EXPOSURE TO HEXABROMOCYCLODODECANES FROM BOXED SUSHI

Sato T[1, 2], Tobiishi K[1], Hori T[1], Tsutsumi T[3], Matsui T[2], Akiyama H[3]

[1]Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano, Dazaifu, Fukuoka 818-0135, Japan; t-satou@fihes.pref.fukuoka.jp; [2]Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School of Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan; [3]National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan

Introduction

Flame retardants are widely used as additives in high molecular weight organic materials such as plastics, rubbers, and fibers. Among these additives, halogenated flame retardants are widely used in plastic products because of their low cost and excellent flame retardant effect. However, there have been serious concerns about hexabromocyclododecanes (HBCDs), a brominated flame retardant, particularly regarding its environmental persistence and/or health degradation through long-term bioaccumulation. HBCDs are thus internationally regulated by Annex A of the Stockholm Convention on Persistent Organic Pollutants; their manufacture, use, and import/export are highly prohibited.

Thus far, there have been many reports on HBCD contents in foods[1-5]. Previously we reported the estimated intake of HBCDs in total diet study based on a market basket method, and mentioned their unusual accumulation in seafood, suggesting that humans may suffer from HBCD exposure by eating seafood diets[6, 7].

Ready-made meals are currently in demand in Japan, making it difficult to estimate HBCD intake by a conventional total diet study, since the estimation is based on average food consumption for a general population according to the National Health and Nutrition Survey. Thus, in the present study we investigated HBCD exposure in boxed sushi, a typical ready-made raw seafood dish, using LC-tandem MS analysis.

Materials and methods

Sample: from June to September 2020, 25 boxed sushi meals (including 3 seafood bowls) that were each sold as a single serving at supermarkets and commercial facilities were subjected to a fact-finding investigation in this study. After the seafood portion was separated from the remaining portion (the nonseafood portion: rice, etc.) from a boxed sushi meal, individual portions of each type were homogenized using a food processor or a hand mixer. The seafood portions included fish, fish eggs, and shellfish, including shrimp, squid, salmon, tuna, salmon roe, scallops, and so on. Edible parts were used for this study.

Materials: α-, β-, γ-HBCD standard; $^{13}C_{12}$-labeled α-, β-, γ-HBCD standard for cleanup spike; and γ-HBCD-d_{18} standard for syringe spike were obtained from Wellington Laboratories. Each standard was mixed and diluted with acetonitrile as appropriate.

The following were obtained from Kanto Chemical: acetone, n-hexane, toluene, dichloromethane, and n-nonane for dioxins analysis grade; cyclohexane and distilled water with an n-hexane wash for residual pesticide analysis grade; and acetonitrile and distilled water for LC/MS grade. Fluvalinate standard for residual pesticide analysis grade, sulfuric acid for analysis of poisonous metals, 44% sulfuric acid−impregnated silica gel for dioxins analysis grade, and 1 mol/L ammonium acetate solution for HPLC grade were purchased from Wako Pure Chemical Industries. Diatomaceous earth was ISOLUTE HM-N, manufactured by Biotage.

Analysis of HBCDs: Figure 1 presents the analytical method used in this study. A 10 g sample was mixed with diatomaceous earth (10 g) and lyophilized. After freeze drying, each sample was extracted by an ASE-350 extractor (Thermo Fisher Scientific) using a mixture of n-hexane: dichloromethane (3+1) as extraction solvent with 2 cycles of 10 min at 100°C and 1,500 psi. The recovered extraction solvent was evaporated to 5 mL under a reduced vacuum and diluted in a 20 mL measuring flask to prepare the extract. Then, 1 mL of the extract was measured and concentrated to dryness, and the residue was weighed to measure the fat content (w/w, %).

The test solution was prepared by the following procedure. α-, β-, γ-$^{13}C_{12}$-HBCDs (5 ng) were each spiked to 5 mL of extract. The extract was then treated with sulfuric acid, after which it was washed with distilled water and concentrated to dryness. The residue was redissolved in 5 mL of a mixture of acetone: cyclohexane (3+7) and spiked with 50 μg of fluvalinate as an indicator of elution time in gel permeation chromatography (GPC). Two milliliters of solution was loaded on a GPC column to remove the fat under the following conditions: flow rate, 5 mL/min mobile phase; acetone, cyclohexane (3+7); column, CLNpak EV-G AC + EV-2000 AC (Showa Denko); column temperature, 40°C. The eluate was collected for 20 min immediately after the indicator substance was eluted and evaporated under a reduced vacuum. The residue redissolved in n-hexane was passed through a Pasteur pipette filled with 44% sulfuric acid−impregnated silica gel (1 g) and eluted with 8 mL of 30% dichloromethane/n-hexane. Finally, the eluate was concentrated to dryness under a light stream of nitrogen and redissolved in 0.1 mL of acetonitrile containing γ-HBCD-d_{18} (2 ng) as syringe spike to prepare the test solution.

The HBCD concentrations were determined using a Waters Acquity UPLC H-Class Plus Binary equipped with a Waters Xevo TQ-XS (LC/MS/MS). Details of the operating conditions of the system are shown in Table 1. The limit of detection for each isomer was 10 pg/g (S/N=3) on a wet weight basis.

Results and discussion

It is important to confirm the recovery rates of HBCDs under set ASE extraction conditions. Therefore, the recovery rates of $^{13}C_{12}$-labeled α-, β-, and γ-HBCDs were confirmed without sample matrix. The recovery rates were 82-94%, which were satisfactory values.

Figure 2 shows MRM chromatograms of the HBCDs by LC-tandem MS analysis. No interfering peak was observed, and chromatograms with good peak separation were obtained. The recovery rates of $^{13}C_{12}$-labeled HBCDs added to the extracts of 25 seafood portion samples and 25 nonseafood portion samples were as follows: α-isomer, 85-103% and 89-115%; β-isomer, 81-102% and 79-104%; γ-isomer, 87-113% and 96-120%. These were good results and met the target values (70-120%).

Figure 3 shows the HBCD concentrations found in the seafood portion samples. The concentrations of ΣHBCDs in the seafood portion samples were 33-1,922 pg/g. By isomer, the concentrations were as follows: α-HBCD, 33-1,808 pg/g (detected in all 25 samples); β-HBCD, ND (<10)-12 pg/g (detected in 1 of 25 samples); γ-HBCD, ND (<10)-102 pg/g (detected in 6 of 25 samples). The ratios of α-HBCD concentration to that of ΣHBCDs were 94-100%; α-HBCD was the main component in all the samples. When β-HBCD was detected (Sample 15), α- and γ-HBCDs were also detected at relatively high concentrations. On the other hand, HBCDs were ND (<10 pg/g) in all of the nonseafood samples.

Table 2 shows the results of the measurement of fat contents and the intake of HBCDs per meal. The fat content of the 25 seafood portion samples averaged 6.1% (1.2-11%), and the nonseafood samples averaged 0.5% (<0.1-1.8%). The fat content of the seafood portion was about 12 times that of the nonseafood portion.

The intake of HBCDs per meal was calculated based on the weight of one serving of boxed sushi. In addition, the estimate assumed a value of zero when the concentration was under the detection limit (ND=0). Per meal, the average intake of HBCDs was 34 ng, the median was 22 ng, and the range was 2-190 ng. Samples 14 and 15, which had high concentrations of ΣHBCDs, also had relatively high fat contents. This suggested that seafood fats and oils contained HBCDs, similar to the case with PCBs, which are typical halogenated compounds[8].

Finally, we compared the obtained HBCD value with the health-based guideline value (NOAEL: 10 mg/kg/day divided by an uncertainty factor of 200)[9]. The intake of HBCDs per meal for a person weighing 50 kg is 0.00008 to 0.008% of this value. Even if the boxed sushi with the highest contents of HBCDs per meal was eaten 3 times a day, the ratio of the health-based guideline value would be only 0.022%. We concluded that the risk to human health due to HBCDs exposure from a boxed sushi meal is negligible.

Acknowledgments

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Table 1. LC-tandem MS conditions

LC conditions	Column	Waters Acquity UPLC BEH C18 (2.1×100 mm, 1.7 μm)
	Column temperature	40 ℃
	Injection volume	2 μL
	Mobile phase	A: 2mM Ammonium acetate aqueous solution, B: Acetonitrile A/B(%) : 45/55-8 min-5/95(6 min)-1 min-45/55(5 min)
	Flow rate	0.2 mL/min
MS conditions	Ionization mode	ESI-Negative
	Desolvation temperature	400 ℃
	Capillary voltage	2.0 kV
	Cone voltage	20 V
	Collision energy	20 eV
	Scan type	SRM
	SRM transition	HBCD: 638.6 > 78.9 (quantifier ion), 640.6 > 78.9 (qualifier ion)
		$^{13}C_{12}$-HBCD: 650.7 > 78.9 (quantifier ion), 652.7 > 78.9 (qualifier ion)
		HBCD-d_{18}: 658.7 > 78.9

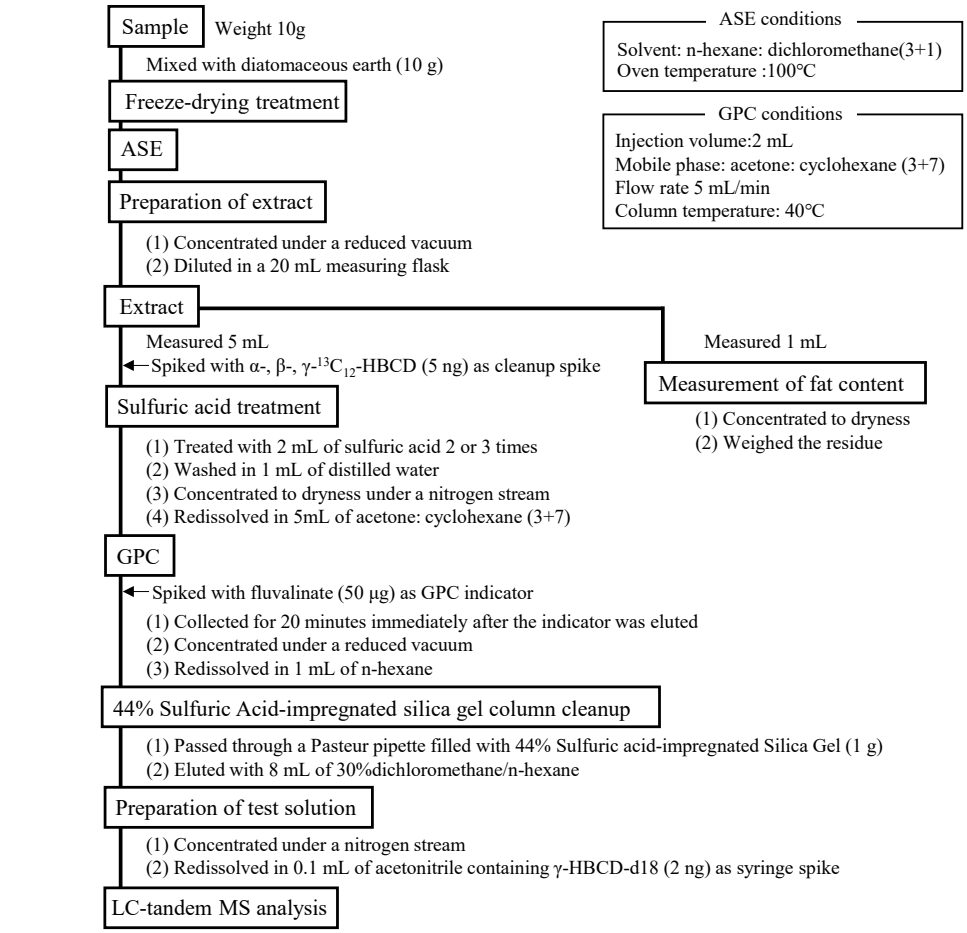

Figure 1. The analytical method for quantifying HBCDs

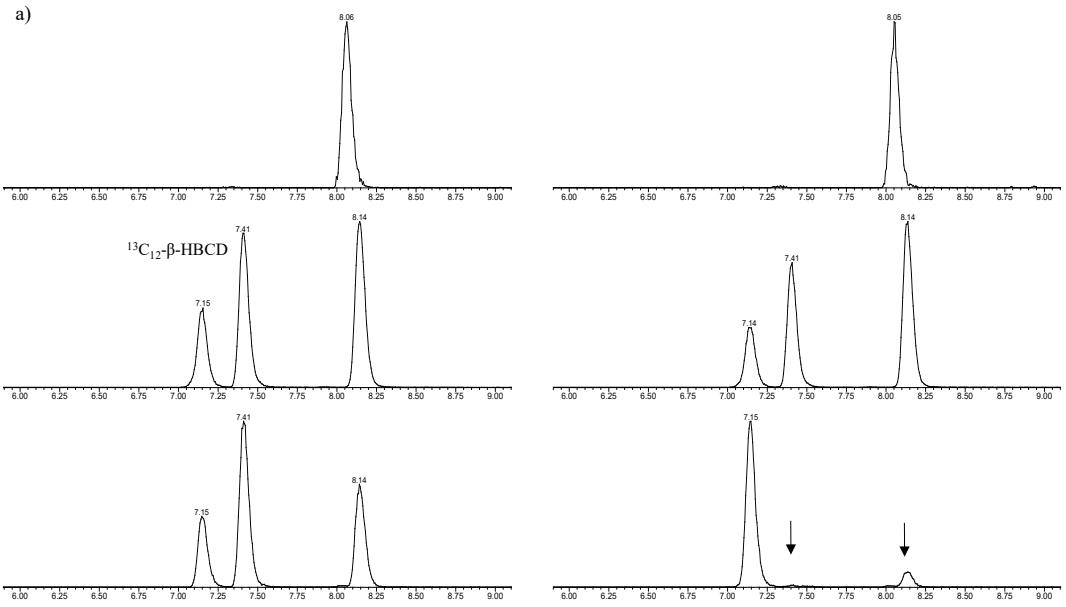

Figure 2. MRM chromatograms of HBCDs
Upper: γ-HBCD-d_{18} (syringe spike), Middle: $^{13}C_{12}$ labeled HBCDs (cleanup spike), Lower: HBCDs
a) Standard solution
b) Seafood portion sample 15

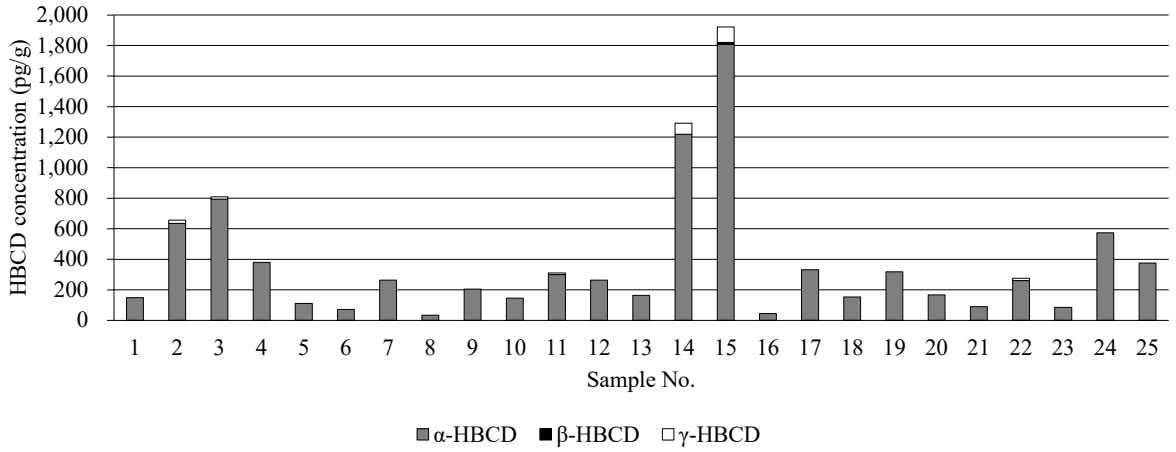

Figure 3. HBCD concentrations in seafood portion samples

Table 2. Fat contents and HBCDs intake

Sample No.	Store	Weight of one serving (g)*		Fat content (%)		HBCDs intake (ng/meal)		
		Seafood portion	Nonseafood portion	Seafood portion	Nonseafood portion	Seafood portion	Nonseafood portion	Total
1	A	66	218	4.9	0.8	10	0	10
2	A	101	226	5.5	0.6	66	0	66
3	B	64	225	11	0.6	52	0	52
4	B	82	199	5.8	0.4	31	0	31
5	C	99	260	4.4	0.8	11	0	11
6	C	59	183	8.3	0.7	4	0	4
7	D	96	176	5.1	0.2	25	0	25
8	D	66	283	4.5	0.3	2	0	2
9	E	95	127	2.1	0.5	19	0	19
10	E	74	215	5.1	0.1	11	0	11
11	F	86	201	9.1	0.4	27	0	27
12	G	89	174	8.8	0.1	23	0	23
13	G	59	202	5.6	0.9	10	0	10
14	H	147	253	8.9	0.3	190	0	190
15	H	87	182	9.3	0.1	167	0	167
16	I	98	269	4.0	0.4	4	0	4
17	I	72	204	7.2	0.5	24	0	24
18	J	80	225	2.7	<0.1	12	0	12
19	J	68	220	2.5	0.5	22	0	22
20	J	143	211	6.4	0.2	24	0	24
21	K	134	214	1.2	0.1	12	0	12
22	K	63	198	5.6	1.8	17	0	17
23	L	69	189	10	0.7	6	0	6
24	L	82	177	5.5	0.2	47	0	47
25	M	82	200	9.1	1.5	31	0	31
Average	-	-	-	6.1	0.5	-	-	34
Minimum	-	-	-	1.2	0.1	-	-	2
Median	-	-	-	5.6	0.5	-	-	22
Maximum	-	-	-	11	1.8	-	-	190

* Average of 3 to 4 identical products

The intake of HBCDs per meal calculated based on the weight of one serving of boxed sushi.
The estimate is assumed to be zero when the concentration was under the detection limit (ND=0).