

ANALYSIS OF TRACE ORGANIC CONTAMINANTS IN SEDIMENT, PORE WATER, AND WATER SAMPLES FROM ONSAN BAY, KOREA: INSTRUMENTAL ANALYSIS AND IN VITRO GENE EXPRESSION ASSAY

Chul-Hwan Koh,*† Jong Seong Khim,†‡ Daniel L. Villeneuve,‡ Kurunthachalam Kannan,‡ and John P. Giesy‡

†School of Earth and Environmental Sciences (Oceanography), College of Natural Sciences, Seoul National University, Seoul 151-742, South Korea

National Food Safety and Toxicology Center, Department of Zoology, and Institute for Environmental Toxicology, Michigan State University, East Lansing, Michigan 48824-1311, USA

(Received 24 October 2001; Accepted 7 March 2002)

Abstract—Persistent organic pollutants and alkylphenols (APs) were determined in sediment and water samples from Onsan Bay, Korea, by using instrumental analysis and in vitro gene expression cell bioassay. Polycyclic aromatic hydrocabons (PAHs) were the predominant compounds in sediments with concentrations as great as 573 ng/g dry weight. The PAH concentrations were elevated in sediment from inland rivers that flow through Onsan City (mean: 116 ng/g dry wight. The PAH concentrations discharge into Onsan Bay. Concentrations of polychlorinated biphenyls (PCBs) in sediments ranged from <1.00 to 56.2 ng/g dry weight. Among different organochlorine (OC) pesticides analyzed (hexachlorobenzene, hexachlorocyclohexanes, chlordanes, and DDTs), DDT concentrations were the greatest, ranging from <0.01 to 7.58 ng/g dry weight. The spatial gradient of contaminant concentrations suggested that streams and rivers are the major sources of PCBs, PAHs, and APs to the bay. Maximum concentrations of nonylphenol, octylphenol, and bisphenol A in sediments were 860, 11, and 204 ng/g dry weight, respectively. Screening of Onsan Bay sediment samples for dioxinlike activity with the H4IIE-luc in vitro cell bioassay revealed that 17 of 22 samples contained significant dioxinlike activity. Further fractionation of sediment extracts indicated that mid-polar and more polar fractions were responsible for the significant dioxinlike activity. Based on a mass balance analysis, PAHs apparently accounted for only a small portion of dioxinlike responses elicited by sediment extracts. Only one raw extract of sediment elicited a significant estrogenic response by MVLN cells. The combination of instrumental analysis and in vitro bioassay was useful to assess sediment quality and characterize the causative agents or potential toxic compounds present.

Keywords-Sediment Persistent organic pollutants Polycyclic aromatic hydrocarbons Xenoestrogen In vitro bioassay

INTRODUCTION

Onsan Bay, located on the east coast of Korea, contains several industrial complexes and a large commercial harbor. Industrial complexes contain several heavy metal (zinc, aluminum, and copper) and petrochemical industries, paper mills, and the largest nonferrous metal industry in Korea. Heavy industrial and shipping activities around the Onsan Bay region and rapid urbanization of Onsan City have resulted in environmental contamination since the 1980s. Approximately 340,000 t of industrial and domestic wastes from more than 100 industrial complexes are discharged daily into the bay through Woihwang River and Daejeong Stream (Fig. 1). About one half (150,000 t) of the wastes receive primary sewage treatment, whereas the rest are discharged directly into the bay. Pollution in this bay has been suspected to be related to Onsan disease among inhabitants who consumed seafood collected from the bay in 1985. The disease is characterized by nausea, skin discoloration, and in extreme cases, death. The Korean government planned to relocate 27,000 inhabitants who lived around the bay to other areas because the bay was a serious environmental and health problem. Heavy metals such as cadmium were suspected contaminants for Onsan disease; however, the actual cause of the disease has never been determined.

Despite the potential for direct and accidental releases of organic contaminants into Onsan Bay, little was known about contamination in this region. This study represents one of the first efforts to examine the concentrations, distribution, and biological potency of organic contaminants present in Onsan Bay sediments and waters. The compounds examined in this study were polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, nonylphenol (NP), octylphenol (OP), and bisphenol A (BPA).

The PAHs are derived from not only anthropogenic sources such as waste incineration, coal gasification, and accidental oil spills, but also are combustion products of fossil fuels and wood [1]. Because of their mutagenicity, carcinogenicity, and toxic effects similar to that of dioxins, their concentrations have been evaluated in a variety of environmental matrices including sediment [2–4]. Polycyclic aromatic hydrocarbons have been identified as prominent contaminants in sediments from several Korean coasts [5–7].

Recently, concern has increased about contamination by halogenated aromatic hydrocarbons, such as DDTs, PCBs, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans in Korea [5–11]. Although sources, emission, transport, fate, and effects of persistent organic pollutants have long been studied worldwide, little is known about these compounds in Korea because of the lack of analytical facilities.

Alkylphenols (APs) such as NP and OP are another class

^{*} To whom correspondence may be addressed (kohch@snu.ac.kr).

Fig. 1. Map of the study area. Samples (22 sediment, 10 water, and 11 pore water) were collected from 23 locations from Woihang River (W0–2 locations), Daejeong Stream (D0–6 locations), and Onsan Bay (ON1–3, OS1–3, and O4–10 locations), Korea, in April 1999.

of contaminants found frequently in sediments from Korea [5,6]. Alkylphenols are degradation products of AP ethoxylates, which are nonionic surfactants widely used for industrial and household purposes [12]. Bisphenol A is another environmental contaminant released through its use in polycarbonate plastics, epoxyresins, and phenoxy resins, which are utilized in food storage containers and in dental sealants [5]. These compounds enter the aquatic environment via industrial and municipal wastewater effluents. Information on their distribution in sediments is necessary to understand their effects on sediment bound organisms and to elucidate their environmental fate. Nonylphenol, OP, and BPA were analyzed in this study.

Because of the complex nature of contaminants in sediments, sample extracts were fractionated according to polarity to isolate and identify target contaminants. Both instrumental analyses and in vitro recombinant cell bioassays that used H4IIE-luc cells for dioxinlike activity and MCF-7-luc (MVLN) cells for estrogenic activity were performed to quantify target contaminants and to evaluate dioxinlike and estrogenic potencies [5,13]. Assessment based solely on instrumental analysis may over or underestimate potential hazards of sediment contamination. Thus, a combination of instrumental analysis and in vitro bioassay were used to assess sediment contamination [13–15].

MATERIALS AND METHODS

Sampling

Sediment and water samples were collected from 23 locations in Onsan Bay during April 1999 (Fig. 1). Sampling was designed to determine potential sources of contaminants from inland regions such as the Woihwang River and Daejeong Stream (designated as W and D locations) and to elucidate the distribution in the outer bay (Onsan Bay; ON, OS, and O locations). Whole sediment samples (three replicates) were collected from 22 locations except for one (D5) by a Van Veen grab sampler ($25 \times 40 \times 30$ cm) and surface sediment (0–5 cm) samples subcollected for chemical analysis and bioassay. Samples were homogenized in precleaned aluminum containers and pebbles and twigs were removed. Samples were stored in precleaned amber glass bottles (250 ml) and transported on dry ice to the laboratory. Samples were freeze-dried and ground with a mortar and pestle and stored in precleaned highdensity polyethylene bottles at -20° C until extraction. Total organic carbon was analyzed with a CHN analyzer [6]. Surface water samples (about 16 L) were collected from 10 locations (W0, W1, W2, D2, D4, D5, ON1, OS1, O4, and O10) in precleaned 4-L amber glass bottles and stored on ice. Eleven pore-water samples (about 1 L) were obtained from corresponding sediment samples (W0, W1, W2, D1, D2, D3, D4, ON1, OS1, O4, and O10) by using a pressurized (squeezing) method immediately after sampling surface sediment [16]. Water and pore-water samples were stored in 4-L amber glass bottles and transported on ice to laboratory. Water samples were further separated into particulate matter and dissolved fraction by using glass fiber filters and membrane filters (final pore size: 0.45 µm). Particulate matter samples were freezedried and stored in precleaned aluminum foil at -20°C until extraction.

Sample preparation

Polycyclic aromatic hydrocarbons, PCBs, OC pesticides (DDTs, hexachlorocyclohexanes, chlordanes, and hexachlorobenzene), NP, OP, and BPA were analyzed by previously described methods [5]. Sediment and particulate matter samples were Soxhlet extracted for 20 h with dichloromethane (Burdick & Jackson, Muskegon, MI, USA). Solid-phase samples were treated with activated copper granules to remove residual sulfur from the extracts. Aqueous phase samples such as water and pore water were passed through Empore^(B) solidphase disks (3M, St. Paul, MN, USA) and then extracted with hexane and dichloromethane. Raw extracts (REs) were concentrated and the volume was adjusted to 2 ml in hexane.

One milliliter of RE was used for in vitro bioassay. The remaining 1 ml was passed through a 10-g activated Florisil column (60–100 mesh size; Sigma, St. Louis, MO, USA) for further fractionation. Three fractions (F1, F2, and F3) were collected according to polarity and used for both instrumental analyses and bioassay. Florisil separation was confirmed by using a spike recovery test and standard reference material, 1974a sediment (National Institute of Science and Technology, Gaithersburg, MD, USA). Recoveries of target analytes through the analytical procedures were between 90% and 105%. Procedural blanks were analyzed with every set of six samples to check for interference or contamination arising from solvents or glassware.

Instrumental analysis

The PAHs were quantified by using a Hewlett-Packard 5890 series II gas chromatograph equipped with a 5972 series mass spectrometer detector (Avondale, PA, USA). Organochlorine pesticides and PCBs were quantified by using a gas chromatograph (Perkin-Elmer series 600, Norwalk, CT, USA) equipped with ⁶³Ni electron-capture detector. An equivalent mixture of 98 individual PCB congeners (AccuStandard, New Haven, CT, USA) and a mixture of OC pesticides (CLP-023R



Table 1. Concentrations (ng/g dry wt) of target organic compounds in sediments from Onsan Bay, Korea^a

Region	Location	PAHs	NP	OP	BPA	PCBs	DDTs	CHLs	HCHs	HCB
Woihwang River	W0	<10.0	<1.00	<1.00	<1.00	6.87	0.07	< 0.01	0.02	0.03
U	W1	77.0	20.8	<1.00	<1.00	10.1	0.02	< 0.01	1.02	0.69
	W2	573	4.78	<1.00	1.59	5.47	0.35	0.04	3.37	0.55
Daejeong Stream	D0	<10.0	6.23	<1.00	<1.00	<1.00	7.55	< 0.01	0.04	0.03
	D1	113	860	11.01	204	NA	7.58	0.95	0.79	NA
	D2	<10.0	14.4	<1.00	<1.00	<1.00	0.02	< 0.01	0.02	1.69
	D3	34.5	100	1.39	1.21	19.6	1.20	0.13	0.26	2.58
	D4	214	96.4	5.75	8.93	56.2	1.67	0.81	3.17	4.44
	D6	21.4	<1.00	<1.00	<1.00	3.33	3.06	< 0.01	0.78	0.21
Onsan Bay	ON1	<10.0	1.86	<1.00	<1.00	NA	2.54	0.08	1.12	NA
-	ON2	NAj	1.26	<1.00	<1.00	7.79	< 0.01	< 0.01	< 0.01	1.57
	ON3	NA	<1.00	<1.00	<1.00	<1.00	0.47	< 0.01	0.02	0.08
	OS1	38.7	3.46	<1.00	<1.00	<1.00	0.61	< 0.01	0.76	< 0.01
	OS2	35.9	1.39	<1.00	<1.00	<1.00	0.76	< 0.01	0.02	0.15
	OS3	19.3	NA	NA	NA	<1.00	0.20	0.03	0.68	0.20
	O4	17.9	<1.00	4.33	<1.00	<1.00	0.25	0.05	0.26	0.05
	O5	41.6	<1.00	<1.00	<1.00	<1.00	0.33	0.05	0.44	0.05
	O6	27.0	<1.00	<1.00	<1.00	<1.00	0.05	< 0.01	0.10	0.04
	07	<10.0	<1.00	<1.00	<1.00	<1.00	0.06	< 0.01	0.02	0.13
	O8	<10.0	2.68	<1.00	<1.00	NA	0.05	< 0.01	0.12	NA
	O9	10.4	3.80	<1.00	<1.00	<1.00	0.03	< 0.01	0.02	0.04
	O10	27.7	<1.00	<1.00	<1.00	<1.00	0.15	< 0.01	0.41	< 0.01

^a PAHs = polycyclic aromatic hydrocarbons, sum of 16 priority components; NP = nonylphenol; OP = octylphenol; BPA = bisphenol A; PCBs = polychlorinated biphenyls, sum of 98 individual congeners; DDTs = sum of p,p'-DDT, p,p'-dichlorodiphenyldichloroethane, and p,p' dichlorodiphenyldichloroethylene; CHLs = sum of α and γ -chlordanes; HCHs = sum of α -, β -, and γ -hexachlorocyclohexanes; HCB = hexachlorobenzene; NA = not analyzed.

and CLP-024R, AccuStandard) were used as a standard [17]. Reverse-phase high-performance liquid chromatography with fluorescence detection was used to quantify NP and OP. Further details of sample preparation and instrumental analysis are described elsewhere [5,18].

In vitro bioassay

Each sample was tested as both RE and fractionated extract in the in vitro bioassays [19]. Luciferase and protein assays were conducted after 72 h incubations. Sample responses, expressed as mean relative luminescence units over three replicate wells, were converted to relative response units, expressed as a percentage of the maximum response observed for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; %-TCDDmax) or 17\beta-estradiol (E2, %-E2-max) standard curves generated on the same day [13,18–19]. This was done to normalize responses for day-to-day variability in response magnitude. The mean solvent control response was subtracted from both sample and standard responses on a plate-by-plate basis before conversion to a percentage, to scale values from 0 to 100%standard-max. Significant responses were defined as those outside the range defined by three times the standard deviation (expressed in %-standard-max) of the mean solvent control response (0%-standard-max). Total protein in the wells was used as an index of cell number to detect outliers that were not apparent by simple visual inspection.

Mass balance analysis (or potency balance analysis) was used to examine whether or not the known concentration or composition of a sample (identified by instrumental analyses) could account for the magnitude or potency of biological response observed [19]. Further details of in vitro bioassay and mass balance analysis have been described elsewhere [13,14,19,20].

RESULTS AND DISCUSSION

Concentration and distribution of persistent organic pollutants

The relative abundance of organic residues in sediment was in the order PAHs \approx APs > BPA > PCBs > OC pesticides (Table 1). The PAHs were detected in 14 of 20 sediment samples analyzed (Table 1). The PAH concentrations were as great as 573 and 214 ng/g dry weight in sediment from locations W2 and D4, which correspond to Woihwang River and Daejeong Stream, respectively. Mean PAH concentrations in sediments from the Woihwang River (mean: 218 ng/g dry wt) and Daejeong Stream (mean: 65.4 ng/g dry wt) locations were approximately 10- and threefold greater than those from outer Onsan Bay sites (mean: 19.2 ng/g dry wt), respectively. No spatial gradient was found in concentrations of PAHs in river or stream sediment. This suggests localized inputs of PAHs from sources along the river and stream. Four-ring aromatic hydrocarbons, such as fluoranthene and pyrene, were the predominant PAHs in Onsan Bay sediment. Molecular ratios of specific PAH compounds, such as fluoranthene to pyrene ratio and indeno[1,2,3-cd]pyrene to benzo[ghi]perylene ratio, were calculated to evaluate the potential sources of PAHs [3]. The fluoranthene to pyrene ratios for Onsan Bay sediment samples varied depending on the locations with an overall mean value of 1.18. The ratio of fluoranthene to pyrene in D0, OS1, and O8 locations (range: 0.83-0.99) were less than 1.0, whereas the other parts (range: 1.06-2.13) had fluoranthene to pyrene ratios of greater than 1.0. The indeno[1,2,3-cd]pyrene to benzo[ghi]perylene ratios were less than 1.0 for W2, D1, D3, and O5 locations (range: 0.12–0.69). These results suggest that the sources of PAHs to Onsan Bay were both petrogenic and pyrolytic. The northern locations, which are near petrochemical industries, may receive more petrogenic inputs, whereas the outer bay locations may be influenced by pyrolytic inputs.

Atmospheric transportation and deposition of PAHs from the nearby Ulsan Bay, where PAH concentrations as great as 3,100 ng/g dry weight were detected in sediments, may be a source of PAHs to Onsan Bay [6].

Concentrations of PCBs in sediments were generally less than 10.0 ng/g dry weight (Table 1). Locations D3 and D4 in Daejeong Stream contained relatively great concentrations of PCBs (19.6 and 56.2 ng/g dry wt, respectively). The spatial gradient of PCB concentrations in sediments suggests the presence of sources along Daejeong Stream. Lesser chlorinated congeners such as tetra- and penta-CBs were the most prevalent homologs in Onsan Bay sediments. Previous studies have also reported the presence of lower chlorinated PCB congeners in sediments collected from Masan and Ulsan bays [5,6]. Among different OC pesticides analyzed, DDT concentrations (sum of p, p'-dichlorodiphenyldichloroethane, -dichlorodiphenyldichloroethylene, and -DDT) were the greatest, ranging from <0.01 to 7.58 ng/g dry weight. Concentrations of target organic compounds in the streams were greater than those in the bay (Table 1). The likely sources of these persistent organic pollutants are from industrial activities or municipal wastewaters discharged from Onsan City.

Nonylphenol, OP, and BPA were detected at some locations in Onsan Bay (Table 1). However, concentrations of these contaminants were generally close to the method detection limit (1.00 ng/g dry wt). Maximum concentrations of NP and OP in sediments were 860 and 11 ng/g dry weight, respectively, at location D1 in Daejeong Stream. The greater concentrations of APs at locations D1, D3, and D4 can be explained by its proximity to sewage waste input near D1 location. The BPA was found in sediment from the inland river and stream only and ranged from <1.00 to 204 ng/g dry weight. Similar to that for APs, the greatest concentration of BPA was measured in sediment from location D1.

Comparison to other studies

Several studies have examined the occurrence and distribution of persistent organic pollutants such as PCBs, OC pesticides, and PAHs in Korean coastal areas [5-11,18]. The PCB concentrations in sediment samples from Onsan Bay (n = 3)collected in 1995 ranged from 4.5 to 8.2 ng/g dry weight [21]. Sedimentary PCBs and PAHs in Korea have been reported to range from a few nanograms per gram to several micrograms per gram dry weight (Table 2). Concentrations of PCBs and PAHs in Onsan Bay were approximately 1.5- to 10-fold less than those reported in other industrialized coastal areas such as Kyeonggi, Masan, Ulsan, and Yeongil bays. Concentrations of APs and BPA in sediment from Onsan Bay were similar to those in Ulsan Bay but less than those in other bay areas (Table 2). Greater concentrations of APs and BPA in Lake Shihwa and Masan and Yeoingil bays are consistent with greater input from heavily populated cities such as Shiheung, Masan, and Pohang. The data presented here establish the baseline for future monitoring of these compounds in Onsan Bay areas.

Potential for biological and ecological effects

Sixteen priority PAH compounds including aryl hydrocarbon receptor (AhR)-active compounds such as benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene were detected in sediments. Nonortho-coplanar PCB congeners 77 (3,3',4,4'-tetraCB), 126 (3,3',4,4',5-pentaCB), and 169 (3,3',4,4',5,5'-hexaCB) also

$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	IP OP	BPA	PCBs	DDTs	CHLs	HCHs	HCB	References
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	860 <1.00-11.0 <1	1.00 - 204	<1.00-56.2	< 0.01 - 7.58	< 0.01 - 0.95	< 0.01 - 3.37	< 0.01 - 4.44	This study
Kyeonggi Bay, 19959.1-1,400NANANA(n = 66)(120)NANANA<0.99-580	3.4) (1.48)	(10.7)	(6.07)	(1.23)	(0.10)	(0.61)	(0.66)	•
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	A NA	NA	< 0.99 - 580	1.70 - 32.0	0.53 - 130	0.38 - 0.69	NA	[7, 10, 11]
Namyang Bay, 1996 NA NA NA NA NA (0.99-2.50 <0.09-0.38 (0.128)) = (0.28) = (0.28) = (0.28) = (0.28) = (0.28) = (0.28) = (0.28) = (0.28) = (0.28) = (0.28) = (0.28) = (0.29) = (0.120) = (0.120) = (0.120) = (0.120) = (0.120) = (0.120) = (0.120) = (0.120) = (0.120) = (0.120) = (0.10) = (0.120) = (0.10) = (0.120) = (0.10) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) = (0.28) = (0.01) =				(9.40)	(29.00)	(0.58)		
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	A NA	NA	< 0.99 - 2.50	< 0.09 - 0.38	< 0.07 - 0.40	0.15 - 0.67	NA	[11]
Lake Sinihwa, 1998<10.0-30.820.2-1,8204.69-50.5NA<1.00-12.30.45-1.94<(n = 11)(23.3)(616)(17.9)(17.9)(7.18)(1.29)(n = 11)(23.3)(616)(17.9)(17.9)(7.18)(1.29)Masan Bay, 199841.5-1,100113-3,8903.97-1792.70-50.310.3-1480.40-12.6<				(0.28)	(0.30)	(0.46)		
	-1,820 4.69–50.5	NA	<1.00-12.3	0.45 - 1.94	< 0.01 - 0.04	0.11 - 0.29	< 0.01 - 4.78	[18]
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	(17.9)		(7.18)	(1.29)	(0.03)	(0.19)	(2.39)	
	3,890 3.97–179 2	2.70 - 50.3	10.3 - 148	0.40 - 12.6	< 0.01 - 0.34	0.09 - 1.65	< 0.01 - 1.87	[5]
Ulsan Bay, 1999 <10.0-3,100 <1.00-1,040 <1.00-120 <1.00-53.5 <1.00-64.6 <0.01-41.9 < (n = 32) (391) (391) (94.2) (17.8) (10.9) (9.03) (3.26) (3.26) (3.26) (17.8) (10.9) (10.9) (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6 < (0.01-81.6	(18.1)	(11.5)	(38.3)	(3.45)	(0.11)	(0.30)	(0.70)	
	-1,040 <1.00-120 <1	.00-53.5	<1.00-64.6	< 0.01 - 41.9	< 0.01 - 3.91	< 0.01 - 4.55	< 0.01 - 3.23	[9]
Yeongil Bay, 2000 <10.0-7,690 <1.00-6,760 <1.00-99.1 <1.00-191 <1.00-171 <0.01-8.26 < $(n = 34)$ (644) (422) (12.0) (72.4) (41.0) (0.53) (0.53) $(n = 11)$ (644) (422) (12.0) (72.4) (41.0) (0.53) $(n = 11)$ (644) (12.0) (12.0) (72.4) (41.0) (0.53) $(n = 11)$ (36.4) (18.2) (1.44) (28.3) (4.67) (0.07) Mean ^b 261 286 11.5 26.8 17.7 2.44 PAHs = polycyclic aromatic hydrocarbons, sum of 16 priority components; NP = nonylphenol; OP = octylphenol; BPA = bisph 2.64 2.04 2.04 • PAHs = polycyclic aromatic hydrocarbons, sum of 16 priority components; NP = nonylphenol; OP = octylphenol; BPA = bisph 2.04 2.04 2.04	1.2) (17.8)	(10.9)	(9.03)	(3.26)	(0.50)	(4.55)	(0.41)	
	-6,760 <1.00–99.1 <1	00 - 191	<1.00-171	< 0.01 - 8.26	< 0.01 - 0.53	< 0.01 - 46.8	< 0.01 - 1.97	[27]
Kwangyang Bay, 2001 10.1–105 9.90–35.0 <1.00–1.81	2) (12.0)	(72.4)	(41.0)	(0.53)	(0.14)	(2.46)	(0.18)	
(n = 11) (36.4) (18.2) (1.44) (28.3) (4.67) (0.07) Mean ^b 261 286 11.5 26.8 17.7 2.44 ^a PAHs = polycyclic aromatic hydrocarbons, sum of 16 priority components; NP = nonylphenol; OP = octylphenol; BPA = bisph congeners; DDTs = sum of p,p' -DDT, p,p' -dichlorodiphenyldichloroethane, and p,p' -dichlorodiphenyldichloroethylene; CHLs =	-35.0 <1.00-1.81 <1	.00-28.3	<1.00-4.67	0.01 - 0.14	< 0.01 - 0.05	< 0.01 - 0.91	0.02 - 0.47	[27]
Meanb26128611.526.817.72.44a PAHs = polycyclic aromatic hydrocarbons, sum of 16 priority components; NP = nonylphenol; OP = octylphenol; BPA = bisphcongeners; DDTs = sum of p, p' -DDT, p, p' -dichlorodiphenyldichloroethane, and p, p' -dichlorodiphenyldichloroethylene; CHLs =	3.2) (1.44)	(28.3)	(4.67)	(0.01)	(0.05)	(0.33)	(0.11)	
^a PAHs = polycyclic aromatic hydrocarbons, sum of 16 priority components; NP = nonylphenol; OP = octylphenol; BPA = bisph congeners; DDTs = sum of p,p' -dichlorodiphenyldichloroethane, and p,p' -dichlorodiphenyldichloroethylene; CHLs =	5 11.5	26.8	17.7	2.44	3.78	1.19	0.74	
hexachlorocyclohexanes; HCB = hexachlorobenzene; NA = not analyzed. \mathfrak{M} and concontrotion of commonial in <i>Exercise</i> actimates ($n = 200$)	of 16 priority components; NP hlorodiphenyldichloroethane, an zene; NA = not analyzed.	= nonylpheno hd <i>p,p'-</i> dichlor	ıl; OP = octylph odiphenyldichlo	aenol; BPA = bi roethylene; CHL	sphenol A; PCBs $\omega = \sin \alpha \sigma$	= polychlorinate and γ -chlordanes	ed biphenyls, sum ; HCHs = sum c	of 98 individual f α -, β -, and γ -

Table 3. Instrumentally derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) and predicted and observed percentage of the maximum response for TCDD (%-TCDD-max) of Onsan Bay sediments, Korea

	TEQs (pg/g dry wt) ^a			%-T	CDD-max-calcu	lated	%-]	CDD-max-obs	erved ^b
Location	TEQ _{PCB}	TEQ _{PAH}	TEQs	PCB	PAH	Total	F1	F2	RE
W0	3.276	0.009	3.285	24.1	< 0.00	24.2	0.47	22.8	14.2
W1	0.019	0.340	0.359	< 0.00	< 0.00	< 0.00	2.43	48.7	7.8
W2	0.016	4.928	4.944	< 0.00	31.9	31.9	9.76	85.0	17.1
D0	8.859	ND	8.859	42.6	NAC	42.6	0.60	29.1	16.9
D1	ND^{c}	0.414	0.414	NAC ^d	< 0.00	< 0.00	< 0.00	2.31	< 0.00
D2	6.113	ND	6.113	27.2	NAC	27.2	3.59	4.14	< 0.00
D3	0.003	0.151	0.154	< 0.00	< 0.00	< 0.00	8.54	23.6	< 0.00
D4	3.249	1.352	4.601	23.2	7.30	29.5	14.5	18.7	< 0.00
D6	0.009	0.242	0.252	< 0.00	< 0.00	< 0.00	5.54	32.7	21.5
ON1	ND	ND	ND	NAC	NAC	NAC	0.00	2.61	3.06
ON2	0.348	NAe	0.348	< 0.00	NA	NA	NA	NA	12.5
ON3	ND	NA	NA	NAC	NA	NA	NA	NA	9.1
OS1	ND	0.260	0.260	NAC	< 0.00	< 0.00	2.49	15.9	14.6
OS2	ND	0.461	0.461	NAC	< 0.00	< 0.00	NA	NA	20.9
OS3	ND	0.159	0.159	NAC	< 0.00	< 0.00	NA	NA	18.3
O4	0.037	0.252	0.289	< 0.00	< 0.00	< 0.00	2.61	38.0	20.1
O5	0.029	0.434	0.463	< 0.00	< 0.00	< 0.00	3.41	44.9	36.6
06	ND	0.150	0.150	NAC	< 0.00	< 0.00	1.51	28.4	15.7
O7	ND	0.003	0.003	NAC	< 0.00	< 0.00	NA	NA	11.1
08	ND	ND	ND	NAC	NAC	NAC	NA	NA	12.7
O9	ND	0.028	0.028	NAC	< 0.00	< 0.00	NA	NA	14.7
O10	ND	0.170	0.170	NAC	< 0.00	< 0.00	0.60	22.6	18.1

^a Instrumentally derived TEQs of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) associated with sediment samples. Refer to the TEQs generated from assay-specific median relative potency factor values and selected PCB concentrations (PCBs 81, 77, 118, 105, 126, 167 + 185, 156, and 169) and PAH concentrations (benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and dibenz[*a*,*h*]anthracene).

^b F1 = least polar fraction (contains PCBs); F2 = mid-polar fraction (contains PAHs); RE = raw extract.

 $^{\circ}$ ND = not detected.

 d NAC = not available for calculation.

 $^{\circ}$ NA = not analyzed.

were detected in a few samples. Based on the concentrations of dioxinlike compounds, 2,3,7,8-TCDD equivalents (TEQs) were estimated by using relative potency factors specific to the H4IIE-luc assay for selected PAHs and non- and monoortho-PCB congeners [19]. Concentrations of total TEQs ranged from less than the method detection limit to 8.86 pg/ g dry weight (Table 3). The $TEQ_{\mbox{\tiny PCB}}s$ for 50% of the locations were not detected, whereas $TEQ_{\mbox{\scriptsize PAH}}s$ for all the locations except for four (D0, D2, ON1, and O8) were detected. Contributions of PCBs and PAH concentrations to total TEQs varied among locations. This result suggested that both dioxinlike PCBs and PAHs were responsible for the H4IIE-luc responses observed in sediment RE samples. The TEOs estimated for selected PCB and PAHs in Onsan sediment were at the lower end of the sediment quality guideline of 0.014 to 210 pg/g dry weight reported for dioxin equivalents [22].

Sediment quality guidelines such as effect range low (ERL) and threshold, median, and extreme effects concentrations for PAHs and PCBs were applied to further evaluate the quality of sediment in Onsan Bay [23–25]. The total organic carbon– normalized concentrations of 16 individual PAHs, total PAHs, and total PCBs were calculated. None of the locations exceeded the ERLs for 16 PAHs and PCBs. When threshold effects concentrations reported for total PCBs were compared, only one location (D4) exceeded the limit of 35 ng/g dry weight. Because the ERL determined for PAHs and PCBs is influenced by co-occurring toxicants in field studies, it may be an over estimate of the toxicity attributable to PAHs or PCBs. This results in an artificially low value for the ERL. Thus, calculation of hazard quotients based on ERLs would overestimate the effects. In any case, PAH or PCB concentrations did not exceed the ERLs, thus neither PAHs nor PCBs would be expected to have adverse effects on benthic invertebrates.

Dioxinlike activity in vitro

Extracts of sediment, water (dissolved fraction and particulate matter), and pore water were screened for their ability to induce AhR-mediated gene expression in vitro with H4IIEluc cells [19]. Based on the initial screening of raw extracts, 17 of 22 sediment samples showed significant dioxinlike activity in the H4IIE-luc bioassay (Table 4). Surface water and pore-water samples were less active. No pore-water samples produced a significant response in the H4IIE-luc bioassay. Among the surface water samples, only samples from locations D2 and D5 elicited a significant response. Response magnitudes ranged from 11.0%-TCDD-max to 17.5%-TCDD-max and responses for the particulate matter did not differ significantly from those from the dissolved fraction sample. The activity of the D2 and D5 water samples could not be explained based on the instrumental data available. The unusual bioassay responses suggest that these areas may warrant further study as potential locations containing point sources or relatively hydrophilic AhR agonists. Overall, the RE screening results suggested that most dioxinlike organic contaminants in the Onsan Bay area aquatic environment were associated with sediment. This is consistent with the relatively hydrophobic properties of most known AhR agonists.

To examine potential cause–effect relationships between the target (known) AhR agonists quantified in this study and the AhR-mediated bioassay responses observed, sediment extracts were divided into three fractions and each fraction was ana-

Table 4.	Luciferase bioassay	response	(%-TCDD-	max) ^a o	f H4IIE-lı	ic cells	for the	sediment	raw	extracts	(RE) a	nd f	fractionated	samples	(F1,	F2,
				an	d F3) from	n Onsa	n Bay,	Korea								

Location	RE	F1 ^b	F2°	F3 ^d
W0	14.22 ± 4.38	0.47 ± 2.63	13.02 ± 0.63	60.32 ± 5.97
W1	7.78 ± 4.49	2.43 ± 2.02	24.56 ± 5.33	13.91 ± 1.85
W2	17.10 ± 1.04	9.76 ± 2.90	29.28 ± 5.55	54.09 ± 6.70
D0	16.87 ± 3.28	0.60 ± 4.91	4.61 ± 2.92	52.14 ± 8.83
D1	$<0.00 \pm 0.40$	$<0.00 \pm 4.73$	25.22 ± 1.19	$<0.00 \pm 0.32$
D2	$<0.00 \pm 0.72$	3.59 ± 3.10	4.80 ± 0.76	$<0.00 \pm 0.69$
D3	$<0.00 \pm 3.21$	8.54 ± 5.11	6.76 ± 3.86	23.07 ± 6.06
D4	$<0.00 \pm 4.99$	14.52 ± 2.67	15.55 ± 2.15	42.12 ± 15.98
D6	21.47 ± 2.61	5.54 ± 2.22	$<0.00 \pm 6.14$	27.47 ± 3.29
ON1	3.06 ± 1.20	0.00 ± 2.49	$<0.00 \pm 3.23$	1.39 ± 1.45
ON2	12.51 ± 3.72	NA ^e	NA	NA
ON3	9.09 ± 6.86	NA	NA	NA
OS1	14.60 ± 5.45	2.79 ± 1.13	5.31 ± 16.39	16.47 ± 3.15
OS2	20.90 ± 4.92	NA	NA	NA
OS3	18.28 ± 1.98	NA	NA	NA
O4	20.11 ± 1.98	2.61 ± 1.99	7.60 ± 9.38	9.21 ± 0.87
05	36.64 ± 4.09	3.41 ± 2.33	6.25 ± 7.46	12.14 ± 1.70
06	15.65 ± 1.98	1.51 ± 2.73	9.89 ± 6.61	8.41 ± 5.93
07	11.12 ± 4.88	NA	NA	NA
08	12.73 ± 2.79	NA	NA	NA
09	14.68 ± 3.79	NA	NA	NA
O10	18.13 ± 0.72	0.60 ± 2.60	4.05 ± 2.30	12.87 ± 4.36
Significant level ^f	4.56	2.87	2.87	2.87

^a Response magnitude presented as percentage of the maximum response observed for a 2,000 pM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) standard.

 b F1 = fraction 1 of sediment samples (contains PCBs).

 $^{\circ}$ F2 = fraction 2 of sediment samples (contains PAHs).

 d F3 = fraction 3 of sediment samples (contains alkylphenols and bisphenol A).

 $^{\circ}$ NA = not analyzed.

f Significant level is defined as 99% confidence interval around the mean solvent control response (set to 0%-TCDD-max).

lyzed in the H4IIE-luc assay. Previously reported spike-recovery tests [5] have shown AhR-active PCBs to partition to the least polar fraction (F1) and AhR-active PAHs to partition to the mid-polar fraction (F2). High-resolution mass spectrometer analysis suggests that polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans partition primarily to F2, with only a small percentage occurring in F3 [13]. Thus, if the target, AhR-active analytes for this study were the predominant causes of AhR-mediated reporter gene expression in vitro, one would expect F1 and F2 to have the greatest activity, whereas that of F3 should be minimal. Furthermore, the magnitude of activity expected in F1 and F2 could be predicted (Table 3) based on the measured concentrations of AhR-active PCBs, AhR-active PAHs, and their previously reported, H4IIEluc-specific, relative potencies [19,26]. Predicted magnitudes of response were based on the assumption that TEQs would behave in the bioassay as if they were pure 2,3,7,8-TCDD standard and that all TEQs acted in a simple additive fashion (no synergistic or antagonistic interactions). Predicted and observed magnitudes of response were compared in a qualitative mass (potency) balance to evaluate whether or not the target analytes quantified in this study could account for the responses observed.

Based on TEQ_{PCB} concentrations present in the samples, 4 of the 15 F1 samples analyzed should have yielded a significant H4IIE-luc response (Table 3). Each of those significant responses was predicted to be 20%-TCDD-max or greater (Table 3). However, this predicted profile of responses was not observed. Instead, 6 of the 15 F1 samples analyzed produced a significant response (Table 4). Only two of these (D2 and D4) were samples predicted to yield a significant F1 response, based on TEQ_{PCB} concentrations. Thus, four samples (W2, D3,

D6, and O5) that should not have yielded a F1 response did yield a response, and two samples (W0 and D0) that should have yielded F1 responses did not do so. Furthermore, the maximum magnitude of response observed was 14.5%-TCDDmax (Table 3), which was significantly lower than the magnitude expected for all four predicted responses. Thus, responses of F1 did not conform well to the additive, TEQ_{PCB}, model applied. This suggests that either non–AhR-active PCB components of F1 were modulating the activity of the AhRactive PCBs, or that the AhR-active PCBs present were acting in a nonadditive fashion in the H4IIE-luc bioassay.

If target analytes were the primary cause of the AhR-mediated bioassay responses observed for Onsan area sediment samples, TEQ_{PAH} should have resulted in activity in F2. The additive TEQ_{PAH} model predicted that only two F2 samples (W2 and D4) contained sufficient concentrations of dioxinlike PAHs to induce a significant response in the H4IIE-luc bioassay (Table 3). Contrary to this prediction, 13 of the 15 F2 samples analyzed yielded a significant response (Table 3). Twelve of the 14 responses were in excess of 20%-TCDDmax (Table 3). The F2 samples for both W2 and D4 yielded significant responses, as predicted, but the response magnitudes observed were approximately 2.6-fold greater than predicted (Table 3). The strong and consistent deviation from the additive TEQ_{PAH} model suggests that PAHs were not the primary cause of activity in F2 or that AhR-active PAHs were acting in a greater than additive manner. Because polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans are known to partition predominantly to F2, one likely explanation for the greater than predicted responses is that AhRactive dioxins and furans were present in the Onsan area sediment samples. Polychlorinated naphthalenes are another class

Table 5.	Luciferase	bioassay	response	$(\%-E_2-max)$	^a of N	ACF-7-luc	(MVLN)	cells f	or the	e sediment	raw	extracts	(RE)	and	fractionate	ed sample
					(F1, F	F2, and F3) from Or	isan Ba	ay, Ko	orea						

Location	RE	F1 ^b	F2 ^c	F3 ^d
W0	<0.00 ± 11.2	1.62 ± 2.63	13.0 ± 1.11	<0.00 ± 1.19
W1	$<0.00 \pm 10.7$	5.50 ± 2.02	24.6 ± 3.93	$< 0.00 \pm 0.80$
W2	$<0.00 \pm 2.37$	8.86 ± 2.90	29.3 ± 4.65	$<0.00 \pm 1.44$
D0	0.15 ± 9.84	4.75 ± 4.91	4.61 ± 1.83	$<0.00 \pm 2.41$
D1	$<0.00 \pm 2.15$	$<0.00 \pm 4.73$	25.2 ± 4.98	$<0.00 \pm 0.69$
D2	$<0.00 \pm 18.9$	3.86 ± 3.10	4.80 ± 4.21	$<0.00 \pm 4.28$
D3	10.44 ± 9.19	0.50 ± 5.11	6.76 ± 1.01	$<0.00 \pm 4.33$
D4	$<0.00 \pm 4.42$	2.65 ± 2.67	15.5 ± 3.32	$<0.00 \pm 1.34$
D6	$<0.00 \pm 2.31$	1.48 ± 2.22	$<0.00 \pm 0.92$	$<0.00 \pm 5.37$
ON1	$<0.00 \pm 22.8$	0.83 ± 2.49	$<0.00 \pm 1.54$	$<0.00 \pm 0.32$
ON2	$<0.00 \pm 4.80$	NA ^e	NA	NA
ON3	$<0.00 \pm 4.62$	NA	NA	NA
OS1	$<0.00 \pm 1.51$	3.44 ± 1.13	5.31 ± 2.88	$<0.00 \pm 2.45$
OS2	$<0.00 \pm 1.87$	NA	NA	NA
OS3	$<0.00 \pm 4.74$	NA	NA	NA
O4	$<0.00 \pm 6.17$	3.44 ± 1.99	7.60 ± 3.31	1.67 ± 2.07
O5	$<0.00 \pm 3.54$	5.03 ± 2.33	6.25 ± 0.53	$<0.00 \pm 2.60$
06	$<0.00 \pm 4.15$	8.77 ± 2.73	9.89 ± 6.11	1.99 ± 1.17
07	$<0.00 \pm 6.58$	NA	NA	NA
08	0.001 ± 2.80	NA	NA	NA
09	$<0.00 \pm 5.85$	NA	NA	NA
O10	$<0.00 \pm 5.55$	1.62 ± 2.60	4.05 ± 1.46	$<0.00 \pm 1.09$
Significant level ^f	6.60	1.32	1.32	1.32

^a Response magnitude presented as percentage of the maximum response observed for a 1,000 pM 17β-estradiol (E₂) standard.

 b F1 = fraction 1 of sediment samples (contains PCBs).

 $^{\circ}$ F2 = fraction 2 of sediment samples (contains PAHs).

 d F3 = fraction 3 of sediment samples (contains alkylphenols and bisphenol A).

^e NA = not analyzed.

^f Significant level is defined as 99% confidence interval around the mean solvent control response (set to 0%-E₂-max).

of ubiquitous, dioxinlike compounds that may partition to F2 [15] and may have contributed to the F2 responses. Such compounds should be investigated in future studies of persistent organic pollutants in the Onsan area.

No known AhR-agonists were expected to partition into F3. Despite this, 12 of the 15 F3 samples analyzed produced a significant response in the H4IIE-luc bioassay (Table 4). The prevalence and magnitude of AhR activity in F3 suggests the presence of unidentified, relatively polar, AhR agonists in sediment from the Onsan area. The results are consistent with previous studies, which examined sediments from other Korean coastal areas [13,18,19]. Thus, the unidentified agonists seem to have a fairly ubiquitous distribution near Korea. Future studies should employ extensive bioassay-directed fractionation and chemical analysis in an effort to identify the causative agents in F3 samples from this study and other locations in Korea. Additional research also could be aimed at determining whether similar F3 responses would be observed for sediments from other areas, both contaminated and relatively uncontaminated, worldwide.

Comparison of RE and fractionated extract sample responses suggested that a simple additive model considering presently characterized AhR agonists is probably not sufficient to characterize the AhR-mediated toxic potency of the complex mixtures of compounds found in Korean sediment. Among the 15 samples tested as both RE and fractionated extract, 4 of F1, 14 of F2, and 9 of F3 samples yielded fractionated extract responses that were greater than the corresponding RE responses, respectively (Table 4). This implies antagonistic interactions within the total extract that were relieved by separating components of the complex mixture. Current additive models for predicting dioxinlike potency based on congenerspecific chemical concentrations and previously determined relative potencies do not account for the type of interactions implied by these results. Additional identification and characterization of novel AhR agonists and antagonists may improve future evaluations.

Estrogenic activity in vitro

Onsan area sediment extracts also were screened for their ability to induce estrogen receptor-mediated gene expression in vitro [19]. Only one RE sample (D3) yielded a significant response in the MVLN bioassay (Table 5). The general lack of response was consistent with the relatively low concentrations of known estrogen receptor agonists in extracts of Onsan area sediment (Table 1). Based on MVLN-specific relative potencies previously determined for NP, OP, BPA, and two weakly estrogenic PAHs (benzo[*a*]anthracene and dibenz[*a*,*h*]anthracene), concentrations of E₂ equivalents based on these residues in the extracts were not predicted to elicit a response in the MVLN bioassay.

Analysis of fractionated extracts complicated the picture somewhat. Twelve out of 15 F1 samples and 13 of 15 F2 samples caused significant estrogenic responses (Table 5). Magnitudes of MVLN response for F2 samples as great as 29.3%-E₂-max (W2) were observed (Table 4). This is equivalent to a response elicited by 21 pM of E₂. Based on the PAH analysis in sediment samples, concentrations of estrogenic PAHs such as benzo[*a*]anthracene (65.3 ng/g dry wt) and dibenz[*a*,*h*]anthracene (8.73 ng/g dry wt) in W2 were greatest among samples analyzed. The E₂ equivalent for estrogenic PAHs in sediment from W2 was 4.01 pM of E₂ based on the reported PAH concentrations and their relative potency values [26]. This mass balance suggested that concentrations of estrogenic PAHs account only for a portion of estrogenic responses observed in F2. Thus, the F2 results suggest that Onsan area sediments, from at least some locations, contain estrogen receptor-active compounds that elute in the same Florisil fraction as do PAHs. Additional bioassay-directed fractionation and chemical analysis would be necessary to identify the causative agents. No F3 samples yielded a significant MVLN response. Instead, W1, D1, D3, and D4 F3 samples, all river or stream sediments, were cytotoxic to the MVLN cells. The F3 results suggest a significant difference in the chemical profile of inland river and stream sediment and coastal sea sediment. Overall, in vitro bioassay applied in conjunction with instrumental analysis provides a powerful tool for characterizing mechanism-specific agonists present in the environment.

SUMMARY

The relative abundance of organic contaminants measured in Onsan Bay was in the order PAHs \approx APs > BPA > PCBs > OC pesticides. Spatial distributions of these contaminants in Onsan Bay suggested that their sources were independent of each other. Overall, the target organic contaminants examined in this study do not seem to be a severe problem in Onsan Bay. Bioassay responses to F1 in the H4IIE-luc cell bioassay were consistent with the relatively low concentrations of dioxinlike PCBs detected in Onsan Bay sediments. Mass balance analysis suggested that PAHs accounted for only a portion of dioxinlike activity in F2 samples. The undefined components of F2 and F3 seem to be responsible for the majority of the dioxinlike responses. The lack of responses of MVLN cells to samples was consistent with the low concentrations of estrogenic compounds such as NP, OP, and BPA in Onsan Bay sediments. Based on mass balance analysis, the known estrogenic PAH and AP composition of F3 probably accounts for less than 20% of the estrogenic activity observed in the MVLN bioassay. Overall, these results suggest the need for more extensive fractionation and instrumental analysis to identify uncharacterized AhR and estrogen receptor agonists and cytotoxic compounds in F3 samples.

Acknowledgement—This study was financially supported by the National Institute of Environmental Research, Ministry of Environment, Korea, Sediment Organic Compounds Bioassay Study 1998–2000.

REFERENCES

- 1. Neff JM. 1979. *Polycyclic Aromatic Hydrocarbons in the Aquatic Environment, Sources, Fates, and Biological Effects.* Applied Science, London, UK.
- Sanderson JT, Aarts, JMMJG, Brouwer A, Froese KL, Denison MS, Giesy JP. 1996. Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-O-deethylase induction in H4IIE cells: Implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. *Toxicol Appl Pharmacol* 137:316–325.
- Baumard P, Budzinski H, Garrigues P. 1998. Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean Sea. *Environ Toxicol Chem* 17:765–776.
- 4. Giesy JP, 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.
- Khim JS, Kannan K, Villeneuve DL, Koh CH, Giesy JP. 1999. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea: 1. Instrumental analysis. *Environ Sci Technol* 33:4199–4205.
- Khim JS, Lee KT, Kannan K, Villeneuve DL, Giesy JP, Koh CH. 2001. Trace organic contaminants in sediment and water from Ulsan Bay and its vicinity, Korea. *Arch Environ Contam Toxicol* 40:141–150.
- Koh CH, Kim GB, Maruya KA, Anderson JW, Jones JM, Kang SG. 2001. Induction of the P450 reporter gene system bioassay

by polycyclic aromatic hydrocarbons in Ulsan Bay (South Korea) sediments. *Environ Pollut* 111:437–445.

- Chang YS, Kong SB, Ikonomou MG. 1999. PCBs contributions to the total TEQ released from Korean municipal and industrial waste incinerators. *Chemosphere* 39:2629–2640.
- Kim SK, Lee DS, Oh JR, Kahng SH. 2000. Effects of extreme tidal range on characteristics of polychlorinated biphenyl distribution in sediment of industrial Incheon North Harbor, Korea. *Environ Toxicol Chem* 19:2448–2456.
- Lee KT, Tanabe S, Koh CH. 2001. Contamination of polychlorinated biphenyls (PCBs) in sediments from Kyeonggi Bay and nearby areas, Korea. *Mar Pollut Bull* 42:273–279.
- Lee KT, Tanabe S, Koh CH. 2001. Distribution of organochlorine pesticides in sediments from Kyeonggi Bay and nearby areas, Korea. *Environ Pollut* 114:207–213.
- Nimrod AC, Benson WH. 1996. Environmental estrogenic effects of alkylphenol ethoxylates. *Crit Rev Toxicol* 26:335–364.
- Khim JS, Villeneuve DL, Kannan K, Koh CH, Giesy JP. 1999. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea: 2. In vitro gene expression assays. *Environ Sci Technol* 33:4206–4211.
- Hilscherova K, Machala M, Kannan K, Blankenship AL, Giesy JP. 2000. Cell bioassays for detection of aryl hydrocarbon receptor (AhR) and estrogen receptor (ER) mediated activity in environmental samples. *Environ Sci Pollut Res* 7:159–171.
- Kannan K, Villeneuve DL, Yamashita N, Imagawa T, Hashimoto S, Miyazaki A, Giesy JP. 2000. Vertical profiles of dioxin-like and estrogenic activities associated with a sediment core from Tokyo Bay, Japan. *Environ Sci Technol* 34:3560–3567.
- Carr RS, Chapman DC. 1995. Comparison of method for conducting marine and estuarine sediment porewater toxicity tests— Extraction, storage, and handling techniques. Arch Environ Contam Toxicol 28:69–77.
- Khim JS, Villeneuve DL, Kannan K, Hu WY, Giesy JP, Kang SG, Song KJ, Koh CH. 2000. Instrumental and bioanalytical measures of persistent organochlorines in blue mussel (*Mytilus edulis*) from Korean coastal waters. *Arch Environ Contam Toxicol* 39: 360–368.
- Khim JS, Villeneuve DL, Kannan K, Lee KT, Snyder SA, Koh CH, Giesy JP. 1999. Alkylphenols, polycyclic aromatic hydrocarbons (PAHs), and organochlorines in sediment from Lake Shihwa, Korea: Instrumental and bioanalytical characterization. *Environ Toxicol Chem* 18:2424–2432.
- Khim JS, Lee KT, Villeneuve DL, Kannan K, Giesy JP, Koh CH. 2001. In vitro bioassay determination of dioxin-like and estrogenic compounds in environmental samples from Ulsan Bay and its vicinity, Korea. Arch Environ Contam Toxicol 40:151–160.
- Yamashita N, Kannan K, Imagawa T, Villeneuve DL, Hashimoto S, Miyazaki A, Giesy JP. 1999. Vertical profile of polychlorinated dibenzo-p-dioxins, dibenzofurans, naphthalenes, biphenyls, polycyclic aromatic hydrocarbons, and alkylphenols in a sediment core from Tokyo Bay, Japan. *Environ Sci Technol* 34:3560–3567.
- Kim GW, Lee Y. 1996. Concentrations of PCBs (polychlorinated biphenyls) in coastal sediments of Korea. *Korean J Sanit* 11:9– 12.
- Iannuzzi TJ, Bonnevie NL, Wenning RJ. 1995. An evaluation of current methods for developing sediment quality guidelines for 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch Environ Contam Toxicol 28:366–377.
- Long ER, MacDonald DD, Smith SL, Calder FD. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ Manag* 19: 81–97.
- Swartz R. 1999. Consensus sediment quality guidelines for polycyclic aromatic hydrocarbon mixtures. *Environ Toxicol Chem* 18: 780–787.
- MacDonald DD, Dipinto LM, Christopher JF, Ingersoll CG, Long ER, Swartz R. 2000. Development and evaluation of consensusbased sediment effect concentrations for polychlorinated biphenyls. *Environ Toxicol Chem* 19:1403–1413.
- Villeneuve DL, Khim JS, Kannan K, Giesy JP. 2002. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxin-like and estrogenic responses in three different cell lines. *Environ Toxicol* 17:128–137.
- Ministry of Environment. 2002. Final report on public related technology development process: Sediment Organic Compound Study. SORGBIOS 98–2000. National Institute of Environmental Research, Inchon, Korea.