CHARACTERIZATION OF DIOXIN-LIKE AND ESTROGENIC COMPOUNDS IN ENVIRONMENTAL SAMPLES FROM ONSAN BAY AND ITS INLAND REGION, KOREA

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Introduction

The Onsan Bay area, located on the east coast of Korea, contains several industrial complexes and a large commercial harbor. Rapid and heavy industrialization near this area has been accompanied by environmental deterioration, which has led to social and environmental health problems[1]. Despite the potential for direct and accidental release of organic contaminants into the bay, little is known regarding the current status of contamination in this region. This study represents one of the first efforts to examine the concentrations, fate, distribution, and biological potency of toxic organic contaminants in the Onsan Bay area. Surface sediment, pore water, and water samples were collected from Onsan Bay, Woihwang River, and Daejeong Stream (Figure 1). Due to the complex nature of contaminants in environmental matrices, samples were fractionated based on polarity to isolate and identify target contaminants[2,3]. Instrumental analyses and *in vitro* recombinant cell bioassays (H4IIE-luc cells for dioxin-like activity and MVLN cells for estrogenic activity) were performed to quantify target contaminants and evaluate dioxin-like and estrogenic potencies of each fraction[2,3].

Methods

Instrumental Analysis: The target compounds measured in this study were polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides (HCB, HCHs, CHLs, DDTs), polycyclic aromatic hydrocarbons (PAHs), nonylphenol (NP), octylphenol (OP), and bisphenol A (BPA). Sediment, water, and pore water samples were collected from 23 locations in the Onsan Bay area. Samples were either Soxhlet extracted and Empore™ disk filtered, and then passed through 10 g activated Florisil column for further fractionation[3,4]. Three fractions (F1, F2, and F3) differing in polarity were collected using solvents appropriate for both instrumental analyses and bioassay. PCBs and OC pesticides were quantified using a gas chromatograph (Perkin Elmer series 600) equipped with ^{63}Ni electron capture detector (GC-ECD). PAHs were quantified using a Hewlett Packard 5890 series II gas chromatograph equipped with a

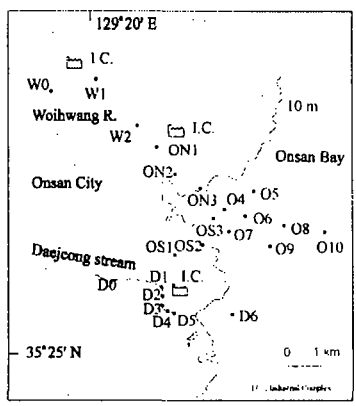

Figure 1. Map of the location in Onsan Bay, Korea

5972 series mass spectrometer detector. Reverse phase high performance liquid chromatography (HPLC) with fluorescence detection was used to quantify NP, OP, and BPA[3].

***In Vitro* Bioassay**: Each sample was tested as raw extract (RE) and fractionated extract (FE) using *in vitro* bioassays. Luciferase and protein assays were conducted after 72 h of exposure. Sample responses, expressed as mean RLU 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; %-TCDD-max.) or 17-β-estradiol (E2, %-E2-max.) standard curves generated on the same day. Where appropriate, sample potency relative to the TCDD or E2 standards, was estimated. Mass balance analysis (or potency balance analysis) was used to examine whether or not the known concentration and/or composition of a sample (identified by instrumental analyses) could account for the magnitude or potency of biological response observed.

Table 1. Concentrations (ng/g dry wt) of the target organic contaminants in sediment from Onsan Bay areas, Korea

Location	PCBs	DDTs	PAHs	NP	OP	BPA
W0	6.87	0.07	<10.0	<1.00	<1.00	0.23
W1	10.1	0.02	77.0	20.8	<1.00	<1.00
W2	5.47	0.35	573	4.78	<1.00	1.59
D0	<1.00	7.55	<10.0	6.23	<1.00	<1.00
D1	NA[a]	7.58	113	860	11.0	204
D2	<1.00	0.02	<10.0	14.4	<1.00	0.09
D3	19.6	1.20	34.5	100	1.39	1.21
D4	56.2	1.67	214	96.4	5.75	8.93
D6	3.33	3.06	21.4	<1.00	<1.00	0.35
ON1	NA	2.54	<10.0	1.86	<1.00	<1.00
ON2	7.79	<0.01	NA	1.26	<1.00	<1.00
ON3	<1.00	0.47	NA	<1.00	<1.00	<1.00
OS1	<1.00	0.61	38.7	3.46	<1.00	<1.00
OS2	<1.00	0.76	35.9	1.39	<1.00	<1.00
OS3	<1.00	0.20	19.3	NA	NA	NA
O4	<1.00	0.25	17.9	<1.00	4.33	<1.00
O5	<1.00	0.33	41.6	<1.00	<1.00	<1.00
O6	<1.00	0.05	27.1	<1.00	<1.00	1.01
O7	<1.00	0.06	<10.0	<1.00	<1.00	<1.00
O8	NA	0.05	<10.0	2.68	<1.00	<1.00
O9	<1.00	0.03	10.4	3.80	<1.00	<1.00
O10	<1.00	0.15	27.7	<1.00	<1.00	<1.00
Mean	15.6	1.29	89.3	85.9	5.62	27.1
SD	18.6	2.25	149	235	4.03	71.4

W: Woihwang River locations, D: Daejeong Stream locations, ON, OS, O: Onsan Bay locations
[a] NA: not analyzed.

Results and Discussion

Instrumental Analysis: The relative abundance of organic contaminants in sediment was generally PAHs ≈ alkylphenols (APs) > PCBs > OC pesticides (Table 1). PAHs were detected in nearly all the samples. PAH concentrations were as great as 573 ng/g dry wt. PAH concentrations were localized and elevated in inland locations (mean: 172 ng/g dry wt). Maximum concentrations

of NP, OP, and BPA in sediments were 860, 11, and 204 ng/g dry wt, respectively. Concentrations of APs were greater in inland location, where as those in the bay area were generally below the method detection limit (1 ng/g dry wt). Sediment PCB concentrations ranged from <1.00 to 56.2 ng/g dry wt. Lower chlorinated congeners such as tetra- and penta-CBs were the most prevalent. Previous studies also reported the presence of lower chlorinated PCB congeners in sediments collected from Masan and Ulsan Bay and other coastal areas in Korea[3,4]. Among different OC pesticides analyzed, DDT concentrations (sum of *p,p'*-DDD, DDE, DDT) were the greatest ranging from <0.01 to 7.58 ng/g dry wt. Overall, the concentrations of target organic compounds in the streams were greater than those in the bay. The likely sources of these contaminants are from industrial activities and/or municipal wastewaters discharged from Onsan City (Figure 1). Although the mean concentrations of PAHs and PCBs in sediments were less than the consensus sediment quality guidelines (SQGs), their concentrations in some locations were close to or above the SQGs for toxic effects in benthic organisms[5,6].

***In Vitro* Bioassay**: Extracts of sediment, water, and pore water were screened for their ability to induce aryl hydrocarbon receptor (AhR)- and estrogen receptor (ER)- mediated gene expression *in vitro*. Based on the initial screening of RE, 17 of 22 sediment samples showed significant dioxin-like activity in H4IIE-luc bioassay, where as, most water and pore water samples did not elicit response. Analysis of FE samples indicated that the mid polar (F2) and most polar (F3) fractions were responsible for the significant reporter gene expression in H4IIE-luc bioassay (Table 2). F2 and F3 samples yielded greater magnitudes than corresponding REs. This suggests that compounds present in F1 may have depressed the activities of AhR agonists in F2 and F3. The F2 samples were the most active fraction. The sediment sample containing the greatest PAH concentration (location W2, 573 ng/g dry wt.) produced magnitudes of response similar to those induced by a TCDD standard (Table 1,2). Based on a qualitative mass balance analysis, PAHs appeared to account for only a portion of dioxin-like responses observed. Magnitudes of dioxin-like activity elicited by Onsan Bay sediment F2 and F3 extracts were similar to those observed for Korean sediment from other locations[3,4]. These observations add to a growing body of evidence, which suggests that there may be unidentified, relatively polar, AhR-active compounds in sediment from some areas. Over half RE samples were either cytotoxic or caused morphological changes in MVLN cells. Only one of the RE samples produced a significant MVLN response. FE samples also showed low estrogenic activity in MVLN cells, which was consistent with low concentrations of known ER agonists, such as APs and BPA in F3. *In vitro* bioassay was a useful and rapid screening tool to characterize the causative agents and/or potential toxic compounds presented in Onsan Bay samples[2,7].

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Table 2. Luciferase induction in the H4IIE-luc and MVLN cell bioassays elicited by Onsan Bay sediment raw extract (RE) and fraction samples (F1, F2, and F3).

Location	H4IIE-luc responses[a]				MVLN responses[b]			
	RE	F1	F2	F3	RE	F1	F2	F3
W0	14.22	0.47	22.83	60.32	< 0.00	1.62	13.02	< 0.00
W1	7.78	2.43	48.72	13.91	< 0.00	5.50	24.56	< 0.00
W2	17.10	9.76	84.99	54.09	< 0.00	8.86	29.28	< 0.00
D0	16.87	0.60	29.12	52.14	0.15	4.75	4.61	< 0.00
D1	< 0.00	< 0.00	2.31	< 0.00	< 0.00	< 0.00	25.22	< 0.00
D2	< 0.00	3.59	4.14	< 0.00	< 0.00	3.86	4.80	< 0.00
D3	< 0.00	8.54	23.62	23.07	10.44	0.50	6.76	< 0.00
D4	< 0.00	14.52	18.73	42.12	< 0.00	2.65	15.55	< 0.00
D6	21.47	5.54	32.66	27.47	< 0.00	1.48	< 0.00	< 0.00
ON1	3.06	0.00	2.61	1.39	< 0.00	0.83	< 0.00	< 0.00
ON2	12.51	NA[c]	NA	NA	< 0.00	NA	NA	NA
ON3	9.09	NA	NA	NA	< 0.00	NA	NA	NA
OS1	14.60	2.49	15.93	16.47	< 0.00	3.44	5.31	< 0.00
OS2	20.90	NA	NA	NA	< 0.00	NA	NA	NA
OS3	18.28	NA	NA	NA	< 0.00	NA	NA	NA
O4	20.11	2.61	38.03	9.21	< 0.00	3.44	7.60	1.67
O5	36.64	3.41	44.87	12.14	< 0.00	5.03	6.25	< 0.00
O6	15.65	1.51	28.44	8.41	< 0.00	8.77	9.89	1.99
O7	11.12	NA	NA	NA	< 0.00	NA	NA	NA
O8	12.73	NA	NA	NA	0.01	NA	NA	NA
O9	14.68	NA	NA	NA	< 0.00	NA	NA	NA
O10	18.13	0.60	22.58	12.87	< 0.00	1.62	4.05	< 0.00
Sig. level[d]	4.561	2.866	2.866	2.866	6.602	1.318	1.318	1.318

[a,b] Response magnitude presented as percentage of the maximum response observed for a 2000 pM TCDD standard (%-TCDD-max.) and for a 1000 pM E2 standard (%-E2-max.).
[c] NA: not analyzed
[d] Sig. level is defined as 99% confidence interval around the mean solvent control response (set to 0%-TCDD or E2-max.

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