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Are styrene oligomers in coastal sediments of an industrial area aryl hydrocarbon-receptor agonists?☆

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ABSTRACT

Effect-directed analysis (EDA) was performed to identify the major aryl hydrocarbon receptor (AhR) agonists in sediments collected from a highly industrialized area (Lake Shihwa, Korea). Great AhR-mediated potencies were found in fractions containing aromatic compounds with log K_{ow} values of 5–8, and relatively great concentrations of styrene oligomers (SOs) and polycyclic aromatic hydrocarbons (PAHs) were detected in those fractions. Until now, there was little information on occurrences and toxic relative potencies (RePs) of SOs in coastal environments. In the present study; i) distributions and compositions, ii) AhR binding affinities, and iii) contributions of SOs to total AhR-mediated potencies were determined in coastal sediments. Elevated concentrations of 10 SOs were detected in sediments of inland creeks ranging from 61 to 740 ng g^{-1} dry mass (dm), while lesser concentrations were found in inner (mean = 33 ng g^{-1} dm) and outer regions (mean = 25 ng g^{-1} dm) of the lake. Concentrations of PAHs in sediments were comparable to those of SOs. 2,4-diphenyl-1-butene (SD3) was the predominant SO analogue in sediments. SOs and PAHs were accumulated in sediments near sources, and could not be transported to remote regions due to their hydrophobicity. RePs of 3 SOs could be derived, which were 1000- to 10,000-fold less than that of one representative potent AhR active PAH, benzo[*a*]pyrene. Although concentrations of SOs in sediments were comparable to those of PAHs, the collective contribution of SOs to total AhR-mediated potencies were rather small (<1%), primarily due to their smaller RePs. Overall, the present study provides information on distributions and AhR binding affinities for SOs as baseline data for degradation products of polystyrene plastic in the coastal environment.

1. Introduction

Effect-directed analysis (EDA) has been increasingly recognized as a powerful ecotoxicological tool for identification of key toxicant(s) in complex mixtures of crude oils and in environmental matrices, such as sediments, soils, and biota (Brack, 2003; Hong et al., 2015, 2016a; Simon et al., 2015). EDA is initially conducted by biological analyses such as *in vitro* and/or *in vivo* bioassays on environmental samples. If a significant biological response is observed in raw materials, the sample is subject to fractionation to reduce complexity and separate chemicals from the original mixture (Brack, 2003; Hecker and Hollert, 2009). Biological effects of given fractions are measured by the same testing methods to identify fraction(s) with measurable toxic potencies. Complexities of samples can be reduced though rigorous fractionation, then major toxicants are isolated and finally identified by use of instrumental quantification (Hong et al., 2016a). Several studies have successfully identified key toxicants in environmental and/or

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biological samples by use of EDA in recent years (Legler et al., 2011; Simon et al., 2011, 2013; Vrabie et al., 2012; Yue et al., 2015).

Lake Shihwa is an artificial lake which has been isolated from the sea by construction of a dike in 1994. The original purpose of the lake was to supply freshwater for use in industry and agriculture (Khim and Hong, 2014; Lee et al., 2014) (Fig. 1). However, Lake Shihwa environments had deteriorated due to inadequate wastewater treatment facilities and runoff of contaminants from surrounding industrial complexes and densely populated cities (Lee et al., 2014). Consequently, the Korean government abandoned its original plan of a freshwater lake and constructed a water gate in 1999 enabling circulation of seawater. Also, more recently the government constructed the Lake Shihwa Tidal Power Station (TPS) in 2011 as part of developmental policy (Lee et al., 2014). Although the water quality has been improving in the vicinity of the gate and TPS, various organic pollutants have been found in sediments of inland creeks and the Lake Shihwa. Thus, the opening of the gate to the sea has not fully resolved the issue of pollution (Khim et al., 1999; Hong et al., 2010; Khim and Hong, 2014; Lee et al., 2014).

In the present study, EDA combining the *in vitro* H4IIE-*luc*, transactivation bioassay with gas chromatography-mass selective detector (GC-MSD) analysis was performed to identify major AhR agonists in sediments collected from the Lake Shihwa. Results of EDA indicated that the greatest AhR-mediated potencies were found in fractions F2.6 to F2.8, which contained aromatic compounds with log K_{ow} values between 5 and 8, such as polycyclic aromatic hydrocarbons (PAHs) and styrene oligomers (SOs) (details in Results and discussion).

PAHs are widely distributed in sediments, with comparatively greater concentrations in industrial area than in rural areas and are well-known aryl hydrocarbon receptor (AhR) agonists (Hong et al., 2012a). SOs, including styrene dimers (SDs) and styrene trimers (STs) are known to originate from polystyrene plastic materials (Ohyama et al., 2001; Yanagiba et al., 2008; Kwon et al., 2014, 2015). Various SOs analogues are known to be derived from degradation of polystyrene, particularly during thermal decomposition at temperatures of 240–300 °C (Kitamura et al., 2003; Kwon et al., 2014). Polystyrene has been widely utilized for food containers (Ohyama et al., 2001), and SOs are known to migrate from polystyrene containers into foods (Hirano et al., 2001). Thus, studies on toxic effects of SOs have been concentrated in the fields of Food Chemistry and Health Sciences. However, there was little information on occurrences of SOs in coastal environments and their potential toxic effects on wildlife.

Synthetic polymers used in plastics have been thought to be chemically stable and resistant to biodegradable in aquatic environments (Carpenter and Smith, 1972; Lucas et al., 2008; Andrady, 2011; Kwon et al., 2015). Few studies have reported distributions of only a few SOs in selected areas (Kwon et al., 2015). In addition, there was no information on the physico-chemical properties of SOs. SOs have been reported to cause adverse or toxic effects on cells and organisms, such as endocrine-disrupting effects (Hirano et al., 2001; Date et al., 2002; Kitamura et al., 2003), genotoxicity (Nakai et al., 2014), proliferative activity (Ohyama et al., 2001), thyrogenic activity (Yanagiba et al., 2008), and inhibition of reproduction of daphnids (Tatarazako et al., 2002). STs have three aromatic rings, in structures similar to AhR-active compounds (Yanagiba et al., 2008), however, no experimental evidence has been documented for their AhR-binding potencies.

Specific objectives of the present study were to: i) screen and identify the major AhR-mediated potencies and agonists in sediments of inland creek flowing into Lake Shihwa by use of EDA; ii) investigate distributions and compositions of SOs in coastal sediments, iii) determine relative potency values (RePs) for AhR-mediated activities for 10 SOs, and iv) assess and compare relative contributions of SOs and PAHs to overall induced AhR-mediated potencies in sediments. To the best of our knowledge, this is the first report describing occurrences of SOs in sediments

Fig. 1. Map showing the sampling sites of surface sediments from inland creeks and inner and outer regions of the Lake Shihwa.

and AhR-mediated potency of individual SOs.

2. Materials and methods

2.1. Collection and preparation of sediments

Sediments were collected from inland creeks (C1-C6) and inner (1–11) and outer (12–15) regions of Lake Shihwa, South Korea (Fig. 1). Surface sediments of inland creeks were collected by a hand shovel in April 2015. In inner and outer regions of Lake Shihwa, surface sediments were collected by use of a Van Veen grab in May 2015. Samples were transferred into pre-cleaned glass jars, and then immediately transported to the laboratory where they were stored at - 20 °C until analysis.

Sample preparation methods for bioassay and chemical analysis are described elsewhere (Hong et al., 2012b, 2015, 2016b). In brief, a 30 g of freeze-dried sediment was extracted with 350 mL of dichloromethane (Burdick & Jackson, Muskegon, MI) on a Soxhlet extractor for 24 h. Elemental sulfur in extracts was removed by use of activated copper (Merck, Darmstadt, Germany), and organic extracts were concentrated to 3 mL (~10 g sediment mL^{-1}). Solvent of the extract being used for the bioassay was exchanged with dimethyl sulfoxide (DMSO, Burdick & Jackson).

2.2. Silica gel and RP-HPLC fractionations

One milliliter of raw extract (RE) was passed through 10 g of activated silica gel (70–230 mesh, Merck) in a packed glass column for fractionation (Hong et al., 2015, 2016b). The first fraction (F1), containing non-polar compounds, was eluted with 40 mL hexane (Burdick & Jackson). The aromatic fraction (F2) was collected by elution with 50 mL of 20% DCM in hexane (v/v). The third fraction (F3), which contained polar compounds, was eluted in 50 mL of 60% DCM in acetone (Burdick & Jackson). The fourth fraction (F4), which contained column residues was eluted by use of 50 mL of acetone. All elutriates were concentrated, by use of a rotary evaporator and nitrogen concentrator, to 1 mL for use in the bioassay, instrumental analysis, or further fractionation.

The F2 and F3 fractions which contained the majority of AhR agonists were further separated into 10 sub-fractions, using reversed phase (RP)-HPLC (Agilent 1260 HPLC, Agilent Technologies, Avondale, PA) with multiple wavelength detector (Fig. S1 of Supplementary Materials (S)) (Hong et al., 2016a). Gradient conditions for fractionation on the C18 column (PrepHT XBD-C18, 21.2 × 250 mm, 7 μm, Agilent Technologies) were optimized by use of calibration curves between log K_{ow} values of known chemicals and HPLC retention times. Gradient conditions for HPLC were optimized by use of the calibration curve (Eq. (1)) using 34 polychlorinated biphenyls (PCBs), 16 PAHs, 7 alkylphenols, and 5 phthalates with water/methanol as the mobile phase (Vrabie et al., 2012; Hong et al., 2016a) (Fig. S1).

$$\text{HPLC retention time (min.)} = 6.48 \times \log K_{ow} + 1.81 \quad (1)$$

Based on this calibration curve, 10 fine fractions were collected at intervals of 1 for log K_{ow} units (such as < 1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, and >9) of compounds in fractions (Table S1). Injection volume of each fraction was 1 mL and flow rate of mobile phase was 10 mL min^{-1}. Sub-fractions (about 65 mL of water/methanol) were collected, and extracted with hexane, which was concentrated to 1 mL and exchanged to DMSO for further bioassay.

2.3. In vitro bioassay

H4IIE-*luc* bioassay was performed according to previously reported methods (Hong et al., 2012b). Sediments REs, fractions (silica gel column and RP-HPLC), and 10 individual SOs were tested in the bioassay with two different durations of exposure (4 or 72 h), in order to identify metabolically labile compounds (e.g., PAHs) or stably AhR binding compounds (e.g., polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and coplanar PCBs) (Louiz et al., 2008; Lee et al., 2013; Larsson et al., 2014). Dilution factors of each sample were determined (% live cell > 80%) by use of cell proliferation assay (WST).

Trypsinized cells (~8.0×10^4 cells mL^{-1}) were seeded into the 60 interior wells of 96 micro-well plates at a volume of 250 μL per well. After 24 h incubation, test and control wells were dosed with the appropriate standards (benzo[*a*]pyrene (BaP) for 4 h exposure and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for 72 h exposure; 0.1% dose), sample (REs, fractions, or SOs; 0.1% dose), and solvent controls (0.1% DMSO). After 4 h or 72 h of exposure, luminescence of luciferase was quantified by use of a Victor X3 multi label plate reader (Perkin-Elmer, Waltham, MA). Responses of the H4IIE-*luc* bioassay (expressed as mean relative luminescence units) were converted to percentages of the maximum response (%BaP_{max} or % $TCDD_{max}$) observed for a 50 nM (=100% BaP_{max}) of BaP or 300 pM (= 100% $TCDD_{max}$) of TCDD. Finally, AhR-mediated potency at 4 h exposure was expressed as a BaP equivalent concentration (BaP-EQ) for direct comparison to instrumentally-derived BaP equivalent concentrations (BEQs).

2.4. GC-MSD screening

The most potent fractions, based on the results of the H4IIE-*luc* bioassay, were used to identify major AhR agonists by use of an Agilent 7890 GC coupled to a model 5975C MSD (Agilent Technologies) (details in Fig. S2). A capillary column DB-5MS (30 m long × 0.25 mm i.d.; film thickness: 0.25 μm, J&W Scientific, Folsom, CA) was utilized for the separation. The mass spectrometer was operated in electron impact ionization mode at 70 eV with the full scan mode (m/z = 50–550).

2.5. Analyses of SOs and PAHs

SOs and PAHs in sediments of inland creeks and inner and outer regions of the Lake Shihwa were quantified. Four SDs and six STs (full chemical name and abbreviations given in Table 1) as target SOs were obtained from the Wako Pure Chemical Ind. (Osaka, Japan) and Hayashi Pure Chemical Ind. (Osaka, Japan) (Table 1 and Fig. S3). A total of 15 parent PAHs including 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 (DbahA), and benzo[*g*,*h*,*i*]perylene (BghiP) were quantified, mixture standards were obtained from ChemService (Chester, PA).

In the EDA procedure, the SOs and PAHs were detected in F2 of sediments and standard materials, with no measurable detections in F1 or F3, which indicated that SOs have similar physico-chemical properties with PAHs. Thus, it is suggested that quantification of SOs, including methods of extraction, clean-up, and GC-MSD analysis (except for monitoring ions) could be conducted simultaneously with PAHs. However, due to interferences of peak integrations and great difference of peak heights during GC/MSD analysis, concentrations of SOs and PAHs were quantified in separately.

Extraction and clean-up procedures for SOs and PAHs in sediments were performed as described above. Isotopically-labeled surrogate standards (Ace-d10, Phe-d10, Chr-d12, and Pery-d12, ChemService) were added for assessment of recovery of target

Table 1
Chemical properties and GC/MSD retention times and mass fragment ions of 10 styrene oligomers.

Name	Abb. [a]	CAS number	Company	Molecular mass	Molecular formula	GC RT [b] (min.)	Mass fragment ions (*m/z*)
Styrene dimers							
1,3-Diphenylproane	SD1	1081-75-0	Wako	196.29	$C_{15}H_{16}$	21.10	*92*, 196, 105
cis-1,2-Diphenylcyclobutane	SD2	7694-30-6	Hayashi	208.30	$C_{16}H_{16}$	21.92	*104*, 208, 78
2,4-Diphenyl-1-butene	SD3	16,606-47-6	Wako	208.30	$C_{16}H_{16}$	22.35	*91*, 208, 104
trans-1,2-Diphenylcyclobutane	SD4	20,071-09-4	Wako	208.30	$C_{16}H_{16}$	22.93	*104*, 208, 78
Styrene trimers							
2,4,6-Triphenyl-1-hexene	ST1	18,964-53-9	Wako	312.45	$C_{24}H_{24}$	33.52	*91*, 117, 194, 207
1e-Phenyl-4e-(1-phenylethyl)-tetralin	ST2	26,681-79-8	Wako	312.45	$C_{24}H_{24}$	34.76	*91*, 129, 207, 105
1a-Phenyl-4e-(1-phenylethyl)-tetralin	ST3	26,681-79-8	Wako	312.45	$C_{24}H_{24}$	34.93	*91*, 129, 207, 105
1a-Phenyl-4a-(1-phenylethyl)-tetralin	ST4	26,681-79-8	Hayashi	312.45	$C_{24}H_{24}$	35.06	*91*, 129, 207, 105
1e-Phenyl-4a-(1-phenylethyl)-tetralin	ST5	26,681-79-8	Hayashi	312.45	$C_{24}H_{24}$	35.19	*91*, 129, 207, 105
1,3,5-Triphenylcyclohexane (isomer mix)	ST6	28,336-57-4	Wako	312.45	$C_{24}H_{24}$	36.87	*91*, 104, 130, 312
						37.33	*91*, 117, 104, 312

[a] Abb.: abbreviations.
[b] GC RT: gas chromatography retention time.

analytes before extraction. Instrumental internal standard (2-fluorobiphenyl, ChemService) was added both in SOs and PAHs quantifications before GC injection. SOs and PAHs were identified and quantified by use of GC-MSD with selected ion monitoring (SIM) mode (Table 1 and Figs. S2–S4).

As QA/QC purpose, the matrix spike test was performed for five sediments in a separate experiment using 10 SOs, and recoveries were generally acceptable with an average of 92% (details in Table S2). Recoveries of surrogate standards ranged from 64 to 94% (mean = 78%) for Ace-d10, from 67 to 128% (mean = 88%) for Phe-d10, from 74 to 127% (mean = 101%) for Chr-d12, and 92–126% (mean = 105%) for Pery-d12, respectively. Method detection limits (MDL) for individual SOs and PAHs ranged from 0.10 to 0.42 and from 0.10 to 0.50 ng g^{-1} dry mass (dm), respectively (Table S2).

2.6. Relative potency values

RePs for AhR-mediated potencies were developed by use of the H4IIE-*luc* bioassay based on the effective concentration at 20% of maximum level achieved by BaP (EC-20) of each SOs analogue. A total of 10 individual SOs were selected to evaluate novel RePs for AhR-mediated potency (Table 1). The chemicals were prepared at six concentrations using 5-fold serial dilution (viz., 1000, 200, 40, 8.0, 1.6, and 0.32 μg mL^{-1}), and were tested as described above. The RePs were estimated by fitting the curves of the dose–response relationship according to the method reported previously (Lee et al., 2013).

2.7. Potency balance analysis

Potency balances between bioassay-derived BaP-EQs and instrument-derived BEQs were conducted to determine the contribution of each known chemical to total induced AhR-mediated potency. Concentrations of BEQs were calculated as the sum of the equivalent concentrations of individual SOs and PAHs multiplied by their ReP values, either previously reported or newly obtained from this study (Louiz et al., 2008) (Table S3).

3. Results and discussion

3.1. Effect-directed analysis of sediments

Significant AhR-mediated potencies were observed in all of sediment REs from six inland creeks for both 4 and 72 h exposures in the H4IIE-*luc* bioassay (Fig. S5). AhR-mediated potencies reached saturating efficacy for the REs at 4 h exposure, ranging from 95 to 120% BaP_{max} (mean = 109% BaP_{max}), while responses of REs at 72 h exposure greatly varied among samples, ranging from 37 to 104% $TCDD_{max}$ (mean = 77% $TCDD_{max}$). Among silica gel fractions of selected organic extracts, such as C2 (Shingil), C3 (Shiheung), C5 (Gunja), and C6 (Okgu), relatively great AhR-mediated potencies were commonly found in F2 (aromatics) and F3 (polar), while smaller responses were observed in F1 (non-polar) and F4 (column residues elutriate) for both exposures of 4 and 72 h (Fig. 2). In RP-HPLC sub-fractions of F2, greater AhR-mediated potencies were found in F2.6, F2.7, and F2.8 at 4 h exposure, however, lesser responses were observed at 72 h exposure, except for F2.1 of C2 and C6.

In general, comparison of AhR-mediated potencies between 4 and 72 h exposures in the H4IIE-*luc* bioassay provides useful metabolic information on major AhR agonists in environmental mixture samples. For example, PAHs can be easily degraded due to metabolism by H4IIE-*luc* cells during the longer exposure (48 or 72 h) (Villeneuve et al., 2002; Louiz et al., 2008; Larsson et al., 2014). However, strong AhR agonists, PCDD/Fs and coplanar PCBs are relatively stable as a function of exposure duration in the H4IIE-*luc* bioassay (>48 h) (Lee et al., 2013). Thus, results of the present study suggested that major AhR agonists in coastal sediments of Lake Shihwa were aromatic compounds, including PAHs with log K_{ow} values between 5 and 8, and PCDD/Fs and coplanar PCBs did not seem to be major AhR agonists.

In order to identify the major AhR agonists in more potent fractions, such as F2, F2.6, F2.7, and F2.8 of sediments from inland creeks, these fractions were analyzed by use of GC-MSD (full scan, *m/z* 50–550). Several peaks were found in GC-MSD chromatograms in corresponding fractions. Based on peak patterns and mass fragment ions reported in previous studies (Nakada et al., 2000; Suzuki et al., 2003; Saido et al., 2014), we could narrow suspected chemicals to be SOs. Since then, individual SOs were confirmed by use of commercial standard materials (GC retention time and mass fragment ions) (Table 1 and Figs. S3 and S4). The SDs (SD1 – SD4) and STs (ST1 – ST6) were detected in F2.6 and F2.7, respectively, together with PAHs, which indicates that SOs are non-polar aromatic compounds. Log K_{ow} values estimated by use of their retention times of RP-HPLC (Eq. (1)) range from 5.30 to 5.53 for SDs and from 6.37 to 6.75 for STs, respectively.

Meanwhile, complexities of F3 and even its sub-fractions were still very great in the results of GC-MSD screening. Thus, it is challenging to identify causative compounds in F3 of sediment extracts. Elevated AhR-mediated potencies were also found in F3, however, there were no significant responses detected in F3 sub-fractions (F3.1 to F3.10) (data not shown). This phenomenon seemed to be due to mixture toxic effects (e.g., synergism) across the compounds in F3, say between known and/or unknown

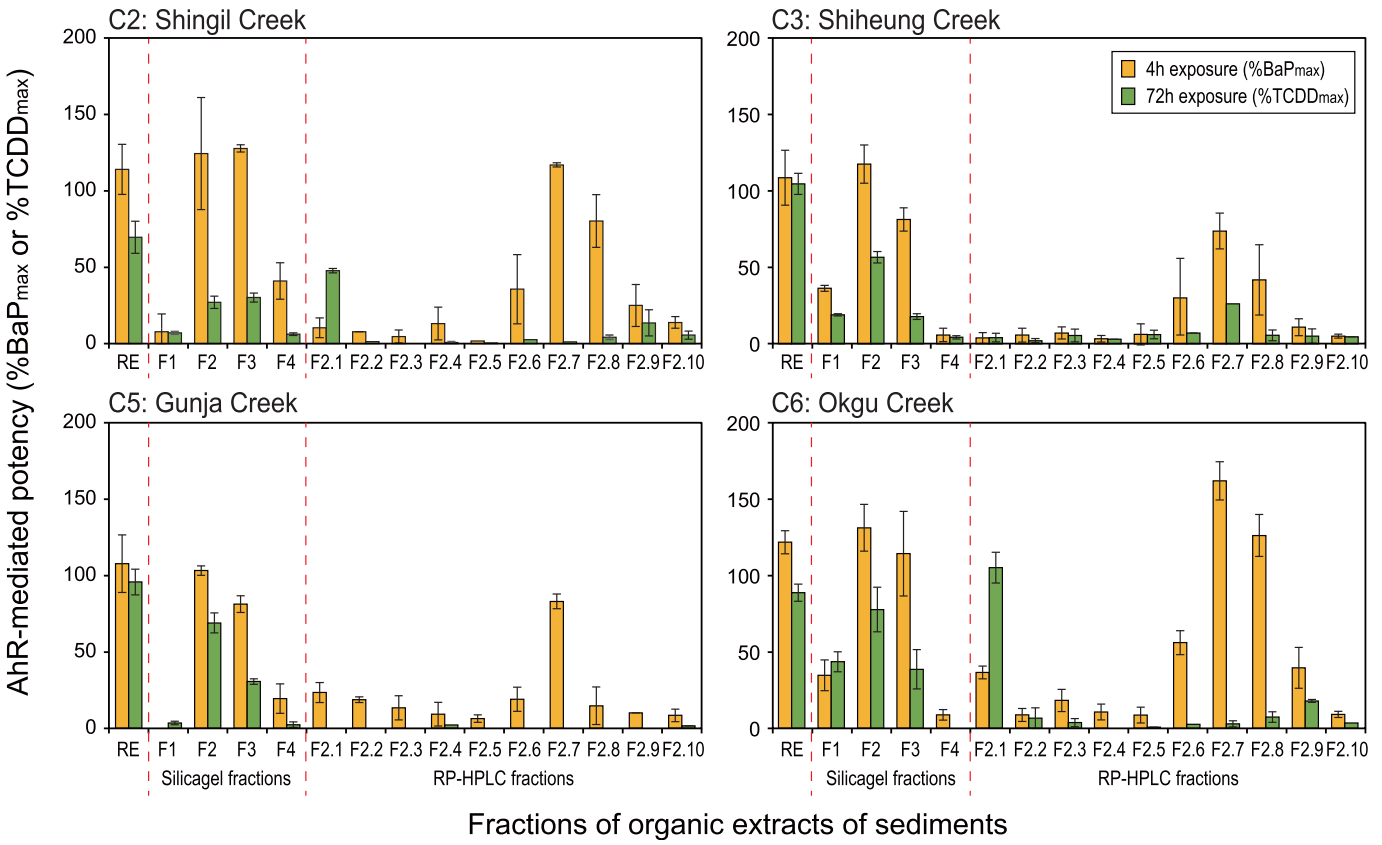

Fig. 2. AhR-mediated potencies of raw extracts (RE) and fractions (silica gel and RP-HPLC) of selected inland creeks sediments determined at 4 and 72 h exposure durations in the H4IIE-*luc* bioassay (Error bar: mean ± SD (n = 3)).

agonists in sediments. However, to verify such mixture effects among polar compounds in F3 subfractions of sediments, it is deemed absolutely necessary to prove that synergism occurred through further confirmation (e.g., iterative testing for combined subfractions).

3.2. Distributions and compositions of styrene oligomers

SOs and PAHs were detected in all sediments from inland creeks and inner and outer regions of Lake Shihwa (Fig. 3). Concentrations of SOs in sediments ranged from 61 to 740 ng g^{-1} dm (mean = 400 ng g^{-1} dm) in inland creeks (n = 6), from 10 to 70 ng g^{-1} dm (mean = 33 ng g^{-1} dm) in inner regions (n = 11), and from 20 to 35 ng g^{-1} dm (mean = 25 ng g^{-1} dm) in outer regions (n = 4), respectively (details in Table S4). Concentrations of PAHs in sediments were comparable to those of SOs, which were 690 ng g^{-1} dm (210–1900 ng g^{-1} dm), 45 ng g^{-1} dm (25–79 ng g^{-1} dm), and 42 ng g^{-1} dm (29–62 ng g^{-1} dm) in inland creeks, inner and outer regions, respectively (details in Table S5). Concentrations of SOs and PAHs decreased drastically from inland creeks to Lake Shihwa (Fig. 3a and b). Spatial distributions of SOs and PAHs indicated that chemicals seemed to be accumulated in sediments near the sources, and could not be transported to remote regions due to their hydrophobic nature.

Compositions of SOs in sediments of inland creeks of Lake Shihwa were relatively consistent among samples (Fig. 3c). Among 10 SOs, SD3 (21%) was the predominant chemical in creeks sediments, followed by ST3 (20%), ST2 (17%), ST1 (11%), ST4 (11%), and ST5 (11%). Minor SOs, such as SD4, SD1, SD2, and ST6 contributed less than 5% to total concentrations of SOs in inland creeks. Relative contributions of SD3 in sediments increased greatly in the inner (mean = 66%) and outer regions (mean = 75%) of Lake Shihwa. This result indicated that the SD3 were widely distributed in the coastal area and seemed to be transported relatively far from land. Relative great contributions of STs were found in some sites located in inner and outer regions which seemed to be affected by direct inputs from local sources and/or a wastewater treatment plant (WWTP) outfall (Fig. S6). Concentration ratios of SDs and STs were 0.40, 3.2, and 5.2 in sediments of inland creeks and inner and outer regions of Lake Shihwa, respectively. Thus, we suggest that the SDs/STs ratio can be used as an indicator of sources of polystyrene plastic pollution in coastal sediments.

Meanwhile, compositions of PAHs in sediments were relatively constant among sites from inland creeks to coastal areas (Fig. 3d). These patterns of relative concentrations of PAHs could be explained by the different sources, transport pathways, and compound-specific degradability of PAHs in the coastal environments (Simcik et al., 1999; Yunker et al., 2002; Haritash and Kaushik, 2009; Hong et al., 2012a).

Few studies have been conducted on occurrences and distributions of SOs in coastal sediments with only reports for three SOs, including SD1, SD3, and ST1 (Kwon et al., 2014, 2015; Saido et al., 2014). Major SOs in sediments found in the present study, including ST2, ST3, ST4, and ST5, which accounted for over 60% of total concentrations of SOs in inland creeks were excluded past studies. Although lacking some SOs, previous studies demonstrated that SOs are distributed globally in sand beaches, where they originated mainly from polystyrene and may persist in sand for an unknown period of time (Kwon et al., 2015). SOs are also derived from decomposition of polystyrene polymer as well as from marine

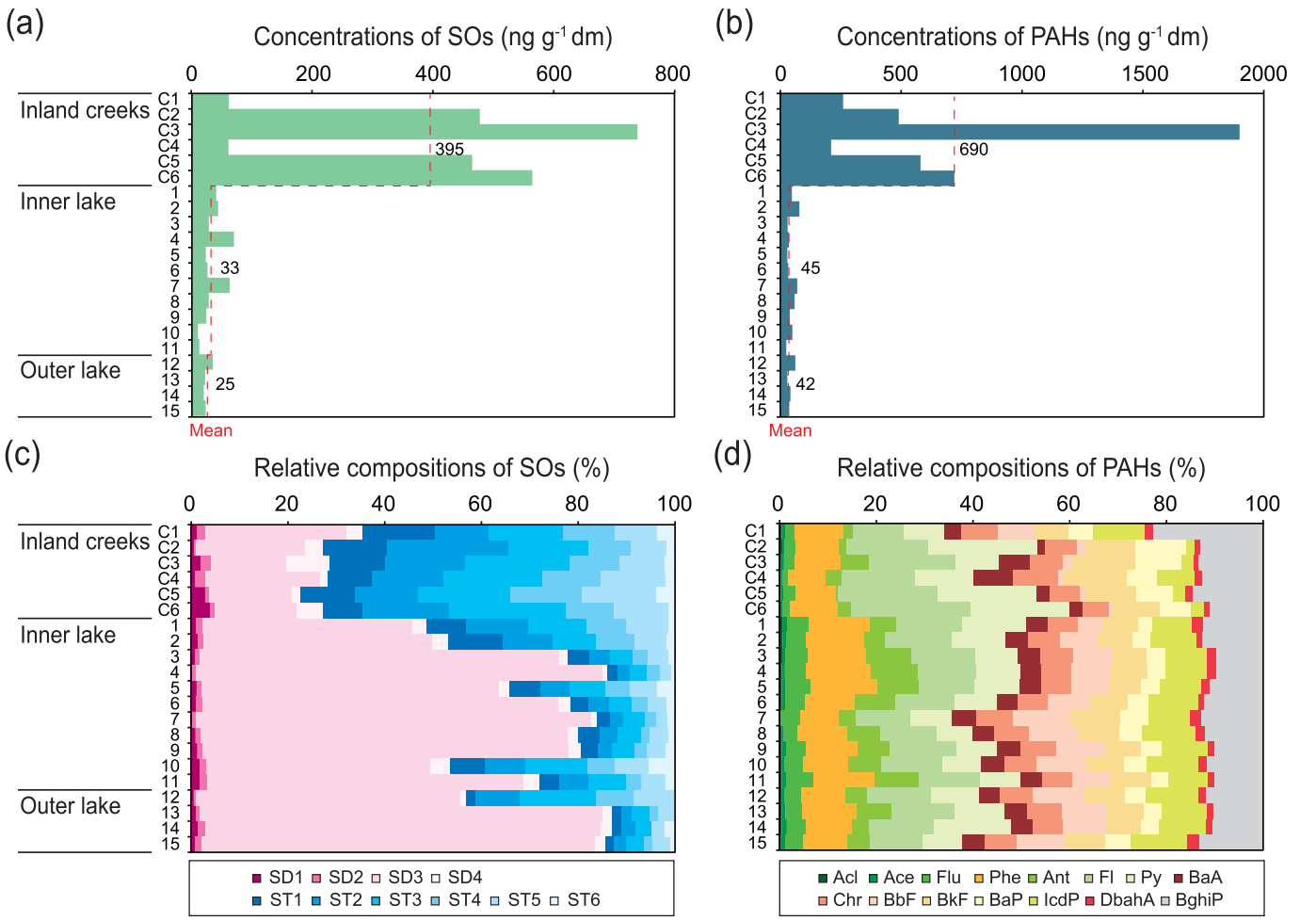

Fig. 3. Distributions of (a) 10 styrene oligomers and (b) 15 PAHs and relative compositions of (c) styrene oligomers and (d) PAHs in sediments from inland creeks and inner and outer regions of the Lake Shihwa.

plastic debris (Kwon et al., 2015). Synthetic plastic polymers have been recognized to be chemically stable and less biodegradable in environments (Carpenter and Smith, 1972; Lucas et al., 2008; Andrady, 2011; Kwon et al., 2015). Thus, SOs should be considered to be new pollutants of emerging concern, particularly in more industrialized, coastal areas.

Overall, the present study provides useful information on concentration, composition, and distribution of SOs as baseline data for the future study of plastic pollution. We suggest the distribution of SOs in sediments could be useful indicators of chemical pollution by polystyrene polymers in coastal environments considering the environmental chemo-dynamic properties evidenced from this study.

3.3. Relative potency of styrene oligomers as AhR agonists

In order to assess affinities of SOs to bind to the AhR, 10 individual SOs, including 4 SDs and 6 STs were tested by use of H4IIE-*luc* bioassay. Out of 10 chemicals tested, 8 SOs (SD1-ST4 and ST1-ST4) exhibited significant AhR-mediated responses (significant level = 5% BaP_{max}), in maximal efficacy, at concentrations tested (Fig. 4). Among these chemicals, 3 SOs, SD1, SD3, and ST2, elicited sufficiently strong responses to enable calculation of corresponding RePs based on EC-20 concentrations (Fig. 4). ReP values of these 3 SOs relative to the AhR-mediated potency of BaP were 2.3×10^{-3}, 3.0×10^{-4}, and 2.7×10^{-3} for SD1, SD3, and ST2, respectively. ReP values of SD1, SD3, and ST2 were one to three orders of magnitude less than those of well-known AhR active PAHs, such as Flu (1.44×10^{-2}), BaA (2.58×10^{-1}), Chr (2.92×10^{-1}), BbF (6.94×10^{-1}), BkF (2.94), IcdP (8.43×10^{-1}), and DbahA (3.66) (Table S3) (Louiz et al., 2008). ReP values of these three SOs were comparable to Py (3.58×10^{-3}) and Acl (5.56×10^{-3}) (Louiz et al., 2008). Overall, some SOs have an AhR binding potential, but affinities are generally less than those of PAHs.

SOs did not show significant AhR-mediated potencies during 72 h exposures in the H4IIE-*luc* bioassays (data not shown). This result indicated that SOs could be easily metabolized in H4IIE-*luc* cells during the exposure periods, similarly to PAHs. Rates of metabolisms of chemicals during exposures in cell-based bioassays showed a compound-specific manner and might be dependent on their molecular structures (Larsson et al., 2014). Thus, it is suggested that risk assessment of potential toxic effects of SOs by use of cell-based bioassays should consider metabolism by testing time-dependent responses during the longer duration of exposure.

3.4. Potency balance analysis

Results of the potency balance between bioassay-derived BaP-EQs and instrument-derived BEQs was conducted to address chemical specific contribution to total induced AhR-mediated responses, targeting the most potent fractions, such as F2.6 and F2.7 of inland creek sediments (Fig. 5). Instrument-derived BEQs were

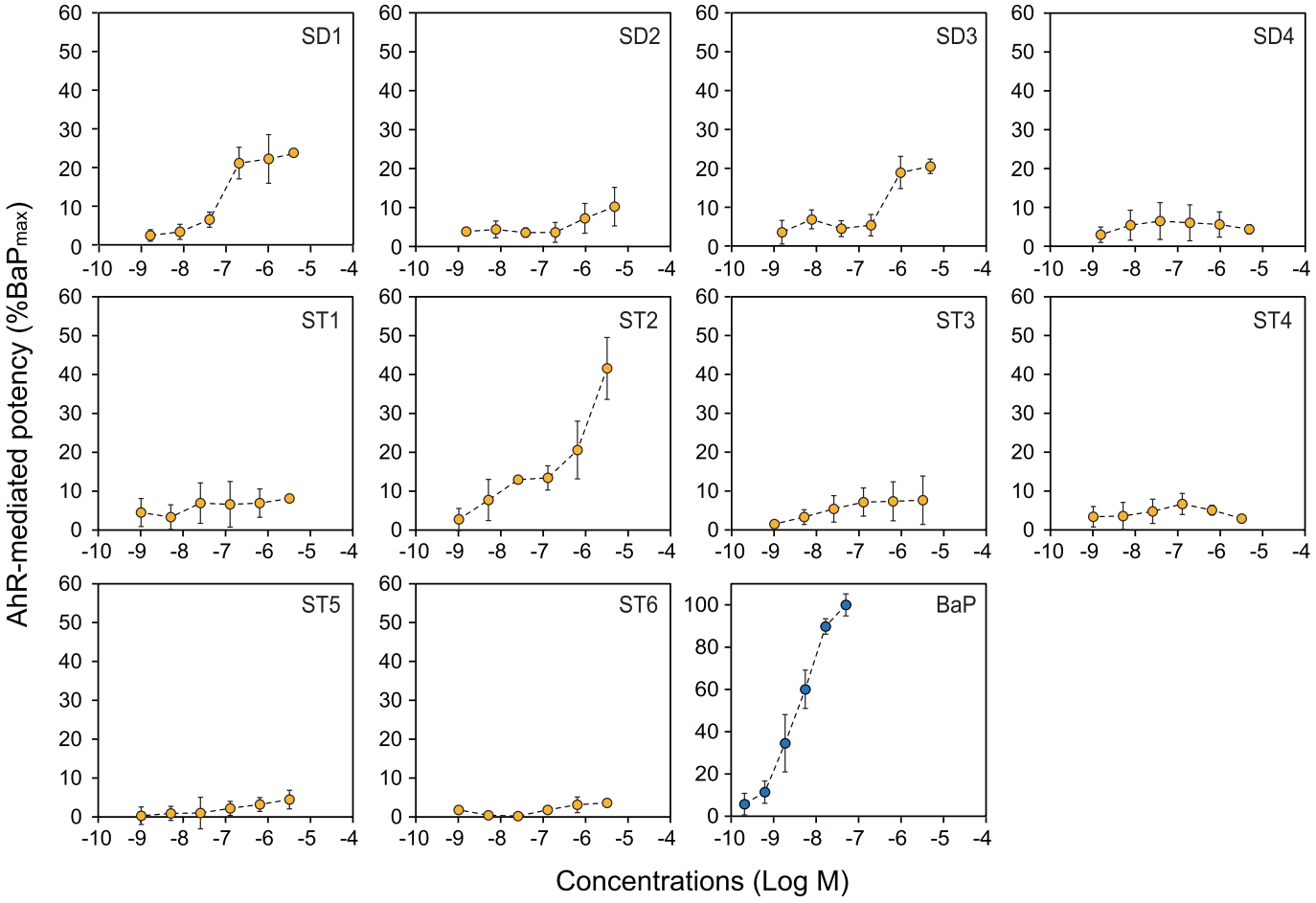

Fig. 4. Dose-response relationships for AhR-mediated activities of styrene oligomers and benzo [*a*]pyrene in the H4IIE-*luc* bioassays. Each point is the mean ± SD of three independent assays in triplicate.

calculated based on reported RePs of 10 AhR-active PAHs determined previously (Louiz et al., 2008) and newly obtained RePs of 3 SOs from this study. In F2.6 fractions of inland creek sediments, concentrations of BEQs of four- to five-ring PAHs, such as Fl, Py, BaA, and Chr and SDs (SD1 and SD3) explained 4.4–94% of the total induced BaP-EQs measured in the H4IIE-*luc* bioassay. In fraction F2.7, BEQs contributed by five- to six-ring PAHs, such as BbF, BkF, BaP, IcdP, and DbahA and ST2 accounted for about 10–67% of BaP-EQs (Fig. 5). PAHs, such as Chr (~25% of BaP-EQ) and BkF (~32% of BaP-EQ) were the most potent AhR agonists in fractions F2.6 and F2.7, accounting for the majority of BaP-EQs. AhR-active SOs, SD1, SD3, and ST2 explained only a small portion of concentrations of BaP-EQs in sub-fractions of F2, ranging from 0.01 to 0.32% for F2.6 (mean = 0.13%) and from 0.12 to 0.22% for F2.7 (mean = 0.16%), respectively. Due to their lesser ReP values, contributions of SOs to overall AhR-mediated potencies in sediments were not very great even though the sedimentary concentrations were comparable those of PAHs. Accordingly, the power of explanation for BaP-EQs by measured AhR-active compounds in sediments was not improved by additional quantification of selected SOs.

Targeted PAHs and SOs explained 41% and 48% of BaP-EQs in F2.6 and F2.7 measured by use of the H4IIE-*luc* bioassay at 4 h exposure, on average. This result, in turn, indicates that other unknown AhR agonists might exist in Lake Shihwa sediments which seem to be aromatic compounds with log K_{ow} values between 5 and 7. For example, several untargeted PAHs and/or oxygenated-, methylated-, and N-containing derivatives (e.g., benzo[*a*]fluorenone, naphthacene, 9,10-dihydrobenzo[*a*]pyren-7(8H)-none, 7H-benz[*d*,*e*]anthracen-7-one, dibenzo[*a*,*h*]acridine, and 2-methylanthracene-9,10-dione) have also been reported to show AhR binding affinity in the H4IIE-*luc* bioassay, some of which could be possible unknown or unmeasured agonists in the given area (Larsson et al., 2014). Well-known, strong, and traditional AhR agonists such as PCDD/Fs and coplanar PCBs did not seem to be major contributors in Lake Shihwa, because small AhR-mediated potencies were observed in the H4IIE-*luc* bioassay for 72 h exposures.

Although less AhR-mediated potencies of SOs were observed in the present study, due to their persistent and hydrophobic characteristics, SOs could affect other adverse effects on benthic invertebrates. Results of a previous study indicated that some SOs, such as SD2 (as named in this study), SD4, ST1, ST3, ST4, and ST5 exhibit estrogenic activity in the MCF-7 cell bioassay (Ohyama et al., 2001). Relative estrogenic potencies of these chemicals were reported to be comparable to that of bisphenol A, a well-known potent environmental estrogen (Ohyama et al., 2001). In addition, SDs and STs could affect fertility (25% reduction) of daphnids (*Ceriodaphnia dubia*) and have a toxic potential on crustacean in aquatic environments (Tatarazako et al., 2002). Thus, more complementary studies to address ecotoxicological effects of SOs with various toxicological endpoints would be necessary in terms of effect (or risk)-based management for the given emerging pollutants in coastal environments.

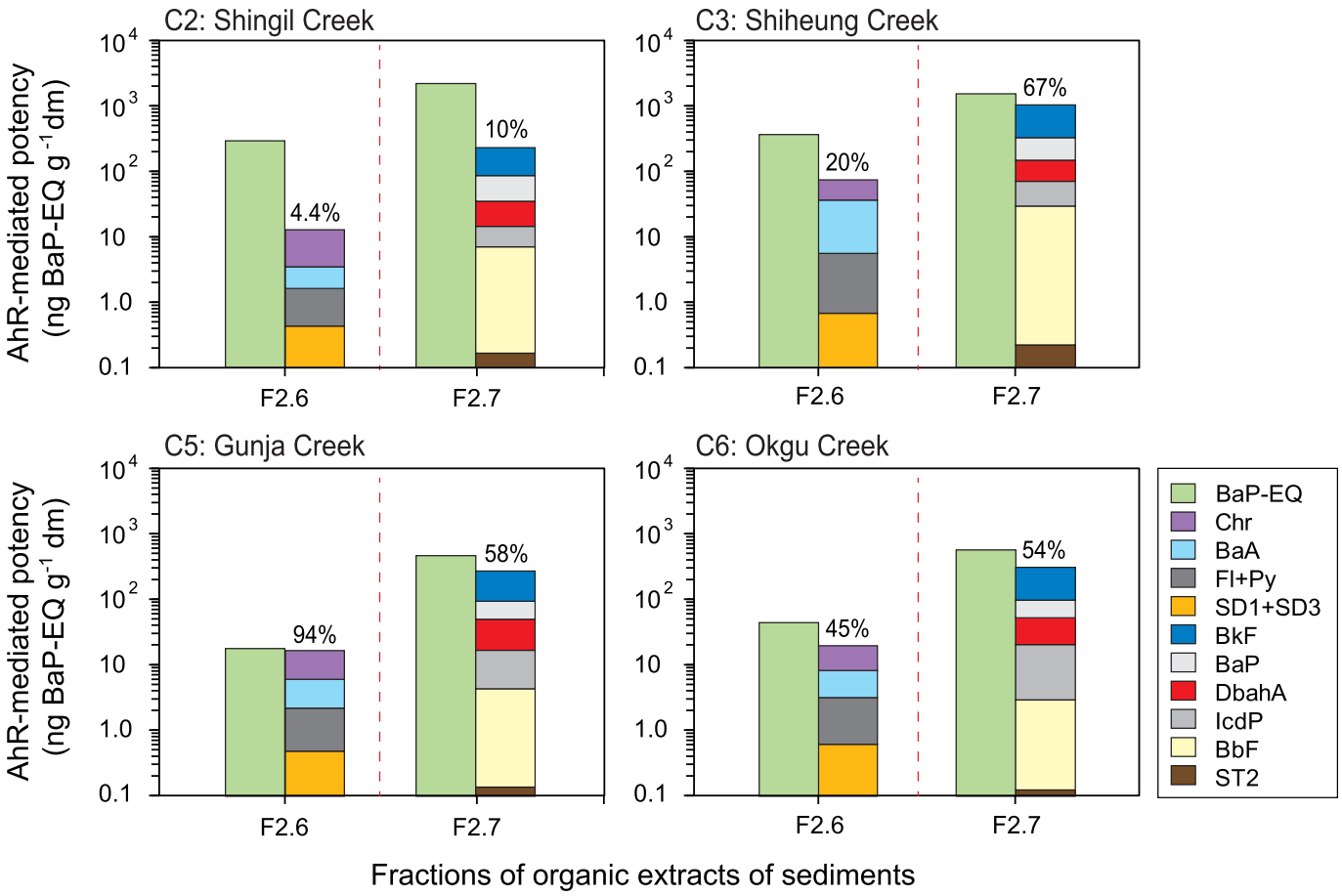

Fig. 5. Potency balance between bioassay-derived BaP-EQs and instrument-derived TEQs in the RP-HPLC fractions (F2.6 and F2.7) of selected inland creeks sediments (Error bar: mean ± SD (n = 3)).

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This work was supported by the project entitled "Development of Techniques for Assessment and Management of Hazardous Chemicals in the Marine Environment" funded by the Ministry of Oceans and Fisheries of Korea given to JSK. JPG was supported by the Canada Research Chair program, a Visiting Distinguished Professorship in the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, the 2012 "High Level Foreign Experts" (#GDW20123200120) program, funded by the State Administration of Foreign Experts Affairs, the P.R. China to Nanjing University and the Einstein Professor Program of the Chinese Academy of Sciences.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.03.025.

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<Supplementary Materials>

Are styrene oligomers potential aryl hydrocarbon-receptor agonists in coastal sediments of an industrial area?

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Supplementary Tables

Supplementary Figures

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Supplementary Tables

Table S1. Sampling times and log K_{ow} intervals of RP-HPLC fractionation.

RP-HPLC Sub-fraction	Starting sampling time (min.)	End sampling time (min.)	Log K_{ow}
1	1.81	8.30	< 1
2	8.30	14.78	1 – 2
3	14.78	21.27	2 – 3
4	21.27	27.75	3 – 4
5	27.75	34.24	4 – 5
6	34.24	40.73	5 – 6
7	40.73	47.21	6 – 7
8	47.21	53.70	7 – 8
9	53.70	60.18	8 – 9
10	60.18	66.67	> 9

Table S2. Summary of QA/QC results for analysis of styrene oligomers.

Styrene oligomers	**Abbreviation**	**Recovery (%, n = 5)**	**Method detection limits (%, n = 5)**
1,3-Diphenylproane	SD1	84	0.24
cis-1,2-Diphenylcyclobutane	SD2	81	0.16
2,4-Diphenyl-1-butene	SD3	91	0.40
trans-1,2-Diphenylcyclobutane	SD4	101	0.06
2,4,6-Triphenyl-1-hexene	ST1	91	0.10
1e-Phenyl-4e-(1-phenylethyl)-tetralin	ST2	91	0.26
1a-Phenyl-4e-(1-phenylethyl)-tetralin	ST3	96	0.12
1a-Phenyl-4a-(1-phenylethyl)-tetralin	ST4	91	0.33
1e-Phenyl-4a-(1-phenylethyl)-tetralin	ST5	97	0.42
1,3,5-Triphenylcyclohexane (isomer mix)	ST6	96	0.19

Table S3. Relative potency values for AhR-mediated activity of PAHs reported previously.

PAHs	ReP values	Reference
Acenaphthylene	5.56×10^{-3}	Louiz et al., 2008
Fluoranthene	1.44×10^{-2}	
Pyrene	3.58×10^{-3}	
Benz[*a*]anthracene	2.58×10^{-1}	
Chrysene	2.92×10^{-1}	
Benzo[*b*]fluoranthene	6.94×10^{-1}	
Benzo[*k*]fluoranthene	2.94	
Benzo[*a*]pyrene	1.00	
Indeno[*1,2,3-c,d*]pyrene	8.43×10^{-1}	
Dibenz[*a,h*]anthracene	3.66	

Louiz, I., Kinani, S., Gouze, M.E., Ben-Attia, M., Menif, D., Bouchonnet, S., Porcher, J.M., Ben-Hassine, O.K., Aït-Aïssa, S., 2008. Monitoring of dioxin-like, estrogenic and anti-androgenic activities in sediments of the Bizerta lagoon (Tunisia) by means of in vitro cell-based bioassays: Contribution of low concentrations of polynuclear aromatic hydrocarbons (PAHs). Sci. Total Environ. 402, 318-329.

Table S4. Concentrations of styrene oligomers in sediments of inland creeks and inner and outer regions of the Lake Shihwa.

Regions	**Sites**	**Styrene oligomers (ng g^{-1} dry mass)**										**ΣSDs**	**ΣSTs**	**D/T**[b]	**ΣSOs**
		SD1	SD2	SD3	SD4	ST1	ST2	ST3	ST4	ST5	ST6				
Inland	C1	0.91	1.1	18	1.9	9.3	6.9	9.4	6.6	5.4	2.1	22	40	0.55	61
creeks	C2	3.7	1.8	108	18	63	120	79	41	36	7.4	130	350	0.38	480
	C3	17	15	120	67	83	120	160	76	70	14	210	530	0.41	740
	C4	0.66	1.2	15	0.9	5.5	9.1	12	7.3	8.2	1.1	17	44	0.40	61
	C5	14	4.1	79	8.6	52	60	89	68	72	17	110	360	0.30	470
	C6	24	4.9	96	31	47	100	140	57	63	7.0	150	410	0.38	570
Inner	1	0.48	0.56	18	1.2	3.3	5.2	4.9	3.3	3.5	0.49	20	21	0.96	40
Lake	2	0.69	0.55	21	1.4	5.0	4.5	4.0	3.4	2.9	0.63	23	20	1.1	44
Shihwa	3	0.30	0.29	21	0.52	1.2	1.2	1.3	0.88	1.2	0.33	22	6.1	3.6	28
	4	0.36	0.49	59	0.61	1.5	1.7	2.6	1.9	1.6	0.44	60	9.7	6.2	70
	5	0.31	0.26	14	0.54	1.4	1.4	1.7	1.2	1.3	0.81	15	7.8	2.0	23
	6	0.32	0.32	19	0.66	0.93	1.1	1.3	0.83	1.0	0.39	20	5.5	3.7	26
	7	0.31	0.47	51	0.66	1.8	1.7	2.9	1.4	1.5	0.90	53	10	5.2	63
	8	0.32	0.31	21	0.62	1.2	1.1	1.3	0.50	1.1	0.35	22	5.5	4.1	28
	9	0.36	0.28	18	0.57	0.82	0.72	1.1	0.64	0.93	0.40	19	4.6	4.2	24
	10	0.21	0.12	4.7	0.41	0.71	0.86	1.3	0.58	0.69	0.53	5.4	4.7	1.2	10
	11	0.25	0.19	8.1	0.40	0.51	0.74	0.97	0.37	0.64	0.20	8.9	3.4	2.6	12
Outer	12	0.26	0.16	19	0.40	0.70	3.1	5.5	2.7	2.9	< DL	20	15	1.3	35
Lake	13	0.28	0.17	18	0.46	0.41	0.48	0.78	0.43	0.71	< DL	19	2.8	6.8	22
Shihwa	14	0.31	0.29	16	0.46	0.39	0.58	0.63	< DL[a]	0.55	0.38	17	2.5	6.8	20
	15	0.26	0.28	19	0.46	0.44	0.55	0.86	0.38	0.84	0.20	20	3.3	6.0	23

[a] < DL: below detection limits.
[b] D/T: ΣSDs / ΣSTs.

Table S5. Concentrations of PAHs in sediments of inland creeks and inner and outer regions of the Lake Shihwa.

Regions	**Sites**	**Polycyclic aromatic hydrocarbons (ng g^{-1} dry mass)**															**ΣPAHs**
		Acl	Ace	Flu	Phe	Ant	Fl	Py	BaA	Chr	BbF	BkF	BaP	IcdP	DbahA	BghiP	
Inland	C1	1.2	2.0	5.0	26	5.5	27	22	8.9	20	20	18	13	28	4.6	59	260
creeks	C2	2.6	1.9	12	43	7.4	83	110	7.2	32	9.9	49	51	8.8	5.6	63	490
	C3	2.0	15	42	180	110	340	170	120	130	42	240	180	49	21	250	1900
	C4	0.42	0.75	2.4	16	7.0	31	25	17	19	2.3	27	13	16	2.9	26	210
	C5	1.4	2.8	15	49	2.0	120	120	15	37	6.1	61	45	15	9.2	84	580
	C6	1.1	3.1	11	74	20	180	150	20	40	4.1	73	46	21	8.9	80	720
Inner	1	0.27	0.46	2.2	6.1	2.6	6.6	6.4	2.1	3.0	2.5	3.5	1.5	3.9	1.1	6.0	48
Lake	2	0.48	0.58	3.3	9.5	3.5	11	8.8	3.7	5.2	6.1	5.9	3.0	7.5	0.93	10	79
Shihwa	3	0.16	0.21	1.4	3.7	2.8	4.0	2.7	1.4	2.1	2.4	2.2	1.2	2.6	0.59	2.9	30
	4	0.17	0.23	1.6	4.9	3.5	4.4	3.4	1.5	2.3	2.9	2.8	1.4	3.1	0.73	3.5	36
	5	0.13	0.24	1.5	4.0	2.5	3.3	2.7	1.3	1.8	2.2	2.0	1.1	2.5	0.49	3.2	29
	6	0.14	0.28	1.3	3.4	2.8	4.0	2.9	1.4	2.1	2.9	2.5	1.4	3.4	0.43	4.0	33
	7	0.12	0.05	2.5	5.0	2.2	7.1	5.4	3.2	4.7	7.5	6.4	3.8	5.5	1.5	8.0	70
	8	0.24	0.28	1.7	6.1	4.0	6.9	4.4	2.7	4.2	6.2	4.9	2.8	6.5	1.2	7.1	59
	9	0.17	0.39	1.6	4.2	2.6	5.4	3.3	1.9	2.9	3.9	3.0	1.7	3.7	0.59	3.9	39
	10	0.20	0.33	1.9	4.7	3.4	6.3	4.0	2.4	3.3	5.1	4.0	2.3	5.4	0.84	5.8	50
	11	0.12	0.24	1.4	3.2	2.3	3.2	2.1	1.1	1.6	2.0	2.1	0.87	2.1	0.36	2.5	25
Outer	12	0.23	0.43	2.1	5.6	2.8	8.1	6.2	2.6	4.1	6.7	5.1	2.7	6.7	1.0	7.2	62
Lake	13	0.11	0.25	1.0	3.2	2.1	3.7	3.0	1.3	2.1	2.6	2.2	1.1	2.7	0.39	2.9	29
Shihwa	14	0.18	0.45	1.7	4.0	1.5	5.6	6.8	1.9	2.6	3.8	3.0	1.6	4.1	0.56	4.4	42
	15	0.11	0.32	1.4	3.4	1.8	4.0	3.1	1.7	2.5	3.8	3.0	2.0	4.3	1.0	4.9	37

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.

Supplementary Figures

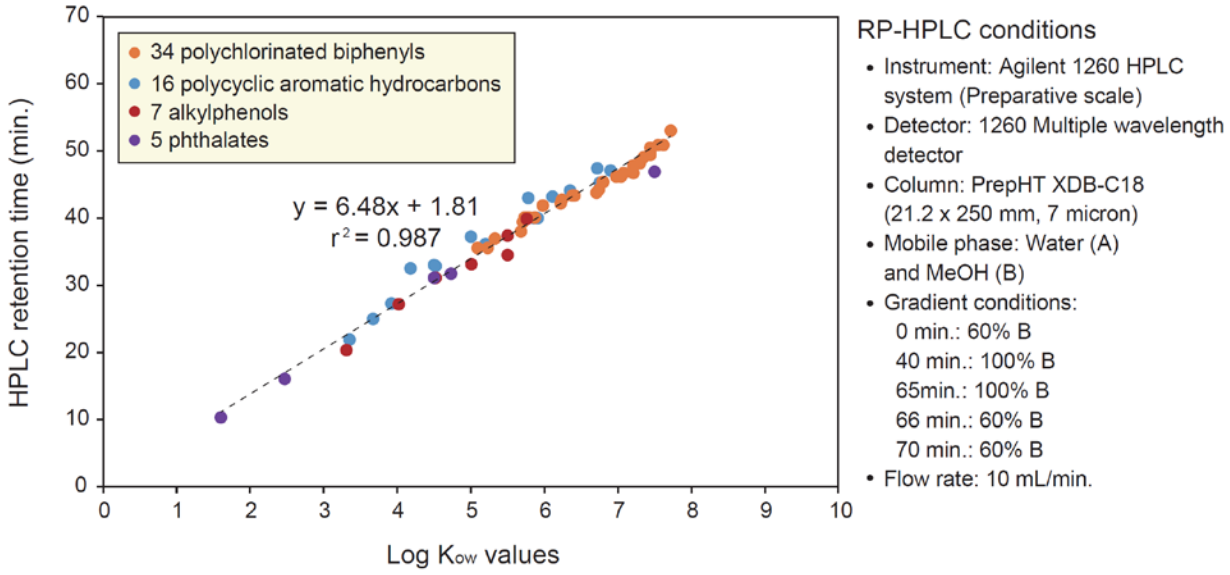

Fig. S1. Instrumental conditions of reverse phase-HPLC (XDB-C18 column) for fractionation of organic extracts. Retention times of various organic chemicals (n = 62) as a function of the chemical's log K_{ow} (Hong et al., 2016).

Hong, S., Giesy, J.P., Lee, J.-H., Khim, J.S., 2016. Effect-directed analysis: Current status and future challenges. Ocean Sci. J. Submitted.

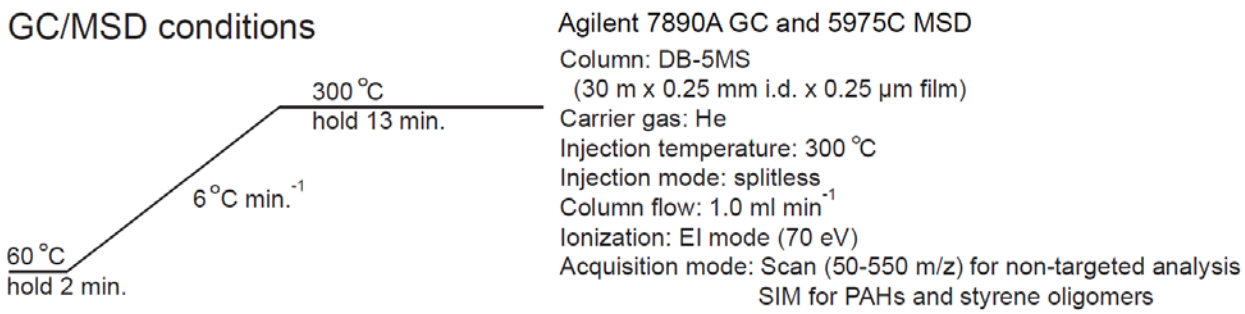

Fig. S2. Instrumental conditions of GC/MSD for targeted analyses of PAHs and styrene oligomers.

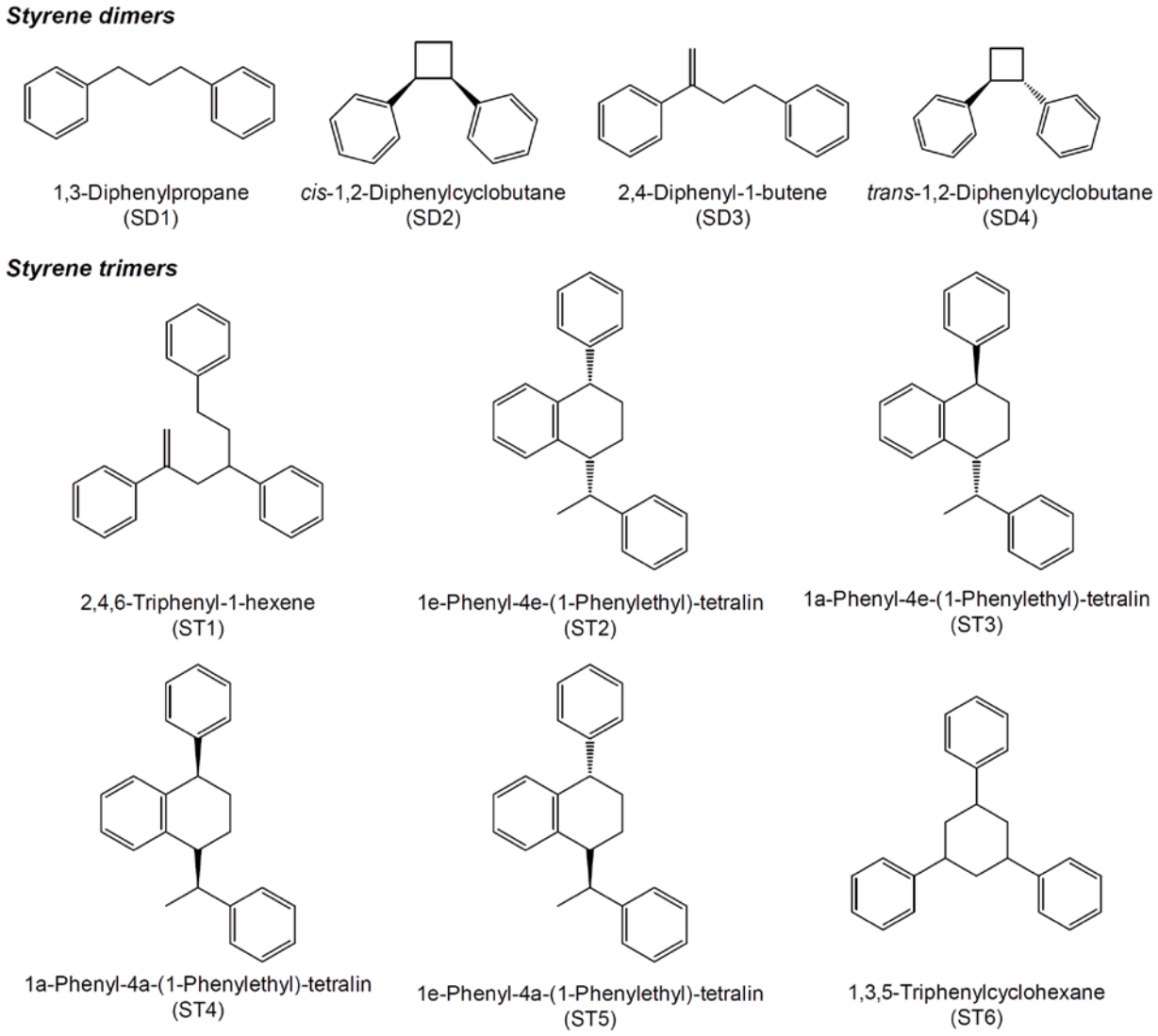

Fig. S3. Chemical structures of 10 styrene oligomers.

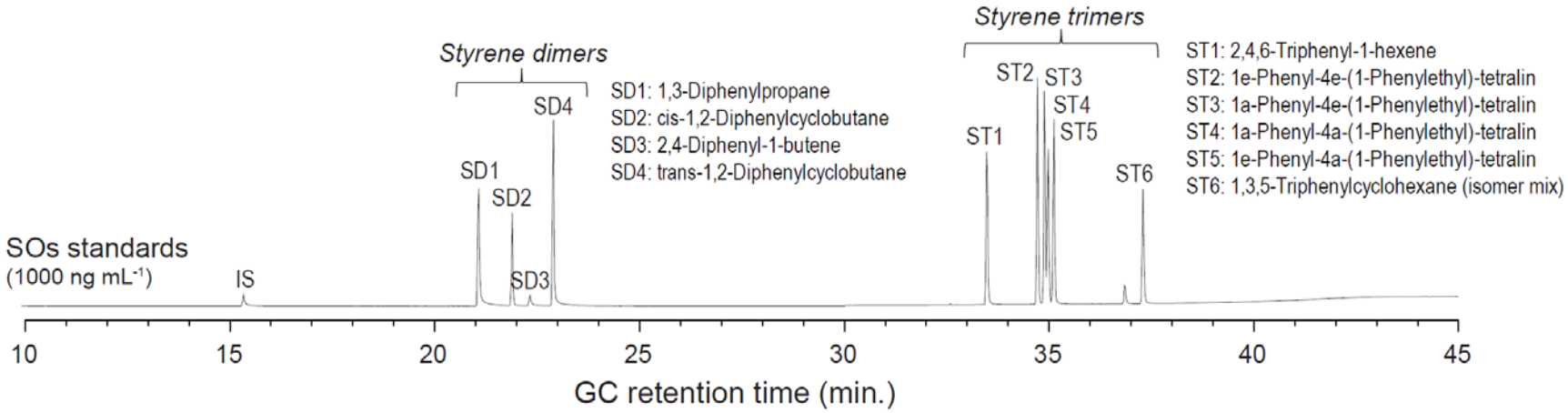

Fig. S4. GC-MSD chromatograms of 10 styrene oligomer standard materials.

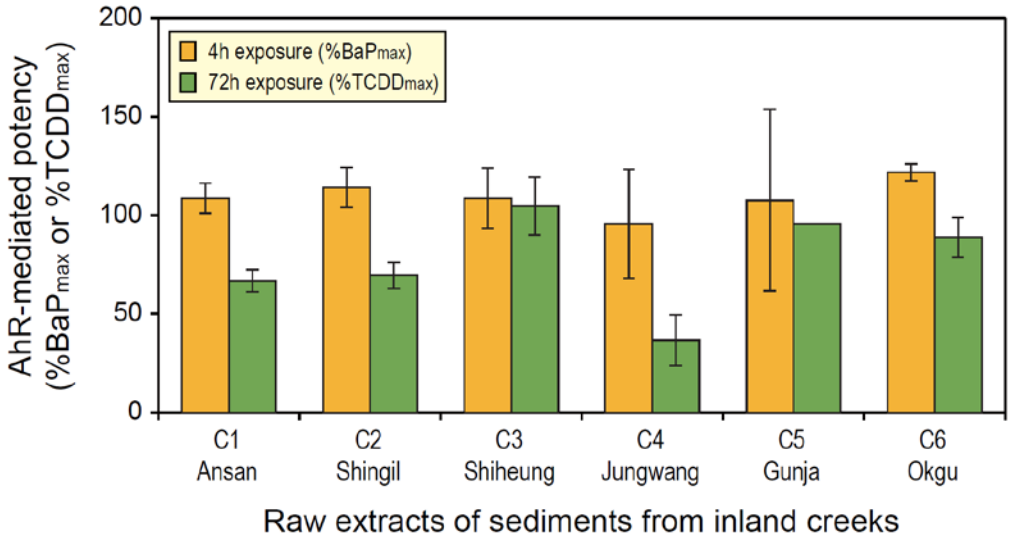

Fig. S5. AhR-mediated potencies of raw extracts of inland creeks sediments at 4 and 72 h exposure durations in H4IIE-*luc* bioassay.

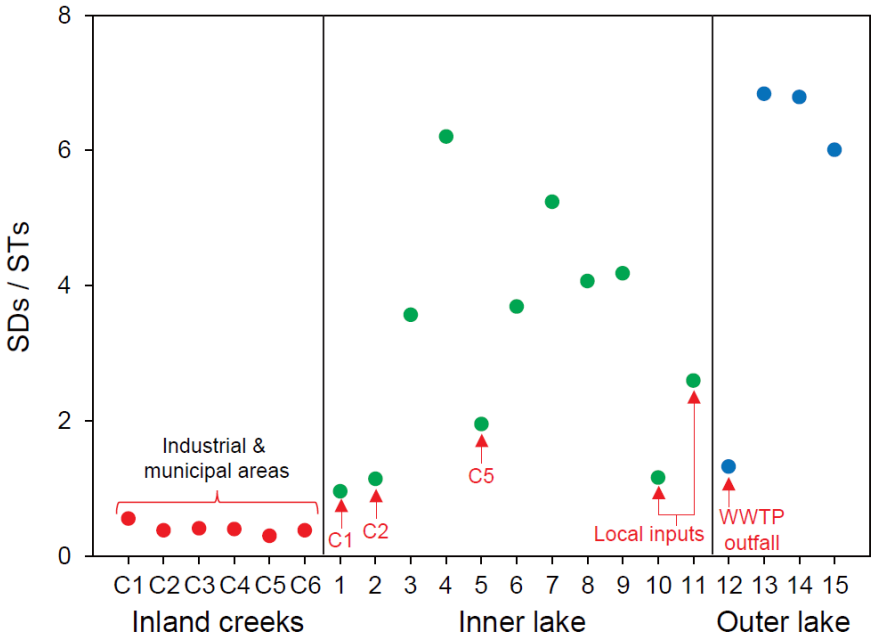

Fig. S6. Ratios of concentrations of styrene dimers and styrene trimers in sediments of inland creeks and inner and outer regions of Lake Shihwa (Locations of sites were shown in Fig. 1 in the main text).