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Major AhR-active chemicals in sediments of Lake Sihwa, South Korea: Application of effect-directed analysis combined with full-scan screening analysis

Jihyun Cha[a], Seongjin Hong[a,*], Jaeseong Kim[a], Junghyun Lee[b], Seo Joon Yoon[b], Sunggyu Lee[c], Hyo-Bang Moon[c], Kyung-Hoon Shin[c], Jin Hur[d], John P. Giesy[e,f], Jong Seong Khim[b]

[a] Department of Ocean Environmental Sciences, Chungnam National University, Daejeon 34134, Republic of Korea
[b] School of Earth and Environmental Sciences & Research Institute of Oceanography, Seoul National University, Seoul 08826, Republic of Korea
[c] Department of Marine Science and Convergence Engineering, Hanyang University, Ansan 15588, Republic of Korea
[d] Department of Environment & Energy, Sejong University, Seoul 05006, Republic of Korea
[e] Department of Veterinary Biomedical Sciences & Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada
[f] Department of Environmental Science, Baylor University, Waco, TX 76798-7266, United States

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ABSTRACT

This study utilized effect-directed analysis (EDA) combined with full-scan screening analysis (FSA) to identify aryl hydrocarbon receptor (AhR)-active compounds in sediments of inland creeks flowing into Lake Sihwa, South Korea. The specific objectives were to (i) investigate the major AhR-active fractions of organic extracts of sediments by using H4IIE-*luc in vitro* bioassay (4 h and 72 h exposures), (ii) quantify known AhR agonists, such as polycyclic aromatic hydrocarbons (PAHs) and styrene oligomers (SOs), (iii) identify unknown AhR agonists by use of gas chromatography-quadrupole time-of-flight mass spectrometry (GC-QTOFMS), and (iv) determine contributions of AhR agonists to total potencies measured by use of the bioassay. FSA was conducted on fractions F2.6 and F2.7 (aromatics with log K_{ow} 5–7) in extracts of sediment from Siheung Creek (industrial area). Those fractions exhibited significant AhR-mediated potency as well as relatively great concentrations of PAHs and SOs. FSA detected 461 and 449 compounds in F2.6 and F2.7, respectively. Of these, five tentative candidates of AhR agonist were selected based on NIST library matching, aromatic structures and numbers of rings, and available standards. Benz[*b*]anthracene, 11H-benzo[*a*]fluorene, and 4,5-methanochrysene exhibited significant AhR-mediated potency in the H4IIE-*luc* bioassay, and relative potencies of these compounds were determined. Potency balance analysis demonstrated that these three newly identified AhR agonists explained 1.1% to 67% of total induced AhR-mediated potencies of samples, which were particularly great for industrial sediments. Follow-up studies on sources and ecotoxicological effects of these compounds in coastal environments would be required.

1. Introduction

Currently available biological tests to detect toxicants remain limited and are failing to provide sufficient information on the compounds responsible for measurable toxic effects (Brack, 2003). Instrumental analyses do not consider how chemicals interact in complex mixtures; thus, are providing little information on potential effects on biota (Brack, 2003; Rijk et al., 2009; Wang et al., 2014). Effect-directed analysis (EDA) represents a powerful tool for identifying chemicals in environmental samples, such as sediments, crude oil, wastewater, and biota (Brack, 2003; Brack et al., 2016; Hong et al., 2016a; Muschket et al., 2018). A typical EDA study consists of a bioassay, fractionation, and instrument analyses. The approach has been successfully utilized to identify and address key toxicants in environmental matrices (Brack, 2003; Brack et al., 2016; Muschket et al., 2018). For instance, EDA was used to investigate major aryl hydrocarbon receptor (AhR) and estrogen receptor (ER) agonists in sediments of the west coast of South Korea (Jeon et al., 2017). Another EDA study using combined *in vitro* and *in vivo* assays identified toxicologically active compounds in wetland sediments from North-Eastern Spain (Regueiro et al., 2013). Although

* Corresponding author at: Department of Ocean Environmental Sciences, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea.
E-mail address: hongseongjin@cnu.ac.kr (S. Hong).

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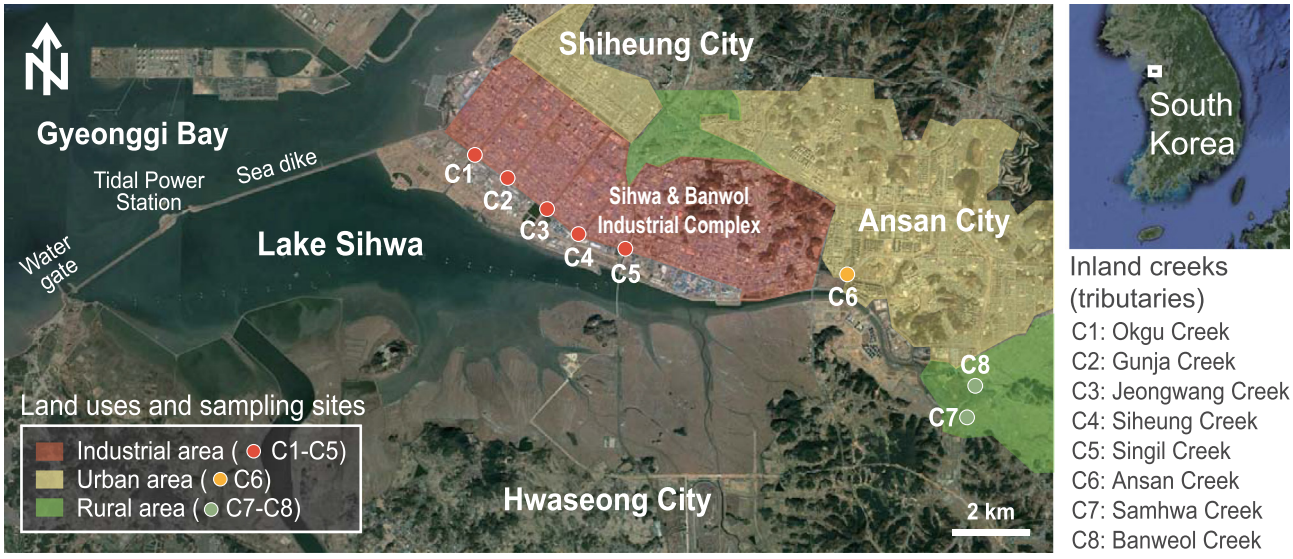

Fig. 1. Sites from which sediments were collected from the inland creeks (tributaries) of Lake Sihwa, South Korea.

EDA is useful for identifying causative agents in environmental samples, use of targeted chemical analysis alone, cannot fully explain responses of bioassays (Hong et al., 2016b; Jeon et al., 2017; Kim et al., 2019; Lee et al., 2017).

Full-scan screening (untargeted) analysis (FSA) has become a widely used technique to find previously unidentified compounds in environmental samples by use of high-resolution mass spectrometry (HRMS) such as time-of-flight mass spectrometry (TOFMS) (Gomez et al., 2009; Ibáñez et al., 2008; Schymanski et al., 2015; Tousova et al., 2018; Zedda and Zwiener, 2012). Because samples might include hundreds to thousands of chemicals, HRMS isolates neighboring peaks that have not been separated by low-resolution mass spectrometry because of the similar *m/z*; and thus increases selectivity for screening unknown toxicants (Hernandez et al., 2012; Zedda and Zwiener, 2012). However, it is laborious finding key toxicants by use of FSA. Thus, a stepwise approach is necessary, i.e., checking the matching score, identifying molecular formulas in libraries or databases, and then confirmation by use of authentic standards (Booij et al., 2014; Hollender et al., 2017; Muz et al., 2017). When supported by EDA, FSA became more powerful to identify candidate compounds and previously undescribed toxicants in complex environmental media. For example, this approach successfully identified AhR agonists in sediments of the Three Gorges Reservoir in China (Xiao et al., 2016), and previously undescribed anti-androgenic compounds in surface water of a nearby upstream wastewater treatment plant (WWTP) of Silstedt, Germany (Muschket et al., 2018).

Lake Sihwa is an artificial lake surrounded by the cities of Siheung, Ansan, and Hwaseong on the west coast of South Korea (Khim et al., 1999; Khim and Hong, 2014; Lee et al., 2014, 2017). Sihwa industrial complexes include a variety of businesses, such as metal, petrochemical, biochemical, and engineering manufacturing. During the past 20 years, various chemicals originating from industrial areas have been discharged to inland creeks, which has resulted in deterioration of the environment of Lake Sihwa (Hong et al., 2016b; Lee et al., 2014, 2017; Yoo et al., 2006). To improve the environment around Lake Sihwa, in 2011, the Korean Government designated a special coastal management zone, and in 2013, implemented a system to manage total loadings of contaminants (Hong et al., 2016b; Lee et al., 2014). Although, in recent years, management efforts have been applied to the environment of Lake Sihwa, studies over the last five years have repeatedly reported that sediments of inland creeks remain polluted by various persistent toxic substances, such as polycyclic aromatic hydrocarbons (PAHs), styrene oligomers (SOs), and alkylphenols (APs) (Hong et al., 2016b, 2019; Jeon et al., 2017; Lee et al., 2017; Meng et al., 2017). Results of several studies have shown that concentrations of PAHs and APs in sediments of inland creeks of Lake Sihwa exceeded interim sediment quality guidelines (ISQGs) established by the Canadian Council of Ministers of the Environment (CCME) (CCME, 2002; Hong et al., 2016b; Lee et al., 2017). Among these classes of compounds, PAHs and SOs are AhR agonists (Eichbaum et al., 2014; Hong et al., 2016b; Xiao et al., 2017).

The AhR is a ligand-activated transcription factor that mediates a wide range of biological and toxicological effects (Giesy et al., 2002; Mitchell and Elferink, 2009). Binding of xenobiotics to the AhR initiates a variety of biochemical, physiological, and toxicological effects, including carcinogenicity, developmental toxicity, and the development of tumors in some organisms (Mimura and Fujii-Kariyama, 2003; Quintana, 2013). Due to such complexity in toxicity assessment relating to PAHs, AhR binding affinity in organic extracts of sediments contaminated by AhR-active PAHs could be screened *in vitro*. Furthermore, identification of unknown AhR-mediated potency in environmental matrices addresses overall sample toxicity which gives important information for protection of aquatic life as well as human health. Screening for AhR-mediated potency by use of *in vitro* bioassay can be useful to evaluate contamination by toxic substances and potential adverse effects in environments.

The present study investigated AhR-active compounds in sediments of an industrialized area of Lake Sihwa. Specific objectives were to: (i) investigate AhR-active fractions in organic extracts of sediments by use of the H4IIE-*luc* bioassay; (ii) measure concentrations of known AhR agonists, such as PAHs and SOs; (iii) identify previously untargeted AhR agonists by use of a combination of the H4IIE-*luc* bioassay and GC-QTOFMS; and (iv) determine contributions of traditional and newly identified AhR agonists to total induced potencies.

2. Materials and methods

2.1. Collection and preparation of samples

Sediments were collected from inland creeks (tributaries) of industrial (C1–C5) and urban (C6) areas in April 2015 and from rural areas (C7–C8) in September 2017 at Lake Sihwa, South Korea (Fig. 1). Although samples were collected in different years, sediments record a relatively long history of contamination by persistent toxic substances, including the target compounds of the present study, compared to that of water samples. Thus, in this study, sampling time was not considered during the interpretation of data. Surface sediments were collected with

a hand shovel and transferred to pre-cleaned glass jars. Sediments were transported to the laboratory and stored at −20 °C until analysis. Detailed descriptions of preparation of samples for bioassay and chemical analysis have been described previously (Hong et al., 2015, 2016b; Lee et al., 2017). In brief, sediments were freeze-dried, passed through a 1-mm sieve, and homogenized. Approximately 60 g of homogenized sediments were placed into the thimble and extracted on a Soxhlet extractor with 350 mL dichloromethane (DCM, J.T. Baker, Phillipsburg, NJ) for 16 h. To remove elemental sulfur from organic extracts, activated copper was added for about 1 h. Organic extracts were concentrated to 4 mL with a rotary evaporator and N_2 gas flow (~15 g sediment equivalent (SEq) mL^{-1}). Four milliliters of raw extract (REs) were divided into 2 mL volumes for silica gel column fractionation and H4IIE-*luc* bioassay. The REs separated for bioassay were exchanged into dimethyl sulfoxide (DMSO, Sigma-Aldrich, Saint Louis, MO).

2.2. Silica gel and RP-HPLC fractionations

To perform fractionation based on polarity, approximately 8 g of activated silica gel (70–230 mesh, Sigma-Aldrich) was added to a glass column. Two milliliters of organic extract was passed through the column and divided into non-polar (F1), aromatics (F2), and polar (F3) fractions. F1 was eluted with 30 mL hexane (Honeywell, Charlotte, NC). F2 was collected with 60 mL of 20% DCM in hexane. F3 was eluted with 50 mL of 60% DCM in acetone (J.T. Baker). Eluted fractions were evaporated on a rotary evaporator and concentrated to 2 mL using N_2 gas flow. Of the 2 mL silica gel fraction samples, 1 mL was further fractionated into ten finer fractions based on log K_{ow} values using reverse-phase (RP)-HPLC (Agilent 1260 HPLC; Agilent Technologies, Santa Clara, CA) (for details, see Hong et al., 2016a, 2016b).

2.3. H4IIE-luc in vitro bioassay

AhR-mediated potencies were measured by use of the H4IIE-*luc* bioassay in REs, the silica gel fractions, and RP-HPLC fractions of sediments. The bioassay was performed following the methods of previous studies (Hong et al., 2016b; Lee et al., 2017). In brief, trypsinized cells (~7.0×10^4 cells mL^{-1}) were seeded on a 96-well plate at 250 μL per well. After seeding, the cells were incubated at 37 °C in a 5% CO_2 incubator for 24 h. Dosing was carried out by adding the sample to the well-cultured cells. Plates contained a positive control, such as benzo[*a*]pyrene (BaP) or 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD), a solvent control (0.1% DMSO), and a media control. BaP (for 4 h) and TCDD (for 72 h) were diluted three times with 50 nM (=100 $\%BaP_{max}$) and 300 pM (=100 $\%TCDD_{max}$) as the first concentration, respectively. Dosing was performed at six concentrations. Luciferase luminescence was quantified using a Victor X3 multi-label plate reader (PerkinElmer, Waltham, MA) after 4 h or 72 h of exposure. Responses were converted to percentages of the maximum responses of BaP and TCDD, respectively. Finally, magnitude-based BaP-EQ values and potency-based BaP-EQ values (ng BaP-EQ g^{-1} dm) for AhR-mediated potency at 4 h were calculated. Potency-based BaP-EQ values were obtained from sample dose-response relationships elicited by sediment samples at six dilutions.

2.4. Quantifications of PAHs and SOs

Authentic standards 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 Ind. (Osaka, Japan), and included 1,3-diphenylproane (SD1), *cis*-1,2-diphenylcyclobutane (SD2), 2,4-diphenyl-1-butene (SD3), *trans*-1,2-diphenylcyclobutane (SD4), 2,4,6-triphenyl-1-hexene (ST1), 1e-phenyl-4e-(1-phenylethyl)-tetralin (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). Target compounds present in sediments of organic extracts were analyzed in selected ion monitoring mode by use of an Agilent 7890B GC coupled to a 5977 MSD (Agilent Technologies). Volume injected onto a DB5-MS column (30 m × 0.25 mm i.d. × 0.25 μm film) was 1 μL. Instrumental conditions used to detect target compounds are provided in Fig. S1 of Supplementary Materials (S). Isotopically-labeled surrogate standards (Ace-d10, Phe-d10, Chr-d12, and perylene-d12) were not added to subsamples used for bioassay, but were added to samples used for targeted analysis. Internal standard (2-fluorobiphenyl) was added before GC-MSD analysis. Recovery rates of surrogate standards ranged from 48% to 102% (mean = 72%) for Ace-d10, from 79% to 98% (mean = 91%) for Phe-d10, from 62% to 109% (mean = 86%) for Chr-d12, and from 82% to 96% (mean = 87%) for perylene-d12.

2.5. Full-scan screening analysis

FSA using GC-QTOFMS was performed on F2.6 and F2.7 of the organic extract of sediment from Siheung Creek (C4), where AhR-mediated potency was greater. The gas chromatograph Agilent 7890B coupled with a 7200 QTOFMS (Agilent Technologies) was used for FSA. The carrier gas was 1.2 mL min^{-1} He. A DB-5MS UI column (30 m × 0.25 mm i.d. × 0.25 μm film) was used for separation. Instrumental conditions are detailed in Table S1. The selection criteria of candidates for AhR agonists from the GC-QTOFMS analysis consisted of four steps. The first step involved matching the compounds with the NIST library (ver. 2014) (Booij et al., 2014; Zedda and Zwiener, 2012), and removing targeted PAHs and SOs. The second step involved selecting only compounds with a score of ≥70 by identifying the library matching factors (Muz et al., 2017). The third step involved identifying aromatic compounds. A previous study showed that compounds with the structure of aromatic rings had AhR binding affinity (Mekenyan et al., 1996). The fourth step involved selecting compounds with three or more aromatic rings. Because AhR-active PAHs have more than three benzene rings, compounds with three or more rings were selected (Louiz et al., 2008; Xiao et al., 2016). From the FSA, five commercially available compounds including benz[*b*]anthracene (BbA), cyclopenta[*cd*]pyrene (CcdP), 11*H*-benzo[*a*]fluorene (11BaF), 4,5-methanochrysene (4,5MC), and 1-methylpyrene (1MP) were selected to conduct chemical and toxicological confirmations. BbA and 1MP were obtained from Sigma-Aldrich and 11BaF and CcdP were purchased from Accustandard (NewHaven, CT). 4,5MC was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The five putative AhR agonists in fractions were quantified using GC-MSD.

2.6. Relative potency values of putative AhR-active compounds

To determine relative potencies (RePs) for AhR-mediated potencies of the five candidate compounds, H4IIE-*luc* bioassay was performed at 4 h. Each compound was prepared at a total of eight concentrations (viz., 1000, 333, 111, 37, 12, 4.1, 1.4, and 0.46 ng mL^{-1}), and was analyzed using the *in vitro* bioassay method described in Section 2.3. Effective concentrations (EC) and RePs were measured based on previous studies (Villeneuve et al., 2000), with minor modifications. In brief, ReP_{20}, ReP_{50}, and ReP_{80} of the candidate compounds were calculated based on the EC_{20}, EC_{50}, and EC_{80}, respectively. If the deviation between ReP_{20}, ReP_{50}, and ReP_{80} was within an order of magnitude, ReP_{50} was generally considered reliable (Horii et al., 2009).

2.7. Potency balance analysis

Potency balance analyses were performed between instrument-derived BEQ and bioassay derived BaP-EQs (potency-based concentrations) to determine contributions of each compound to AhR-mediated potency. Instrument-derived BEQ concentrations were calculated as the sum of products of measured concentrations of individual compounds multiplied by their RePs. RePs of AhR-active PAHs and SOs are presented in Table S2 (Hong et al., 2016b; Kim et al., 2019). The BEQ concentrations of newly identified AhR agonists were calculated using chemical concentrations, with this study delineating the ReP values. The contribution of instrument-derived BEQs of individual compounds (PAHs, SOs, and newly identified AhR agonists) to bioassay derived BaP-EQs was determined.

2.8. QSAR modeling using VEGA

The VEGA platform is an *in silico* program that contains tens of quantitative structure-activity relationship (QSAR) models for various endpoints (Marzo et al., 2016). *In silico* techniques are used to predict the toxicological endpoints of a chemical-based on its physicochemical properties and structure (Pizzo et al., 2013). Five candidate compounds identified by FSA were evaluated for mutagenicity, carcinogenicity, developmental toxicity, and estrogen receptor activity (Benfenati et al., 2013).

3. Results and discussion

3.1. AhR-mediated potencies in sediments

Detectable AhR-mediated potencies were found in extracts of sediments from inland creeks at both 4 h and 72 h exposures in the H4IIE-*luc* transactivation bioassay with luciferase as the reporter gene (Fig. S2). All REs of sediments reached saturation efficiency (≥100 % BaP_{max}) after 4 h exposure, while the AhR-mediated potencies of REs at 72 h exposure varied among sites. In the three silica gel fractions of eight sediment REs, F2 (aromatics) and F3 (polar) generally exhibited greater AhR-mediated potencies than did F1 (non-polar) at both 4 h and 72 h exposure durations. This result was expected because F2 mainly contains compounds capable of binding to the AhR, including PCDD/Fs, coplanar PCBs, and PAHs (Hong et al., 2014, 2016b; Kinani et al., 2010; Lee et al., 2017; Louiz et al., 2008). Industrial areas of Siheung Creek (C4) and Singil Creek (C5), an urban area of Ansan Creek (C6), and a rural area of Samhwa Creek (C7) were then subjected to RP-HPLC fractionation, in which the AhR response of F2 was the greatest in each area. Significant AhR-mediated potencies were commonly observed in F2.6–F2.8 at 4 h exposure duration at sites C4–C7 (Fig. 2). These fractions contained aromatic compounds with 5–8 log K_{ow} values, indicating that these compounds are major AhR agonists. For example, the well-known AhR agonists (such as PAHs and SOs) were detected in these fractions. BaA, Chr, SD1, and SD3 occurred in F2.6, and BbF, BkF, BaP, IcdP, DbahA, and ST2 occurred in F2.7 (Hong et al., 2016b; Kim et al., 2019; Lee et al., 2017). Similar patterns have been observed in previous studies conducted in Lake Sihwa and the west coast of South Korea (Hong et al., 2016b; Jeon et al., 2017). However, it was not possible to determine whether the greater AhR responses found in F2.6 and F2.7 in different sediment samples were caused by the same substances across regions, or to identify the common physico-chemical characteristics of AhR-active compounds.

Simultaneous tests at durations of exposure of 4 h or 72 h in the H4IIE-*luc* bioassay provided metabolic information on the AhR agonists present in sediments. Labile compounds (such as PAHs) tended to be metabolized as a function of increasing exposure time; however, refractory compounds (such as PCDD/Fs and coplanar-PCBs) were relatively stable during the exposure of 72 h (Xiao et al., 2017). The present study identified strong AhR-mediated potencies after exposure for either 4 h or 72 h in the H4IIE-*luc* bioassay. Due to the relatively strong AhR-mediated potency, this study focused on identifying causative compounds during 4 h exposures. In addition, F3 exhibited significant AhR-mediated potency. In the RP-HPLC fractions of F3, significant AhR-mediated potencies were observed in F3.5–F3.8 after 4 h exposure, while weaker responses were observed after 72 h exposure (Fig. S3). Thus, AhR agonists occurred in polar fractions and were actively metabolized during the longer exposure. Because it is difficult and challenging to identify AhR-active compounds in the polar fraction (Creusot et al., 2013; Hong et al., 2016b; Lubcke-von Varel et al., 2011), further studies focusing on polar AhR agonists in sediments are needed.

The full dose-response curves of selected fractions (F2.6 and F2.7 of C4–C7 sediment extracts) were obtained (Fig. S4). Responses of some fraction samples were insufficient (< 50 %BaP_{max}) to calculate EC_{50} values (Lee et al., 2013). Thus, potency-based BaP-EQ concentrations were calculated based on EC_{20} values. These concentrations of potency-based BaP-EQs were used for the potency balance analysis (vs. instrument-derived BEQ concentrations).

3.2. Absolute and relative concentrations of PAHs and SOs

PAHs and SOs were detected in extracts of all sediments collected from inland creeks of Lake Sihwa. Concentrations of sedimentary PAHs ranged from 160 to 1400 ng g^{-1} dm (mean: 460 ng g^{-1} dm) in industrial areas (C1–C5), 310 ng g^{-1} dm in more urban areas (C6), and from 14 to 120 ng g^{-1} dm in rural areas (C7–C8) (Table S3). Concentrations of PAHs in extracts of sediments from industrial areas were generally greater than those in extracts of sediments from more urban and rural areas. Thus, contamination by sedimentary PAHs seems to be associated with land use and other surrounding activities at nearby industrial complexes. Concentrations of PAHs measured in sediments from inland creeks of Lake Sihwa were compared with several existing SQGs, such as effect-range-low and -median values (ERL and ERM) (Long et al., 1995), threshold and probable effect concentrations (TEC and PEC) (Macdonald et al., 1996), and ISQGs and probable effect levels (PEL) (CCME, 2002). Some PAHs including Ace, Flu, Phe, Fl, Py, and DbahA in the C4 sediment exceeded threshold effect concentration guidelines (i.e., ERL, TEC, and ISQG), but none exceeded probable effect concentration guidelines (i.e., ERM, PEC, and PEL) (Fig. S5). To assess potential sources of PAHs, diagnostic paired ratios were applied, including Ant/(Ant + Phe), Fl/(Fl + Py), and IcdP/(IcdP + BghiP) (data not shown). Ratios calculated indicated that sources of sedimentary PAHs from the inland creeks of Lake Sihwa were pyrogenic, including grass, wood, coal, and fossil fuel combustion (Yang et al., 2014). Patterns of distributions of sedimentary SOs were generally similar to those of PAHs. Concentrations of SOs ranged from 89 to 870 ng g^{-1} dm (mean: 590 ng g^{-1} dm) in more industrial areas, 230 ng g^{-1} dm in urban areas and from 150 to 300 ng g^{-1} dm in rural areas, respectively (Table S4). In industrial areas, C5 sediment had the greatest concentration of SOs, followed by C4, C1, C2, and C3. Unlike the unwanted by-product PAHs, SOs are mainly derived from polystyrene plastic products; thus, the types of industries present around the inland creek might have influenced the distribution of SOs (Hong et al., 2019).

Greater concentrations of AhR-active PAHs and SOs were found in sediments of inland creeks in the industrial areas (C4 and C5) compared to those from urban (C6) and rural (C7) areas (Fig. 3a). To determine contributions of known AhR agonists, instrument-derived concentrations of BEQs were calculated using concentrations of AhR-active PAHs and SOs and their ReP values (Fig. 3b and Table S5). Potency balance analysis between instrument-derived BEQs and bioassay-derived BaP-EQs was performed on F2.6 and F2.7 of C4–C7 sediments (Fig. 3b). The results showed that known AhR agonists (such as PAHs and SOs) explained only a small portion of total AhR-mediated activities, ranging from 1.1 to 39% for F2.6 and 1.2 to 6.4% for F2.7, respectively. Unknown (i.e., untargeted) AhR-active compounds were largely present in

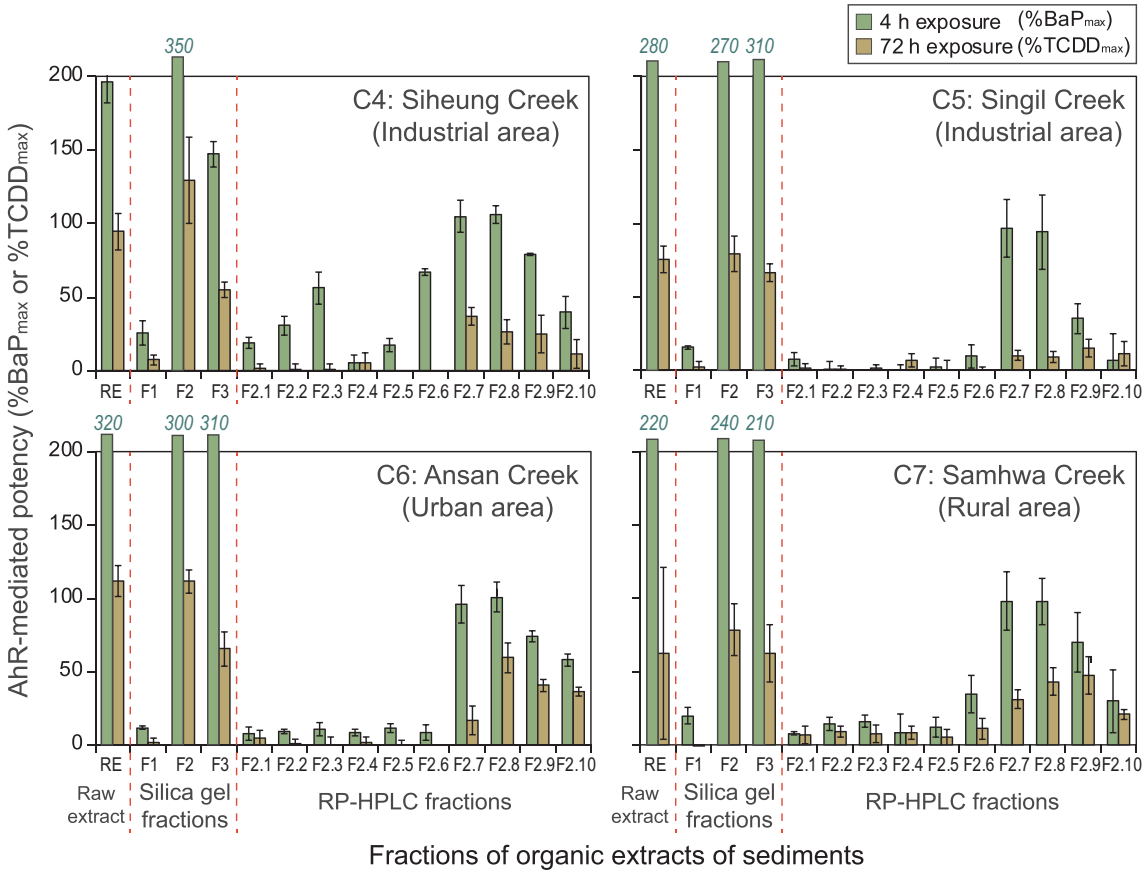

Fig. 2. AhR-mediated potencies of raw extracts (RE) and fractions (silica gel and RP-HPLC) of selected inland creeks (C4–C7) determined, at the end of 4 h and 72 h exposures, in the H4IIE-*luc* bioassay (Error bar: mean ± SD; n = 3).

the inland creek sediments of Lake Sihwa. In F2.6 of C4, Chr (18%) and BaA (5.6%) showed the greatest contribution to AhR-mediated potencies. In comparison, BbF (2.1%), BkF (1.9%), and BaP (1.9%) were the major AhR agonists in F2.7 of C4. Although concentrations of SOs were comparable to concentrations of PAHs, their contributions were small (< 1%) because of their small RePs. Overall, potency balance analysis showed that known AhR-active PAHs and SOs did not fully explain the bioassay results for sediments. Thus, FSA was performed to search for previously unidentified AhR agonists present in more potent fractions.

3.3. Full-scan screening analysis of highly potent fractions

FSA was performed on F2.6 and F2.7 of C4 sediment (Siheung Creek) using GC-QTOFMS. The process for selecting the causative compounds consisted of four steps (Fig. 4a). In the first step (NIST library matching, ver. 2014), 461 and 449 compounds were detected in F2.6 and F2.7 of C4 sediment extracts, respectively. Among them, the number of compounds with a matching score of ≥70 (i.e., second step) was 267 in F2.6 and 214 in F2.7. The third step involved identifying aromatics, with 129 and 93 compounds being selected, respectively.

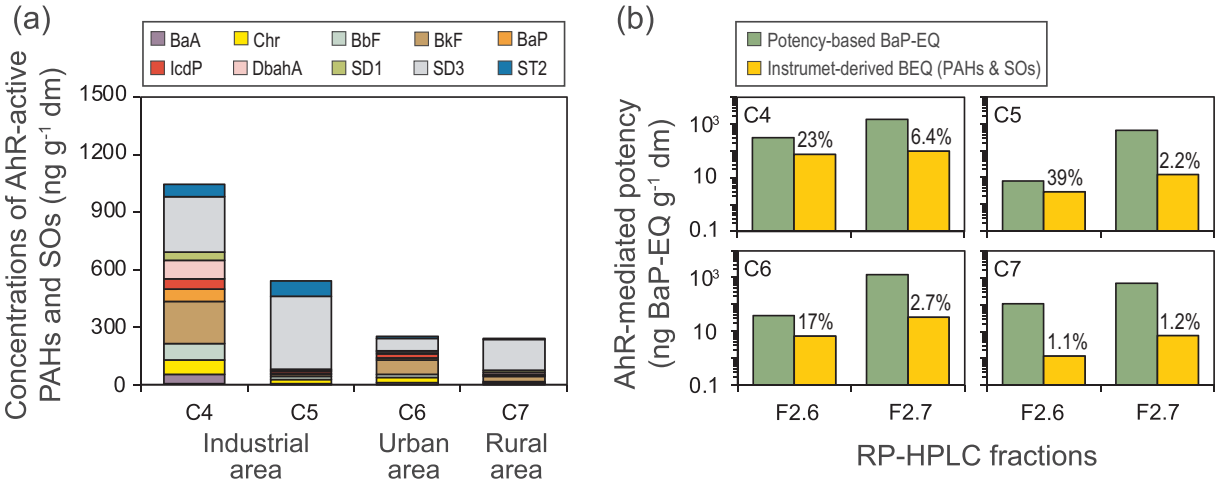

Fig. 3. (a) Concentrations of AhR-active PAHs and SOs in sediments of selected inland creeks (C4–C7) in Lake Sihwa and (b) contributions of instrument-derived BEQs (PAHs and SOs) to bioassay-derived BaP-EQs (potency-based) in RP-HPLC fractions (F2.6 and F2.7).

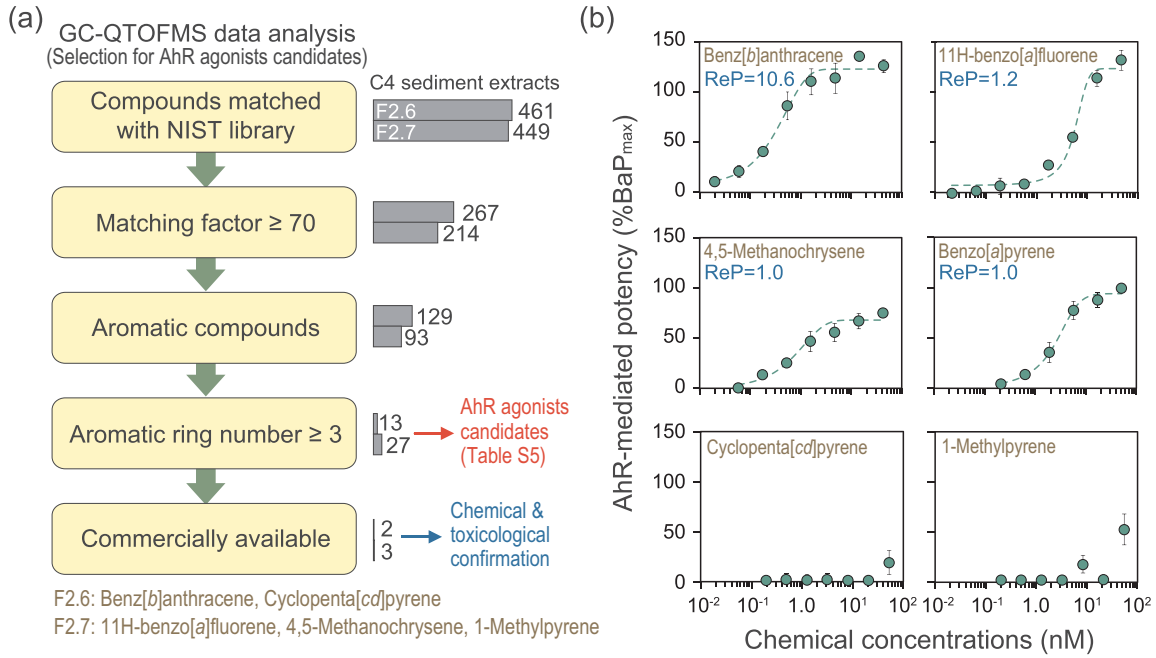

Fig. 4. (a) Stepwise approach for GC-QTOFMS data analysis to select AhR agonist candidates and (b) dose-response relationships for AhR-mediated potency of five tentative AhR agonists and benzo[*a*]pyrene in the H4IIE-*luc* bioassay (Error bar: mean ± SD; n = 3; ReP: relative potency value).

The fourth step involved selecting compounds with three or more aromatic rings, with a total of 13 and 27 compounds being selected, respectively (Table S6). Six compounds were detected both in F2.6 and in F2.7. Thus, a total of 34 compounds were selected as tentative candidates of AhR agonists from the organic extracts of sediment (Table S6 lists these compounds). The common characteristics of these compounds are (i) molecular mass 200–300 (average 240), (ii) log K_{ow} 5–7, and (iii) methyl-substituted structures. Due to the lack of authentic standards, among the 34 compounds, five commercially available compounds including BbA, CcdP (in F2.6), 11BaF, 4,5MC, and 1MP (in F2.7) were tested for identification of compounds and toxicological confirmations. Overall, this study successfully applied FSA to select candidate AhR agonists in more potent fractions. However, the approach had several limitations, including being dependent on library matching. Using a high-resolution mass spectrometer, the molecular formula is somewhat reliable; however, the chemical structure cannot be characterized. To solve this problem, it would be necessary to confirm the structure using standards, but available standards are limited.

3.4. Chemical and toxicological confirmation

Chemical and toxicological confirmation of the five compounds (BbA, CcdP, 11BaF, 4,5MC, and 1MP) were conducted to determine concentrations in sediments and AhR binding potencies (Fig. 4b and Table 1). GC retention time and mass fragment ions of the candidate compounds with those of samples were matched using GC-MSD. Concentrations of BbA, CcdP, 11BaF, 4,5MC, and 1MP in inland creek sediments were quantified. For toxicological confirmation, dose–response tests for five candidates were performed after exposure for 4 h of H4IIE-*luc* cells (Fig. 4b). Among the five candidates, three compounds (BbA, 11BaF, and 4,5MC) showed significant AhR-mediated potencies (significant level = 5%BaP_{max}), thus ReP values relative to that of BaP could be obtained. RePs of BbA, 11BaF, and 4,5MC were 10.6, 1.2, and 1.0, respectively (Fig. 4b). Variation among ReP_{20}, ReP_{50}, and ReP_{80} was relatively small, and thus, the use of ReP_{50} was considered reliable (Table S7) (Lee et al., 2013). BbA was a strong AhR agonist, with its ReP being 10-fold greater than that of BaP. ReP values of 11BaF and 4,5MC were comparable to that of BaP. BbA and 11BaF were previously reported as AhR-active chemicals (Larsson et al., 2014), but have not been reported in environmental samples to date. BbA and 11BaF showed AhR-mediated potencies along exposure durations of 24, 48, and 72 h in the H4IIE-*luc* bioassay, which indicated that these chemicals are not easily metabolized (Larsson et al., 2014).

3.5. Distribution and sources of newly identified AhR agonists in sediments

In this study, three AhR-active chemicals were newly identified from sediment fractions through EDA combined with FSA, which were all detected in sediments from the inland creeks of Lake Sihwa (Table S8). Relatively great concentrations of 11BaF (253 ng g^{-1} dm), 4,5MC (49 ng g^{-1} dm), and BbA (19 ng g^{-1} dm) were detected in sediment of C4, followed by C5, C6, and C7. Similar to distributions of PAHs and SOs, these newly identified AhR agonists predominated in industrial areas. Of the three AhR agonists, 11BaF was the most frequently detected with the greatest concentrations among sampling sites. In comparison, BbA was primarily detected in sediments from industrial areas. Greatest concentrations of 11BaF were observed in the industrial, urban, and rural areas, and mainly originated from gasoline engines, tobacco smoke, and oil-fired heating (Snook et al., 1978). Previous studies reported that 4,5MC is derived from urban particulate matter and tobacco smoke (Agarwal et al., 1999; Lee-Ruff et al., 1984). 4,5MC concentrations were comparable in C4 and C5 sediments (industrial area) and were 6–8 times greater than those in urban and rural areas (C6 and C7). BbA is an essential element for the active layer of high-performance organic field-effect transistors (OFETs) and organic light-emitting diodes (OLEDs). This compound was identified as the active material, because of its high hole OFET mobility in the single-crystal form (Gundlach et al., 2002; Takahashi et al., 2007; Yamamoto and Takimiya, 2007). BbA was primarily distributed in the C4 and C5 sediments of the industrial areas compared to urban and rural areas.

Studies on occurrences and distributions of 11BaF, 4,5MC, and BbA in sediments remain limited globally (Kishida et al., 2007). The concentration of BbA in sediment from C4 was greater than those recorded in the suburban and rural areas of Vietnam, but was lesser than those observed in some industrial areas (Kishida et al., 2007). Unlike 4,5MC and 11BaF, which originate from combustion processes, BbA originates from transistor film, which was widely used in the industrial area. BbA has a very high affinity to bind AhR and contributes to the total induced

Table 1
AhR agonist candidates in the RP-HPLC fractions (F2.6 and F2.7) of sediment extracts from Siheung Creek of Lake Sihwa, South Korea.

Fractions and compounds	Abb.[a]	Molecular formula	CAS number	Structure	MW.[b]	Matching factor[c]	AhR-active[d]	Uses/origins (References)
F2.6 fraction								
Benz[*b*]anthracene	BbA	$C_{18}H_{12}$	92-24-0		228.288	85	+	Film layer of OFETs and OLEDs (Takahashi et al., 2007)
Cyclopenta[*cd*]pyrene	CcdP	$C_{18}H_{10}$	27208-37-3		226.272	90	–	Automobile exhaust and atmospheric soot (Eisenstadt and Gold, 1978)
F2.7 fraction								
11*H*-benzo[*a*]fluorene	11BaF	$C_{17}H_{12}$	238-84-6		216.277	73	+	Gasoline engines and tobacco smoke (Snook et al., 1978)
4,5-Methanochrysene	4,5MC	$C_{19}H_{12}$	202-98-2		240.299	72	+	Urban particulate matters and tobacco smoke (Agarwal et al., 1999)
1-Methylpyrene	1MP	$C_{17}H_{12}$	2381-21-7		216.277	87	–	Crude oil and meat and charcoal smoke (Dyremark and Westerholm, 1995)

[a] Abb.: abbreviations.
[b] MW.: Molecular weight.
[c] National Institute of Standards and Technology (NIST) library matching score (Ver. 2014).
[d] +: significant; −: not significant.

AhR-mediated potency (see Section 3.6 for more details); thus regulations and monitoring would be of immediate concern.

3.6. Contributions of newly identified AhR agonists to total induced potencies

To address relative contributions of traditional and newly identified AhR agonists to total AhR-mediated potencies, instrument-derived BEQs using all the identified AhR-active chemicals were calculated (Fig. 5 and Table S5). The potency balance suggested varying contribution among chemicals and sites. For example, BbA was the greatest contributor, explaining 67% of the total AhR-mediated potency in F2.6 of the C4 sediment organic extract. 11BaF and 4,5MC explained 22% and 2.9% AhR-mediated potency of F2.7 in the C4 organic sediment extract. Thus, these three newly identified AhR agonists contributed significantly to the total induced AhR-mediated potencies compared to the known traditional AhR agonists, such as BaA, Chr, BkF, etc. Addition of these three AhR agonists increased the explanatory power of total induced AhR-mediated potencies for the F2.6 and F2.7 fractions of inland creek sediments. Meantime, it should be noted that compositions and contributions of AhR agonists are site-specific. The three AhR agonists were observed in F2.6 and F2.7 fractions of the C4 sediment extract (industrial area). These compounds occurred predominantly in industrial areas and appeared to act as major AhR agonists. However, BbA, 11BaF, and 4,5MC showed relatively minor contributions (< 3%) to the fractions of organic sediment extracts from urban and rural areas.

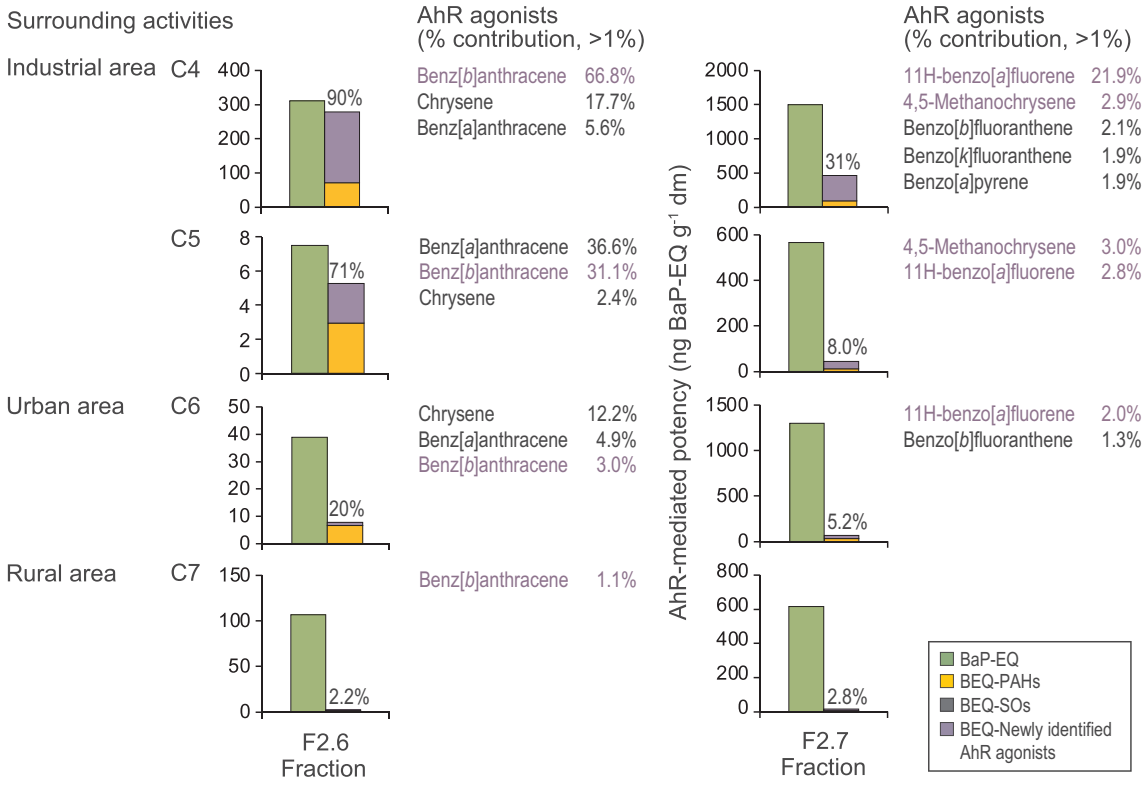

Fig. 5. Improved potency balance between bioassay-derived BaP-EQs and instrument-derived BEQs (PAHs, SOs, and newly identified AhR agonists) in the RP-HPLC fractions (F2.6 and F2.7) of selected inland creek sediments (C4–C7) (The percentage number indicates total contributions of AhR agonists to BaP-EQs).

Although large AhR-mediated potencies were detected in the sediments of urban (C6) and rural (C7) areas, a large proportion of such elevated AhR potencies has yet to be identified.

The five candidate compounds selected in this study (BbA, CcdP, 11BaF, 4,5MC, and 1MP) occurred widely in sediments from inland creeks (Table S8). Whether these compounds had other potential toxicities, such as mutagenicity, carcinogenicity, developmental toxicity, and estrogen activity using VEGA QSAR was further evaluated (Table S9). Consequently, it was postulated that these compounds have other potential toxicities in aquatic environments. Specifically, it was likely that all these compounds exhibited mutagenicity, carcinogenicity, and developmental toxicity. CcdP and 1MP were prominent in sediments of the inland creeks, mainly originated from automobile exhaust, atmospheric soot (Eisenstadt and Gold, 1978), crude oil, and meat and charcoal smoke (Dyremark and Westerholm, 1995). In addition, these substances have been reported to have specific potential toxicities in several previous studies (Table S9). For example, BbA is known to have potential AhR activity (Larsson et al., 2014; this study) and mutagenicity (Pahlman, 1988), CcdP has potential genotoxicity and tumorgenicity (Nesnow et al., 1994), 11BaF has AhR activity (Pahlman, 1988; Larsson et al., 2014; this study), 4,5MC has AhR activity (this study) and mutagenicity (Lee-Ruff et al., 1987), and 1MP has mutagenicity and tumorgenicity (Rice et al., 1987). Since these emerging substances are widely distributed in sediments near industrial complexes, further studies on their sources, fate, potential adverse effects, and reduction measures are necessary.

4. Conclusions

Results of the present study indicated that EDA combined with FSA enhanced the understanding of the distributions of bioactive chemicals and potential toxicities associated with environmental mixture samples. We have identified and addressed the contributions of known and newly identified AhR-active compounds from sediment extracts in a highly complexed area of varying land uses and activities. This study demonstrated that three newly identified AhR agonists from sediments act as strong AhR agonists and contributed a large proportion of sample activities, particularly for the samples collected from highly industrialized areas. However, the results of the H4IIE-*luc* bioassay used in the present study do not allow direct extrapolation to ecotoxicological responses, but provide baseline information on pollution of AhR-active compounds in sediments for further risk evaluation. Thus, further studies are needed to confirm whether the newly identified AhR agonists have toxic effects on living aquatic organisms, which will provide better understanding of ecologically relevant predictions of risk in the contaminated sediments. Meanwhile, the stepwise screening approach throughout non-target analysis applied in this study would benefit to identify and address unknown causative agents in environmental samples, elsewhere. In addition, candidate compounds that are not AhR agonists might exhibit other toxicity mechanisms; thus it is crucial to characterize all the aspects of sources, distribution, fate, and ecotoxicological effects of target compounds in the aquatic environment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105199.

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<Supplementary Materials>

Major AhR-active chemicals in sediments of Lake Sihwa, South Korea: Application of effect-directed analysis combined with full-scan screening analysis

Jihyun Cha, Seongjin Hong*, Jaeseong Kim, Junghyun Lee, Seo Joon Yoon, Sunggyu Lee, Hyo-Bang Moon, Kyung-Hoon Shin, Jin Hur, John P. Giesy, Jong Seong Khim

Supplementary Tables

Supplementary Figures

*__Corresponding Author.__ *E-mail address*: hongseongjin@cnu.ac.kr (S. Hong).

Supplementary Tables

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

Instrument	GC: Agilent Technologies 7890B
	QTOFMS: Agilent Technologies 7200
Samples	C4 (Siheung 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. Relative potency values for AhR-mediated activity of previously reported PAHs and SOs.

Compounds	**Abb.**[a]	**Molecular formula**	**CAS number**	**Molecular weight**	**GC RT**[b] **(min.)**	**Mass fragment ions (*m/z*)**	**ReP value**	**References**
Benz[*a*]anthracene	BaA	$C_{18}H_{12}$	56-55-3	228	33.87	***228***, 226, 229	3.2×10^{-1}	Kim et al. (2019)
Chrysene	Chr	$C_{18}H_{12}$	218-01-9	228	34.01	***228***, 226, 229	8.5×10^{-1}	
Benzo[*b*]fluoranthene	BbF	$C_{20}H_{12}$	205-99-2	252	37.85	***252***, 253, 250	5.0×10^{-1}	
Benzo[*k*]fluoranthene	BkF	$C_{20}H_{12}$	207-08-9	252	37.94	***252***, 253, 251	4.8×10^{-1}	
Benzo[*a*]pyrene	BaP	$C_{20}H_{12}$	50-32-8	252	38.90	***252***, 253, 126	1.0	
Indeno[*1,2,3-c,d*]pyrene	IcdP	$C_{22}H_{12}$	193-39-5	276	42.33	***276***, 138, 137	5.8×10^{-1}	
Dibenz[*a,h*]anthracene	DbahA	$C_{22}H_{14}$	53-70-3	278	42.47	***278***, 276, 279	6.6×10^{-1}	
1,3-Diphenylpropane	SD1	$C_{15}H_{16}$	1081-75-0	196	21.10	***92***, 196, 105	2.3×10^{-3}	Hong et al. (2016)
2,4-Diphenyl-1-butene	SD3	$C_{16}H_{16}$	16606-47-6	208	22.35	***91***, 208, 104	3.0×10^{-4}	
2,4,6-Triphenyl-1-hexene	ST1	$C_{24}H_{24}$	18964-53-9	312	33.52	***91***, 117, 194, 207	2.7×10^{-3}	

[a] Abb.: Abbreviations.

[b] GC RT: Gas chromatography retention time.

Table S3. Concentrations of PAHs in sediments of inland creeks (industrial, urban, and rural areas) in Lake Sihwa, South Korea.

Sites	**Polycyclic aromatic hydrocarbons (ng g^{-1} dry mass)**															
	Acl	Ace	Flu	Phe	Ant	Fl	Py	BaA	Chr	BbF	BkF	BaP	IcdP	DbahA	BghiP	ΣPAHs
C1	0.80	1.4	7.7	16	4.0	68	46	9.8	19	17	6.4	15	14	2.2	17	240
C2	0.54	1.2	4.0	13	3.1	53	43	5.4	10	7.1	2.2	6.9	4.9	0.7	6.0	160
C3	0.40	0.89	4.2	12	2.7	61	42	11	11	9.5	10	10	9.8	1.8	9.0	200
C4	1.4	51	76	130	42	200	200	52	74	87	220	69	51	96	69	1400
C5	1.6	1.2	11	15	3.2	94	88	7.0	20	13	11	9.1	12	1.4	17	310
C6	0.92	2.2	3.1	15	1.8	41	38	11	23	19	73	12	20	13	35	310
C7	0.43	0.79	1.9	6.7	0.86	15	20	3.2	7.6	6.2	24	4.4	6.1	14	14	120
C8	0.05	0.21	1.1	2.1	0.30	2.6	5.1	0.09	0.35	0.20	0.19	1.0	0.20	0.04	0.22	14

Abbreviations. Acl: acenaphthylene; Ace: acenaphthene; Flu: fluorene; Phe: phenanthrene; Ant: anthracene; Fl: fluoranthene; Py: pyrene; BaA: benzo[*a*]anthracene; Chr: chrysene; BbF: benzo[*b*]fluoranthene; BkF: benzo[*k*]fluoranthene; BaP: benzo[*a*]pyrene; IcdP: indeno[*1,2,3-cd*]pyrene; DbahA: dibenz[*a,h*]anthracene; BghiP: benzo[*g,h,i*]perylene.

Table S4. Concentrations of SOs in sediments of inland creeks (industrial, urban, and rural areas) in Lake Sihwa, South Korea.

Sites	**Styrene oligomers (ng g^{-1} dry mass)**										
	SD1	SD2	SD3	SD4	ST1	ST2	ST3	ST4	ST5	ST6	ΣSOs
C1	33	3.4	200	43	63	89	120	40	48	33	670
C2	13	9.5	300	12	49	19	22	17	24	28	490
C3	1.2	0.80	43	5.4	11	6.2	8.2	3.0	5.8	4.6	89
C4	43	14	290	76	110	63	76	38	44	73	820
C5	7.0	8.8	380	47	69	79	110	36	40	100	870
C6	5.2	0.85	65	3.9	65	9.0	12	6.1	11	53	230
C7	6.9	1.4	160	5.5	74	6.3	8.1	5.0	8.8	17	300
C8	0.32	0.41	140	1.1	0.25	0.55	0.71	0.89	0.78	2.3	150

Abbreviations. SD1: 1,3-Diphenylproane; SD2: cis-1,2-Diphenylcyclobutane; SD3: 2,4-Diphenyl-1-butene; SD4: trans-1,2-Diphenylcyclobutane; ST1: 2,4,6-Triphenyl-1-hexene; ST2: 1e-Phenyl-4e-(1-phenylethyl)-tetralin; ST3: 1a-Phenyl-4e-(1-phenylethyl)-tetralin; ST4: 1a-Phenyl-4a-(1-phenylethyl)-tetralin; ST5: 1e-Phenyl-4a-(1-phenylethyl)-tetralin; ST6: 1,3,5-Triphenylcyclohexane (isomer mix).

Table S5. Potency balance between instrument-derived BEQs and bioassay-derived BaP-EQs in the RP-HPLC fractions (F2.6 and F2.7) of selected inland creek sediments (C4–C7).

Compounds	C4 (Siheung Creek)		C5 (Singil Creek)		C6 (Ansan Creek)		C7 (Samhwa Creek)	
	F2.6	F2.7	F2.6	F2.7	F2.6	F2.7	F2.6	F2.7
Instrument-derived BEQs (ng BEQ g^{-1} dm)								
PAHs and SOs								
Benz[*a*]anthracene	17		2.8		1.9		0.24	
Chrysene	55		0.18		4.8		0.96	
1,3-Diphenylpropane	0.06		0.01		0.01		0.02	
2,4-Diphenyl-1-butene	0.02		0.02		0.004		0.004	
Benzo[*b*]fluoranthene		32		4.8		16		3.0
Benzo[*k*]fluoranthene		28		1.9		3.8		1.4
Benzo[*a*]pyrene		28		5.2		12		2.3
Indeno[*1,2,3-c,d*]pyrene		4.3		0.06		0.38		0.14
Dibenz[*a,h*]anthracene		3.4		0.63		1.7		0.31
2,4,6-Triphenyl-1-hexene		0.03		0.03		0.01		0.005
BEQ-PAHs and SOs[a]	73	96	3.0	13	6.7	34	1.2	7.1
Newly identified AhR agonists								
Benz[*b*]anthracene	210		2.3		1.2		1.2	
4,5-methanochrysene		43		17		6.0		5.4
11H-benzo[*a*]fluorene		330		16		26		4.8
BEQ-newly identified AhR agonists[b]	210	370	2.3	33	1.2	32	1.2	10
Bioassay-derived BaP-EQs (ng BaP-EQ g^{-1} dm)								
Magnitude-based BaP-EQ[c]	2.9×10^2	1.4×10^3	6.0	1.1×10^3	6.0	1.1×10^3	28	8.8×10^2
Potency-based BaP-EQ[d]	3.1×10^2	1.5×10^3	7.5	5.7×10^2	39	1.3×10^3	1.1×10^2	6.2×10^2
Contribution (%)	90	31	71	8.0	20	5.2	2.2	2.8

[a] BEQ-PAHs and SOs concentrations were calculated from the concentrations of BaA, Chr, BbF, BkF, BaP, IcdP, DahA, SD1, SD3, and ST2 multiplied by their ReP values obtained from previous studies (Hong et al., 2016; Kim et al., 2019).

[b] BEQ-newly identified AhR agonists concentrations were calculated from the concentrations of BbA, 4,5MC, and 11BaF multiplied by their ReP values obtained from this study.

[c] Magnitude-based BaP-EQ was calculated as the percentage of the maximum response observed for a 50 nM BaP standard elicited by 100% sediment organic extracts.

[d] Potency-based BaP-EQ was obtained from sample dose-response relationships elicited by sediments samples at 6 levels of dilution (EC50 based).

Table S6. List of candidates for AhR-active compounds in the fraction samples (F2.6 and F2.7) of organic extracts from C4 sediment using GC-QTOFMS.

Fractions and compounds	Molecular formula	CAS number	Molecular weight	RT (min.)	Matching factor	AhR activity
F2.6 fraction						
Triphenylmethane	$C_{19}H_{16}$	519-73-3	244.330	16.779	95	
2-Pyrenol	$C_{16}H_{10}O$	78751-58-3	218.250	18.795	92	
2-Methylnaphtho[*2,1-b*]furan	$C_{13}H_{10}O$	2000150-17-4	182.218	12.386	89	
9-Ethylanthracene	$C_{16}H_{14}$	605-83-4	206.282	16.950	88	
3-Methylphenanthrene	$C_{15}H_{12}$	832-71-3	192.256	15.591	86	
Benz[*b*]anthracene	$C_{18}H_{12}$	92-24-0	228.288	23.447	85	+[a]
2,3-Dimethylphenanthrene	$C_{16}H_{14}$	3674-65-5	206.282	17.586	77	
F2.6 and F2.7 co-occurring						
o-Terphenyl	$C_{18}H_{14}$	84-15-1	230.304	15.2	96	
m-Terphenyl	$C_{18}H_{14}$	92-06-8	230.304	18.913	95	
2-Phenylnaphthalene	$C_{16}H_{12}$	612-94-2	204.266	16.498	93	
4,5-Dimethylphenanthrene	$C_{16}H_{14}$	3674-69-9	206.282	16.718	92	
Cyclopenta[*cd*]pyrene	$C_{18}H_{10}$	27208-37-3	226.272	22.434	90	-[b]
2,5-Dimethylphenanthrene	$C_{16}H_{14}$	3674-66-6	206.282	17.298	79	
F2.7 fraction						
1-Methyl-7-(1-methylethyl)phenanthrene	$C_{18}H_{18}$	483-65-8	234.335	19.805	89	
4'-Phenyl-1,1':2',1''-Terphenyl	$C_{24}H_{18}$	1165-53-3	306.400	25.469	88	
1-Methylpyrene	$C_{17}H_{12}$	2381-21-7	216.277	20.549	87	-
Methylbis(phenylmethyl)benzene	$C_{21}H_{20}$	2000440-25-8	272.384	20.743	84	
1,9-Dimethylpyrene	$C_{18}H_{14}$	74298-70-7	230.304	22.596	84	
Methyldiphenyl(methyldiphenylsilyl)oxysilane-	$C_{26}H_{26}OSi_2$	807-28-3	410.655	26.325	84	
2-EthylAnthracene	$C_{18}H_{14}$	52251-71-5	206.304	16.958	82	
3,6-Dimethylphenanthrene	$C_{18}H_{14}$	1576-67-6	206.304	17.588	81	
9-propylanthracene	$C_{17}H_{16}$	1498-77-7	220.309	18.014	79	
2,3,5-Trimethylphenanthrene	$C_{17}H_{16}$	3674-73-5	220.309	18.822	77	
9,10-Diethyl-9,10-dihydrotransanthracene,	$C_{18}H_{20}$	23660-32-4	236.351	16.876	76	
9-Ethyl-9,10-dihydroanthracene	$C_{16}H_{16}$	605-82-3	208.298	14.428	76	
p-Terphenyl	$C_{18}H_{14}$	92-94-4	230.304	18.910	75	
9H-Tribenzo[*a,c,e*]cycloheptene	$C_{19}H_{14}$	213-10-5	242.315	25.053	75	
11H-indolo[*3,2-c*]quinoline	$C_{15}H_{10}N_2$	239-09-8	218.253	18.100	74	
Benzene,1,1'-[*2-methyl-2-(phenylthio)cyclopropylidene*]bis-	$C_{22}H_{20}S$	56728-02-0	316.459	24.522	73	
11H-benzo[*a*]fluorene	$C_{17}H_{12}$	238-84-6	216.277	20.112	73	+
12H-benzo[*a*]xanthen-9-ol	$C_{17}H_{12}O_2$	2000357-90-2	248.276	24.468	72	

4,5-Methanochrysene	$C_{19}H_{12}$	202-98-2	240.299	25.523	72	+
8,8'-Diiodo-1,1'-binaphthalene	$C_{20}H_{12}I_2$	2000896-64-6	506.118	29.213	71	
9-Allyl-10-methylphenanthrene	$C_{18}H_{16}$	69258-19-1	232.320	21.091	71	

[a] +: significant response in the H4IIE-*luc* bioassay.

[b] –: not significant response in the H4IIE-*luc* bioassay.

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

Compound	**Maximum concentration[a] (nM)**	**%BaP$_{max}$**	**Slope**	**Relative potency$_{20\text{-}50\text{-}80}$[b]**	
				ReP$_{50}$	**ReP$_{20\text{-}80}$**
BaP	50	100	45	1.0	1.0-1.0
11BaF	46	133	54	1.2	0.9-1.5
4,5MC	42	87	32	1.0	0.6-1.9
BbA	44	134	46	10.6	10.1-11.0

[a] 0.1% dosing concentration.

[b] RePs reported as the range of ReP estimates generated from multiple points over a response range from 20 to 50 to 80%BaP$_{max}$.

Table S8. Concentrations of five candidate compounds in the sediments of inland creeks in Lake Sihwa, South Korea.

Sites	**Concentrations of five candidates for AhR agonists (ng g^{-1} dm)**				
	Benz[*b*]anthracene	Cyclopenta[*cd*]pyrene	11H-benzo[*a*]fluorene	4,5-Methanochrysene	1-Methylpyrene
C1	14	12	31	22	22
C2	16	13	29	22	63
C3	6.6	14	6	6.1	4.9
C4	18	73	250	49	130
C5	12	11	48	50	71
C6	1.3	18	46	8.1	3.9
C7	0.60	9.6	21	5.9	4.3
C8	1.0	0.49	1.4	1.2	0.39

Table S9. Predicted potential toxicity of five candidate compounds using VEGA QSARs and their potential toxicities reported from the previous studies.

Toxicity	Model or bioassay	Compounds				
		BbA	CcdP	11BaF	4,5MC	1MP
Predicted potential toxicity						
Mutagenicity	CAESAR	+ [a]	+	+	+	+
	ISS	+	+	+	+	+
	CONSENSUS	+	+	+	+	+
	KNN/Read-Across	+	+	–	+	+
	SarPY/IRFMN	+	+	+	+	+
Carcinogenicity	CAESAR	+	+	+	+	+
	ISS	+	+	+	+	+
	IRFMN/Antares	– [b]	+	+	+	+
	IRFMN/ISSCAN-CGX	+	+	+	+	+
Developmental toxicity	CAESAR	+	+	+	+	+
	PG	–	–	–	–	–
Estrogen receptor activity	IRFMN	+	+	–	+	+
	IRFMN/CERAPP	Not predicted	–	–	–	–
Measured potential toxicity						
AhR activity	H4IIE-*luc* cell [c,d]	+ [c,d]		+ [c,d]		
Mutagenicity	Ames test [e,f]	+ [e]			+ [f]	+ [g]
	HPC/DNA repair test [g]					
Genotoxicity	^{32}P-postlabeling assay [h]		+ [h]			
Tumorgenicity	HPC/DNA repair test [g]		+ [h]			+ [g]
	^{32}P-postlabeling assay [h]					

Abbreviations. BbA: benz[*b*]anthracene; CcdP: cyclopenta[*cd*]pyrene; 11BaF: 11H-benzo[*a*]fluorene; 4,5MC: 4,5-Methanochrysene; 1MP: 1-Methylpyrene.
[a] +: result of the prediction is active.
[b] –: result of the prediction is non-active.
[c] This study.
[d] Larrson et al. (2014).
[e] Pahlman et al. (1988).
[f] Lee-Ruff et al. (1987).
[g] Rice et al. (1987).
[h] Nesnow et al. (1994).

Supplementary Figures

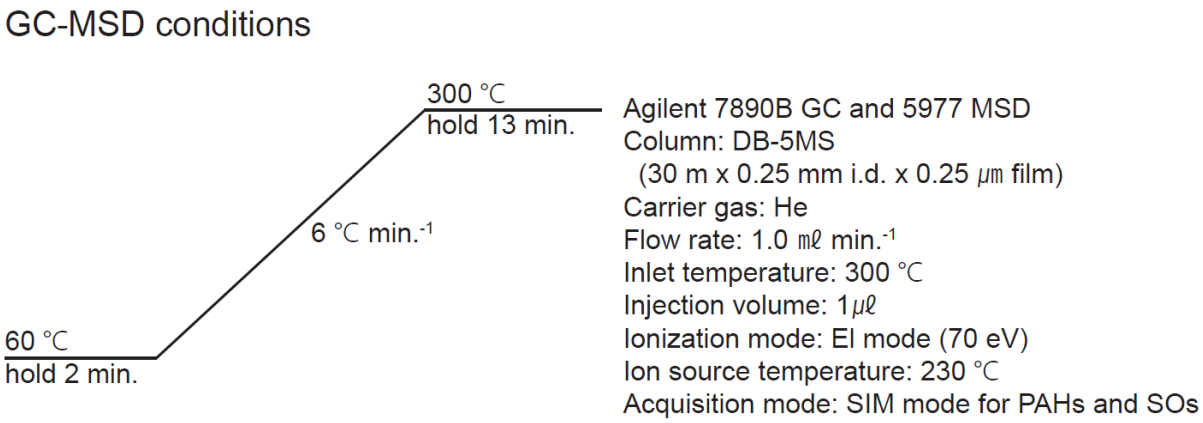

Fig. S1. Instrumental conditions of GC-MSD for PAH and SO analyses.

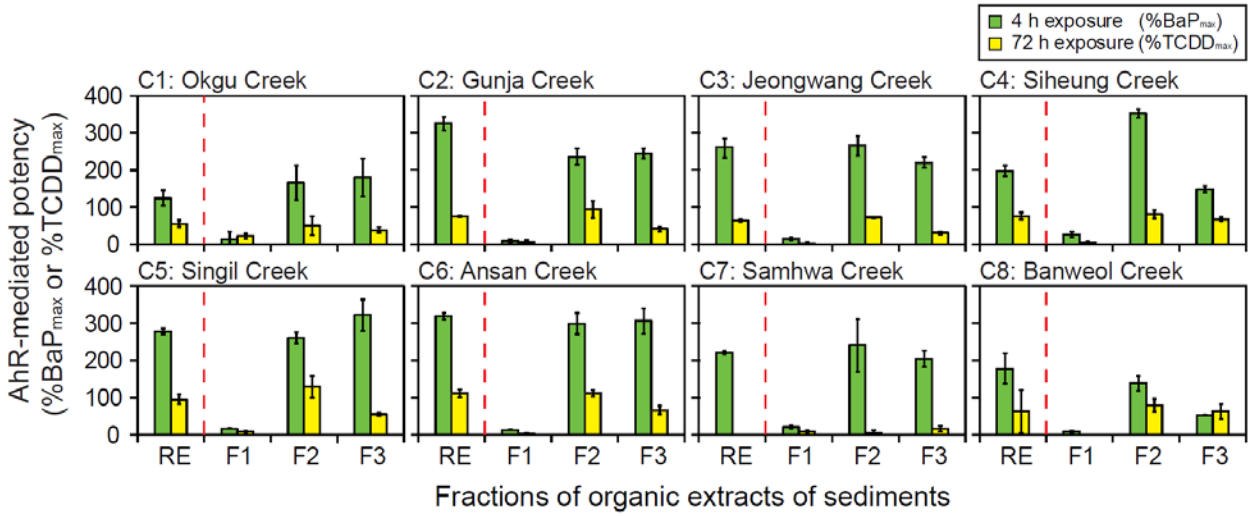

Fig. S2. AhR-mediated potencies of raw extracts (RE) and silica gel fractions from inland creeks of Lake Sihwa determined at 4 h and 72 h exposure durations in the H4IIE-*luc* transactivation bioassay.

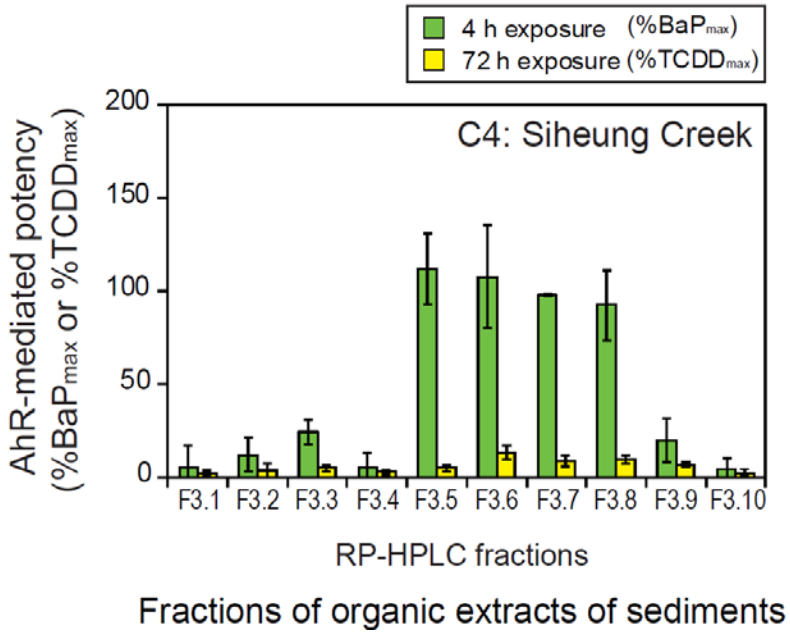

Fig. S3. AhR-mediated potency of RP-HPLC fractions (F3.1–F3.10; subfractions of F3) of sediment extracts from Siheung Creek, South Korea, determined at 4 h and 72 h exposure durations in the H4IIE-*luc* transactivation bioassay.

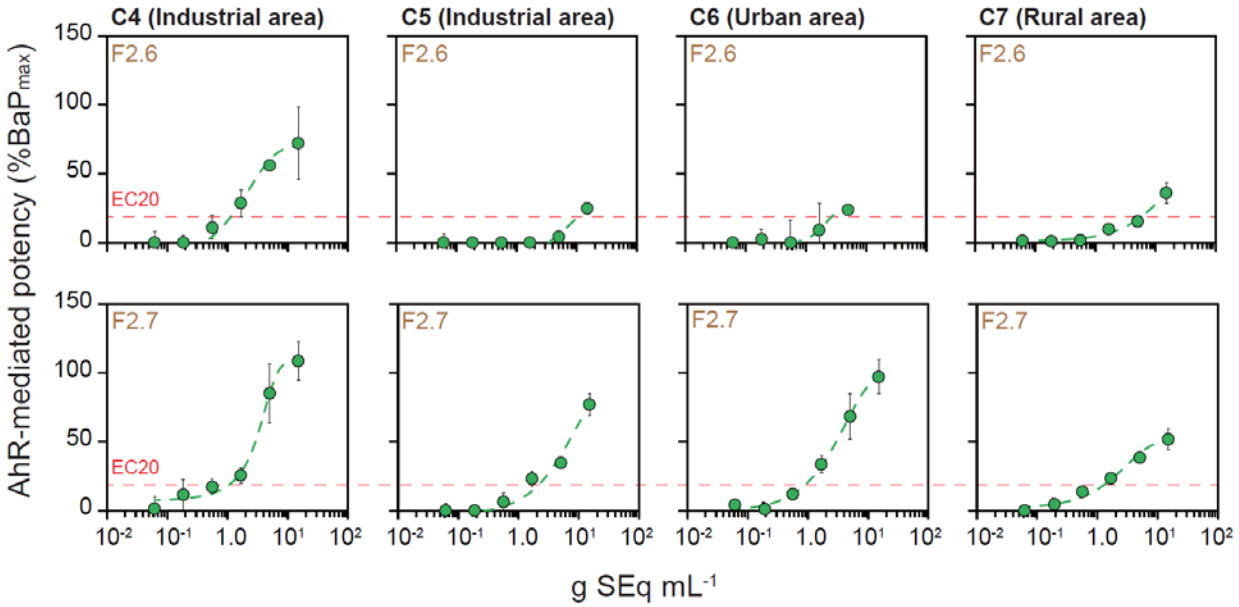

Fig. S4. Dose-response curves for the AhR-mediated potency of the selected RP-HPLC fractions (F2.6 and F2.7 of C4–C7 sediment extracts) from the inland creeks of Lake Sihwa, South Korea (SEq: sediment equivalents).

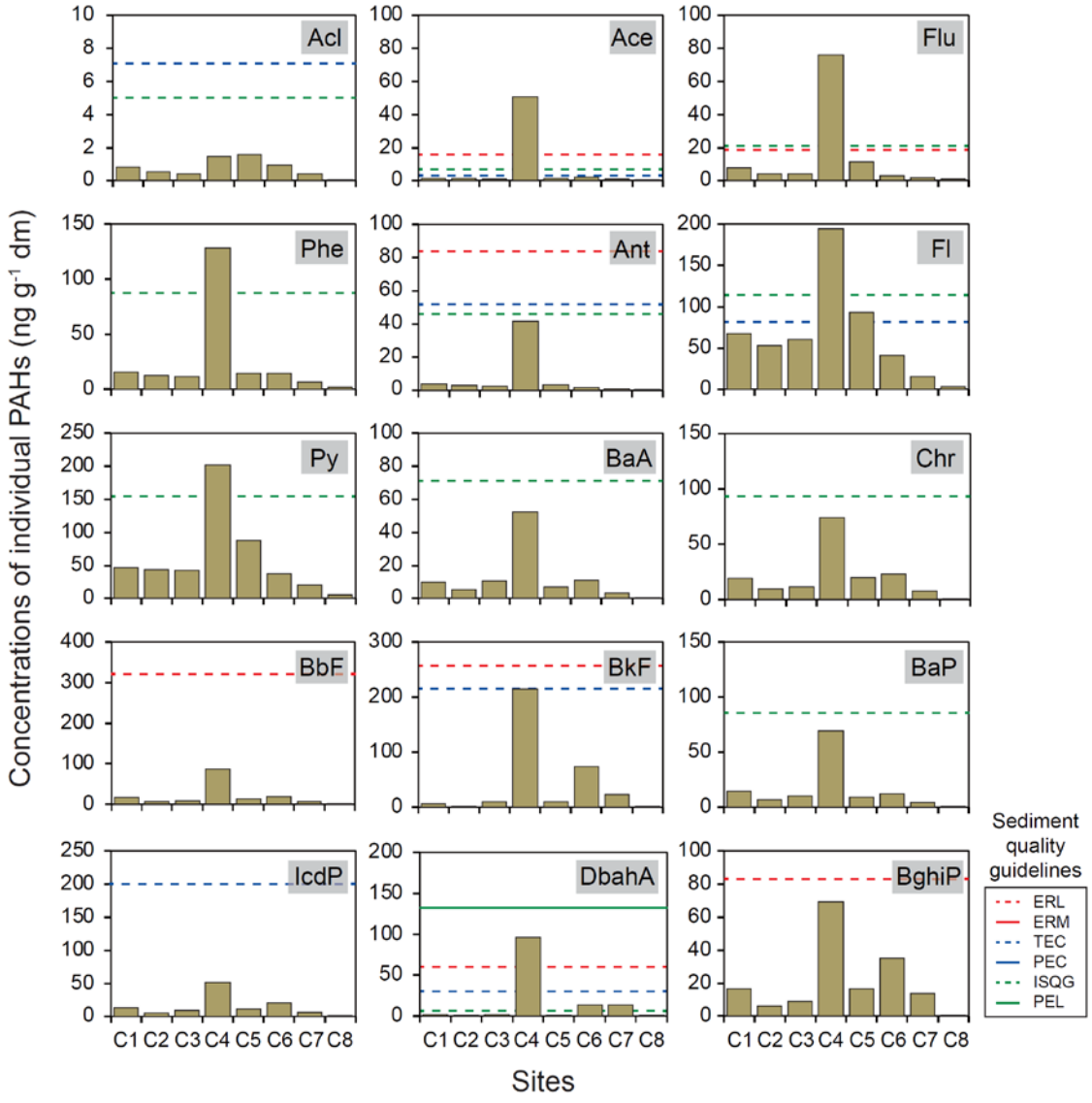

Fig. S5. Concentrations of individual PAHs in sediment samples collected from the inland creeks of Lake Sihwa, South Korea. Dotted lines indicate existing sediment quality guidelines (ERL and ERM: effect range low and median values (Long et al., 1995); TEC and PEC: threshold and probable effect concentrations (Macdonald et al., 1996); and ISQG: interim sediment quality guidelines, PEL: probable effect levels (CCME, 2002)).

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