HEXABROMOCYCLODODECANES IN MARINE PRODUCTS COLLECTED FROM FOUR REGIONS OF JAPAN

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Introduction

The Japan domestic consumption of hexabromocyclododecane (HBCDs) has recently increased as it is used to replacce or supplement other brominated flame retardants (BFRs). The demand in 2004 was reported to be 35,000 tons for tetrabromobisphenol A (TBBPA), 2,000 tons for decabrominated diphenyl ether (OCDE) and 2,600 tons for HBCDs. HBCDs are extraordinarily residual and accumulative in aqueous mammals, however, there is still little information about the extent of human exposure. At Dioxin 2007, we reported that the Japanese populace would be exposed to HBCDs mostly via fish among the market basket food group samples investigated [1)]. Therefore, it is critical to clarify the status of seafood pollution by HBCDs. We determined the levels of α-, β-, and γ-HBCDs in fish (natural feeding habitat) collected from four regions of Japan and compared them with the previously reported levels of TBBPA and PBDEs[2)], from which the harmful brominated dioxins could be generated during the formulation process of BFRs and when recycled plastic waste containing BFRs is burned.

Materials and Methods

Fish samples: Forty-three fish samples belonging to 33 kinds having natural feeding habitats were collected from four regions of Japan (Kyushu, Seto Inland Sea, Nagoya and Tohoku) between 2004 and 2005. Each of homogenized fish sample was prepared as to weigh 200-300 g with edible parts of single or plural fish caught at the same time and location. Samples were stored below -20 ^{o} C until analysis.

Sample preparation: Five grams of a homogenized sample with an addition of $^{13}C_{12}$ –α-,β- and γ-HBCD were extracted twice with 20mL of dichloromethane (DCM) using a POLYTRON®. The extracts were dried over anhydrous sodium sulfate and concentrated. The residue was dissolved in 10% DCM/n-hexane and was treated twice with 5mL of sulfuric acid. After centrifuging at 2000 rpm, the upper hexane layer was collected and evaporated. The residue was dissolved in 0.2mL of acetone, and half of this solution was subjected to GPC. HBCD was fractionated over 12 to 14 min after large molecules such as crude fatty acids were eluted in 10 to 12 min. The fraction was re-purified with a cartridge mini-column (Varian BOND ELUT-PSA, 500mg) and then was reconstituted to 50μL using methanol. For determination of HBCDs, a Waters Quatro Micro API was used in the ESI negative operation mode. The HPLC column was a 150mm x 2.1mm i.d. 5μ Inertsil ODS-3 (GL-Science, Tokyo). The detection limit of both α- and γ-HBCD was 0.02 ng/g ww. That for β-HBCD was 0.01 ng/g ww.

Results

HBCD levels in fish collected from four regions of Japan

In this study, fish that have natural feeding habitats and are available at market stores were investigated for HBCD levels. Most of the fish samples in this study belonged to a relatively small fish category, and the others were mollusks (cuttlefish and octopus). From the view point of guaranteeing a precise analysis, the recoveries of the objectives and the negative controls were checked though the protocol. The recoveries of spiked ^{13}C-labeled HBCDs were obtained at above ca 40 %.

The fish species differed among the regions. However, every fish monitored in this study was familiar to the Japanese populace.

Figure 1 showed the distribution of the levels of ΣHBCDs and ΣPBDEs in the samples for each region.

The median levels of detected ΣHBCDs were 0.04ng/g ww (1.4ng/g lw) for the Kyushu (K) region ＜0.06 ng/g ww, (11 ng/g lw) for the Seto (S) region ＜2.9 ng/g ww (150 ng/g lw) for the Nagoya (N) region＜3.6 ng/g ww (170 ng/g lw) for the Tohoku (T) region (Table 1). In the K region, the extent of HBCD pollution is generally considered to be small. In the S region, one extremely polluted sample (small herring) was found and in the N region, three polluted samples (two seer fish and sea bass) were found. In those fish samples, γ-HBCD was dominantly or highly detected more than α- and β-HBCDs. Γ-HBCD is reported to be the main ingredient in HBCD formula (83.9 %)[3], while α- and β-HBCD are reported to be minor ingredients at 8.5% and 7.9%, respectively. Therefore, the highly polluted fish sample would have been intensively influenced by a neighboring heavy pollution source. For the N and T regions, the detection rates of ΣHBCDs in fish samples were both 100 %, and the level range of ΣHBCDs were higher than in the K and S regions.

On the other hand, referring to our previous report, the median levels of ΣPBDEs were 0.10 ng/g ww for the K region ＜ or ＝ 0.11 ng/g ww for the S region ＜0.33 ng/g ww for the N region and ＜0.37 ng/g ww for the T region. With the exception of the T region of which the sample number (n=5) was small, the difference in the medians of ΣHBCDs and ΣPBDEs between the K and N regions were 73 and 3.3 times, respectively. Between the S and N regions, these differences were 48 and 3 times. Therefore, on near coast of Japan, HBCD pollution of fish is considered to be increasing, particularly in the N region, which is a commercialized and industrial area. Due to their large bioconcentration factor, HBCDs should be received more attention than PBDEs and TBPPA.

Statistical analysis of ΣHBCDs in all the fish

Figure 2 shows the correlations among ΣHBCDs, ΣPBDEs and fat content in 39 samples excepting 4 samples considered to be affected by a nearby pollution source. The Spearman's rank correlation coefficients were 0.9809 between ΣHBCDs and ΣPBDEs and 0.9432 between ΣHBCDs and fat content. By a one-side test of both coefficients, it was estimated that positive correlations would exist among them at $P<0.01$. However, between TBBPA and ΣHBCDs there was no correlation (data not shown).

Discussions

An U.K. research report on the levels of HBCDs in the blubber of harbor porpoises demonstrated that there is a time trend of pollution from PBDEs to HBCDs[4]. However, there are few data on the pollution trend in Japan, where the domestic consumption of ΣHBCDs was announced as 2,600 tons in 2004 exceeding that of DeBDE (2,000 tons). On the other hand, PBDEs have been identified as related chemicals of polybrominated dioxins (PBDDs/DFs), because they could be generated unintentionally during the formulation of PBDEs or the burning of plastics and textiles to which PBDEs are added. The transfer of PBDEs to HBCDs in BFR use might contribute to the decrease of the emission of PBDDs/DFs into the environment. For that purpose, HBCDs would be acceptable. Recently, the non-observed adverse effect level (NOAEL) of HBCD has been reported to be 10.2 mg/kg/day, which means that the use of HBCDs is safe at present. However, HBCDs are too accumulative to allow entering the environment without further attention. Considering the matter of epigenesis in humans, intensive HBCD pollution in fish should also be avoided and it is necessary to continue the monitoring of

HBCDs in fish and food as is done for other harmful residual chemicals.

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Table 1 HBCD levels of marine products from four regions of Japan

Region		Fat(%)	α-HBCD ng/g ww	β-HBCD ng/g ww	γ-HBCD ng/g ww	ΣHBCDs ng/g ww	ΣHBCDs ng/g lw
K	Median	1.2	0.04	ND	ND	0.04	1.4
	Max	20.4	0.73	ND	0.27	1.0	40
	Min	0.2	0.00	ND	ND	ND	ND
S	Median	0.9	0.05	ND	ND	0.06	11
	Max	12.7	18.3	2.4	57	77	1700
	Min	0.3	0.00	ND	ND	ND	ND
N	Median	1.3	1.94	ND	1.1	2.9	153
	Max	13.7	14	0.35	18	24	3300
	Min	0.3	0.07	ND	0.08	0.15	12
T	Median	2.4	2.1	ND	1.5	3.6	170
	Max	3.4	3.2	ND	2.3	5.5	230
	Min	1.3	1.2	ND	0.80	2.2	110

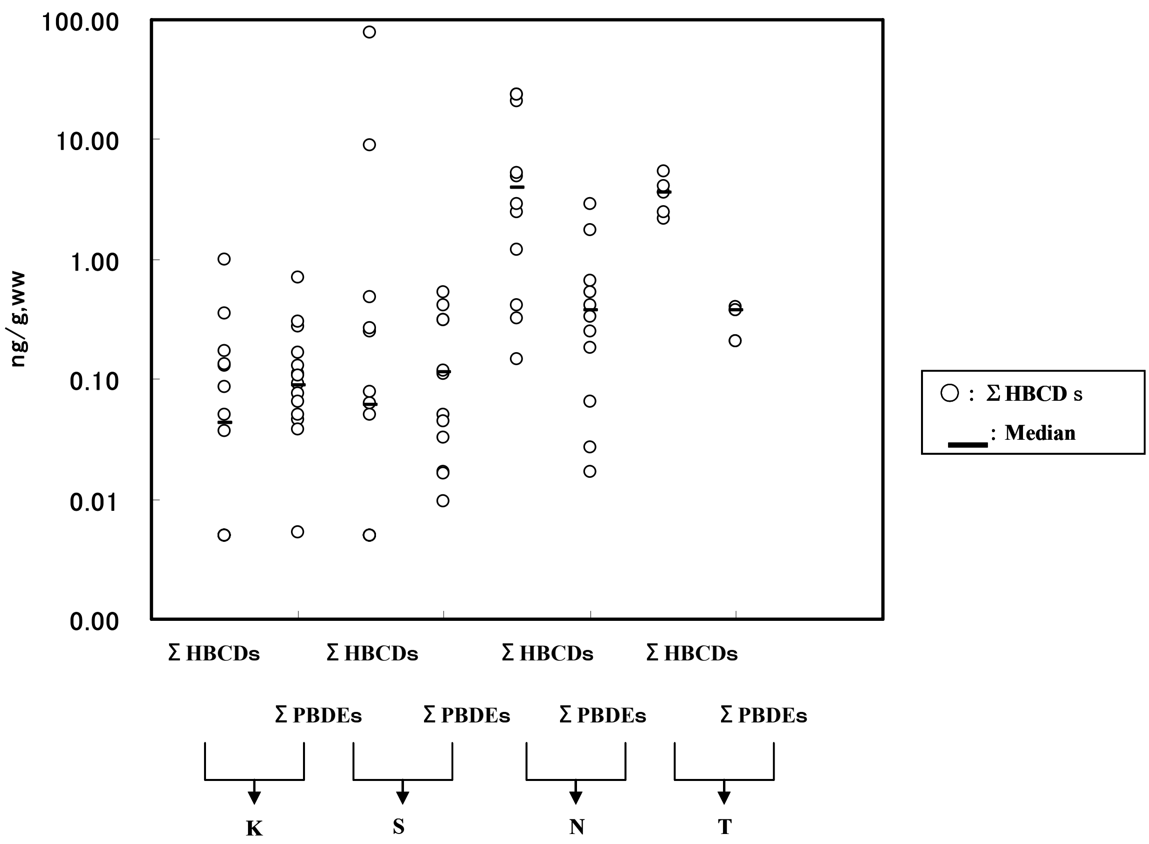

Fig.1 Distribution of ΣHBCDs and ΣPBDEs in marine products from four regions of Japan

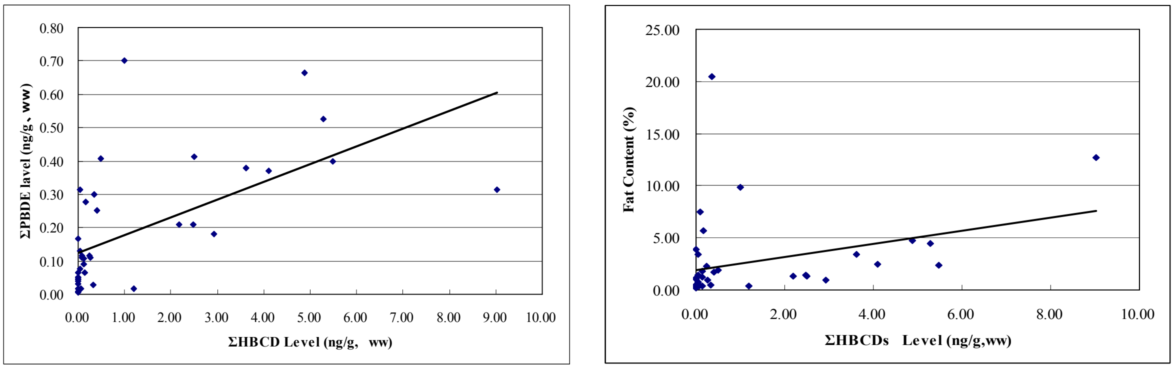

Fig.2 Correlations between ΣHBCDs and ΣPBDEs and between ΣHBCDs and fat content (%)in marine products in Japan