DIETARY EXPOSURE TO HEXABROMOCYCLODODECANES IN JAPAN

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Introduction

The economic benefit of adding various flame retardants to polyurethane products, such as home insulation, curtains, and plastics in PCs and other electrical appliances, has been proved. However, the disposal of these products has led to environmental pollution, and they pose a potential risk to human health. Hexabromocyclododecanes (HBCDs) are brominated flame retardants, and their high residuality in the environment and marine wildlife are problematic. Therefore, HBCDs, PBDEs, and PBBs are specified substances under the European Union Restriction of Hazardous Substances Directive and the Japanese Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., (2004), and must be monitored. The consumption of HBCDs in Japan in 2003 was about 2400 t/year, which is close to that of decabromodiphenyl ether (2000 t/year), the only PBDE currently used in Japan. Human exposure to HBCDs through dietary intake has been studied by European, American, Chinese, and Japanese researchers. We have previously estimated the Japanese dietary intake of HBCDs based on the HBCD content of fish collected from the coast near Japan (1). In this paper, we present an analysis of the Japanese dietary intake of HBCDs by region and year as determined by an alternative method called total diet study (TDS). In the TDS, 13 food groups were used raw, or were prepared simply by boiling or grilling, depending on the food. The food was collected from 4 regions in 2007, 2008 and 2010 for HBCD isomeric analysis. Recently, the importance of HBCD enantiomeric analysis has been demonstrated (2,3). Therefore, the enantiomer fractions (EF) of fish which had a high HBCD content were determined.
In this paper, we report the geographical changes in the average daily intake (EDI) of ΣHBCDs of the Japanese population and the enantiomeric profile of selected fish samples.

Materials and Methods

Food samples: The total diet (TD) food samples were prepared independently from 2007 to 2010 at four public laboratories located in Sendai City, Miyagi Prefecture, population ~1,000,000; Saitama City, Saitama Prefecture, population ~12,000,000; Osaka City, Osaka Prefecture, population ~8,800,000; and Fukuoka City, Fukuoka Prefecture, population ~1,450,000. The food samples were taken from 13 groups (I-XIII). However, the total number in one set of the TD food samples was 16, because additional samples were added from the X', XI' and XII' groups, which were prepared with alternative foods. More than 100 food items from 99 categories of foods commonly consumed in Japan were purchased for the preparation of food samples. The number of food items in each food group (from I to XIII) are shown in Table 1, and were determined based on actual Japanese nutrition surveillance data. In our study, group XIIII was not analyzed. Eight fish samples were collected from nearby markets for the determination of EF values.
Chemicals: Pesticide residue analysis grade dichloromethane (DCM), *n*-hexane (Hx), acetone and anhydrous sodium sulfate, (Kanto Chemical Co. Ltd., Tokyo), and reagent grade sodium chloride were used. The anhydrous sodium sulfate and sodium chloride were baked at 600 °C before use to reduce contamination. Native and $^{13}C_{12}$-labeled standards of α-, β- and γ-HBCD were purchased from Wellington Laboratories Inc. (Guelph, ON). LC/MS analysis grade methanol and distilled water were used (Kanto Chemical Co. Ltd., Tokyo), and the 44% sulfuric acid-impregnated silica gel was dioxin analysis grade (Wako Pure Chemical Industries Ltd., Tokyo).
Analysis of HBCDs: A clean-up spike of distilled water (5 mL), methanol (20 mL), and $^{13}C_{12}$-labeled HBCDs (1 ng) was added to each sample (5 g), and the mixture was homogenized. The mixture was filtered and the filtrate was collected in a 300 mL separating funnel. The residues on the filter were re-homogenized and filtered using methanol/10% DCM/Hx (20 mL, 1:1, v/v) and 10% DCM/Hx (20 mL). 5% NaCl (120 mL) was added to the collected filtrates and shaken. The organic layer was poured through anhydrous sodium sulfate into a 200 mL flask. The water layer was re-extracted twice more with 10% DCM/Hx. The collected extracts were concentrated and adjusted to 10 mL with acetone/cyclohexane (3:7, v/v). A portion of the extracts was volumetrically loaded onto a gel permeation column and fractionated at a flow rate of 5 mL/min with a mobile phase of acetone/cyclohexane. The HBCD fraction was re-purified through a mini column packed with 44% sulfuric acid impregnated silica gel prior to analysis by LC/MS/MS (Table 2). The detection limits of α-and γ-HBCD were 0.02 pg/g ww that for β-HBCD was 0.01 ng/g ww.

Table 1 Average composition of diet (2007).

Group	Foods in group	Wt, g/day
I	Rice and rice products,	343.9
II	Grains, seeds and potatoes	169.1
III	Sugar and confectionary	32.2
IV	Oils	10.3
V	Legume and legume products	59.2
VI	Fruits	125.7
VII	Carrots and green leafy vegetables	102.1
VIII	White leafy vegetables, mushrooms and seaweeds	208
IX	Beverages	601.5
X	Fish and fish products	84.1
XI	Meat and eggs	114.4
XII	Milk and milk products	125
XIII	Seasonings and other products	104.6
XIIII	Water	-
	Total	2080.1

Results and Discussion

Satisfactory recovery of HBCDs was achieved from food group X (fish and marine products) of the 2007 Kyushu TDS sample. HBCDs were detected in all the foods in group X of the TDS samples and the α- and γ- HBCD isomers were the most common. The α-isomer was the most abundant, although where the level of ΣHBCDs was very high, as in the food group X for Kyushu (24.7 ng/g), the γ-isomer was more abundant. We have previously observed this pattern in fish samples (1), and it may arise from a point source such as HBCD-contaminated sludge discharged from industrial facilities.

The mean of the ΣHBCD levels in each of the two food groups X and X' in 2007 was 1.16 ng/g (0.67, 1.64) for Kansai, 1.47 ng/g (0.62, 2.31) for Kanto and 1.71 ng/g (1.19, 2.23) for Kyushu. In 2010, the mean of the ΣHBCDs levels for Kansai, Kanto and Kyushu in each of the two food groups X and X' were 1.58 ng/g (1.27, 1.89) and 15.47 ng/g (6.22, 24.72), respectively. There was a slight increase from 2007 to 2010. The mean of the ΣHBCDs in the food groups X and X' for Tohoku prepared in 2008 was 0.97 ng/g (1.76, 0.17). The intake of HBCDs via food can be calculated by multiplying the level of ΣHBCDs by the average daily consumption per person of each food group. The daily estimated intake of HBCD in 2007 was 1.8 ng/kg bw/day for Kansai, 2.4 ng/kg bw/day for Kanto and 3.1 ng/kg bw/day for Kyushu, when assuming ND (not detected) = 0. These levels are very similar to those we estimated from the analysis of fish samples (Table 3) (1). The average EDI for ΣHBCDs in the three regions was 2.3 ng/kg in 2007 and 9.4 ng/kg in 2010, when assuming ND (not detected) = 0. The EDI of ΣHBCDs in three Japanese regions has on average increased 4.1-fold (Table 4). To clarify which marine item in food group X for Kyushu had the highest ΣHBCD level, the levels of HBCDs in all the food items were determined. Mackerel caught in the sea near Ishikawa Prefecture had the highest level (61.9 ng/g), followed by salmon (9.56 ng/g), and young yellowtail (2.54 ng/g). The Ishikawa Prefecture is located on the coast to the east of Korea and China, however, the pollution source of the mackerel was unclear.

Recently, the value of analyzing enantiomeric HBCD isomers has been demonstrated (4); therefore the EF values((+)E/((+)E+(-)E) of the HBCD enantiomers were also determined for several fish samples. A chiral column was used, and the EF values were obtained from the areas of the LC/MS/MS chromatograms (Table 5). However, there was no evidence of enantiomeric enrichment, in contrast to the pattern observed in human blood (3). This suggests that fish do not selectively metabolize one HBCD enantiomer.

The lowest observed adverse effect level (LOAEL) for HBCDs is 11.2 mg/kg bw/day in rat (4). The LOAEL was divided by a safety factor of 100, thus the tolerable daily intake should be 112 μg/kg bw/day. Therefore, the Japanese dietary intake of HBCDs is unlikely to present a serious threat to human

health. However, it is important to continue environmental monitoring of HBCDs, particularly in seafood, because they are persistent pollutants and may accumulate in the food chain.
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Table 2 LC/MS/MS analysis conditions.

Column	1.GL Sciences, Intertsil ODS-4(150×2.1 mm i.d., 5μm) 2.Marcherey-Nagel, Nucleodex b-PM(200×4.0 mm i.d., 5μm)
Column Temp. & Injection dose,	40℃; 5 μL
Mobile phase & flow rate	1. 10 mM Ammonium acetate:Methanol:Acetonitri＝20:50:30（2min）～（linear gradient, 5min）～0:70:30, 0.2 μL/min 2. Methanol/ water（1：1）with 2 mM ammonium acetate：Methanol/ acetonitrile（3：7）＝50：50(7min) ～（linear gradient, 8min)～20:50:30, 0.5 μL/min
Aquisition mode, ESI negative MRM ; Monitor ions, 653>79、641>79, 639>79,	

Table 3 Estimated dietary intakes for an average Japanese person calculated from the HBCD concentration of fish.

Samples from which daily intake was estimated	Median of samples	EDI (ng/kg bw/day)
Five fish samples (Wild Anguilliformes, two species)	2.09	3.7
Five fish samples (farmed Salmoniformes)	1.29	2.3
30 fish samples (wild Performes, 10 species)	0.75	1.3

Table 4 Estimated intake (ng/kg bw/day) of ΣHBCDs for an average Japanese person, 2007-2010.

Region	2007		2008		2010		2007-2010	
	ND=0	ND=1/2× LOD	ND=0	ND=1/2× LOD	ND=0	ND=1/2× LOD	ND=0	ND=1/2× LOD
Kyushu	3.1	4.2			29.4	30.5	3.1-29.4	4.2-30.5
Kanto	2.4	3.4			3.6	4.7	2.2-3.6	3.4-4.7
Kansai	1.8	3.0			2.4	3.5	1.8-2.4	3.0-3.5
Tohoku			2.2	2.9			2.2	2.9

Table 5 HBCD levels and EF values for several fish samples.

na; not analyze

Fish name	Status	Sampling location	Fat (%)	α-HBCD ng/g	β-HBCD ng/g	γ-HBCD ng/g	Total HBCD ng/g	EF values
Saury 1	natural	Kanto, Japan	17.4	0.58	0.01	0.03	0.62	0.54
Saury 2	salted	Hokkaido, Japan	15.8	0.70	0.01	0.03	0.74	0.47
Salmon 1	salted	Russia, Imported	5.9	1.93	0.01	0.48	2.43	0.52
Salmon 2	salted	North America	5.9	0.13	0.00	0.00	0.13	0.50
Salmon 3	natural	Norway	9.6	0.21	0.00	0.03	0.23	0.66
Mackerel 1	salted	Norway	28.1	0.17	0.00	0.00	0.17	0.50
Mackerel 2	natural	Ishikawa, Japan	na	15.2	0.20	46.5	61.9	0.50
Young yellowtail	natural	Fukuoka, Japan	na	1.19	0.02	1.33	2.54	0.54