TIME TREND STUDIES ON PBDEs CONTAMINATION IN JAPANESE SEA BASS AND GREY MULLET FROM OSAKA BAY IN JAPAN (from 1986 to Present)

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives for many applications such as television sets, computers, radios, textiles, new synthetic building materials and car etc. There is growing evidence that the large amounts of PBDEs in the environment are due to released during the manufacturing of these chemicals or consumer products containing these[1-3]. In addition, there is sufficient evidence that the incineration of consumer products containing such flame-retardant chemicals results in the formation of polybrominated dibenzo-p-dioxins and –furans[4, 5]. These chemicals, as well as the PBDEs, have been found to occur throughout the environment. And the intake of these contaminants from food, air and water is suspected to be the primary route of human.

The PBDEs were first discovered in pike, eel and sea trout samples from Sweden in 1981 [6]. Since then, many researchers in Canada and in European countries, have confirmed the presence of PBDEs in various environmental samples, detecting primary the lower brominated isomers such as tetra- and penta- BDEs[7-10]. However, little is known about the pollution of environmental samples by brominated flame-retardants in Japan[11,12].

In this study, we investigated that the time-dependent alteration of PBDEs contamination observed in the muscle of Japanese sea bass and grey mullet as the long-term stock-fish samples from 1986 to present, which was collected from Osaka Bay and the mouse of Yamato River flowing to Osaka Bay, respectively. Further, it was also surveyed that the pollution levels in nine sediment samples collected from the coastal area of Osaka Bay.

Materials and Methods

1) Samples

Japanese sea bass and grey mullet were collected from Osaka Bay and the mouth of Yamato River flowing into Osaka Bay, respectively. After dissecting muscle from the fishes, the samples were stored at –80 °C during 1986-1999. The surface sediment samples were collected from 9 points around the coastal area of Osaka Bay in 1999.

2) Analytical method

In this study, the isotope dilution methods typically used to quantify dioxins in environmental samples was for the analysis of PBDEs. Quantification of PBDE congeners was performed by the method of relative calibration curves using five different $^{13}C_{12}$-labelled BDE isomers (2,2',4,4'-TeBDE(#47), 2,2',4,4'5-PeBDE(#99), 2,2',4,4'6-PeBDE(#100), 2,2',4,4'5, 5'-HxBDE(#153),

2,2',4,4'5,6'-HxBDE(#154) purchased from Wellington Laboratories (Ontario, Canada) and twenty seven unlabelled native standards (2,2',4'-TriBDE (#17), 2,3',4'-TriBDE (#25), 2,4,4'-TriBDE (#28), 2,4,6-TriBDE (#30), 2,4,6'-TriBDE (#32), 2,3,4-TriBDE (#33), 3,3',4-TriBDE (#35), 3,4,4'-TriBDE (#37), 2,2',4,4'-TeBDE(#47), 2,2',4,5'-TeBDE(#49), 2,3',4,4'-TeBDE(#66), 2,3',4',6-TeBDE(#71), 2,4,4'6-TeBDE(#75), 3,3',4,4'-TeBDE(#77), 2,2',3,4,4'-PeBDE(#85), 2,2',4,4'5-PeBDE(#99), 2,2',4,4'6-PeBDE(#100), 2,3,3',4,4'-PeBDE(#105), 2,3,4,5,6-PeBDE(#116), 2,3',4,4'6-PeBDE(#119), 3,3',4,4'5-PeBDE(#126), 2,2',3,4,4',5'-HxBDE(#138), 2,2',3,4,4',6'-HxBDE(#140), 2,2',4,4'5, 5'-HxBDE(#153), 2,2',4,4'5, 6'-HxBDE(#154), 2,2',4,4'6, 6'-HxBDE(#155), 2,3,4,4'5,6-HxBDE(#166) purchased from Wellington Laboratories and Cambridge Isotope Laboratories (MA, USA).

Sample preparation for analysis of fatty tissues included 10 g of the fish homogenate for five fish samples per year and 2 ng each of the internal $^{13}C_{12}$-labelled BDE standards was performed. Each sample was saponified by 150 ml of 1 mol/L KOH/EtOH containing 10% H_2O for 2 hr with shaking. The PBDEs were partitioned and extracted with 120ml of n-hexane. The lipid content of each sample was determined by the method of Haglund et al[2]. While, 10 g of the sediments was also used for the analysis of PBDEs; the sample was extracted with 250 ml toluene for 5 hr under reflux. Then, after filtration, the extract was cleaned up on the multi-layer and subsequent active carbon dispersed silica-gel column described as below.

The purification method for the PBDEs in fish and sediment sample was the multi-layer column for dioxins purification developed by Ohta et al[12]. After first elution with n-hexane (180 ml), the PBDEs in the extracts were cleaned up on a multi-layer column containing Na_2SO_4 (2.0 g), 10% (w/w) $AgNO_3$-silica (6.0 g), silica (0.9 g), 22% (w/w) H_2SO_4-silica (6.0 g), 44% (w/w) H_2SO_4-silica (4.5 g), silica (0.9 g), and 2% (w/w) KOH-silica (3.0 g), silica (0.9 g). Second eluent was 10% methylene chloride/n-hexane (220 ml). The eluate was concentrated and purified by a active carbon dispersed silica-gel column (Kanto chemicals Co. Inc.) with a first eluent of 25% methylene chloride/n-hexane (200 ml). The purified extract was dissolved in 20 µl of n-nonane and analyzed for PBDEs by the use of HP6890 GC-JEOL JMS700 MS (HRGC-HRMS) at high-resolution condition (R=5000) in EI-SIM mode. The sample extract was analyzed on a Supelco SPB-5 (30 m x 0.32 mm, 0.25 m film thickness) with the rate of the heating condition as follows; held for 2min at 120 programmed to 215 °C at 10 °C /min, to 270 °C at 3 °C /min, and to 310 °C at 10 °C /min, finally held for 10 min at 310 °C. The column was connected directly to the ion source of the mass spectrometer (interface temperature 260°C). Sample introduction was performed by splitless injection (injection temperature 260°C, 1 min splitless time) of a 2-µl aliquat of the sample extract. The ion source was operated in the electron ionization (EI, 38 eV, 600 µA, 260°C) mode. The 504.9696-u fragmention of perfluorokerosene was used as the lock-mass. As the conditions of quality assurance/quality control for analytical data, PBDEs isomers were identified by comparing the retention times and mass spectra with those of authentic standards. The concentrations of PBDE isomers were corrected with the recoveries of their respective ^{13}C-internal standards. The samples, which exhibited high recovery in the range of 70 to 110% of the respective internal standards, were finally used for data collection. The PBDE analysis was performed twice for each sample.

Results and discussion

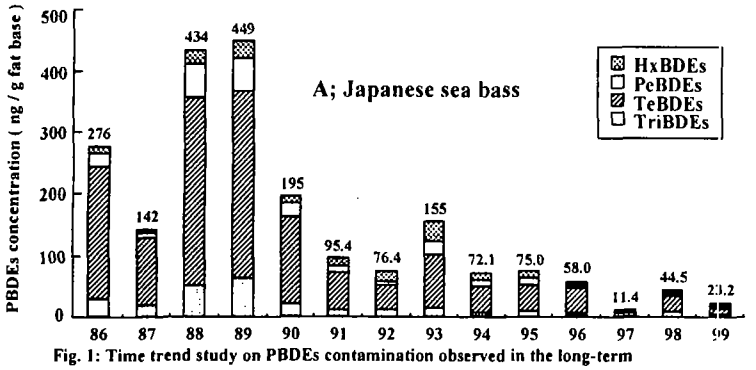

Fig. 1: Time trend study on PBDEs contamination observed in the long-term stock-fish samples (Japanese sea bass; 1986~1999) collected from Osaka Bay

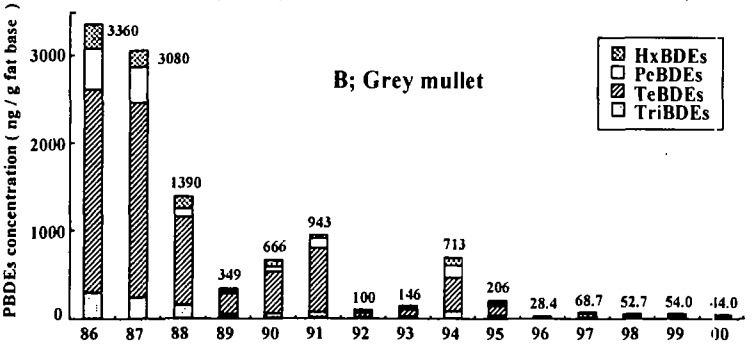

Fig. 2: Time trend study on PBDEs contamination observed in the long-term stock-fish samples (Grey mullet; 1986~2000) collected from the mouse of Yamato River flowing into Osaka Bay

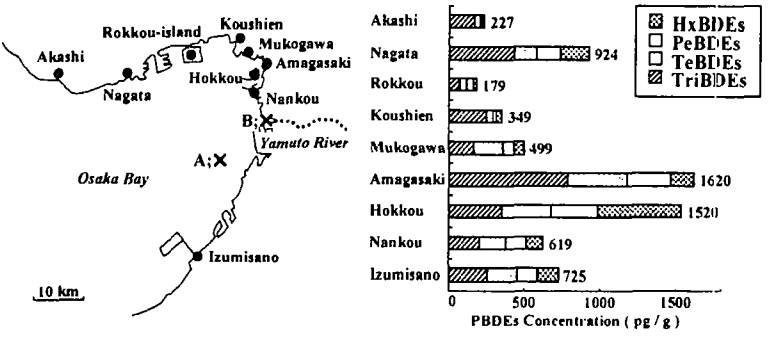

Fig. 3: Levels of PBDEs in surface sediments collected from the coastal area of Osaka Bay
Closed circle and × indicated as sampling points of sediment and fish, respectively

It was surveyed that PBDEs pollution in fishes and sediments at Osaka district as second big city in Japan, where tremendous amount of waste water from many chemical and electric factories and over six million people has been flown into Osaka Bay. As shown in Fig. 1, the time-dependent alteration of PBDEs contamination observed in the long-term stock-fish samples (Japanese sea bass; 1986-1999) from Osaka Bay was investigated. A remarkable pollution was observed in the samples of 1988 and 1989, showing over 400 ng/g fat bases. However, It was found that such accumulation levels gradually decreased from 1990, and the accumulation level in the sample of 1999 reduced to about 5 %, comparing with that of 1989 as the maximum level. Similar tendency was also observed in the samples of Grey mullet, collected at the mouse of Yamato River. Further, the contribution ratio of the PBDE isomers for total concentration in Japanese sea bass (1989 vs. 1999) and grey mullet (1987 vs. 1999) were compared. In the sample of Japanese sea bass of 1989,

the isomer ratios were higher in the order, 2,2',4, 4'-TeBDE (52.8%), 2,4,4'/2',3,4-TriBDE (11.1%), 2,3',4',6-TeBDE (8.0%), 2,3',4,4'-TeBDE (7.0%) and 2,2',4,4',5-PeBDE, whereas the order in the samples of 1999 was 2,2',4,4'-TeBDE (43.4%), 2,2',4,4',5,6'-HxBDE (13.1%), 2,4,4'/2',3,4-TriBDE (7.0%), 2,2',4,4',6-TeBDE (6.5%) and 2,2',4,4',6,6'-HxBDE (6.4%). Similar phenomenon was also recognized by the comparative studies of grey mullet of 1987 and 1999. Thus, the isomer ratios of HxBDE congeners were relatively increased with time alteration. The above observation was supported that PeBDEs congener prohibited applying for the industrial materials in 1990, and DeBDE consumption also decreased year after year in Japan (data not shown). Luross et al[7] reported that PBDEs level in the lake trout from the Great Lake increased with time between 1978 and 1998. It was also observed that PBDEs contamination in sediment cores dated by measuring the isotopes ^{210}Pb and ^{137}Cs from different locations in Europe has a tendency to increase[13]. Therefore, it was estimated that the actual status of PBDEs pollution in Japan was fairy different from that in Canada and European countries. Fig. 3 shows the levels of PBDEs in the surface sediments collected from 9 points around the coastal area of Osaka Bay in Japan. It was apparent that high pollution by PBDEs in sediments from the area of Amagasaki and Hokkou where a lot of factories stand close together and particularly density populated. When the distributions of each PBDE congener were compared, interestingly, the 3,3', 4 -TriBDE and 2,2',4, 4'-TeBDE were the predominant isomers observed in almost samples analyzed.

Hereafter, we plan to clarify the environmental transport and fate of PBDE congeners in Japan.

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