

## VERTICAL PROFILE OF DIOXIN-LIKE AND ESTROGENIC POTENCIES IN A SEDIMENT CORE FROM TOKYO BAY, JAPAN

Kannan K.<sup>1</sup>, Yamashita N.<sup>2</sup>, Villeneuve D. L.<sup>1</sup>, Hashimoto S.<sup>3</sup>, Miyazaki A.<sup>2</sup>, Giesy J. P.<sup>1</sup>

<sup>1</sup> National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI, USA

<sup>2</sup> National Institute for Resources and Environment, Tsukuba, Japan

<sup>3</sup> Tokyo University of Fisheries, Tokyo, Japan

### SUMMARY

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), polychlorinated naphthalenes (PCNs), polycyclic aromatic hydrocarbons (PAHs) and nonylphenol (NP) were measured by gas chromatography-mass spectrometry (GC-MS) or high performance liquid chromatography (HPLC) techniques. NP concentrations were greater in surface sediments (0 to 12 cm) than those in sub-surface (12–30 cm). The maximum observed PCN concentration was 5 ng/g, whereas that of PCBs was 300 ng/g. Concentrations of PCBs in fraction 1 (F1) were not high enough to induce significant luciferase activities in H4IIE-luc cells. Significant dioxin-like activities were found in fractions 2 (F2) and 3 (F3). PAH concentrations were 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.

**Key words:** dioxin-like activity, estrogenicity, sediment, Japan

Address for correspondence: K. Kannan, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824, USA

### INTRODUCTION

The use of data 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 (1–3). 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). 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-passivated 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<sub>2</sub> 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.

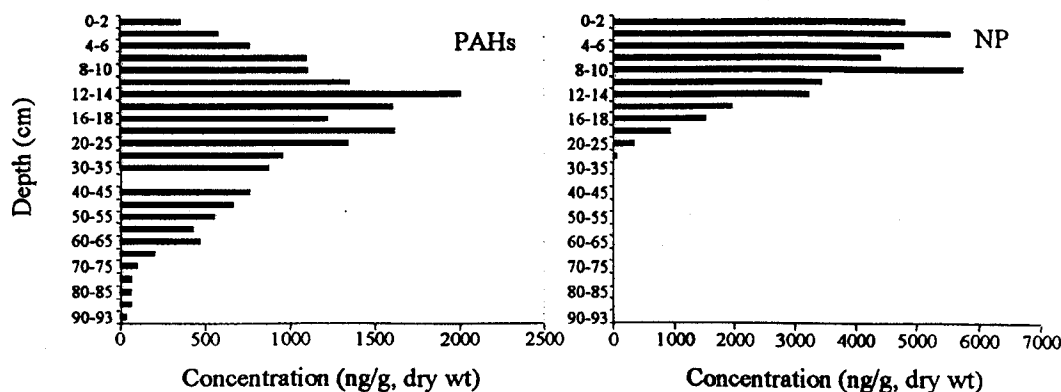


Fig. 1. Vertical profile of PAHs and nonylphenol concentrations in a sediment core from Tokyo Bay, Japan.

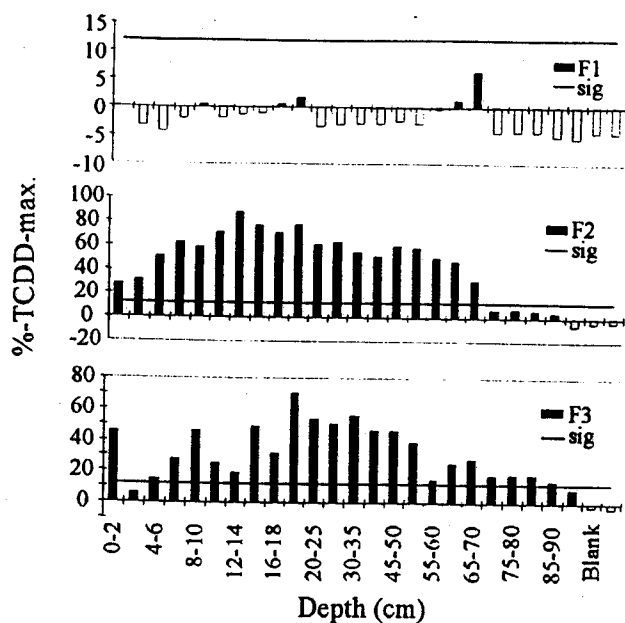


Fig. 2. Luciferase induction in the H4IIE-luc cell bioassay (dioxin responsive) elicited by Tokyo Bay sediment core extract fractions 1, 2 and 3 (F1, F2, F3) and procedural blanks (last two bars). Response magnitude presented as percentage of the maximum response observed for a 2 nM TCDD.

## RESULTS AND DISCUSSION

The sedimentation rate at the sampling location has been reported to be 250 mg/cm<sup>2</sup>/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 2 000 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 (Fig. 1).

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 (Fig. 1). Concentrations of NP ranged from <10 to 5 540 ng/g, dry wt. NP concentrations were high in surface layers, suggesting recent inputs. Concentrations of PCNs were greater in deeper layers corresponding to peak values in the 1960s. Similarly, PCB concentrations in Tokyo Bay sediment core in general were less than 300 ng/g, dry wt.

None of the F1 extracts elicited a significant increase in luciferase expression in H4IIE-luc cells (Fig. 2). However, presence of residual sulfur in the extract caused morphological aberrations in cells. Sulfur was removed by additional treatments with activated copper. Despite this, F1 did not induce H4IIE activity. These bioassay results lead to a hypothesis 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

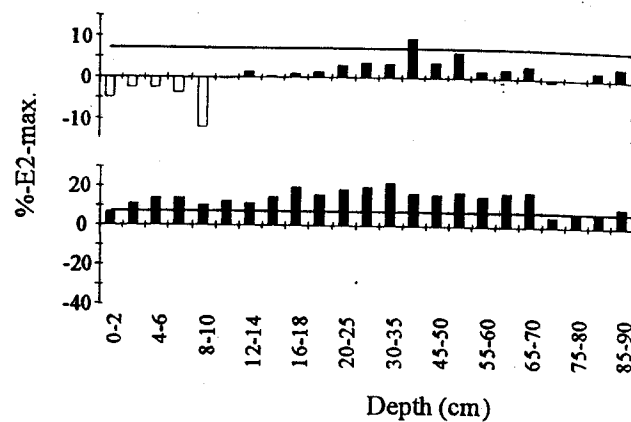


Fig. 3. Luciferase induction in the MCF-7 cell bioassay (estrogen responsive) elicited by Tokyo Bay sediment core extract fractions 1 and 2 (F1, F2) and procedural blanks (last two bars). Response magnitude presented as percentage of the maximum response observed for a 1 nM 17 $\beta$ -estradiol.

F2 extracts very correlated with sediment PAH concentrations and profile.

Several F3 extracts also elicited significant luciferase activity in H4IIE-luc cells (Fig. 2), although the magnitude of induction was slightly 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 present in 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.

Sediment at a depth of 35–40 cm elicited a significant increase in luciferase expression in MCF-7 cells (Fig. 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 therefore their estrogenic potency could not be determined.

## REFERENCES

1. Gevaio, B., Jones, K. C., Hamilton-Taylor, J.: *Sci. Total Environ.* 215, 1998, 231–242.
2. Eisenreich, S. J., Capel, P. D., Robbins, J. A., Bourbonniere, R.: *Environ. Sci. Technol.* 23, 1989, 1116–1126.
3. Hites, R. A., Laflamme, R. E.: *Science* 198, 1977, 829–831.
4. Khim, J. S., Villeneuve, D. L., Kannan, K., Koh, C. H., Giesy, J. P.: *Environ. Sci. Technol.* 33, 1999, 4206–4211.
5. Khim, J. S., Villeneuve, D. L., Kannan, K., Lee, K. T., Snyder, S. A., Koh, C. H., Giesy, J.P.: *Environ. Toxicol. Chem.* 18, 1999, 2424–2432.
6. Matsumoto, E.: *Chikyu Kagaku* 17, 1983, 27–32. (In Japanese.)
7. Clemons, J. H., Allan, L. M., Marvin, C. H., Wu, Z., McCarry, B. E., Bryant, D. W., Zacharewski, T. R.: *Environ. Sci. Technol.* 32, 1998, 1853–1860.
8. Washburn, B. S., Rein, K. S., Baden, D. G., Walsh, P. J., Hinton, D. E., Tullis, K., Denison, M. S.: *Arch. Biochem. Biophys.* 343, 1997, 149–156.