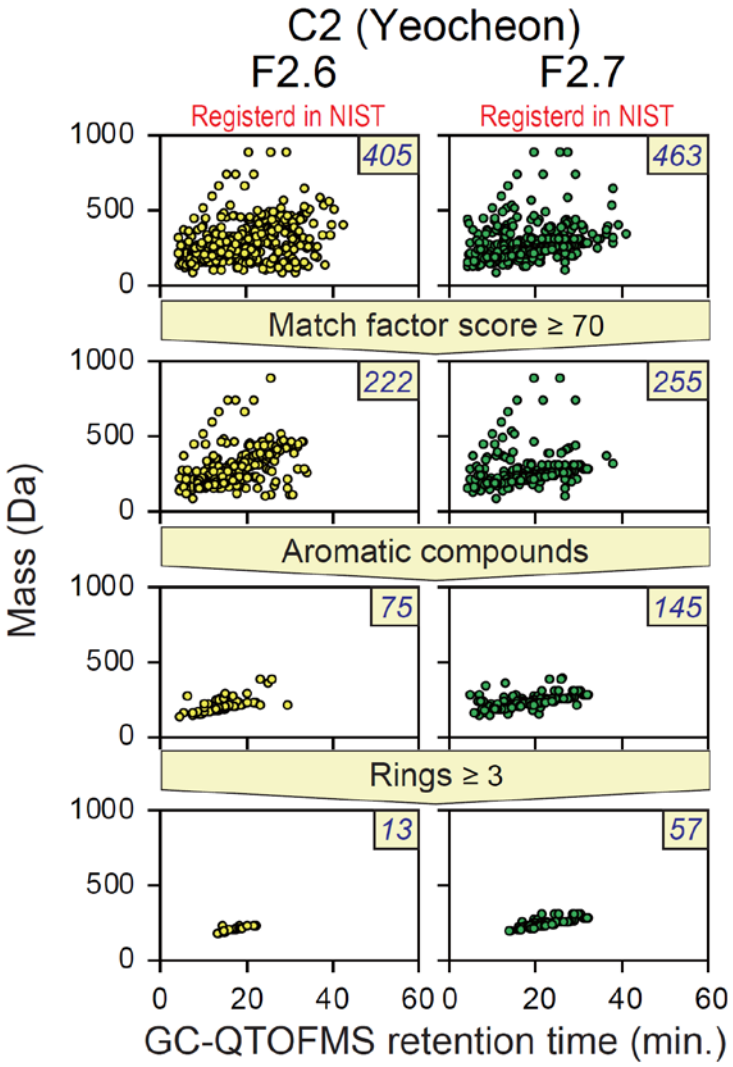

Figure S5. Selection criteria of candidates for AhR agonists and the results of GC-QTOFMS data analysis (The numbers in boxes represent the number of detected compounds).

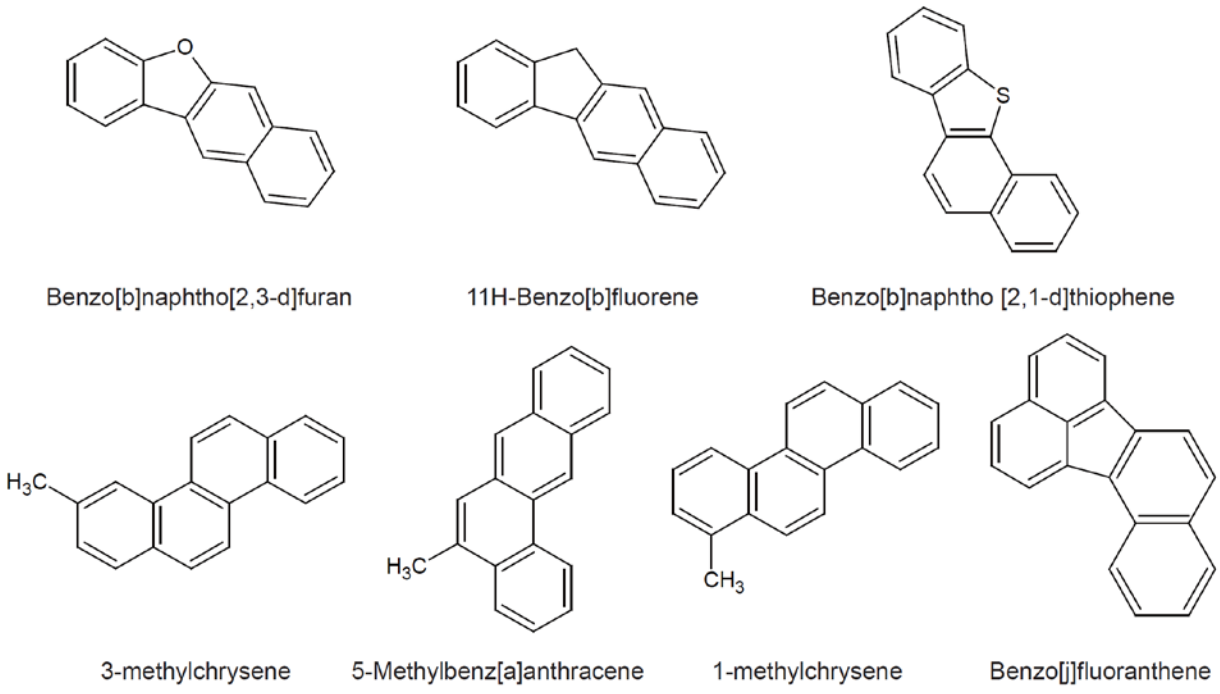

Figure S6. Chemical structures of 7 newly identified AhR agonists in sediments from inland creeks of Ulsan Bay, South Korea.