Arch. Environ. Contam. Toxicol. 48, 209–216 (2005)
DOI: 10.1007/s00244-003-0205-3

ARCHIVES OF
Environmental
Contamination
and Toxicology

Horizontal and Vertical Distribution of Estrogenic Activities in Sediments and Waters from Tokyo Bay, Japan

S. Hashimoto,[1] A. Horiuchi,[2] T. Yoshimoto,[3] M. Nakao,[3] H. Omura,[3] Y. Kato,[4] H. Tanaka,[5] K. Kannan,[6] J. P. Giesy[6]

[1] Institute for Environmental Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
[2] Department of Ocean Sciences, Tokyo University of Fisheries, Tokyo, 108-8477, Japan
[3] Yakult Central Institute for Microbiological Research, Tokyo, 186-8650, Japan
[4] School of Marine Science and Technology, Tokai University, 424-8610, Japan
[5] Independent Administrative Institutions Public Works Research Institute, Ibaraki 305-8516, Japan
[6] National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824, USA

Received: 10 September 2003 /Accepted: 23 June 2004

Abstract. Endocrine-disrupting chemicals with estrogenic activity (*e.g.*, alkylphenols) have been detected in coastal Japan. We aimed to determine estrogenic activity in extracts of river water, seawater, sediments, and sediment cores from Tokyo Bay by *in vitro* gene expression assay. Fifty-one of 57 extracts had some estrogenic activity. E_2 equivalents (ng E_2 equivalents per gram dry weight or per liter above the limit of detection) in river water samples ranged from 0.70 to 4.01 ng/L; in seawater samples from 0.34 to 2.52 ng/L; and in surface sediments from 2.07 to 12.1 ng/g. The relationship between salinity and estrogenic activity in water samples suggested that fresh water is one source of environmental estrogens in Tokyo Bay. Fractionation of sediment extracts showed that the highest estrogenic activity was observed in the midpolar fraction. The observed activities were compared with activities mediated by known concentrations of nonylphenol, bisphenol-A, estrone, and 17β-estradiol. In sediment collected near the sewage treatment plants, the estrogenic activity of the midpolar fraction could be explained about 34% by nonylphenol and estrone contained in this fraction. Core sediment measurements detected estrogenic activity from as far back as the 1960s. The regulations on the industrial wastewater in early 1970s would be one of the main reasons for the lower estrogenic activity in the upper section of the sediment core. The high estrogenic activities as measured in water and sediment samples from Tokyo might be restricted to certain coastal areas.

There has been considerable concern about endocrine-disrupting chemicals (EDCs) detected in the environment and their potential to cause deleterious physiological effects in humans and wildlife (Jobling and Sumpter 1993). Endocrine disruption has a multitude of mechanisms and actions, including effects on growth, behavior, reproduction, and immune function. EDCs are found in the aquatic environment, especially in discharges from sewage treatment plants. For example, alkylphenols such as nonylphenol are known to exert estrogenic activity in aquatic organisms (Gray and Metcalfe 1997; Staples *et al.* 1998), and are present in effluents from sewage treatment plants and also in river water, sediments, and fish tissue (Yamagishi *et al.* 1997; Lye *et al.* 1999).

The appearance of intersex fish in the aquatic environment, especially in wastewater effluents, has led to the hypothesis that substances in sewage effluent might be estrogenic to fish. Estrogens induce vitellogenin, a precursor protein for egg yolk that has been used as a sensitive biomarker for studies of xenoestrogens in aquatic environments (Harries *et al.* 1997; Allen *et al.* 1999a, 1999b; Minier *et al.* 2000; Simpson *et al.* 2000; Oberdörster and Cheek 2001; van Aerle *et al.* 2001). We previously found vitellogenin induction and intersex gonads, two bioindicators of the effects of environmental estrogens, in male flounder, *Pleuronectes yokohamae*, collected from Tokyo Bay, Japan (Hashimoto *et al.* 1998b, 2000). Intersex characteristics, such as the presence of perinucleolar oocytes, have also been found in male konoshiro gizzard shad (*Konosirus punctatus*) collected from inner Tokyo Bay (Cho *et al.* 2003). Contamination of the waters of Tokyo Bay by environmental estrogens may cause vitellogenin induction and the development of intersex gonads in local male fish.

Several assays have been developed to assess environmental samples for estrogenic activity mediated by estrogen receptors. In recent years, estrogenic activity in wastewater (Körner *et al.* 2001; Kirk *et al.* 2002), river water (Fenet *et al.* 2003), seawater (Koh *et al.* 2002), and sediment (Kannan *et al.* 2000; Koh *et al.* 2002; Fenet *et al.* 2003) has been analyzed by *in vitro* bioassays. They demonstrate the total estrogenic activity of environmental samples, regardless of which compounds are responsible for the activity. The results of these studies indicate that estrogenic activity varies widely among sampling sites (Koh *et al.* 2002).

Correspondence to: S. Hashimoto; *email:* hashimos@u-shizuoka-ken.ac.jp

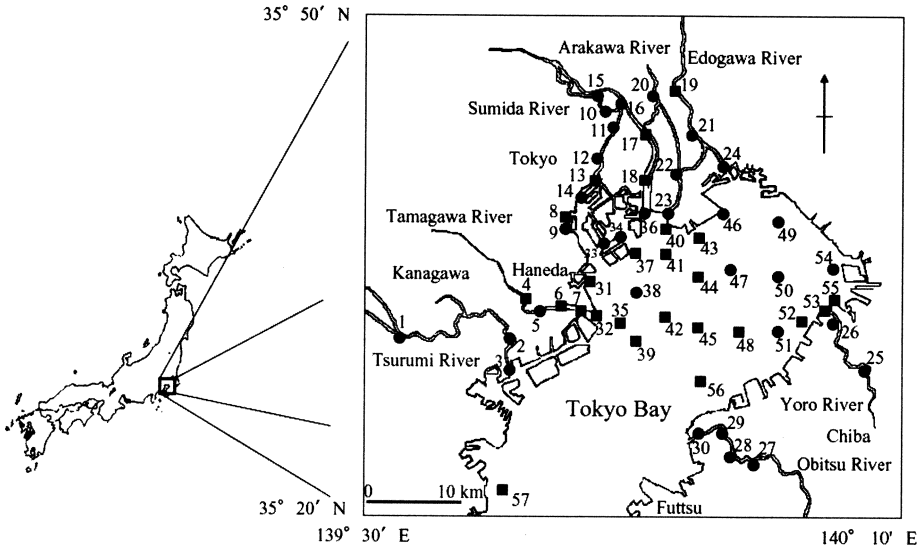

Fig. 1. Locations of water and sediment sampling locations in Tokyo Bay. ●, water sampling points; ■, water and sediment sampling points.

Our objective was to assess the distribution and sources of estrogenic activity in the area where we had collected feminized male *Pleuronectes yokohamae* (Hashimoto *et al.* 1998b, 2000) and *Konosirus punctatus* (Cho *et al.* 2003). We collected samples from river water, seawater, surface sediments, and sediment cores at 57 study sites in the inner part of Tokyo Bay. We report the results of analyses of estrogenic activity in extracts of these samples. The analyses used an *in vitro* gene expression assay using an estrogen-responsive reporter gene cell line. In addition, the sediment extracts were fractionated on a silica column, and the estrogenic activity of each fraction was analyzed in the *in vitro* assay. The concentrations of nonylphenol, bisphenol-A, estrone, and 17β-estradiol of each fraction were also measured.

Materials and Methods

Sampling and Extraction

Water and surface sediment samples were collected from 57 points in inner Tokyo Bay (57 water samples and 25 surface sediment samples, from June to July 1998, Figure 1).

The sampling points are as follows: Tsurumi River, stations 1, 2; Tamagawa River, stations 4–7; Sumida River, stations 10–12, 15; Arakawa River, stations 16, 17; Edogawa River, stations 19–22, 24; Yoro River, stations 25, 26; Obitsu River, stations 27–30; near estuary, stations 3, 8, 9, 13, 14, 18, 23, 32–34, 36, 53; the bay, stations 31, 35, 37–52, 54–57. The annual river flows are as follows: Tsurumi River, 1.08×10^{10} m^3/year (1998); Tamagawa River, 5.25×10^{10} m^3/year (1998); Sumida River, no data; Arakawa River, 2.66×10^9 m^3/year (1998); Edogawa River, 1.53×10^9 m^3/year (1998); Yoro River, 1.38×10^8 m^3/year (1998); Obitsu River, 1.1×10^8 m^3/year (1998). Chemical analysis was done on water (stations 7, 8) and sediment samples (stations 8, 39, 57). Station 8 was selected for chemical analysis of water and sediment because it is exposed to sewage treatment works outflow. Station 7 was selected for chemical analysis of water because the Tamagawa River is one of the biggest rivers flowing into Tokyo Bay. Station 39 was selected for chemical analysis of sediment because the sediment here is carried out of the Tamagawa River. Station 57 was selected to provide data from outside the inner part of Tokyo Bay.

Water samples were collected at about 50-cm depth and extracted as previously described (Snyder *et al.* 1999). The extraction procedure was conducted within 24 h to minimize bacterial degradation of samples. Briefly, each water sample was filtered and extracted by using a poly(styrenedivinylbenzene) Empore Disk (3M Corporation, Tokyo, Japan). First, the Empore Disk was conditioned with 15 ml of dichloromethane and methanol. The 5-L water sample was then pumped through the disk, and the analyte was extracted with 30 ml of dichloromethane. The solvent extract was passed through a cleanup column containing 1 g of anhydrous sodium sulfate. The elute was collected and concentrated to about 5 ml in a rotary evaporator. The sample was solvent-exchanged to 0.1 ml of isooctane under a gentle stream of nitrogen. Samples were stored in precleaned glass bottles at –40°C until analysis.

Sediment core was collected in January 1999 at station 39 in Tokyo Bay (Figure 1) in an acrylic tube (120 cm long × 11 cm i.d.). Core sample was sliced at 2-cm intervals for up to 52 cm with a clean stainless steel slicer. Each section was freeze-dried and stored at –40°C until analysis. The sediment core was dated by measuring ^{210}Pb and ^{137}Cs (Sanada *et al.* 1999). Total organic carbon (TOC) was measured by the high-temperature combustion method using Shimadzu TOC-5000 (Shimadzu Co. Ltd., Tokyo, Japan), as described previously (King *et al.* 1998).

Sampling and extraction of sediments were performed as described previously (Khim *et al.* 1999). Briefly, each surface sediment sample was collected with a grab sampler (Ekman–Birge-type bottom sampler) and homogenized in a precleaned stainless steel container. The sample was freeze-dried and stored in a precleaned glass bottle at –40°C until analysis. Five grams of dry surface sediment and sediment core samples were extracted twice (30 min and 30 min) by ultrasonic extraction with 30 ml of dichloromethane. The solvent extracts were combined and passed through the glass column containing 1 g of anhydrous sodium sulfate. The elute was collected and concentrated to about 5 ml in a rotary evaporator. The sample was solvent-exchanged to 0.1 ml of isooctane under a gentle stream of nitrogen. Samples were stored in precleaned glass bottles at –40°C until further fractionation.

HPLC Fractionation

For fractionation, a Phenomenex Luna 5-μm silica column (250 mm × 4.6 mm, Torrance, CA, USA) was used for normal-phase sepa-

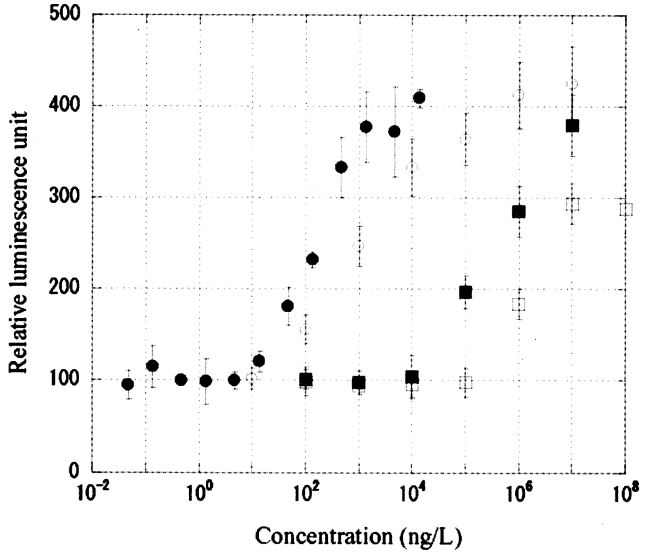

Fig. 2. Concentration response curves of nonylphenol (■), bisphenol-A (□), estrone (○), and 17β-estradiol (●) determined with MVLN cells.

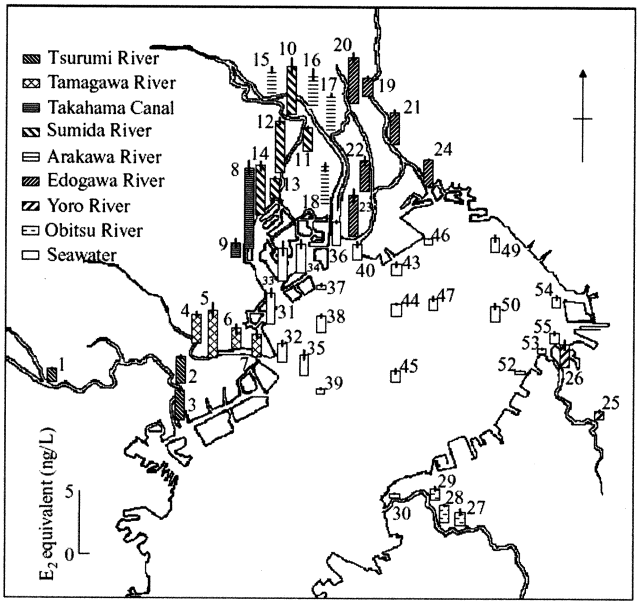

Fig. 3. Distribution of estrogenic activities of river water and seawater samples.

rations with three-step isocratic elution, as described before (Snyder *et al.* 1999). The mobile-phase solvent profile was 30% dichloromethane in hexane for 15 min, dichloromethane for 20 min, and methanol for 20 min at 25°C. The flow rate was 1 ml/min, with no gradient curves. Dichloromethane was then passed into the column for 10 min, followed by 30% dichloromethane in hexane for 35 min. Three fractions were collected, from 0 to 20 min (F1), 20 to 45 min (F2), and 45 to 70 min (F3). Each fraction was concentrated in a rotary evaporator under a nitrogen stream for further assays. To determine recovery during high-performance liquid chromatography (HPLC) fractionation, known amounts (50 ng) of nonylphenol, bisphenol A, estrone, and 17β-estradiol were fractionated by HPLC, and the amounts of the recovered compounds were determined.

Chemical Analysis

We measured the concentrations of nonylphenol, bisphenol-A, estrone, and 17β-estradiol in HPLC fractions in water and sediment samples (Desbrow *et al.* 1998; Tsuda *et al.* 2000). Standards were purchased from Wako Chemical Co. Inc. (Tokyo, Japan) and Kanto Chemical Co. Inc. (Tokyo, Japan). Pesticide-residue-grade solvents and chemicals were obtained from Wako Chemical Co. The extracted sample was concentrated to 0.1 ml of isooctane under a gentle stream of nitrogen. Analysis was performed by gas chromatography (GC: HP 5890 series II, Agilent Technologies, Inc., Tokyo, Japan)–mass spectrometry (MS: HP 5971 MSD, Agilent Technologies, Inc., Tokyo, Japan) using selected ion monitoring. The conditions were as follows: splitless injection mode, injector temperature 250°C; column DB-5MS, 30 m, 0.25-mm i.d., 0.25-μm film thickness (J. & W. Scientific, Folsom, CA, USA), carrier gas, helium at a flow rate of 1 ml/min. The GC oven temperature was programmed with a starting temperature of 50°C for 2 min, followed by a 10°C/min temperature slope to a final temperature of 300°C, which was held for 10 min. Target compounds were identified by their retention times and ion ratios (Cho *et al.* 2003).

Bioassay Using MVLN (MCF-7 ERE-Luc) Cells

Bioassay of the estrogenic activity of each sample using MVLN cells was done according to methods already described for measuring the estrogenic activity of sediment samples (Khim *et al.* 1999). In brief, MVLN cells are MCF-7 human breast carcinoma cells stably transfected with a luciferase reporter gene under the control of estrogen-responsive elements of the *Xenopus* vitellogenin A2 gene. All cells were cultured in 100-mm disposable Petri plates (Corning, Corning, NY, USA) and incubated in a humidified 5% CO_2 atmosphere maintained at 37°C. MVLN cells (Demirpence *et al.* 1993) were grown in Dulbecco's Modified Eagle Medium with Hams F-12 nutrient mixture (Sigma D-2906, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) and 27.3 I.U. insulin (Sigma I-1882)/L and 1.0 mM sodium pyruvate (Sigma). The cells were trypsinized from the plates, diluted to a concentration of about 75,000 cells/ml, and seeded into the 60 interior wells of 96-well-culture ViewPlates (Packard Instruments, Meriden, CT, USA) at 250 μl per well. The plates were incubated overnight. Prior to the exposure of the cells, samples were fivefold diluted in the culture medium. The wells were then dosed with 2.5 μl of the sample (test wells) or isooctane (control wells). All analyses were performed in triplicate. Luciferase assay was conducted after 72 h of exposure. Luciferase assay reagent containing a luciferin substrate was added to the wells. The plates were incubated for 10 min at 30°C and then scanned with a luminescence microplate counter (LumiCount, Packard Japan, Tokyo, Japan). On MVLN cells assay, 100% of transactivation is obtained by 10 nM of 17β-estradiol. The value of control, isooctane solution, was corresponding to the blank baseline. The magnitude of the luciferase bioassay response of the MCF-7-luc (MVLN) cells is presented as the 17β-estradiol standard (E_2) equivalent: for sediment samples, ng/g dry weight; for water samples, ng/L. Figure 2 shows the concentration response curves of nonylphenol, bisphenol-A, estrone, and 17β-estradiol. Differences in concentrations of each between station 8 and stations 39 and 57 were tested by one-way analysis of variance (ANOVA).

Differences in E_2 equivalent values of seawater samples from the west (Tokyo and Kanagawa Prefecture) and east (Chiba Prefecture) sides of the bay were also tested by one-way ANOVA.

Results and Discussion

Estrogenic Activity of Water Samples

The distribution of estrogenic activity in the water samples is shown in Figure 3. Most of the extracts (51 out of 57) had some estrogenic activity (water samples collected at stations 41, 42, 48, 51, 56, and 57 did not have estrogenic activity). Estrogenic activity was detected in all seven main rivers flowing into Tokyo Bay. E_2 equivalents (above the limit of detection) in river water samples ranged from 0.70 to 4.01 ng/L and in seawater samples from 0.34 to 2.52 ng/L. Relatively high estrogenic activity was observed in samples colleted at the sewage treatment works outflow (station 8). The estrogenic activity of river water samples collected on the Chiba Prefecture side of Tokyo Bay (<1.76 ng/L, Yoro and Obitsu rivers; Figure 3) was lower than that of the river water collected on the Tokyo and Kanagawa Prefecture sides of the Bay (about 1.48–4.01 ng/L in the crowded Edogawa, Arakawa, Sumida, and Tamagawa river areas; Figure 3). Low estrogenic activities were observed at some stations with little input of sewage water (stations 52–55; Hashimoto *et al.* 1998a). The E_2 equivalents of seawater samples were higher on the Tokyo and Kanagawa Prefecture side of the Bay than on the Chiba Prefecture side ($p < 0.05$, ANOVA, Figure 3).

Using the dose–response curves of nonylphenol, bisphenol-A, estrone, and 17β-estradiol determined on the MVLN cell lines, the observed activities were compared with activities mediated by known concentrations of nonylphenol, bisphenol-A, estrone, and 17β-estradiol in the samples. According to these comparisons, the natural hormones, estrone and 17β-estradiol, contained in the water samples at stations 7 and 8 (see "Chemical Analysis" below) caused the most (about 78%) of the marked estrogenic response of these samples (Figure 3). This is in agreement with the previous report suggesting that estrogenic activity would mainly be due to steroid hormones in effluent samples of sewage treatment plants (Desbrow *et al.* 1998). The relationship between salinity and the E_2 equivalents of water samples is weakly negative (Figure 4). These results may indicate that one of the sources of the environmental estrogens in Tokyo Bay is fresh water, such as river and canal water containing outflows from sewage treatment plants.

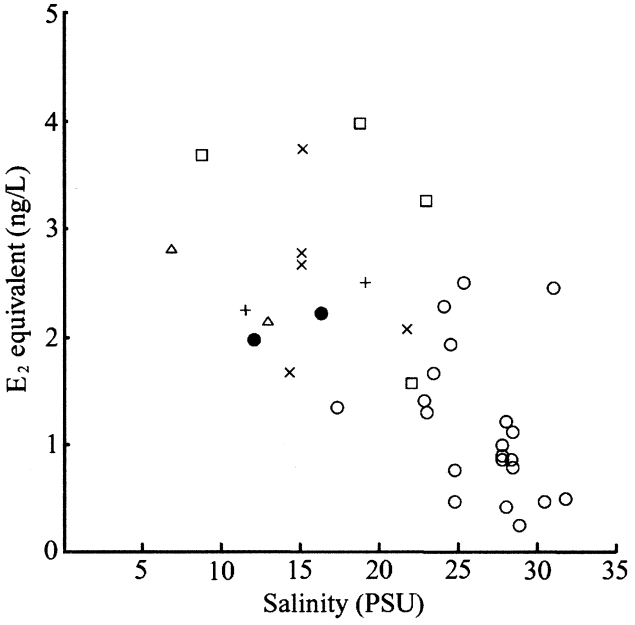

Fig. 4. Relationship between salinity and E_2 equivalents of water samples. ○, seawater; □, Sumida River; ●, Tamagawa River; +, Tsurumi River; Δ, Arakawa River; ×, Edogawa River.

Estrogenic Activity of Sediment Samples

The E_2 equivalent values in the sediment samples are shown in Table 1. All the extracts (25 out of 25) had some estrogenic activity. E_2 equivalents of surface sediments ranged from 2.07 to 12.1 ng/g. E_2 equivalents of sediment samples were relatively high on the Tokyo and Kanagawa Prefecture side of inner Tokyo Bay (Table 1). The highest activity was observed at station 8, at the discharge site of a sewage treatment plant. In combination with the results of the water samples, it can be concluded that the serious problems of estrogenic activity in the aquatic environment of Tokyo Bay might be restricted to certain coastal areas. Hashimoto *et al.* (1998b, 2000) reported that three out of 20 male flounder collected off Haneda (near station 31 in Figure 1) had signs of feminization, such as occurrence of oocytes in the testes. Intersex characteristics, such as the presence of perinucleolar oocytes, were also found in three out of 125 male konoshiro gizzard shads (*Konosirus punctatus*) collected off Haneda (Cho *et al.* 2003). This sexual disruption in fish collected from Tokyo Bay appears to be also restricted to some areas since no abnormal males collected on the outer sides of the Bay were found (off Futtsu; Cho *et al.* 2003).

A linear regression procedure was used to calculate the correlations between TOC and estrogenic activity of the sediment samples from Tokyo Bay. The relationship between TOC and estrogenic activity of the sediment samples is weakly positive ($r = 0.645$). This may mean that most of the EDCs are adsorbed to organic compounds in the sediment. For example, both nonylphenol and octylphenol are hydrophobic, and the bulk of these compounds have a tendency to be attached to particles and sediments containing higher TOC (Fenet *et al.* 2003). In this study, the sediments collected from inner Tokyo Bay having high TOC (around 2.5%) probably have a high potential for EDCs (*e.g.*, nonylphenol) accumulation.

The results of the bioassay measurements of the estrogenic activity using MVLN cells to the fractionated extracts of surface sediment samples from Tokyo Bay are shown in Table 1. Fractionation of sediment extracts showed that the estrogenic activity was mainly present in F2, the midpolar fraction (Table 1). In this fraction, nonylphenol and estrone were detected. In sediment collected at the discharge site of the sewage treatment plant (station 8), the observed estrogenic activity of F2 could be explained about 34% by the nonylphenol and estrone detected in F2 (see "Chemical Analysis" below), but these compounds poorly contribute to the observed estrogenic activity of F2 at stations 39 and 57 by the

Table 1. Estrogenic activities of surface and fractionated sediment samples

Station number	Total	Estrogenic activity (E_2 equivalent, ng/g dry weight)		
		Fraction 1	Fraction 2	Fraction 3
4	5.36 ± 0.68			
6	4.32 ± 0.29	2.20 ± 0.21	2.52 ± 0.28	1.54 ± 0.13
7	5.01 ± 0.53	0.70 ± 0.12	2.46 ± 0.44	1.60 ± 0.23
8	12.1 ± 0.86	Not detected	9.22 ± 0.92	5.33 ± 0.41
13	8.38 ± 0.81			
17	6.22 ± 0.53			
18	5.79 ± 0.41			
19	7.95 ± 0.62			
31	7.99 ± 0.58			
32	7.43 ± 0.35	1.93 ± 0.18	1.98 ± 0.21	1.05 ± 0.08
35	7.69 ± 1.19			
37	2.59 ± 0.38	2.59 ± 0.28	2.90 ± 0.17	2.41 ± 0.32
39	3.46 ± 0.43	0.66 ± 0.16	2.20 ± 0.21	1.54 ± 0.32
40	6.91 ± 0.78			
41	2.76 ± 0.35			
42	2.51 ± 0.42			
43	3.46 ± 0.22			
44	3.20 ± 0.34			
45	5.18 ± 0.35			
48	2.51 ± 0.41	1.30 ± 0.12	2.31 ± 0.31	2.20 ± 0.20
52	2.07 ± 0.19			
53	3.56 ± 0.57			
55	2.85 ± 0.32			
56	4.15 ± 0.62			
57	7.78 ± 0.41	0.83 ± 0.08	5.01 ± 0.34	2.22 ± 0.14

Table 2. Efficiency of extraction and limit of detection of nonylphenol, bisphenol-A, estrone, and 17β-estradiol from water and sediment samples

	Efficiency of extraction[a] Mean and relative standard	Limit of detection[b] deviation (%)
Water samples		
Nonylphenol	84.3, 8.1	0.54, 7.5 (ng/L)
Bisphenol-A	78.3, 6.3	0.32, 5.5
Estrone	80.7, 7.7	0.2, 7.8
17β-Estradiol	86.9, 3.6	0.1, 8.9
Sediment samples		
Nonylphenol	85.6, 7.4	0.72, 8.2 (ng/g)
Bisphenol-A	81.7, 8.5	0.05, 6.3
Estrone	82.1, 4.9	0.1, 8.3
17β-Estradiol	90.5, 5.8	0.1, 9.5

[a] 50 ng each compound was added to 5 L of seawater sample or 5 g of dry sediment sample.
[b] A signal-to-noise ratio of 3:1 was defined as the detection limit.

Table 3. Chemical analysis of water and surface sediment samples collected from Tokyo Bay

Water sample	St. 7		St. 8
Nonylphenol (ng/L)	30.4 ± 3.1		104 ± 6
Bisphenol-A (ng/L)	20.2 ± 1.4		30.1 ± 2.4
Estrone (ng/L)	5.8 ± 1.2		32.0 ± 1.5
17β-Estradiol (ng/L)	0.4 ± 0.2		1.7 ± 0.4
Sediment sample	St. 8	St. 39	St. 57
Nonylphenol (ng/g)	4560 ± 350	5.9 ± 1.2	2.2 ± 0.2
Bisphenol-A (ng/g)	48.0 ± 2.1	0.11 ± 0.03	0.22 ± 0.02
Estrone (ng/g)	10.3 ± 0.7	<0.1	<0.1
17β-Estradiol (ng/g)	4.8 ± 0.2	<0.1	<0.1

calculated activities based on chemical analysis (see "Chemical Analysis" below). In fraction 3, 17β-estradiol and bisphenol-A were detected. The concentration of 17β-estradiol would be responsible for the major (about 90%) estrogenic response of F3 of the sediment extract at station 8 (see "Chemical Analysis" below). On the contrary, at stations 39 and 57, the 17β-estradiol contributed poorly to the activity of F3 in these sites (see "Chemical Analysis" below). The bisphenol-A contributed poorly to the activity of F3 in all sites (see "Chemical Analysis" below). These results suggest the presence of other estrogenic compounds in these samples. There were comparable findings in a study in the Netherlands in which 80% of estrogenic activity in effluent could not be explained by the calculated potencies based on chemical analysis of a number of known (xeno)estrogens (Murk *et al.* 2002). An estimated 4.7 km^3 of industrial and domestic sewage effluent is carried into Tokyo Bay annually (Hashimoto *et al.* 1999). These effluents no doubt contain large amounts of anthropogenic chemicals with the potential to cause endocrine disruption, especially where the rate of water exchange is low, such as in the inner part of Tokyo Bay. In this study, the results of bioassay of the fractionated extracts suggest that compounds responsible for the estrogenic activity exist other than nonylphenol, estrone, 17β-estradiol, and bisphenol-A in sediment samples.

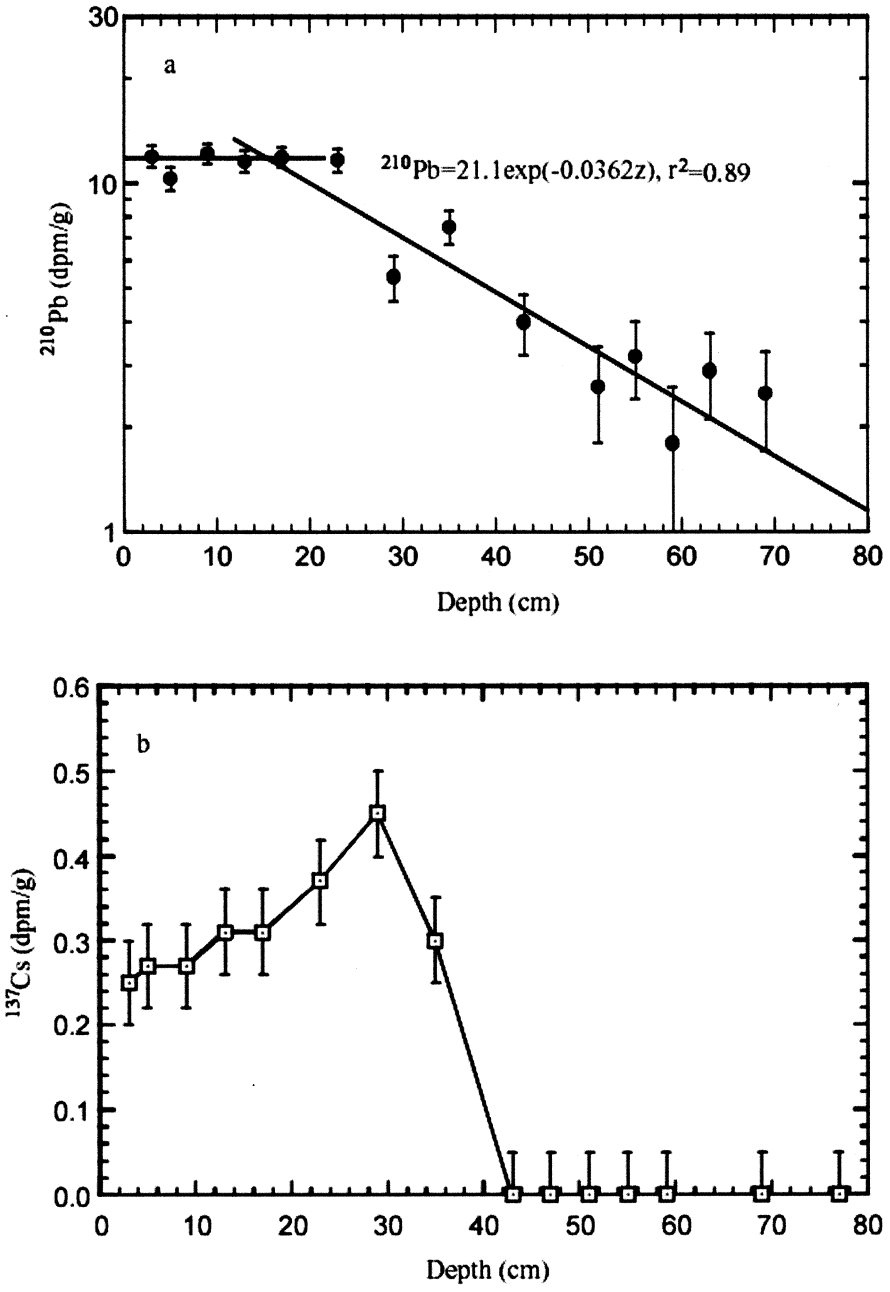

Fig. 5. Vertical profiles of ^{210}Pb and ^{137}Cs in a sediment core from Tokyo Bay. The sampling station is number 39 in Figure 1.

Chemical Analysis

The efficiency of extraction of nonylphenol, bisphenol-A, estrone, and 17β-estradiol in the water and sediment samples is shown in Table 2. All analyses were run in triplicate.

We performed a spiking experiment with seawater collected at station 7 and sediment collected at station 39 using standard dissolved in acetone. Background levels of analyzed compounds in the samples were subtracted in calculating the recoveries. No significant losses occurred during fractionation. Fraction 2 contained estrone and nonylphenol and fraction 3 contained bisphenol A and 17β-estradiol. The limits of detection for each compound are shown in Table 2.

Table 3 shows the results of chemical analysis of water and surface sediment samples, as measured by GC-MS. Station 8 was situated at the discharge gate of a sewage treatment plant; much higher concentrations of nonylphenol, bisphenol-A, estrone, and 17β-estradiol were found in the sediment collected from this station than in those from stations 39 and 57 ($P < 0.01$, one-way ANOVA, Table 3). Station 7 was situated about 200 m downstream from the discharge gate of a sewage treatment plant; water sampling also showed higher concentrations of EDCs at station 8 than those at station 7. In this study, the concentrations of nonylphenol in water and sediment at station 8 were comparable with concentrations found in the Seine watershed up to 550 ng/L and 2870 ng/g, respectively (Fenet *et al.* 2003). The concentrations of estrone in water were also

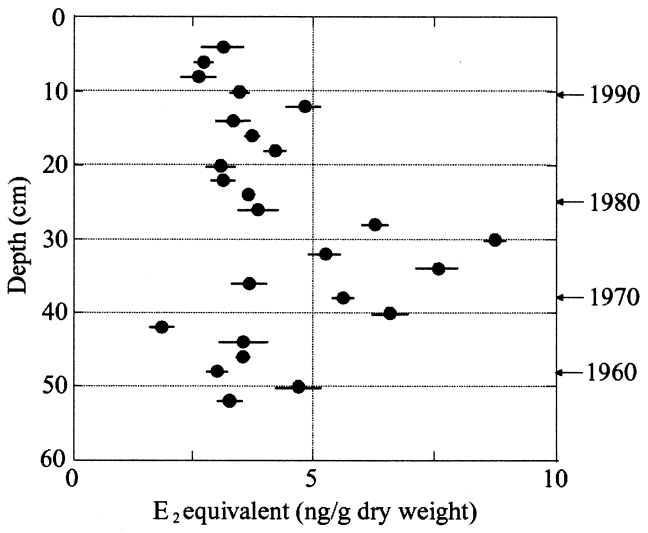

Fig. 6. Vertical profile of estrogenic activities of a sediment core extract from Tokyo Bay. The sampling station is number 39 in Figure 1.

comparable with previously reported concentrations up to 17 ng/L (Xiao *et al.* 2001) in the River Thames in a relatively crowded region. The results might indicate that freshwater that contains effluent from sewage treatment plants is one of the main sources of estrogens in Tokyo Bay.

Estrogenic Activity of Sediment Core Sample

^{210}Pb and ^{137}Cs data of core sample are shown in Figure 5. Measurements of sediment core detected estrogenic activity from as far back as the 1960s (Figure 6). In another study of vertical profiles of sediment cores from Tokyo Bay, the highest observed concentrations of nonylphenol and octylphenol were those in the parts of the cores dating between 1970 and 1975 (station 45, Isobe *et al.* 2001). Our profile of estrogenic activity in the sediment core was consistent with these results. Contaminants with high K_{ow} values have maximum diffusion rates of 1 mm per month in sediments, and the less hydrophobic compounds such as nonylphenol have lower K_{ow} values and so are more likely to be mobilized by pore water and be distributed through the core (Daniels *et al.* 2000). Thus, hydrophobic and less hydrophobic compounds would be transported in sediment via different transport mechanisms. Endocrine-disrupting chemicals are generally not persistent, and their degradation rates are affected by factors such as the redox status of sediment. Thus, interpretation of sediment core data needs the sedimentation conditions as well. In view of the current high levels of concern with environmental xenoestrogen hazards, the signs of decrease in estrogenic activity in the upper section of the sediment core (above 26–28 cm) are very encouraging findings (Figure 6). Regulations on industrial wastewater in the early 1970s would be one of the reasons for the lower estrogenic activity in the upper section of the sediment core collected from Tokyo Bay (Isobe *et al.* 2001).

The horizontal distributions of estrogenic activity in water and sediment samples obtained in this study suggest that the inner part of Tokyo Bay is characterized by high estrogenic activities on the Tokyo and Kanagawa Prefecture sides compared to those on the Chiba Prefecture side. The vertical distribution of estrogenic activity in core sediment shows that the lower estrogenic activities were detected in the upper section of the sediment core. These findings suggest that the problems of environmental estrogens in Tokyo Bay might be restricted to certain coastal areas and the activity in the Bay would show a tendency to decrease in the last 20 years. Nevertheless, more comprehensible studies must be conducted to corroborate these findings. In view of the toxicity in the aquatic environment, further investigations of environmental estrogens are needed to determine the toxic effects in enclosed waters on aquatic organisms such as fish living in Tokyo Bay, Japan.

Acknowledgments. We thank Dr. M. D. Pons, Institut National de la Sante de la Recherche Medicale, Strasbourg, France, for the MVLN cells. This work was supported by the Integrated Research Program for Effects of Endocrine Disrupters on Agriculture, Forestry and Fisheries and their Action Mechanisms on Domestic Animals and Fishes (ED-03-II-1-1).

References

Allen Y, Matthiessen P, Scott AP, Haworth S, Feist S, Thain JE (1999a) The extent of oestrogenic contamination in the UK estuarine and marine environments–further surveys of flounder. Sci Tot Environ 233:5–20

Allen Y, Scott AP, Matthiessen P, Haworth S, Thain JE, Feist S (1999b) Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthys flesus*. Environ Toxicol Chem 18:1791–1800

Cho SM, Kurihara R, Strüssmann CA, Uozumi M, Yamakawa H, Yamasaki T, Soyano K, Shiraishi H, Morita M, Hashimoto S (2003) Histological abnormalities in the gonads of konoshiro gizzard shad (*Konosirus punctatus*) from coastal areas of Japan. Environ Sci 10:25–36

Daniels WM, House WA, Rae JE, Parker A (2000) The distribution of micro-organic contaminants in river bed-sediment cores. Sci Tot Environ 253:81–92

Demirpence E, Duchesne MJ, Badia E, Gagne D, Pons M (1993) MVLN cells: a bioluminescent MCE-7-derived cell line to study the modulation of estrogenic activity. J Steroid Biochem Mol Biol 46:355–364

Desbrow C, Routledge EJ, Brighty GC, Sumpter JP, Waldock M (1998) Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and *in vitro* biological screening. Environ Sci Tech 32:1549–1558

Fenet H, Gomez E, Pillon A, Rosain D, Nicolas JC, Casellas C, Balaguer P (2003) Estrogenic activity in water and sediments of a French river: contribution of alkylphenols. Arch Environ Contam Toxicol 44:1–6

Gray MA, Metcalfe CD (1997) Induction of testis-ova in Japanese medaka *(Oryzias latipes)* exposed to *p*-nonylphenol. Environ Toxicol Chem 16:1082–1086

Harries JE, Sheahan DA, Jobling S, Matthiessen P, Neall P, Sumpter JP, Tylor T, Zaman N (1997) Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. Environ Toxicol Chem 16:534–542

Hashimoto S, Azuma T, Otsuki A (1998a) Distribution, sources and stability of haloacetic acids in Tokyo Bay, Japan. Environ Toxicol Chem 17:798–805

Hashimoto S, Bessho H, Sato K, Hara A, Fujita K (1998b) Vitellogenin in wild male flounder, *Pleuronectes yokohamae*, in Tokyo Bay, Japan. Jpn J Environ Toxicol 1:75–85

Hashimoto S, Bessho H, Hara A, Nakamura M, Iguchi T, Fujita K (2000) Elevated serum vitellogenin levels and gonadal abnormalities in wild male flounder (*Pleuronectes yokohamae*) from Tokyo Bay, Japan. Mar Environ Res 49:37–53

Hashimoto S, Gojo K, Hikota S, Sendai N, Otsuki A (1999) Nitrous oxide emissions from coastal waters in Tokyo Bay. Mar Environ Res 47:213–223

Isobe T, Nishiyama H, Nakashima A, Takada H (2001) Distribution and behavior of nonylphenol, octylphenol, and nonylphenol monoethoxylate in Tokyo metropolitan area: their association with aquatic particles and sedimentary distributions. Environ Sci Technol 35:1041–1049

Jobling S, Sumpter JP (1993) Detergent components in sewage effluent are weakly estrogenic to fish: an *in vitro* study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aquat Toxicol 27:361–372

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 34:3568–3573

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

King P, Kennedy H, Newton PP, Jickells TD, Brand T, Calvert S, Cauwet G, Etcheber H, Head B, Khripounoff A, Manighetti B, Miquel JC (1998) Analysis of total and organic carbon and total nitrogen in settling oceanic particles and a marine sediment: an interlaboratory comparison. Mar Chem 60:203–216

Kirk LA, Tyler CR, Lye CM, Sumpter JP (2002) Changes in estrogenic and androgenic activities at different stages of treatment in wastewater treatment works. Environ Toxicol Chem 21:972–979

Koh CH, Khim JS, Villeneuve DL, Kannan K, Giesy JP (2002) Analysis of trace organic contaminants in sediment, pore water, and water samples from Onsan Bay, Korea: instrumental analysis and *in vitro* gene expression assay. Environ Toxicol Chem 21:1796–1803

Körner W, Spengler P, Bolz U, Schuller W, Hanf V, Metzger JW (2001) Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 2. Biological analysis. Environ Toxicol Chem 20:2142–2151

Lye CM, Frid CLJ, Gill ME, Cooper DW, Jones DM (1999) Estrogenic alkylphenols in fish tissues, sediments, and waters from the U.K. Tyne and Tees estuaries. Environ Sci Technol 33:1009–1014

Minier C, Levy F, Rabel D, Bocquene G, Godefroy D, Burgeot T, Leboulenger F (2000) Flounder health status in the Seine Bay: a multibiomarker study. Mar Environ Res 50:373–377

Murk AJ, Legler J, van Lipzig MMH, Meerman JHN, Belfroid AC, Spenkelink A, van der Burg B, Rijs GBJ, Vethaak D (2002) Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environ Toxicol Chem 21:16–23

Oberdörster E, Cheek AO (2001) Gender benders at the beach: endocrine disruption in marine and estuarine organisms. Environ Toxicol Chem 20:23–36

Sanada Y, Sato F, Kumata H, Takada H, Yamamoto A, Kato Y, Ueno T (1999) Chikyukagaku (Geochemistry) 33:123–138 (in Japanese)

Simpson MG, Parry M, Kleinkauf A, Swarbreck D, Walker P, Leah RT (2000) Pathology of the liver, kidney and gonad of flounder (*Platichthys flesus*) from a UK estuary impacted by endocrine disrupting chemicals. Mar Environ Res 50:283–287

Snyder SA, Keith TL, Verbrugge DA, Snyder EM, Gross TS, Kannan K, Giesy JP (1999) Analytical methods for detection of selected estrogenic compounds in aqueous mixtures. Environ Sci Technol 33:2814–2820

Staples CA, Weeks J, Hall J, Naylor C (1998) Evaluation of aquatic toxicity and bioaccumulation of C_8- and C_9-alkylphenol ethoxylates. Environ Toxicol Chem 17:2470–2480

Tsuda T, Suga K, Kaneda E, Ohsuga M (2000) Determination of 4-nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate and other alkylphenols in fish and shellfish by high-performance liquid chromatography with fluorescence detection. J Chromatogr B 746:305–309

van Aerle R, Nolan M, Jobling S, Christiansen LB, Sumpter JP, Tyler CR (2001) Sexual disruption in a second species of wild cyprinid fish (the gudgeon, *Gobio gobio*) in United Kingdom freshwaters. Environ Toxicol Chem 20:2841–2847

Xiao X, McCalley D, McEvoy J (2001) Analysis of estrogens in river water and effluents using solid-phase extraction and gas chromatography-negative chemical ionization mass spectrometry of the pentafluorobenzoyl derivatives. J Chromatogr A 923:195–204

Yamagishi T, Hashimoto S, Kanai M, Otsuki A (1997) Identification and determination of polyoxyethylene nonionic surfactants by HPLC/atmospheric pressure chemical ionization-MS, and its application to river water samples. Bunseki Kagaku 46:537–547 (in Japanese)