

Rapid communication

# Instrumental and bioanalytical measures of dioxin-like and estrogenic compounds and activities associated with sediment from the Korean coast<sup>☆</sup>

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## Abstract

Sediments from inland areas and open bays along the Korean coast were analyzed to examine the distribution of dioxin-like and estrogenic compounds. Concentrations of target chemicals varied considerably among locations (Lake Shihwa, Masan Bay, and Kwangyang Bay) and between inland and coastal areas. Principal component analysis (PCA) of contaminants measured in sediments showed that all of the inland locations from Lake Shihwa were highly contaminated, and the variations among locations were explained predominantly by the distribution of alkylphenols and polycyclic aromatic hydrocarbons (PAHs). PCA of PAH congener profiles among locations indicated that automobiles were a major source of PAH contamination. Dioxin-like and estrogenic activities associated with sediment from inland sites were approximately three- and six-fold, respectively, greater than those associated with open bay locations. The target dioxin-like and estrogenic compounds measured in raw extracts of sediments accounted for approximately 20% and 40% of the activities measured in the sediment extracts. The results suggest that a combination of instrumental and bioanalytical measurements of dioxin-like and estrogenic compounds is a valuable approach to screen, identify, and prioritize the risks posed by contaminants in complex environmental matrices.

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## 1. Introduction

Contamination of the coastal environment by trace organic contaminants is of increasing concern. Persistent organic pollutants (POPs), such as organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs), are ubiquitous environmental pollutants in coastal marine environments (Loganathan and Kannan, 1994). Recently, several studies have reported the distribution and fate of POPs in Korean coastal environments (Kim et al., 2002; Koh et al., 2002; Oh et al., 2003). Considering the dependence of coastal communities on marine resources for food, contamination by POPs in Korean coastal areas is of great concern. Although the production and/or use of several classes of POPs such as PCBs and OC pesticides have been banned in Korea since the 1970s, these contaminants continue to persist in the environment and enter into the food chain (Kim et al., 2002; Koh et al., 2002).

Some POPs can disrupt normal endocrine function both in aquatic organisms and in humans. At least some of these adverse effects are mediated by aryl hydrocarbon receptor (AhR)- and/or estrogen receptor (ER)-dependent mechanisms (Behnisch et al., 2001; Giesy et al., 2002). Bioassays capable of detecting compounds that act through AhR- and ER-mediated mechanisms are useful for the characterization and evaluation of the toxic potential of sediments and other complex environmental matrices. Earlier studies conducted by our group reported dioxin-like and estrogenic activities associated with sediment and surface water from several locations along the Korean coast (Khim et al., 1999a; Koh et al., 2002, 2004). In addition, chemical analyses were performed to quantify concentrations of selected target contaminants, including POPs and potential xenoestrogens, including polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and bisphenol A (BPA), in the sediment and water extracts. Complementary use of bioassays and instrumental analyses provided a more complete characterization of potential AhR- and ER-active compounds in environmental samples.

Based on the results of past studies, industrial complexes (ICs) and urban areas located near coastal areas have been found to be major sources of organic contamination of coastal waters in Korea (Koh et al., 2002). Some of these pollutants, including xenoestrogens, are introduced into the coastal waters from rivers or streams via discharges of industrial waste, municipal sewage, and urban and agricultural runoff. Thus, coastal contamination by organic compounds is influenced by point and/or non-point sources from inland regions. The present study investigated sediments of Lake Shihwa and Masan Bay to determine the point sources of POPs and xenoestrogens that were identified during more general surveys conducted previously near these highly industrialized urban areas. (Khim et al.,

1999a, b). In addition, sediments collected from Kwangyang Bay, Korea, were analyzed to characterize dioxin-like and estrogenic activities associated with open bay sediment. The objectives of the present study were (1) to determine the spatial distribution of trace organic contaminants in sediment from the inland areas of Lake Shihwa and Masan Bay as well as open bay locations in Kwangyang Bay using instrumental analyses, (2) to elucidate point or non-point sources by applying a principal component analysis (PCA), and (3) to characterize the integrated potency of AhR- and ER-active compounds associated with sediment samples using *in vitro* bioassays. Where appropriate, mass balance analyses were used to help determine whether known chemical components, determined by chemical analysis, could reasonably account for the *in vitro* bioassay responses observed (Sanderson and Giesy, 1998; Villeneuve et al., 2000).

## 2. Materials and methods

### 2.1. Study areas

The study areas included Lake Shihwa, Masan Bay, and Kwangyang Bay, which are located along Korean coasts (Fig. 1). Lake Shihwa is an artificial lake created by the construction of 12.7 km of sea dike in 1994. It was supposed to be used as a freshwater reservoir, but due to serious environmental deterioration, it was recently made into a saltwater lake by opening of the water gate into Kyeonggi Bay. Several ICs and densely populated cities are situated along the northern shore of the lake. The area south of the lake is predominantly agricultural lands. Large amounts of industrial wastewater ( $3 \times 10^5$  ton/day) from inland cities (Shiheung and Ansan) are discharged into the lake via several streams and creeks. Masan Bay, located on the southeast coast, is a long and narrow inlet of semienclosed bay, where the Masan Free Trade Zone and Changwon IC are situated. Due to slow mixing with seawater (10 cm/s) and the long residence time (10–12 h) of wastewater ( $0.5 \times 10^5$  ton/day), the bay serves as a reservoir of various contaminants. Kwangyang Bay, located on the south coast, is a semienclosed bay adjacent to the South Sea. With an average of just 4% tidal exchange volume, the western part of Kwangyang Bay is hydrologically static. Kwangyang Bay opens to the South Sea and is a mesotidal environment with a tidal amplitude of 2 m on average. Posco and Yecheon ICs are located in the northern and southern part, respectively.

### 2.2. Sample collection and preparation

Sediment samples were collected from Lake Shihwa ( $n = 8$ ), Masan Bay ( $n = 8$ ), and Kwangyang Bay

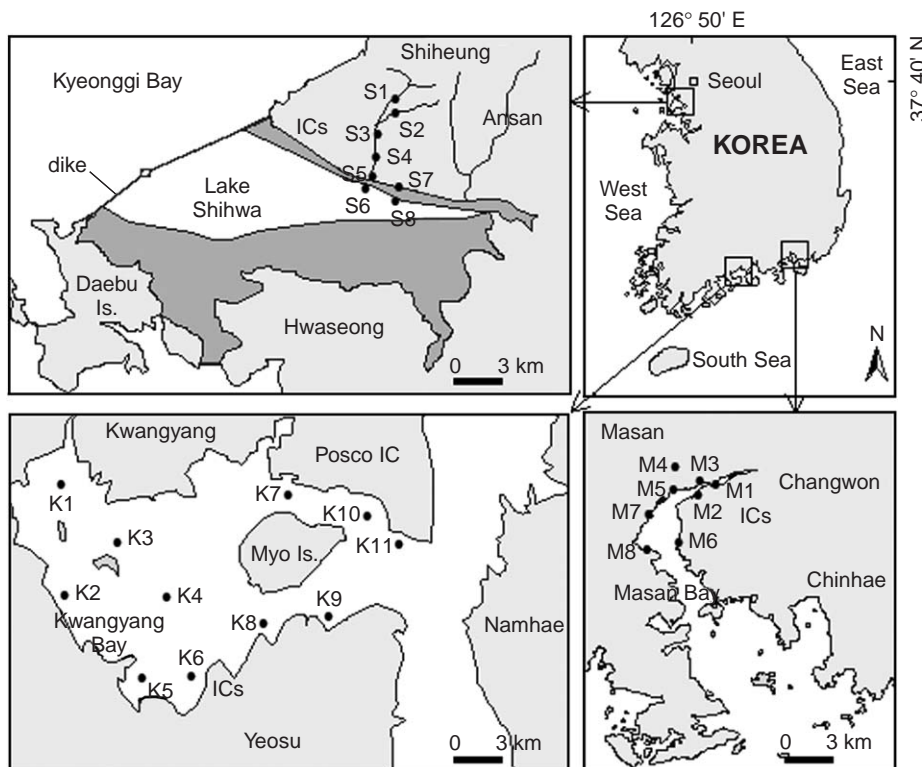


Fig. 1. Map of the Lake Shihwa (S1–S8), Masan Bay (M1–M8), and Kwangyang Bay (K1–K11) sampling sites, Korea.

( $n = 11$ ) in October 2001, November 2000, and February 2001, respectively (Fig. 1). Sampling was designed to determine potential sources of contaminants from river, inland, and marine environments. A global positioning system was employed to identify the precise location of each site. River and coastal inland sediment samples were collected by shovel and spatula, and marine sediment samples were collected aboard ship using a Van Veen grab sampler (25 × 40 × 30 cm). After collection, any pebbles and twigs were removed before samples were freeze-dried and ground with a mortar and pestle. Samples were stored in precleaned, high-density polyethylene bottles at  $-20^{\circ}\text{C}$  until extraction. The total organic carbon (TOC) was analyzed for the sediment samples, allowing the concentrations to be normalized to TOC.

Detailed descriptions of the extraction and fractionation procedures have been presented elsewhere (Khim et al., 1999c; Kannan et al., 2003). Briefly, 40 g of sediment were Soxhlet extracted for 20 h using dichloromethane (DCM; Burdick & Jackson, Muskegon, MI, USA) and concentrated using a rotary evaporator. Extracts were then treated with acid-activated copper granules to remove sulfur and finally concentrated to 2 mL. Aliquots of 1 mL Soxhlet raw extracts (REs) were passed through 10 g of activated Florisil (60–100 mesh size; Sigma Chemical Co., St. Louis, MO, USA) packed in a glass column (10 mm i.d.) for clean-up and

fractionation. The first fraction (F1), which was eluted with 100 mL of hexane (Burdick & Jackson), contained PCBs, hexachlorobenzene (HCB), and *p,p'*-DDE. Remaining OC pesticides, such as hexachlorocyclohexane isomers (HCHs), chlordanes (CHLs), *p,p'*-DDD and -DDT, and PAHs, were eluted in the second fraction (F2) using 100 mL of 20% DCM in hexane. APs such as nonylphenol (NP), octylphenol (OP), butylphenol (BP), and BPA were eluted in the third fraction (F3) with 100 mL of 50% DCM in methanol (Burdick & Jackson). Three fractionated extracts (FEs) were concentrated to 1 mL and used for instrumental quantification. One-milliliter aliquots of three FEs (F1, F2, and F3) as well as 1 mL of REs were allocated for *in vitro* bioassay analysis to determine the dioxin-like (H4IIE-luc cell bioassay) and estrogenic (MVLN cell bioassay) activities associated with each fraction.

### 2.3. Instrumental analysis

Fractions F1 and F2, containing PCBs and OC pesticides, were analyzed using a gas chromatograph (Perkin-Elmer Series 600, Palo Alto, CA, USA) equipped with a  $^{63}\text{Ni}$  electron capture detector. An equivalent mixture of 98 individual PCB congeners (AccuStandard, New Haven, CT, USA) with known composition and content and OC pesticide mixture (CLP-023R, CLP-024R, AccuStandard) were used as

external standards for quantification. Sixteen priority PAHs eluted in F2 samples were quantified using a Hewlett-Packard (Palo Alto, CA, USA) 5890 Series II gas chromatograph interfaced with a 5972 Series mass spectrometer. Reverse-phase high-performance liquid chromatography with fluorescence detection was used to quantify NP, OP, BP, and BPA in the F3 samples. The method detection limits (MDLs) for PCBs, OC pesticides, PAHs, APs, and BPA were 1, 0.01, 10, 1, and 1 ng/g dry wt, respectively. Recoveries of target compounds were between 90% and 105%, and none of the procedural blanks and field blanks contained detectable concentrations of the target compounds analyzed in this study. PCA was conducted using the Statgraphics program (Statistical Graphics Corp., 2001). Concentrations of all residues are reported on a dry weight (dw) basis.

The relative patterns of concentrations of residues were investigated by the use of a PCA. Concentrations of residues in sediments from all of the individual locations were normalized by subtracting the concentration from the average value for all residues from each location and then by dividing the value by the standard deviation [(concentration-average)/standard deviation]. In this study, the principal components (PCs) extracted had eigen values greater than 1.0.

#### 2.4. Cell culture and bioassay

Culturing conditions and assay procedures for H4IIE-luc and MVLN cells have been described previously (Khim et al., 1999b; Villeneuve et al., 2002). In brief, cells for bioassay were plated into the 60 interior wells of 96-well culture plates (250  $\mu$ L per well) at a density of approximately 15,000 cells per well. Cells were incubated overnight prior to dosing. Test and control wells were dosed with 2.5  $\mu$ L of the appropriate extract or solvent. Blank wells received no dose. At least three replicate wells were analyzed for each sample dilution, control, and blank tested. For dose–response characterization, dilution series consisting of six concentrations of sample prepared by three-fold serial dilution were tested (three replicate wells per concentration). Luciferase assays were conducted after 72 h of exposure. Sample responses were converted to relative response units expressed as a percentage of the maximum response observed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; % TCDD-max) or 17- $\beta$ -estradiol (E2; %-E2-max) standard curves generated on the same day. This was done to normalize responses for day-to-day variation in response magnitude and enhance the comparability of these results to other *in vitro* bioassay results reported in the literature (Khim et al., 1999a,b; Villeneuve et al., 2000).

#### 2.5. Mass balance analysis

Mass balance analysis was performed to examine whether or not the known composition of a sample, as identified by instrumental analysis, could account for the magnitude or potency of the biological response observed (Giesy et al., 2002). Instrumentally derived dioxin equivalents (TEQs) or estrogen equivalents (EEQs) were calculated by multiplying the concentrations of known AhR or ER agonists by their assay-specific REP values (or international TEF) and summing the products for the sample or fraction of interest (Giesy and Kannan, 1998; Villeneuve et al., 1998, 2002). Bioassay-derived dioxin equivalents (TCDD-EQs) or estrogen equivalents (E2-EQs) were determined directly from sample dose–response relationships generated by testing samples at multiple levels of dilution. In order to account for uncertainty in the TCDD-EQ and E2-EQ estimates caused by deviations of the sample concentration–response curve from parallelism to the TCDD or E2 standard curve, EQs were calculated for a range of responses from EC<sub>20</sub> to EC<sub>80</sub> (Tables 3 and 4; Villeneuve et al., 2000). Instrumentally derived EQs (TEQs or EEQs) were then compared to the bioassay-derived EQs (TCDD-EQs or E2-EQs). Differences were considered to be relevant when the instrumentally derived TEQ or EEQ estimate was outside of the corresponding TCDD-EQ<sub>20–80</sub> or E2-EQ<sub>20–80</sub> range.

In cases in which a dose–response relationship could not be generated for a sample (mostly for Kwangyang Bay samples), but for which there was sufficient analytical information to derive TEQs or EEQs, regression of TEQs or EEQs against the appropriate standard curve was used to predict the magnitude of bioassay response that should have been elicited by the known agonist/antagonist composition of the sample (Khim et al., 1999b; Villeneuve et al., 2000). Predicted responses were predicated on the assumption that complex mixtures of AhR/ER-active compounds present in the REs or FEs would respond as if they were simply a more dilute or concentrated solution of the standard compound (e.g., 10 pg/mL TCDD-EQs would generate the same response as 10 pg/mL TCDD). In practice, environmental samples rarely conform to this assumption, often generating concentration–response curves with slopes and maximal efficacy that are significantly different from those of the standard (Villeneuve et al., 2000). Thus, magnitude-based comparisons are generally inferior to potency-based comparisons. Nonetheless, broad trends observed when comparing predicted and observed magnitudes of response can be useful for generating hypotheses regarding the potential contribution of the known agonists/antagonists to the bioassay responses observed in cases in which potency-based comparisons are not feasible.

### 3. Results

#### 3.1. Instrumental analysis

TOC contents were different among sediments from Lake Shihwa, Masan Bay, and Kwangyang Bay. Generally, Masan Bay sediment contained relatively greater TOC contents than sediments from Kwangyang Bay and Lake Shihwa. Although the mean TOC content was generally small, some locations (S3 and S4) in Lake Shihwa had a TOC content as great as 2.11% and 1.78% (Table 1). These locations also contained the greatest concentrations of PCBs and APs. Generally, upstream locations of the river or stream contained lesser TOC contents, while sediments from middle to lower reaches contained greater TOC concentrations. TOC concentrations varied more in inland sediments

from Lake Shihwa and Masan Bay than in marine sediment from Kwangyang Bay.

A significant relationship between TOC content and various target organic compounds measured in sediment from Lake Shihwa was observed (Table 2). The correlation between TOC content and NP ( $r = 0.99$ ,  $P < 0.01$ ) was the strongest, whereas TOC content was not significantly correlated with CHLs and BP. In contrast, poor correlation between TOCs and target organic compounds was found for Masan Bay and Kwangyang Bay sediment. Only PAH concentrations ( $r = 0.68$ ,  $P < 0.05$ ) were correlated with TOC content in coastal marine sediment from Masan Bay. Although the sources of organic contaminants in Lake Shihwa are localized, the relatively long hydraulic residence time in the lotic system influences the affinities of lipophilic organic contaminants for sediment (Khim et al., 1999a).

Table 1

Concentrations (ng/g dry wt) of polychlorinated biphenyls (PCBs), organochlorine pesticides (HCB, HCHs, CHLs, DDTs), polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and bisphenol A (BPA) in surficial sediment from inland regions of Lake Shihwa and Masan Bay and from the open ocean of Kwangyang Bay, Korea

Location	PCBs	HCB	HCHs	CHLs	DDTs	PAHs	NP	OP	BP	BPA	TOC
<i>Lake Shihwa</i>											
S1	15.6	0.69	2.45	0.12	0.35	611	1640	42.8	74.8	47.5	0.77
S2	9.35	0.75	1.46	0.06	0.17	121	254	6.10	21.9	26.2	0.35
S3	126	3.49	10.7	0.22	2.14	643	4930	94.2	23.6	74.3	2.11
S4	47.2	2.80	0.97	0.04	0.71	292	3640	99.7	41.1	115	1.78
S5	19.9	0.94	2.76	0.03	0.40	80.2	949	42.6	8.57	<1.00	0.52
S6	10.9	0.10	0.55	<0.01	0.22	25.8	667	20.9	1.15	13.2	0.58
S7	9.37	0.09	2.17	<0.01	0.15	12.8	369	5.27	1.08	17.1	0.48
S8	9.05	0.18	0.88	0.02	0.28	24.9	622	8.59	<1.00	31.0	0.49
Mean	30.9	1.13	2.74	0.08	0.55	226	1634	40.0	24.6	46.3	0.89
<i>Masan Bay</i>											
M1	9.12	0.11	0.01	<0.01	0.23	33.4	184	3.00	7.20	<1.00	0.56
M2	41.9	0.12	0.04	0.02	0.32	128	152	2.44	2.91	<1.00	1.48
M3	92.1	0.22	17.4	0.03	0.14	481	514	7.43	<1.00	<1.00	5.39
M4	11.7	0.61	7.87	0.04	0.28	186	1070	17.5	12.5	18.1	2.24
M5	46.1	0.24	13.0	0.28	5.66	351	179	1.56	3.04	277	2.09
M6	40.9	0.10	0.64	<0.01	0.15	48.3	84.7	1.54	5.93	222	0.66
M7	30.9	0.72	44.4	0.33	1.48	413	526	4.96	6.92	54.6	2.63
M8	11.8	0.32	6.50	<0.01	0.49	85.4	446	8.32	20.0	<1.00	3.67
Mean	35.6	0.30	11.2	0.14	1.09	216	394	5.85	8.36	143	2.34
<i>Kwangyang Bay</i>											
K1	<1.00	0.47	0.02	<0.01	0.06	10.1	19.9	<1.00	<1.00	<1.00	0.83
K2	<1.00	0.08	0.59	<0.01	0.12	28.2	13.4	<1.00	<1.00	<1.00	1.30
K3	<1.00	0.02	0.09	<0.01	0.03	19.6	21.6	<1.00	<1.00	<1.00	1.13
K4	<1.00	0.03	<0.01	<0.01	0.05	17.8	11.5	<1.00	<1.00	<1.00	1.14
K5	<1.00	0.10	0.16	0.05	0.06	25.2	35.0	1.36	<1.00	<1.00	1.20
K6	<1.00	0.05	0.91	0.01	0.01	21.6	21.0	1.17	<1.00	<1.00	1.21
K7	<1.00	0.03	0.29	<0.01	0.10	105	10.8	<1.00	<1.00	<1.00	0.65
K8	<1.00	0.07	0.27	<0.01	0.14	74.9	11.8	<1.00	<1.00	<1.00	1.26
K9	<1.00	0.02	0.10	<0.01	0.11	36.7	9.9	<1.00	<1.00	<1.00	1.08
K10	4.67	0.04	0.24	<0.01	0.09	37.2	33.4	1.81	<1.00	<1.00	1.31
K11	<1.00	<0.01	0.11	<0.01	0.02	24.5	12.3	<1.00	<1.00	28.3	0.57
Mean	4.67	0.09	0.28	0.03	0.07	36.4	18.2	1.44	<1.00	28.3	1.06

PCBs, sum of 98 individual congeners; HCB, hexachlorobenzene; HCHs,  $\alpha$ - +  $\beta$ - +  $\gamma$ -hexachlorocyclohexanes; CHLs,  $\alpha$ - +  $\gamma$ -chlordanes; DDTs,  $p,p'$ -DDE +  $p,p'$ -DDD +  $p,p'$ -DDT; PAHs, sum of 16 priority components; NP, nonylphenol; h OP, octylphenol; BP, butylphenol; BPA, bisphenol A; TOC, total organic carbon content (%); Mean indicates the average of each class of compounds detected.

Table 2  
Relationships ( $r$ ) between total organic carbon content and concentrations of target organic compounds in sediment from the three study sites

Compound	Lake Shihwa	Masan Bay	Kwangyang Bay
PCBs	0.90**	0.56	—
HCB	0.96**	0.27	0.37 (-)
HCHs	0.68*	0.42	0.39
CHLs	0.64	0.22 (-)	0.06 (-)
DDTs	0.88**	0.04 (-)	0.28
PAHs	0.70*	0.68*	0.23 (-)
NP	0.99**	0.43	0.40
OP	0.94**	0.38	0.46
BP	0.29	0.67	—
BPA	0.86**	0.55 (-)	—

PCBs, sum of 98 individual congeners; HCB = hexachlorobenzene; HCHs,  $\alpha$ - +  $\beta$ - +  $\gamma$ -hexachlorocyclohexanes; CHLs,  $\alpha$ - +  $\gamma$ -chlordanes; DDTs,  $p,p'$ -DDE +  $p,p'$ -DDD +  $p,p'$ -DDT; PAHs, sum of 16 priority components, NP, nonylphenol; OP, octylphenol; BP, butylphenol; BPA, bisphenol A, (-) indicates negative correlations between variables.

\*  $p < 0.01$ ; \*\*  $p < 0.05$ .

PCBs were found in all of the sediment samples from streams and coastal inland areas in Lake Shihwa and Masan Bay, where total PCB concentrations were 9.05–47.2 (mean, 30.9) and 9.12–92.1 (mean, 35.6) ng/g dw, respectively (Table 1). Sediment from location S3 in the middle of Shiheung Stream contained the greatest PCB concentrations. This suggests the presence of point sources of PCBs in Shiheung Stream. Paper mill, silk, mechanical, and electronic factories located near S3 are potential sources. Similarly, in Masan Bay sediment PCB concentrations reflected spatial heterogeneity, which indicates point sources. The greatest concentrations of PCBs were observed at locations M2, M3, M5, and M6, all of which were near ICs, whereas in Kwangyang Bay only one location (K10) near Posco IC contained detectable concentrations of PCBs (4.67 ng/g dw) (Table 1). Although several ICs are located in the northern and southern parts of Kwangyang Bay (mainly steel manufacturing), PCB concentrations were not great in sediments collected near those locations.

Among different OC pesticides analyzed, HCH concentrations in sediments were the greatest, accounting for over 60% of total OC pesticide concentrations. The relative abundance of OC pesticides was HCHs > DDTs > HCB > CHLs (Table 1). OC pesticides concentrations were generally 1–2 orders of magnitude lower than PCB concentrations, except at location M7 in Masan Bay, where the HCH concentration (44.4 ng/g dw) was greater than that of PCBs (30.9 ng/g dw). Concentrations of DDTs, HCB, and CHLs were, on average, less than 1.0 ng/g dw (Table 1). The greatest concentrations of HCB, HCHs, CHLs, and DDTs were

found at location S3 in Lake Shihwa, where the sedimentary PCB concentration was also the greatest (126 ng/g dw).

PAHs were detected in all of the locations from river to marine sediment in Lake Shihwa, Masan Bay, and Kwangyang Bay (Table 1). PAH concentrations in river sediment from Lake Shihwa and Masan Bay were greater than those in marine sediment from Kwangyang Bay by an order of magnitude. The greatest concentrations of total PAHs were found at locations S3 (643 ng/g dw) and M3 (481 ng/g dw) in Lake Shihwa and Masan Bay, respectively (Table 1). Sediment samples from those locations contained the greatest concentrations of PCBs as well, which indicates the presence of localized sources. PAH concentrations in Shiheung Stream sediment were greatest at location S1 (611 ng/g dw) and S5 (80.2 ng/g dw). Concentrations at the other downstream locations, S6 and S8, in Lake Shihwa were near the MDL (Table 1). This agrees well with the results of an earlier study, which reported that lesser concentrations of PAHs (16.9–30.8 ng/g dw) were observed in Lake Shihwa sediment (Khim et al., 1999a). Marine sediment from Kwangyang Bay contained detectable concentrations of PAHs (mean, 36.4 ng/g dw), but their concentrations were about six-fold less than those in Lake Shihwa (mean, 226 ng/g dw) and Masan Bay (mean, 216 ng/g dw) (Table 1). When expressed as TEQs, the TEQ<sub>PAH</sub> (mean, 0.49 pg TEQ/g dw) in Kwangyang Bay was approximately three- and four-fold less than those in Lake Shihwa and Masan Bay (Table 3).

APs were detected in sediment samples from Lake Shihwa and Masan Bay, and NP contributed more than 90% of the total AP concentrations (Figs. 2 and 3). NP, OP, and BP were found in nearly all of the locations in Lake Shihwa and Masan Bay, and the greatest concentrations were found in river sediment from Lake Shihwa at 4930, 99.7, and 74.8 ng/g dw for NP, OP, and BP, respectively (Table 1). The marine sediment in Kwangyang Bay contained considerably lower concentrations of NP (mean, 18.2 ng/g dw) and OP (mean, 1.44 ng/g dw). Furthermore, BP was not detected in sediment from any location in Kwangyang Bay. This difference in the distributions of APs between Lake Shihwa (or Masan Bay) and Kwangyang Bay reflects the size and scale of municipal and/or industrial activities in these areas.

BPA, one of the suspected xenoestrogens, was also detected in river and coastal inland sediment and ranged from <1.00 to 277 ng/g dw (Table 1). Coastal marine sediment collected at locations M5 and M6 (Masan Bay) contained high concentrations of BPA of 277 and 222 ng/g dw, respectively. Polycarbonate plastics and synthetic resins from various ICs are likely sources of the BPA found in Masan Bay.

Table 3

Potency-based (TCDD-EQs) and magnitude-based (% TCDD-max) mass balance analysis for PCB and PAH compounds associated with sediment samples from Lake Shihwa, Masan Bay, and Kwangyang Bay

Location	TEQs <sup>a</sup>			TCDD-EQs <sup>b</sup>		% TCDD-max	
	TEQ <sub>PCB</sub>	TEQ <sub>PAH</sub>	Total TEQs	TCDD-EQ <sub>20–80</sub>	TCDD-EQ <sub>50</sub>	Calculated <sup>c</sup>	Observed <sup>d</sup>
<i>Lake Shihwa</i>							
S1	0.78	3.49	4.27	74.3–97.6	85.2	9.35	65.9
S2	2.07	0.83	2.90	45.2–46.7	46.0	2.56	66.0
S3	42.0	3.73	45.7	316–338	327	51.1	82.6
S4	9.23	1.88	11.1	105–204	146	26.2	63.7
S5	2.14	0.54	2.68	13.8–31.7	20.9	1.12	52.2
S6	5.50	0.33	5.82	6.90–24.3	12.9	14.8	46.8
S7	11.1	0.01	11.1	0.87–14.2	3.51	26.2	35.8
S8	2.11	0.25	2.36	41.2–57.3	48.6	<0.00	54.2
<i>Masan Bay</i>							
M1	3.29	0.32	3.61	8.38–49.6	20.4	6.40	46.7
M2	1.04	1.34	2.37	63.3–88.1	74.7	<0.00	76.5
M3	77.7	3.61	81.3	101–169	130	61.2	62.4
M4	0.65	1.40	2.05	107–227	156	<0.00	93.5
M5	0.89	4.09	4.97	256–315	284	12.0	83.8
M6	0.88	0.54	1.42	37.9–42.1	39.9	<0.00	68.7
M7	0.19	3.64	3.83	111–122	117	7.42	47.4
M8	6.84	0.82	7.66	1.01–5.12	2.3	19.7	37.8
<i>Kwangyang Bay</i>							
K1	<0.01	0.02	0.02		NAC <sup>e</sup>	<0.00	10.0
K2	<0.01	0.55	0.55	3.35–3.76	3.55	<0.00	23.3
K3	<0.01	0.35	0.35		NAC	<0.00	17.0
K4	<0.01	0.26	0.26		NAC	<0.00	20.2
K5	<0.01	0.38	0.38		NAC	<0.00	9.05
K6	<0.01	0.31	0.31		NAC	<0.00	8.26
K7	<0.01	0.96	0.96		NAC	<0.00	11.9
K8	<0.01	1.10	1.10	1.43–29.0	6.44	<0.00	37.2
K9	<0.01	0.60	0.60	0.84–14.9	3.53	<0.00	36.9
K10	3.83	0.46	4.30	0.49–13.0	2.54	10.4	35.4
K11	<0.01	0.36	0.36	0.22–8.10	1.34	<0.00	32.6

<sup>a</sup>Instrumentally derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) of PCBs (F1) and PAHs (F2) in sediment extract. Total TEQs are sum of TEQ<sub>PCB</sub> and TEQ<sub>PAH</sub> (pg TCDD/g, dry wt). Refer to the TEQs generated from international TEF or assay-specific REP-50 values (Giesy and Kannan, 1998; Villeneuve et al., 2002) of selected PCBs 81, 77, 118, 105, 126, 167+185, 156, 169) and PAHs (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, and dibenz(a,h)anthracene) and the corresponding concentrations measured.

<sup>b</sup>Bioassay derived TCDD equivalents (TCDD-EQs) of the sediment fraction and raw extracts (pg TCDD/g dry wt). TCDD-EQ<sub>20–80</sub> represents the range of dioxin equivalents generated from multiple point estimates made for responses ranging from 20% to 80% TCDD-max. TCDD-EQ<sub>50</sub> indicates the dioxin equivalents generated from one point estimate for the response of 50% TCDD-max.

<sup>c</sup>Regression of total TEQs against the TCDD standard curve was used to predict the magnitude of the bioassay response.

<sup>d</sup>Observed bioassay response (% TCDD-max) of sediment raw extracts in the H4IIE-luc bioassay.

<sup>e</sup>NAC, data not available for the calculation of TCDD-EQs, i.e., a full dose-response curve could not be obtained.

### 3.2. *In vitro* bioassay

Based on the initial screening of REs, all sediment samples elicited significant dioxin-like activity in the H4IIE-luc bioassay (Fig. 4). Sample responses varied greatly depending on region and/or location, ranging from 8.3% to 94% TCDD-max. Greater bioassay responses were generally observed for the samples from inland areas of Lake Shihwa and Masan Bay, which reflected the general distribution pattern of dioxin-like contaminants. Among 16 sediment REs tested from Lake Shihwa and Masan Bay, 12 REs showed responses

greater than 50% TCDD-max. Particularly, the samples from inland locations at S3, M4, and M5 elicited the greatest dioxin-like activities (over 80% TCDD-max). In contrast, Kwangyang Bay sediment REs showed significantly lower bioassay responses, ranging from 8.3% to 37% TCDD-max (mean, 22). The more modest responses caused by the Kwangyang Bay samples were consistent with the lower concentrations of AhR-active compounds such as PCBs and PAHs present in open bay locations (Table 1).

Total TEQs (sum of TEQ<sub>PCB</sub> and TEQ<sub>PAH</sub>) based on instrumental analyses were generally one order of

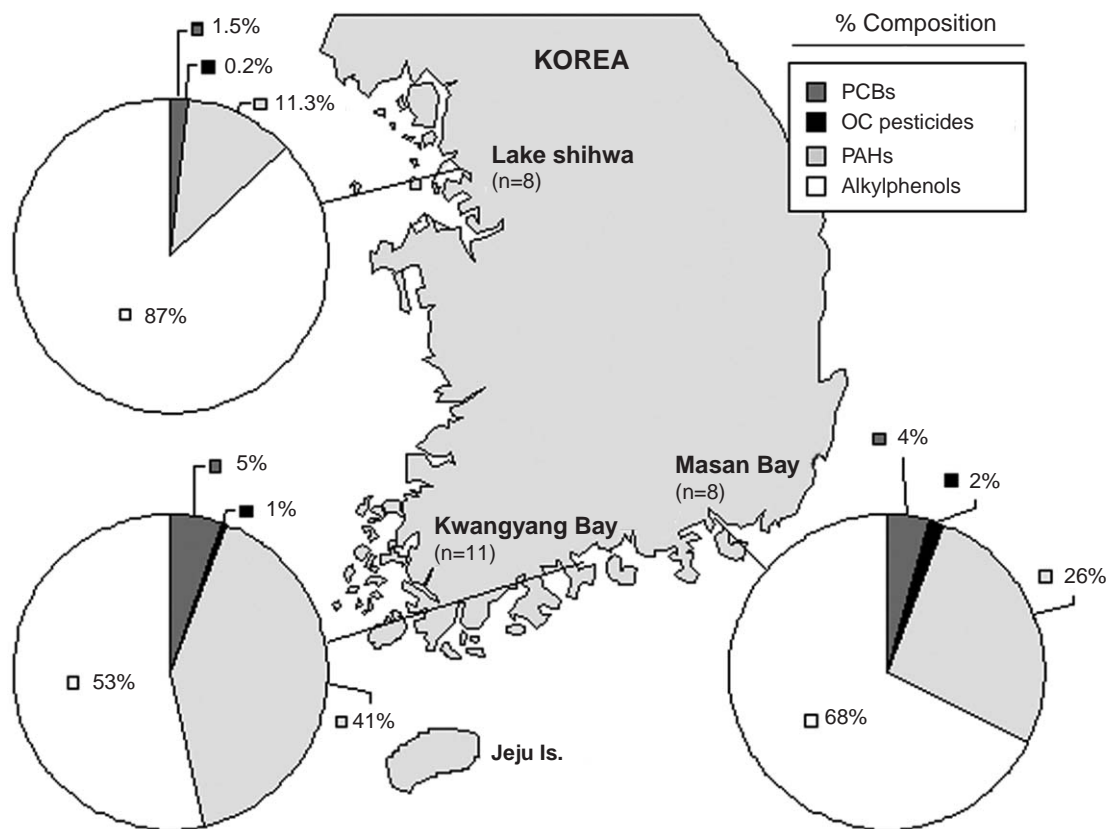


Fig. 2. The chemical composition of target organic contaminants in sediments from Lake Shihwa, Masan Bay, and Kwangyang Bay, Korea. PCBs are the sum of 98 individual PCB congeners; OC pesticides include hexachlorobenzene (HCB), hexachlorocyclohexane isomers (HCHs), chlordanes (CHLs), and DDT and its derivatives (DDTs); PAHs are the sum of 16 priority PAHs; and alkylphenols represent the sum of nonylphenol (NP), octylphenol, (OP), butylphenol (BP), and bisphenol A (BPA).

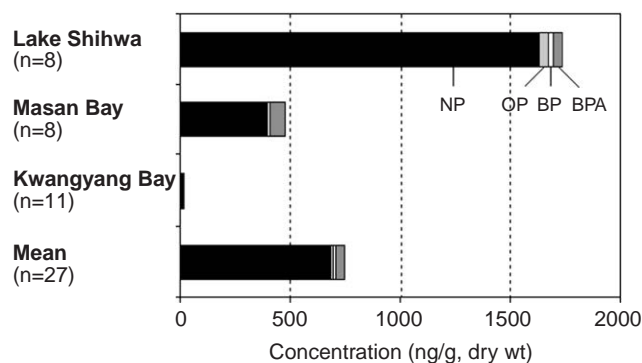


Fig. 3. Mean composition (ng/g dry wt) of nonylphenol (NP), octylphenol (OP), butylphenol (BP), and bisphenol A (BPA) among total alkylphenols in sediments from Lake Shihwa, Masan Bay, and Kwangyang Bay, Korea.

magnitude lower than bioassay-derived TCDD-EQs. Only three locations (S7, M8, and K10) contained greater concentrations of total TEQs than of TCDD-EQs, which indicated that the known AhR agonists in those sediment REs could fully account for the bioassay responses observed in the H4IIE-luc bioassay (Table 3). On average, the known concentrations of AhR-active

compounds present in sediment extracts accounted for approximately 30% of the total bioassay responses observed. Based on the total TEQs concentrations present in the samples, 13 of 27 REs analyzed should have yielded a significant H4IIE-luc response. Each of those significant responses was predicted to be between 1.1% and 61% TCDD-max (Table 3). However, the other 14 samples (primarily from Kwangyang Bay), which were not predicted to elicit significant responses, induced significant dioxin-like activities in all cases. These results strongly suggested that chemical analysis alone, based on the target analytes identified in this study, underestimated the total dioxin-like potency of compounds associated with sediment from these Korean coastal areas.

All of the REs from Lake Shihwa and Masan Bay yielded significant estrogenic responses in the MVLN bioassay (Fig. 5). Among the three areas surveyed, REs of sediment from Lake Shihwa contained the greatest concentrations of EEQs (Table 4) and induced the greatest estrogenic activities (Fig. 5). Based on the instrumental determination, the estrogenic NP, OP, and BPA concentrations in Lake Shihwa sediment were found to be as great as 4930, 99.7, and 115 ng/g dw,



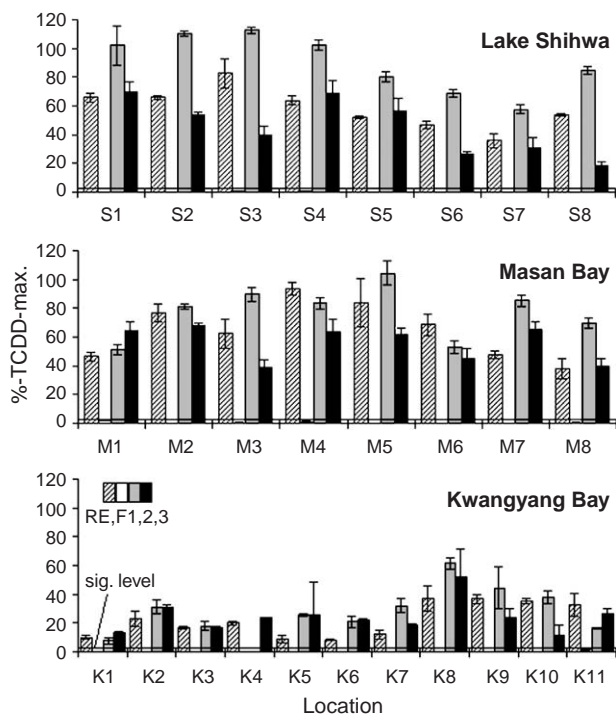


Fig. 4. Luciferase induction in the H4IIE-luc cell bioassay elicited by sediment raw extract (RE) and fractionated extracts (FEs; F1, F2, and F3). The response magnitude is presented as the percentage of the maximum response observed for a 1500 pm 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard (% TCDD-max). The horizontal line equals 3 SD (expressed in % TCDD-max) above the mean solvent control response (set to 0% TCDD-max).

respectively (Table 1). The instrumentally derived EEQs for Lake Shihwa sediment ranged from 3.3 to 64 pg/g dw, with an average of 21 pg EEQ/g dw (Table 4). These EEQs were predicted to yield a significant response of between 19% and 88% E2-max in the MVLN bioassay (Table 4). Significant responses were observed, but in all cases they were greater than predicted (Table 4). Similarly, E2-EQs determined for Lake Shihwa samples were uniformly greater than the corresponding EEQ estimates (Table 4). These results suggest the presence of unidentified estrogenic compounds and/or synergistic effects between quantified estrogenic compounds detected in Lake Shihwa sediment. The relatively great concentrations of EEQs and E2-EQs in sediment from this area, relative to those in Masan Bay, Kwangyang Bay, and other locations studied previously (Khim et al., 1999a, b, 2001; Koh et al., 2002), indicate that Lake Shihwa may be an area of special concern for potential impacts by estrogenic endocrine-disrupting compounds.

Masan Bay sediment REs showed significant estrogenic activities that were more modest than those for observed for Lake Shihwa sediment REs. The estrogenic responses of Masan Bay REs varied greatly among locations, from 9.3% to 113% E2-max. For all locations

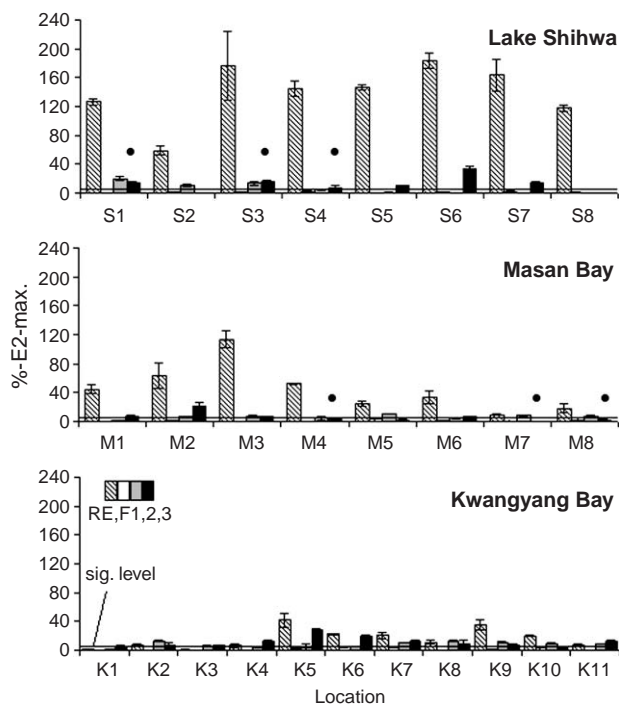


Fig. 5. Luciferase induction in the MVLN cell bioassay elicited by sediment raw extract (RE) and fractionated extracts (FEs; F1, F2, and F3). The response magnitude is presented as the percentage of the maximum response observed for a 1000 pm 17- $\beta$ -estradiol (% E2-max). The horizontal line equals 3 SD (expressed in % E2-max) above the mean solvent control response (set to 0% E2-max). Cells that exhibited an altered or “stressed” morphology (cytotoxicity) are indicated with (●).

except M2 and M3, EEQ concentrations, calculated based on concentrations of estrogenic PAHs, APs, and BPA, were sufficient to reasonably account for the estrogenic potency observed in the MVLN bioassay ( $EEQ \approx E2-EQ$ ), although the less reliable predicted magnitude estimates were still generally less than observed magnitudes (Table 4). As a whole, the results suggest that PAHs, APs, and BPA reasonably account for most of the estrogenic activity of the Masan Bay sediment extracts, but locations M2 and M3 may receive additional inputs of estrogenic compounds that were unaccounted for in this study.

Estrogenic activities associated with Kwangyang Bay sediment REs were weak. This was in general agreement with the lesser concentrations of EEQs detected in open bay locations (Table 4). Nonetheless, REs from 8 of the 11 Kwangyang Bay sites induced significant MVLN responses. The greatest response, 42% E2-max, was observed for the RE associated with location K5, near an IC zone. These results suggest at least some inputs of estrogenic compounds to the open bay area.

The significant correlation ( $r^2 = 0.603$ ,  $P < 0.01$ ) between estrogenic response and the logarithm of APs concentration and the great proportion (99%) of  $EEQ_{AP}$  to total EEQs suggested a significant

Table 4

Potency based (E2–EQs) and magnitude-based (% E2-max) mass balance analysis for selected PAHs compounds, alkylphenols, and bisphenol A associated with sediment samples from Lake Shihwa, Masan Bay, and Kwangyang Bay

Location	EEQs <sup>a</sup>				E2–EQs <sup>b</sup>		%–E2-max.	
	EEQ <sub>PAH</sub>	EEQ <sub>AP</sub>	EEQ <sub>BPA</sub>	Total EEQs	E2–EQ <sub>20–80</sub>	E2–EQ <sub>50</sub>	Calculated <sup>c</sup>	Observed <sup>d</sup>
<i>Lake Shihwa</i>								
S1	0.020	21.3	0.076	21.4	90.4–187	130	62.8	126
S2	0.003	3.29	0.042	3.33	10.1–43.4	20.9	19.7	58.8
S3	0.012	63.4	0.119	63.6	286–762	467	88.0	176
S4	0.005	47.4	0.184	47.6	206–393	285	81.3	145
S5	0.002	12.7	<0.001	12.7	44.5–113	71.0	50.7	146
S6	0.001	8.74	0.021	8.76	51.9–182	97.1	42.1	184
S7	<0.001	4.71	0.027	4.74	32.9–112	60.6	27.9	164
S8	0.001	7.94	0.050	7.99	37.7–73.0	52.4	40.0	118
<i>Masan Bay</i>								
M1	0.001	2.35	<0.001	2.35	1.11–8.31	3.04	11.7	44.3
M2	0.003	1.95	<0.001	1.95	4.04–11.5	6.80	7.33	63.3
M3	0.013	6.56	<0.001	6.58	78.4–135	103	35.5	113.3
M4	0.004	13.7	0.029	13.7	1.79–15.7	5.31	52.5	52.8
M5	0.011	2.71	0.444	2.72	0.002–6.65	0.11	15.0	24.4
M6	<0.001	1.44	0.355	1.44	0.14–5.77	0.90	0.35	34.0
M7	0.008	6.75	0.087	6.76		NAC <sup>e</sup>	36.1	9.3
M8	0.002	5.73	<0.001	5.73	0.004–1.11	0.07	32.3	18.0
<i>Kwangyang Bay</i>								
K1	<0.001	0.27	<0.001	0.27		NAC	<0.00	2.04
K2	<0.001	0.19	<0.001	0.19		NAC	<0.00	7.54
K3	<0.001	0.29	<0.001	0.29		NAC	<0.00	<0.00
K4	<0.001	0.16	<0.001	0.16		NAC	<0.00	6.48
K5	<0.001	0.47	<0.001	0.47	0.97–16.0	3.95	<0.00	41.6
K6	<0.001	0.29	<0.001	0.29	0.02–3.51	0.24	<0.00	22.1
K7	0.003	0.16	<0.001	0.16	0.03–2.94	0.29	<0.00	21.1
K8	0.003	0.17	<0.001	0.17		NAC	<0.00	11.0
K9	0.001	0.14	<0.001	0.15	0.59–10.2	2.44	<0.00	35.3
K10	0.001	0.45	<0.001	0.45		NAC	<0.00	19.8
K11	<0.001	0.22	0.045	0.22		NAC	<0.00	7.28

<sup>a</sup>Instrumentally derived 17- $\beta$ -estradiol (E2) equivalents (EEQs) of PAHs (EEQ<sub>PAH</sub>), alkylphenols (EEQ<sub>AP</sub>), and bisphenol A (EEQ<sub>BPA</sub>) associated with sediment samples. Presented are the EEQs (pg E2/g dry wt) generated from assay-specific REP-50 values (Villeneuve et al., 1998, 2002) of selected PAHs (benzo[a]anthracene, dibenz[a,h]anthracene), nonylphenol, octylphenol, and bisphenol A and the corresponding concentrations measured.

<sup>b</sup>Bioassay-derived estrogen equivalents (E2–EQs) of sediment fractions and raw extracts (pg E2/g dry wt). E2–EQ<sub>20–80</sub> represents the range of estrogen equivalents generated from multiple point estimates made for the responses ranging from 20% to 80% E2-max. E2–EQ<sub>50</sub> indicates the estrogen equivalents generated from one point estimate for response of 50% E2-max.

<sup>c</sup>Regression of total EEQs against the E2 standard curve was used to predict the magnitude of the bioassay response.

<sup>d</sup>Observed bioassay response (% E2-max) of sediment raw extracts in the MVLN bioassay.

<sup>e</sup>NAC; data not available for the calculation of E2–EQs; i.e., a dose–response curve could not be obtained in the full dose–response bioassay.

contribution of NP and OP compounds to estrogenic activities associated with sediment REs in general, although other unidentified estrogenic compounds may contribute to the estrogenic potency associated with sediment from some locations. Overall, the RE screening results suggested a significant difference in the estrogenic response profile between inland rivers/streams and the open ocean.

The cause of the discrepancy between responses induced by the REs and those induced by FEs, particularly F3, was not readily apparent. The discrepancy may indicate that compounds relevant to the RE response were lost during fractionation, but that does

not explain why the concentrations of APs determined from F3 samples did not induce a robust F3 response. Alternatively, the increased cytotoxicity of the F3 samples relative to the REs may indicate that other artifacts associated with the fractionation process may have altered the health and function of the MVLN cells, even where overt cytotoxicity was not readily apparent. As they stand, the MVLN results for the FEs tend to contradict the mass balance conclusions based on RE results. Given the cytotoxicity of at least some of the F3 extract to MVLN cells, the mass balance results for the REs should be considered more reliable. However, without additional study it is not possible to determine

which approach provides a more accurate characterization of causes for the estrogenic activity of these Korean coastal sediment extracts.

## 4. Discussion

### 4.1. Distribution and sources of contaminants

Concentrations of target contaminants measured in sediments from the 27 locations varied among locations. In order to detect general spatial patterns in contaminant distribution, a PCA was performed based on the concentrations of the measured residues (Table 1). Although PCA extracted three components, which collectively accounted for 99.7% of the variability in the original data, the first two PCs explained 93% of the variability of the data set (Fig. 6). PC1 accounted for 74% of the total variance and was significantly correlated with BPA ( $r = -0.48$ ,  $P < 0.05$ ), TOC ( $r = 0.25$ ,  $P < 0.01$ ), BP ( $r = 0.22$ ,  $P < 0.01$ ), HCHs ( $r = 0.20$ ,  $P < 0.05$ ), and PAHs ( $r = 0.20$ ,  $P < 0.01$ ). PC2 accounted for 19% of the total variance and was significantly correlated with OP ( $r = -0.52$ ,  $P < 0.01$ ), NP ( $r = 0.5$ ,  $P < 0.01$ ), HCB ( $r = -0.43$ ,  $P < 0.01$ ), BP ( $r = -0.40$ ,  $P < 0.01$ ), and PCBs ( $r = 0.22$ ,  $P < 0.05$ ). The plot of loading for PC1 against PC2 resulted in three well-defined clusters (Groups A, B, and C; Fig. 6). Greater scores for PC1 suggest greater concentrations of TOC, BP, HCHs, and PAHs in samples, but lesser BPA concentrations, which is inversely correlated with PC1. The small values of PC2 are due to greater concentrations of OP, NP, HCB, BP, and PCBs. This suggests

that cluster C is characterized by greater concentrations of a few of the residues analyzed. All inland locations from Lake Shihwa were grouped in cluster C. These locations contained greater concentrations of HCHs, PAHs, BP, TOC, PCBs, HCB, OP, and BP. The sites in cluster A were characterized by lesser concentrations of several of the residues analyzed. The relatively strong correlations between APs, such as BP, OP, and NP, with scores of either PC1 or PC2 suggest that this class of compounds contributed to the majority of the variance observed among sampling sites.

The majority of PAHs found in sediments contained three to five rings, and these accounted for over 80% of total PAHs. Fluoranthene and pyrene were the predominant compounds, comprising 18% and 19% of the total PAHs. The ratios of fluoranthene-to-pyrene (Fluo/Py) in sediments from Lake Shihwa (0.84) and Masan Bay (0.92) were generally less than 1.0, suggesting that the major PAH sources were petrogenic. In contrast, Fluo/Py ratios in sediments from Kwangyang Bay locations exceeded 1.0 in 6 of the 11 locations, suggesting primary PAH sources were both petrogenic and pyrolytic (Kannan et al., 2001). The composition of individual PAHs to total PAH concentrations was examined by using PCA to identify contaminated sites and probable sources. PAH concentrations were normalized by our logarithmically transforming the data [ $y' = \log(y + 1)$ ] before performing PCA. In this study, components that account for more than 10% of the total variance were chosen. The first two PCs accounted for 89% (PC1 was 77% and PC2 was 12%) of the variation in the profiles of PAH concentrations. The plot of PC1 against PC2 clustered the sampling sites into five groups (Fig. 7), where most of the PAHs were significantly

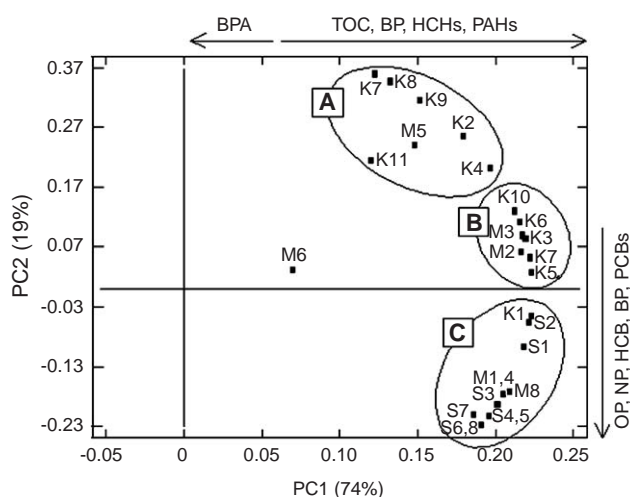


Fig. 6. Principal component analysis of target organic contaminants associated with sediments collected from the 27 locations in Lake Shihwa, Masan Bay, and Kwangyang Bay, Korea. Three well-defined clusters are represented as Groups A, B, and C, in which principal components 1 and 2 accounted for 74% and 19% of the variability of the data set, respectively.

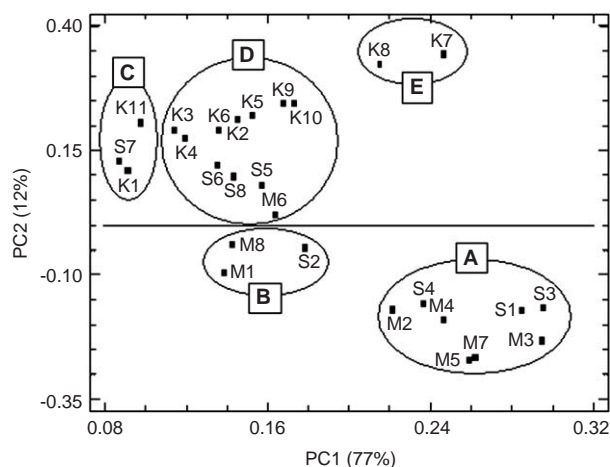


Fig. 7. Principal component analysis of 16 individual PAHs associated with sediments collected from the 27 locations in Lake Shihwa, Masan Bay, and Kwangyang Bay, Korea. Five groups (A–E) were characterized by the specific source profiles of PAHs, for which principal components 1 and 2 accounted for 77% and 12% of the variation in the profiles of PAH concentrations, respectively.

correlated with PC1 ( $r = 0.63\sim 0.90$ ,  $P < 0.01$ ) and PC2 ( $r = -0.76\sim -0.61$ ,  $P < 0.01$ ). Since all PAHs were positively correlated with PC1 and negatively correlated with PC2, the sampling sites that contained the greatest concentrations of PAHs clustered within the four quadrants of the Cartesian system (Fig. 7). Group A (Fig. 7, lower-right cluster) and Group C (Fig. 7, upper-left cluster) contained the greatest and least PAHs concentrations, respectively. PAH profiles reported in the literature (Bzdusek et al., 2004; Li et al., 2003) in various source materials (power plants, gasoline engines, traffic tunnels, etc.) were correlated with the scores of PC1 (diesel engine,  $r = 0.75$ ; gasoline engine,  $r = 0.75$ ), which suggests that automobile traffic is one of the main sources of PAHs in the sampling sites.

APs are degradation products of alkylphenol ethoxylates (APEs), which are widely used in cleaning products. These contaminants could easily be introduced into the river and coastal environments via direct discharge of sewage or municipal wastewater. ICs of over 4000 factories and densely populated cities (ca. 0.7 million) in the Lake Shihwa region discharge large amounts of industrial and municipal wastewaters daily ( $0.5 \times 10^6$  ton/day). A recent study found severe contamination by NP in various environmental samples from Lake Shihwa in 2002, where NP concentrations in dissolved water, particulates, and sediments were as great as 1533 ng/L, 831 ng/L, and 5054 ng/g dw, respectively (Li et al., 2004). Thus, sedimentary NP (254–4930 ng/g dw) found in and around Lake Shihwa suggests continuing input of these compounds via streams and creeks running into the lake. Also, a strong correlation between NP and OP ( $r = 0.95$ ,  $P < 0.01$ ) indicates that their sources are probably related.

Tri- to penta-CBs were the major PCB congeners found in sediments. PCB congeners 18 (2, 2', 5), 40 (2, 2', 3, 3'), and 91 (2, 2', 3,4', 6) were the predominant isomers from most of the locations. The homologue pattern of PCBs found in Lake Shihwa and Masan Bay suggested the source was technical mixtures such as Aroclors 1016 and 1242 (Imamoglu and Christensen, 2002). Non-, mono-, and di-*ortho* PCBs were found at concentrations ranging from 0.25 to 11.2 ng/g dw, a contribution of 6.5% of the total PCB concentrations on average. TEQ<sub>PCB</sub> concentrations in sediments from Lake Shihwa and Masan Bay were estimated to range from 0.19 to 77.7 pg/g dw, whereas those from Kwangyang Bay were mostly less than 0.01 pg/g dw (Table 3). The similarity in the spatial distribution pattern and significant correlation between PCBs and individual OC pesticides (HCB,  $r = 0.90$ ; HCHs,  $r = 0.91$ ; CHLs,  $r = 0.82$ ; DDTs,  $r = 0.99$ ) suggests that their sources are closely related. In contrast, in Masan and Kwangyang Bays no apparent similarity in distribution between PCBs and OC pesticides was found, suggesting that their sources are independent of each other.

#### 4.2. Mass balance analysis

Florisol fractions F1, F2, and F3 of each of the 27 samples were analyzed to characterize dioxin-like contamination associated with sediment from these study locations and to compare the profile of activity to that observed in previous studies of dioxin-like contamination in Korean coastal areas (Fig. 4). Little dioxin-like activity was observed for F1 samples. Congener-specific PCB analysis found total concentrations of TEQ<sub>PCB</sub> to be as great as 77.7 pg/g dw at location M3. Based on regression against the TCDD standard curve, the TEQ<sub>PCB</sub> would have been sufficient to induce a significant response of 55% TCDD-max. However, the PCB-containing fraction (F1) alone did not induce significant H4IIE-luc responses (Fig. 4). This suggests either that nonpolar compounds present in F1 antagonized the activity of AhR-active PCBs in the H4IIE-luc bioassay or that the dioxin-like potency of specific PCB congeners was overestimated. The lack of bioassay responses for F1 samples from the Lake Shihwa and Masan Bay inland region was consistent with previous results that showed low-to-no dioxin-like activities associated with F1 for lake and marine sediments in 1999 (Khim et al., 1999a,b) and for sediment from a variety of other Korean coastal areas (Khim et al., 2001; Koh et al., 2002, 2004).

All F2 and F3 samples elicited significant dioxin-like responses (Fig. 4). The magnitude of induction for F2 samples was as great as 113% TCDD-max, and in many cases F2 induced activities that were greater than those observed for the corresponding RE (Fig. 4). F2 extracts contained detectable levels of AhR-active PAHs, and H4IIE-luc responses were strongly correlated with the logarithm of total PAHs ( $r^2 = 0.65$ ,  $P < 0.01$ ). However, based on the TEQ<sub>PAH</sub> concentrations (0.02–4.09 pg/g dw), bioassay responses were predicted to be lower than 10% TCDD-max. Thus, the concentrations of AhR-active PAHs present in F2 samples account for only a small portion of the F2 responses. This indicates that other unidentified dioxin-like (AhR-active) compounds, probably including PCDD/DFs [polychlorinated dibenzo-*p*-dioxins (PCDDs)/polychlorinated dibenzofurans (PCDFs)], were responsible for much of the activity associated with F2 samples. Furthermore, the fact that many F2 activities were greater than those observed for the corresponding RE suggests that components associated with F1 and/or F3 antagonized the integrated dioxin-like response of components associated with F2. Based on an instrumentally derived TEQ approach alone, it would be difficult to account for this effect and accurately predict the integrated dioxin-like potency of the sediment extracts.

With only a few exceptions (M1, K1, K2, K4, K11), the magnitudes of induction caused by F3 samples were less than those elicited by F2 samples. Response

magnitudes for F3 samples ranged from 12% to 69% TCDD-max (Fig. 4). F3 samples corresponding to Masan Bay sediment, mainly from inland locations, showed relatively greater dioxin-like activities than those of Lake Shihwa and Kwangyang Bay (Figs. 1 and 4). The F3 results were consistent with the previous findings of relatively great dioxin-like activities associated with F3 of Masan Bay sediment extracts (Khim et al., 1999b). F3 results suggest the presence of unidentified, relatively polar AhR-active compounds at numerous locations in the Lake Shihwa, Masan Bay, and Kwangyang Bay regions. PCDDs/DFs, which can carry over into F3 (Khim et al., 1999c; Koh et al., 2004), may account for all or a portion of the F3 responses observed. However, the results could indicate the presence of additional dioxin-like compounds with polarities similar to those of the APs. The similar fraction profiles in the H4IIE-luc bioassay between Masan Bay inland (current data) and bay sediments (Khim et al., 1999b) and for other studies of Korean coastal sediments (Khim et al., 2001; Koh et al., 2002, 2004) suggest broad compositional similarities among dioxin-like contaminants present in sediment from many Korean coastal areas.

A previous study of Hyeongsan River sediments found that PCDDs and PCDFs were the primary source of total TEQs in sediment extracts and that when PCDD/DFs were included instrumentally derived TEQs could reasonably account for the magnitude of bioassay responses observed (Koh et al., 2004). Assuming similar distributions of PCDD/DFs in other industrialized Korean coastal areas, it is likely that PCDD/DFs account for a large portion of the dioxin-like potency that is currently unaccounted for in this study. Quantitative determination of PCDD/DF contamination in the Lake Shihwa, Masan Bay, and Kwangyang Bay regions could help to clarify the contribution of PCDD/DFs versus other unidentified dioxin-like contaminants to the integrated dioxin-like potency (TCDD-EQs) determined in this study.

Estrogenic responses in the MVLN bioassay were markedly less after fractionation (Fig. 5). The lack of estrogenic responses in F1 was explained by there being few ER-active compounds present in F1. Some F2 samples induced significant estrogenic responses, but the magnitudes of response caused by F2 were, on average, 19-fold less than those caused by REs. Significant correlations between the F2 response in the MVLN bioassay and the logarithms of total PAHs ( $r^2 = 0.330$ ,  $P < 0.01$ ), benzo[a]anthracene concentrations ( $r^2 = 0.600$ ,  $P < 0.01$ ), dibenz[a,h]anthracene concentrations ( $r^2 = 0.469$ ,  $P = 0.013$ ), and  $EEQ_{PAH}$  ( $r^2 = 0.598$ ,  $P = 0.001$ ) were found. However, the total  $EEQ_{PAH}$  concentrations ( $< 0.001$ – $0.02$  pg/g dw) measured in the 27 F2 samples were not predicted to yield significant estrogenic responses. It is currently unclear

what additional compounds or interactions may have contributed to the F2 responses, but evidence suggests that these factors were likely correlated with PAH concentrations and may result from similar sources.

All suspected xenoestrogens, such as NP, OP, and BPA, eluted in F3. Consequently, one would have expected F3 samples to induce estrogenic activities similar to those observed for REs. However, the response magnitudes observed for F3 were generally six times lower than those caused by REs (Fig. 5). Furthermore, F3 responses were not significantly correlated with either concentrations of APs and BPA or  $EEQ_{APs}$  ( $P = 0.236$ – $0.889$ ). F3 responses were significantly correlated with RE responses for Lake Shihwa ( $r^2 = 0.766$ ,  $P = 0.0265$ ) and Kwangyang Bay samples ( $r^2 = 0.624$ ,  $P = 0.040$ ), but not for Masan Bay samples ( $r^2 = 0.512$ ,  $P = 0.194$ ). This was in contrast to the fact that there was a reasonable mass balance for the Masan Bay samples, while RE results for Lake Shihwa and Kwangyang Bay suggested that  $EEQ_{APs}$  alone could not readily account for the activity observed. At least for some samples, the lack of response could be attributed to a cytotoxicity associated with F3; however, F3 responses were unexpectedly low even when cytotoxicity was not readily apparent (Fig. 5).

## 5. Conclusion

The relative abundance of organic contaminants measured in sediment from the Korean coast was in the order of APs > PAHs > PCBs > OC pesticides. Concentrations of target contaminants measured in sediments from the 27 locations in Lake Shihwa, Masan Bay, and Kwangyang Bay varied among sampling locations, indicating that their sources of each class of chemicals were independent of each other. Sediment samples from stream and/or inland locations contained the greatest concentrations of POPs and xenoestrogen, which indicated the presence of localized sources. The screening of REs in H4IIE-luc and MVLN cell bioassays indicated a significant difference in the dioxin-like and estrogenic responses between inland rivers/streams and the open ocean, which was in good agreement with instrumental results. Profiles of responses observed for FEs showed a pattern that was fairly consistent with previous studies of Korean coastal sediment contamination, with the greatest dioxin-like activities associated with the midpolar and polar fractions. Overall, the results of this study support the utility of in vitro bioassays in characterizing the occurrence and distribution of potentially adverse contaminants present in the coastal environment.

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## References

- Behnisch, P.A., Hosoe, K., Sakai, S., 2001. Bioanalytical screening methods for dioxins and dioxin-like compounds—a review of bioassay/biomarker technology. *Environ. Int.* 27, 413–439.
- Bzdusek, P.A., Christensen, E.R., Li, A., Zou, Q., 2004. Source apportionment of sediment PAHs in Lake Calumet, Chicago: application of factor analysis with nonnegative constraints. *Environ. Sci. Technol.* 38, 97–103.
- Giesy, J.P., Kannan, K., 1998. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. *Crit. Rev. Toxicol.* 28, 511–569.
- Giesy, J.P., Hilscherova, K., Jones, P.D., Kannan, K., Machala, M., 2002. Cell bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. *Mar. Pollut. Bull.* 45, 3–16.
- Imamoglu, I., Christensen, E.R., 2002. PCB sources, transformations, and contributions in recent Fox River, Wisconsin sediments determined from receptor modeling. *Water Res.* 36, 3449–3462.
- Kannan, K., Kober, J.L., Kang, Y.-S., Masunaga, S., Nakanishi, J., Ostaszewski, A., Summer, C.L., Giesy, J.P., 2001. Polychlorinated-naphthalenes, -biphenyls, -dibenzo-p-dioxins, -dibenzofurans, polycyclic aromatic hydrocarbons and alkylphenols in sediment from the Detroit and Rouge Rivers, Michigan, USA. *Environ. Toxicol. Chem.* 20, 1878–1889.
- Kannan, K., Kober, J.L., Khim, J.S., Szymczyk, K., Falandysz, J., Giesy, J.P., 2003. Polychlorinated biphenyls, polycyclic aromatic hydrocarbons and alkylphenols in sediments from the Odra River and its tributaries, Poland. *Toxicol. Environ. Chem.* 85, 51–60.
- Khim, J.S., Villeneuve, D.L., Kannan, K., Lee, K.T., Snyder, S.A., Koh, C.H., Giesy, J.P., 1999a. Alkylphenols, polycyclic aromatic hydrocarbons (PAHs), and organochlorines in sediment from Lake Shihwa, Korea: Instrumental and bioanalytical characterization. *Environ. Toxicol. Chem.* 8, 2424–2432.
- Khim, J.S., Villeneuve, D.L., Kannan, K., Koh, C.H., Giesy, J.P., 1999b. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 2. In vitro gene expression analysis. *Environ. Sci. Technol.* 33, 4206–4211.
- Khim, J.S., Kannan, K., Villeneuve, D.L., Koh, C.H., Giesy, J.P., 1999c. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 1. Instrumental analysis. *Environ. Sci. Technol.* 33, 4199–4205.
- Khim, J.S., Lee, K.T., Villeneuve, D.L., Kannan, K., Giesy, J.P., Koh, C.H., 2001. In vitro gene expression assays for determination of dioxin-like and estrogenic compounds in environmental samples from Ulsan Bay and its vicinity, Korea. *Arch. Environ. Contam. Toxicol.* 40, 151–160.
- Kim, S.-K., Oh, J.R., Shim, W.J., Lee, D.H., Yim, U.H., Hong, S.H., Shin, Y.B., Lee, D.S., 2002. Geographical distribution and accumulation features of organochlorine residues in bivalves from coastal areas of South Korea. *Mar. Pollut. Bull.* 45, 268–279.
- Koh, C.H., Khim, J.S., Villeneuve, D.L., Kannan, K., Giesy, J.P., 2002. Analysis of trace organic contaminants in environmental samples from Onsan Bay, Korea. *Environ. Toxicol. Chem.* 21, 1796–1803.
- Koh, C.H., Khim, J.S., Kannan, K., Villeneuve, D.L., Senthilkumar, K., Giesy, J.P., 2004. Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-TCDD equivalents (TEQs) in sediments from Hyeongsan River, Korea. *Environ. Pollut.* 132, 489–501.
- Li, A., Jang, J.K., Scheff, P.A., 2003. Application of EPA CMB8.2 model for source apportionment of sediment PAHs in Lake Calumet, Chicago. *Environ. Sci. Technol.* 37, 2958–2965.
- Li, Z., Li, D., Oh, J.R., Je, J.G., 2004. Seasonal and spatial distribution of nonylphenol in Shihwa Lake, Korea. *Chemosphere* 56, 611–618.
- Loganathan, B.G., Kannan, K., 1994. Global organochlorine contamination trends: an overview. *Ambio* 23, 187–191.
- Oh, J.R., Ikononou, M.G., Fernandez, M.P., Hong, S.H., 2003. PCB and PCDD/F totals, TEQs, and congener patterns in Korean coastal marine environment, 1987, 1988, 1990, and 1996–1999. *Arch. Environ. Contam. Toxicol.* 44, 224–236.
- Sanderson, J.T., Giesy, J.P., 1998. Wildlife toxicology, functional response assays. In: Meyers, R.A. (Ed.), *Encyclopedia of Environmental Analysis and Remediation*. Wiley, New York, pp. 5272–5297.
- Statistical Graphics Corp, 2001. *Statgraphics Plus for Windows, Statistical Graphics System, User's Guide Version 5.1*. Statistical Graphics Corp.
- Villeneuve, D.L., Blankenship, A.L., Giesy, J.P., 1998. Interactions between environmental xenobiotics and estrogen receptor-mediated responses. In: Denison, M.S., Helferich, W.G. (Eds.), *Toxicant-Receptor Interactions*. Taylor and Francis, Philadelphia, PA, pp. 69–99.
- Villeneuve, D.L., Blankenship, A.L., Giesy, J.P., 2000. Derivation and application of relative potency estimates based on in vitro bioassay results. *Environ. Toxicol. Chem.* 19, 2835–2843.
- Villeneuve, D.L., Khim, J.S., Kannan, K., Giesy, J.P., 2002. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxin-like and estrogenic responses in three cell lines. *Environ. Toxicol.* 17, 128–137.