Vertical Profile of Dioxin-like and Estrogenic Potencies in a Sediment Core from Tokyo Bay, Japan

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Abstract

Dioxin-like and estrogenic activities were measured in a sediment core collected from Tokyo Bay using *in vitro* bioassays after fractionating sediment extracts into three fractions by florisil column chromatography. Target analytes including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and nonylphenol (NP) were measured by gas chromatography-mass spectrometry (GC-MS) or high performance liquid chromatography (HPLC) techniques. Concentrations of PAHs were highest at a depth of 14 cm. NP concentrations were greater in surface sediments (0 to 12 cm) than those in sub-surface (12-30 cm). NP was not detected at depths above 30 cm. Concentrations of PCBs in fraction 1 (F1) were not high enough (<250 ng/g, dry wt) to induce significant luciferase activities in H4IIE-luc cells. Significant dioxin-like activities were found in fractions 2 (F2) and 3 (F3). While the vertical profile of PAH concentrations correlated with dioxin-like activities measured in F2, compounds that contribute to the dioxin-like activities in F3 were not identified yet. Significant estrogenic activities were observed in F2 samples, which may be related to the presence of certain estrogenic PAHs. F3 samples were cytotoxic to MCF-7 cells and therefore their estrogenic potential could not be estimated. Results of H4IIE-luc responses to F3 suggest the presence of relatively polar Ah-receptor active compounds associated with sediments.

Introduction

The use of dated sediment core is well established as a means of reconstructing historical chronologies of contaminant inputs (1). Several studies have examined vertical profiles of contaminant concentrations using instrumental analyses (1,2,3). While instrumental analyses provide information on the historical inputs of target contaminants, *in vitro* bioassays provide an integrated measure of biological responses due to several contaminants present in a sample (4). Thus, bioassays provide biological relevance of a complex mixture of compounds associated with an environmental matrix. In this study, a sediment core collected from Tokyo Bay, Japan, was analyzed using *in vitro* bioassay and instrumental techniques to evaluate vertical profiles of dioxin-like and estrogenic activities. Two *in vitro* bioassays were employed to screen sediment extracts for compounds able to elicit estrogen- or dioxin-like responses. The first used MVLN cells (hereafter referred as MCF-7) to screen extracts for compounds, which can modulate gene transcription through an estrogen receptor (ER)-mediated mechanism. The second utilized H4IIE-luc cells to screen compounds capable of modulating aryl hydrocarbon receptor (AhR)-mediated gene expression. Instrumental analyses was performed to estimate estrogen- or dioxin-like potency of many of the target compounds.

Materials and Methods

Tokyo Bay, Japan, encompasses major cities like Tokyo and Yokohama in Japan and is heavily contaminated by the disposal of wastes arising from industrial and urban activities. A sediment core was collected in May 1995 from the northern part of Tokyo Bay (35'35"N and 139'55"E) using acrylic tube (120 cm long and 11 cm i.d.). The core was sliced at 2 cm interval for up to 20 cm and then at 5 cm intervals for up to 90 cm using a clean stainless steel slicer. Each section was freeze-dried and stored in a refrigerator until analysis. Sediments were Soxhlet extracted using dichloromethane (DCM) and hexane (3:1, 400 mL). Extracts were treated with acid-activated copper granules to remove sulfur. Concentrated extracts were passed through 10 g of activated Florisil packed in a glass column (10 mm i.d.) for fractionation. First fraction (F1; non-polar) eluted with 100 mL hexane contained PCBs. PAHs and certain organochlorine pesticides were eluted in second fraction (F2; mid-polar) using 100 mL 20% DCM in hexane. Nonylphenol and octylphenol were eluted in third fraction (F3; polar) with 100 mL 50% DCM in methanol. Further details of the fractionation procedure, instrumental analyses of target compounds and bioassay techniques have been described elsewhere (4,5).

H4IIE-luc cells are rat hepatoma cells, which were stably transfected with a luciferase reporter gene under control of dioxin-responsive elements (DREs). MCF-7 cells are human breast carcinoma cells stably transfected with a luciferase reporter gene under control of estrogen response elements (EREs) of the *Xenopus* vitellogenin A2 gene. Cells were cultured in 100-mm disposable Petri plates and incubated in a 37°C humidified 95:5 air:CO_2 atmosphere. H4IIE-luc cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% FBS. MCF-7 were grown in Dulbecco's Modified Eagle Medium with Hams F-12 nutrient mixture supplemented with 10% fetal bovine serum, 27.3 I.U. insulin/L and 1.0 mM sodium pyruvate.

Results and Discussion

The sedimentation rate at the sampling location has been reported to be 250 mg/cm^2/year (6). Based on this, core depths of 0, 10, 20, 30, 40 and 50 cm correspond approximately to the years 1995, 1985, 1975, 1965, 1955 and 1945, respectively. Total concentrations of 16 priority PAHs varied from 38 to 2000 ng/g, dry wt. The highest concentration was recorded at a depth of 12-14 cm and the lowest was at a depth of 90-93 cm, the bottommost section taken for this study. PAH concentration profile was characterized by an exponential increase from background concentrations to a sub-surface maximum and decreasing thereafter to the sediment-water interface (Figure 1). This pattern suggests that PAH inputs to the Tokyo Bay have declined in recent years. Benzo[b]fluoranthene was the most abundant (among 16 PAHs) in the top 10 cm, accounting for 15-20% of the total PAH concentrations. In deeper layers (10-70 cm), indeno(1,2,3-cd)pyrene accounted for a major proportion of the total PAH concentrations. Phenanthrene dominated at depths greater than 70 cm.

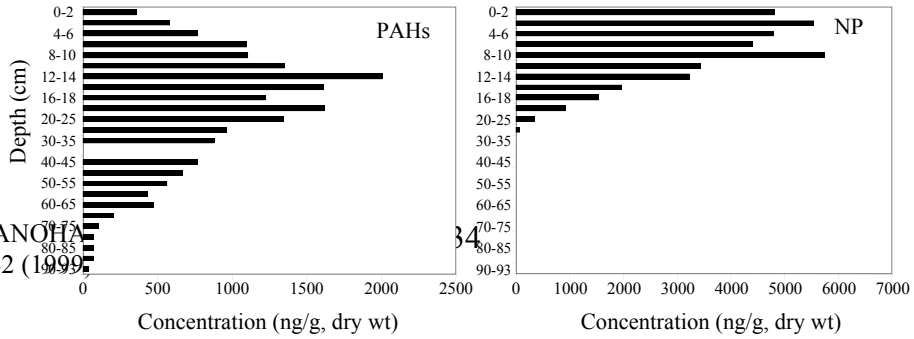

FIGURE 1. Vertical profile of PAHs and nonylphenol concentrations in a sediment core from Tokyo Bay, Japan.

The vertical distribution of nonylphenol (NP) concentrations in sediment was different from that of PAHs. NP was not detected at depths greater than 30 cm (Figure 1). Concentrations of NP ranged from <10 to 5540 ng/g, dry wt. NP concentrations were high in surface layers, suggesting recent inputs. Concentrations of octylphenol were 10-20 fold less than those of NP and ranged from <10 to 190 ng/g, dry wt. Similarly, PCB concentrations in Tokyo Bay sediments in general were less than 250 ng/g, dry wt (Dr. Takada, personal communication).

None of the F1 extracts elicited a significant increase in luciferase expression in H4IIE-luc cells (Figure 2). In fact, most the samples including blanks showed anti-dioxin-like activity. These bioassay results lend to a hypotheses that concentrations of AhR-active compounds in Tokyo Bay core were relatively low. F2 samples exhibited significant dioxin-like activity. Magnitude of induction as high as 90%-TCDD-max was observed. Several PAHs, including benzo[k]fluoranthene, benzo(a)pyrene, benzo[b]fluoranthene, chrysene and anthracene have been shown to upregulate AhR-mediated gene expression and/or induce cytochrome P4501A1 activity *in vitro* (7). The observed responses in F2 extracts very correlated with PAH sediment concentrations and profile.

Several F3 extracts also elicited significant luciferase activity in H4IIE-luc cells (Figure 2), although the magnitude of induction was less than that elicited by F2 samples. Dioxin-like responses were not expected in F3, since there were no target AhR-active compounds, which should have been present in F3. However, recent studies have suggested that the AhR may be capable of binding a wider range of structures than previously suspected (8). This suggests that there may be unidentified, relatively polar, AhR-active compounds in sediments from some areas.

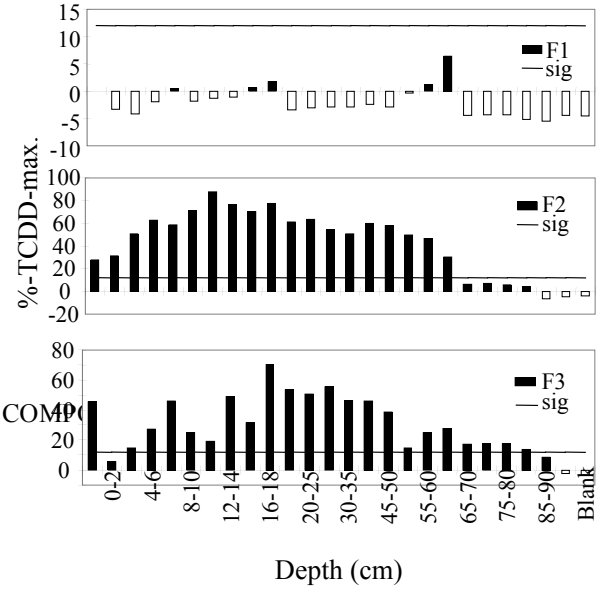

FIGURE 2. Luciferase induction in the H4IIE-luc cell bioassay (dioxin responsive) elicited by Tokyo Bay sediment core extract fractions 1,2 and 3 (F1, F2, F3) and procedural blanks (last two bars). Response magnitude presented as percentage of the maximum response observed for a 2 nM TCDD.

Sediment at a depth of 35-40 cm elicited a significant increase in luciferase expression in MCF-7 cells (Figure 3). The lack of estrogenic response in most F1 samples was expected and consistent with the polarity of estrogen agonists. F2 samples were much more estrogenic than F1. Some PAHs have been shown to elicit estrogen-like responses *in vitro* (7) which suggests that PAHs and their derivatives at least in part account for the estrogenic response observed in MCF-7 cells. F3 extracts were cytotoxic to MCF-7 cells and therefor their estrogenic potency could not be determined.

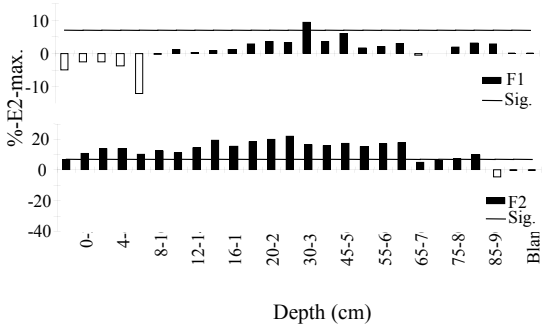

FIGURE 3. Luciferase induction in the MCF-7 cell bioassay (estrogen responsive) elicited by Tokyo Bay sediment core extract fractions 1 and2 (F1, F2) and procedural blanks (last two bars). Response magnitude presented as percentage of the maximum response observed for a 1 nM 17-β-Estradiol.

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References

(1) Gevao, B., Jones, K.C. and Hamilton-Taylor, J. (1998). *Sci. Total Environ.* 215, 231-242.
(2) Eisenreich, S.J., Capel, P.D., Robbins, J.A. and Bourbonniere, R. (1989). *Environ. Sci. Technol.* 23, 1116-1126.
(3) Hites, R.A. and Laflamme, R.E. (1977). Sedimentary polycyclic aromatic hydrocarbons: the historical record. *Science* 198, 829-831.
(4) Khim, J.S., Villeneuve, D.L., Kannan, K., Koh, C.H. and Giesy, J.P. (1999). *Environ. Sci. Technol*. (submitted).
(5) Khim, J.S., Villeneuve, D.L., Kannan, K., Lee, K.T., Snyder, S.A., Koh, C.H. and Giesy, J.P. (1999). *Environ. Toxicol. Chem*. (in press).
(6) Matsumoto, E. (1983). *Chikyu Kagaku* 17, 27-32 (in Japanese).
(7) Clemons, J.H., Allan, L.M., Marvin, C.H., Wu, Z., Mccarry, B.E., Bryant, D.W. and Zacharewski, T.R. (1998). *Environ. Sci. Technol*. 32, 1853-1860.
(8) Washburn, B.S., Rein, K.S., Baden, D.G., Walsh, P.J., Hinton, D.E., Tullis, K. and Denison, M.S. (1997). *Arch. Biochem. Biophys*. 343, 149-156.