Instrumental and Bioanalytical Measures of Dioxin-like Activity in Sediments from Masan Bay, Korea

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Abstract

Sediments collected from 28 locations on Masan Bay were analyzed for trace organic contaminants including alkylphenols, organochlorine pesticides, polychlorinated biphenyl (PCB) congeners, and polycyclic aromatic hydrocarbons (PAHs). Sediment extracts were fractionated through a florisil column chromatography and screened for their ability to induce dioxin-like activity using H4IIE-luc cells. The relative abundance of organic contaminants measured in Masan Bay was in the order, NP>PAHs>PCBs>OP>BPA>OC pesticides. Only a few fraction 1 (F1; non-polar) samples induced significant dioxin-like activity. All 28 fraction 2 (F2; mid polar) samples induced significant dioxin-like activity, which was found to be contributed primarily by PAHs. Several fraction 3 (F3; polar) samples induced significant dioxin-like activity *in vitro*, adding to a growing body of evidence which suggests the presence of unidentified, relatively polar, Ah receptor agonists in sediment from some areas.

Introduction

Due to the complex nature of contaminants in environmental matrices, several schemes have been developed to isolate and identify their toxic potentials. In this study, marine sediment collected from Masan Bay, located at the southeast coast of Korea, was fractionated into three fractions after extraction. Instrumental analyses were performed to quantify target contaminants in each fraction. An *in vitro* bioassay, using H4IIE-luc cells, was used to provide information regarding the biological relevance of a complex mixture of compounds associated with sediments. Qualitative (response based) and quantitative (potency based) mass balance analysis between instrumentally-derived 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (TEQs) and bioassay-derived potency estimates (measured as TCDD-EQs) was used to determine whether non-additive interactions may have been occurring between compounds in the sample or whether unidentified AhR agonists may have been present.

Materials and Methods

Masan Bay, located on the southeast coast of Korea, is a long and narrow inlet of a semi-closed Bay, which receives discharges of industrial and municipal wastewater from Masan and Changwon cities. Surface sediment (0-5 cm) samples were collected from 28 locations on Masan Bay in May 1998 using a Van Veen grab sampler (25 x 40 x 30 cm). Sediments were freeze-dried and Soxhlet extracted using 400 mL dichloromethane (DCM). Extracts were passed through 10 g of activated Florisil packed in a glass column (10 mm i.d.) for fractionation. The first fraction (F1) eluted with 100 mL of high purity hexane contained PCBs, hexachlorobenzene (HCB) and *p,p'*-DDE. Remaining OC pesticides and PAHs were eluted in the second fraction (F2) using 100 mL 20% DCM in hexane. Nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA) were eluted in the third fraction (F3) with 100 mL 50% DCM in high purity methanol. Further details of the fractionation procedure and instrumental and bioassay procedures are presented elsewhere (2). Reverse phase high performance liquid chromatography (HPLC) with fluorescence detection was used to quantify NP, OP, and BPA. PAHs were quantified using a Hewlett Packard 5890 series II gas chromatograph equipped with a 5972 series mass spectrometer detector. OC pesticides and PCBs were quantified using a gas chromatograph (Perkin Elmer series 600) equipped with ^{63}Ni electron capture detector (GC-ECD). H4IIE-luc cells are rat hepatoma cells, which were stably transfected with a luciferase reporter gene under control of dioxin-responsive elements (DREs) (1).

Results and Discussion

Instrumental Analysis:
Target analytes found in F1 were PCBs, HCB and *p,p'*-DDE whereas those in F2 were PAHs, *p,p'*-DDD, *p,p'*-DDT, HCH isomers and chlordane compounds. F3 contained NP, OP and BPA. Concentrations of NP in sediment ranged from 113 to 3890 (mean: 510) ng/g, dry wt. OP concentrations ranged from 3.97 to 179 (mean: 18.1) ng/g, dry wt. Concentrations of PAHs ranged from 36.6 to 1100 ng/g, dry wt (Figure 1). PCBs were detected in all the sediment samples at concentrations ranging from 10.3 to 148 ng/g, dry wt. In general,

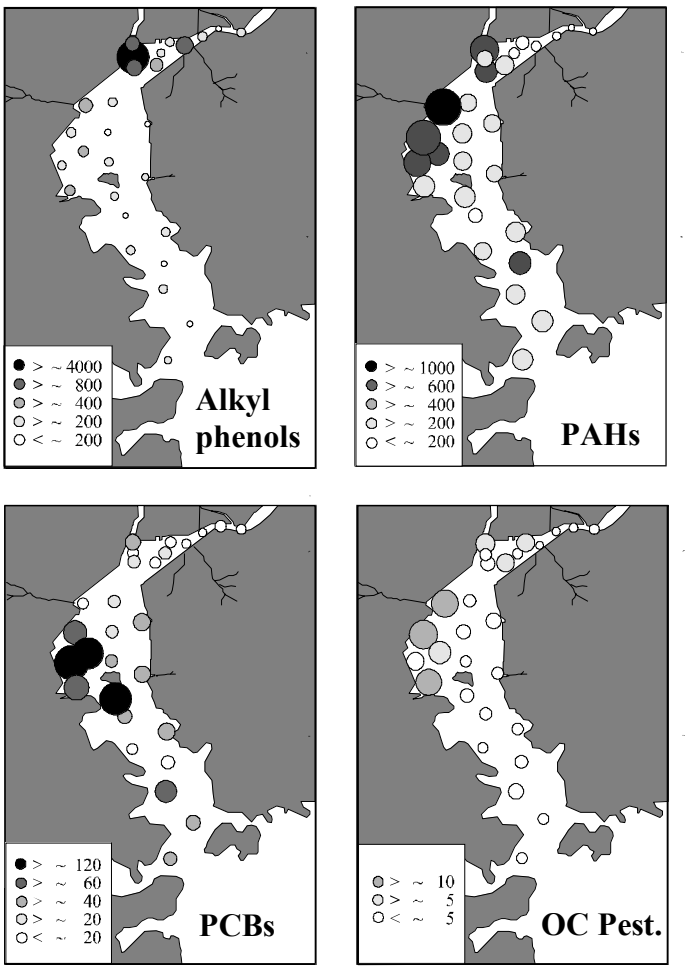

FIGURE. 1. Spatial distribution of alkylphenols (APs), PAHs, PCBs and organochlorine pesticides in sediment from Masan Bay, Korea. Concentration units are in ng/g, dry wt.

congeners 110 (2,3,3',4',6-pentaCB) and 31+28 (2,4',5- and 2,4,4'-triCBs) were prevalent in most samples each accounting for approximately 5% of the total PCB concentrations. Non-*ortho* coplanar PCB congener 77 (3,3',4,4'- tetraCB) was detected in some samples, while other non-*ortho* substituted congeners 126 (3,3',4,4',5-pentaCB) and 169 (3,3',4,4',5,5-hexaCB) were not detected (<20 pg/g, dry wt).

Dioxin-like Activity.
Only seven of the 28 F1 samples elicited a significant increase in luciferase expression (Figure 2). Most of the F1 samples yielded less than 40%-TCDD-max. These bioassay results lend to a hypothesis that concentrations of AhR-active compounds in Masan Bay sediments were relatively low. TEQ_{PCB} estimates based on H4IIE-luc-specific relative potencies ranged from 0.22 to 1.4 pg TEQ_{PCB}/g, dry wt. Based on regression against a TCDD standard curve, the TEQ_{PCB} would not have been sufficient to induce a significant response in the H4IIE-luc assay. This suggests that PCBs were probably not responsible for the dioxin-like responses elicited by F1 samples. Total PCDDs in Masan Bay sediment ranged from 59 to 1190 pg/g, dry wt while total PCDFs ranged from 43 to 5673 pg/g, dry wt (3). Thus, PCDD/DF concentrations may have been great enough to elicit a response as great as 66%-TCDD-max.

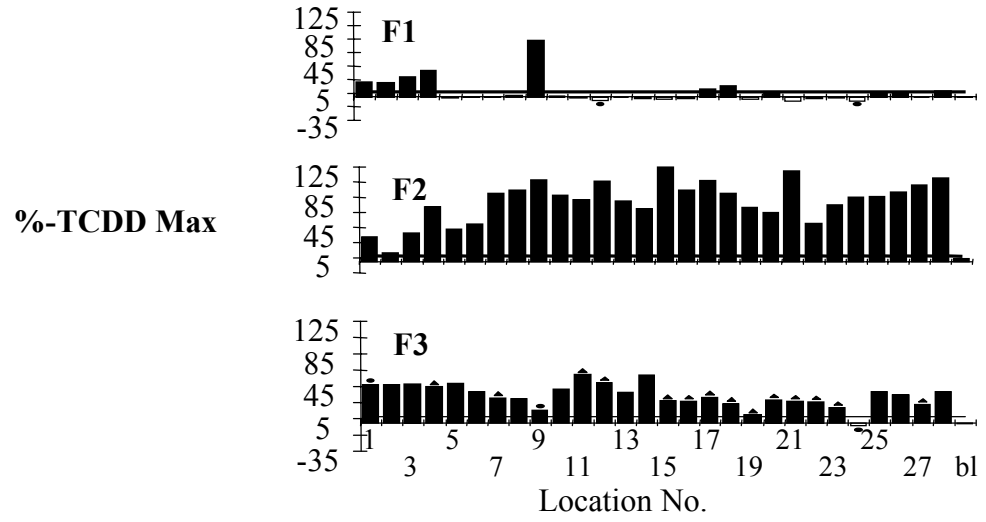

FIGURE 2. Luciferase induction in the H4IIE-luc cell bioassay (dioxin responsive) elicited by Masan Bay sediment extract fractions 1, 2, and 3 (F1, F2, F3) and procedural blank. Response magnitude presented as percentage of the maximum response observed for a 2000 pM 2,3,7,8 tetrachlorodibenzo-*p*-dioxin standard (%-TCDD-max.). Horizontal line equals 3 standard deviations (expressed in %-TCDD-max.) above the mean solvent control response (set to 0%-TCDD-max.). ▲ indicates cells exhibited an altered or "stressed" morphology. ● indicates the sample was toxic to the cells.

All F2 samples, except the blank, elicited significant luciferase expression (Figure 2). Magnitudes of induction were as high as 125%-TCDD-max. Several PAHs, including benzo[k]fluoranthene, bezo(a)pyrene, benzo[b]fluoranthene, chrysene, anthracene and dibenz [a,h]anthracene have been shown to upregulate AhR-mediated gene expression and/or induce cytochrome P4501A1 activity

in vitro (4,5). H4IIE-luc responses were strongly correlated with log total PAH concentration in sediment (R^2 = 0.837). TEQ_{PAH} estimates for F2 ranged from 240 to 3,860 pg TEQ_{PAH}/g, dry wt (4). Based on regression against a TCDD standard curve responses ranging from 70%- to 110%-TCCD-max. were predicted. Thus, the concentrations of AhR-active PAHs present in F2 samples could have accounted for the magnitude of response observed.

Dose-response curves were used to estimate the potencies of F2 samples relative to TCDD. EC-50 based point estimates (TCDD-EQ_{EC-50}) ranged from 170-1110 pg TCDD-EQ/g sediment. On average, there was one order of magnitude of uncertainty in the estimates caused by deviations from parallelism to the TCDD standard curve. Factoring in this range of uncertainty, TCDD-EQ ranged from 60-5050 TCDD-EQ/g, dry wt. Calculated TEQ_{PAH} estimates for the same samples ranged from 830 to 3,860 pg TEQ/g, dry wt. The fair degree of agreement between TCDD-EQ and TEQ_{PAH} estimates and the strong correlation between log PAH concentration and H4IIE-luc responses support the hypothesis that AhR-active PAHs can account for the dioxin-like activity caused by F2 samples. TCDD-EQ_{EC-50} estimates were, on average, 4.5 times less than calculated TEQ and nearly all observed responses were less than predicted. This suggests that interactions with non-AhR-active PAHs or other components of the F2 samples may have slightly antagonized the potency of the AhR-active PAHs.

Nearly all F3 samples elicited significant luciferase activity in H4IIE-luc cells, but the magnitude of induction was less than that elicited by F2 samples. Dioxin-like responses were not expected in F3, since there were no known AhR-active compounds. Recent studies have suggested that the AhR may be capable of binding a wider range of structures than previously suspected, however (6). 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.

Acknowledgements

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