DETERMINATION OF BROMINATED FLAME RETARDANTS IN FOOD FROM JAPANESE MARKETS

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Introduction

Brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs), have been widely used in plastics and textile coatings throughout the world. The major commercial products made with the most commonly used PBDEs are penta-BDE, octa-BDE and deca-BDE (DeBDE). In Japan, although the use of low-brominated PBDEs has decreased, DeBDE is currently in use. PBDEs are additives to polymers such as polystyrene and are not chemically bound to the polymer. They are therefore easily released into the environment from waste products. It is predicted that, in Japan, the amount of waste Br from the plastics used in electrical appliances will increase until at least 2020 due to the increasing size of TV sets there[1)]. This prediction suggests an urgent need to monitor these brominated compounds and to manage them in waste. For PBBs, the commercial products are mixtures containing hexa-BB, octa-BB, nona-BB, and deca-BB. Products made with PBBs have not been produced in Japan, but PBBs have been detected in environmental samples[2)]. It is suspected that the contaminants in these samples have come from imported products or impurities in other BFRs. Decabromodiphenyl ethane (DBDPE) and bis(2,4,6-tribromophenoxy)ethane (BTBPE) are relatively new brominated flame retardants that came to market in the 1990s as alternatives to DeBDE. There is very little information about their toxicity or contamination levels.

In relation to BFRs, it is problematic that *de novo* synthetic compounds such as polybrominated dibenzo-*p*-dioxins, dibenzofurans (PBDD/DFs), and coplanar polychlorinated/brominated biphenyls (Co-PXBs) have been found in market fish[3)4)] and human samples[5)6)]. Co-PXBs may also be formed from BFRs and have toxicity levels similar to those of Co-PCBs due to their structural similarities.

It is important to investigate the levels of these brominated organic compounds in foods and to estimate their effects on humans. In our previous study, we developed a simultaneous-analysis method for brominated compounds, including new BFRs such as DBDPE and BTBPE[7)]. In the present study, we analyzed BFRs in fish samples and market basket samples in Japan.

Materials and methods

Chemicals

DBDPE, BTBPE, and PBDE analytical standards were purchased from Wellington Laboratories. The PBB analytical standards were purchased from Wellington Laboratories and AccuStandard. Dichloromethane, *n*-hexane, and acetone used for extraction and cleanup were of dioxin analysis grade (Kanto Chemical). Silica gel (Wako Pure Chemical Industries) was heated for 3 h at 130°C. A sulfoxide cartridge column (6 g, 20 g glassware) was purchased from Sigma-Aldrich.

Analytical Methods and Instrumentation

The concentrations of DBDPE, PBDEs, Co-PXBs, and PBBs were determined using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The analytical conditions of HRGC/HRMS are shown in Table 1. HRGC/HRMS analysis was performed on a Micromass Autospec Ultima (Waters) connected to an HP6890 GC (Agilent).

Samples

The fish samples for the analysis of brominated compounds were purchased from fish markets in 3 different regions (Chubu, Chugoku-Shikoku, and Kyushu) of Japan. The edible parts of fish samples were blended using a food processor. The food mixtures were kept below –20°C until analysis.

For a market basket study, 120–200 kinds of foods were purchased from markets in 2 different regions (Kanto and Kyushu) of Japan. They were divided into 13 food groups and were weighed and cooked based on the daily consumption data calculated from the Japanese Nutrition Survey carried out by the Ministry of Health, Labor, and Welfare of Japan. The foods were blended with a food processor. The mixtures were kept below –20°C until analysis.

Sample Preparation

The analytical method for the brominated compounds was as follows. Each 50-g sample was freeze-dried using a model AD 2.0ES-BC (VirTis) freeze dryer. Dried samples were extracted with 10% (v/v) dichloromethane/*n*-hexane by an accelerated solvent extractor ASE300 (Dionex). The extraction temperature was 100°C; the time was 10 min. Extracts were treated with sulfuric acid three times and applied to a silica gel column. The column was prewashed with 100 ml *n*-hexane, and brominated compounds were eluted with 150 ml of 10% (v/v) dichloromethane/*n*-hexane. The eluate was evaporated and dissolved in 1 ml of *n*-hexane and treated with a sulfoxide cartridge column to remove the matrix. A sulfoxide column was prewashed with 20 mL of acetone and 20 mL of *n*-hexane. After the sample solution was loaded, the column was washed with 12 mL of *n*-hexane, and the fraction of target compounds was eluted with 25 mL of 50% (v/v) acetone/*n*-hexane. The eluted fraction was concentrated to a final volume of approximately 25 µl, and the samples were analyzed by HRGC/HRMS. The analytical method of BFRs is shown in Fig. 1.

Table 1 Analytical conditions of HRGC/HRMS

Compounds	Column	Injection temp.	Injection type /volume	Oven temp.	HRMS conditions
DBDPE	DB-5 15 m, 0.25 mm i.d., 0.1 µm film	260°C	Splitless 1 µl	100°C–(20°C/min)–200°C– (10°C/min)–320°C (7 min)	Electron energy: 38eV Filament current: 750µA Ion source temp: 270°C Resolution 10,000
PBDEs	DB-5 15 m, 0.25 mm i.d., 0.1 µm film	260°C	Splitless 1 µl	100°C–(20°C/min)–200°C– (10°C/min)–320°C (7 min)	
PBBs Co-PXBs	DB-5 15 m, 0.25 mm i.d., 0.1 µm film	280°C	Splitless 1 µl	130°C(1 min)–(20°C/min)– 170°C(10 min)–(4°C/min)– 210°C–(10°C/min)–300°C (3 min)	

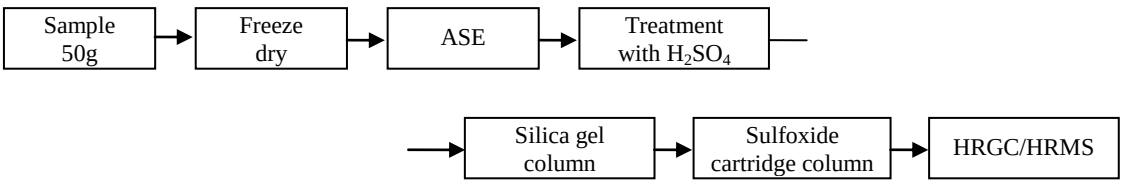

Fig. 1 Analytical method of BFRs (DBDPE, PBDEs, PBBs, and Co-PXBs)

Results and discussion

Concentrations of brominated compounds of fish samples

We have previously analyzed the PBDEs (23 congeners), PBBs (23 congeners), and Co-PXBs (7 congeners) in 12 kinds of fish[8]. In the present study, the other BFR compound, DBDPE was analyzed in the same fish samples by our previously developed method[7]. The results of the analysis are shown in Table 2. DBDPE was detected from 4 samples of fish; sea bream (1), conger eel (2), and flatfish (1). The concentrations of detected DBDPE were 5.86–8.68 pg/g wet weight (ww), 0.0541–0.533 ng/g lipid weight (lw). The detection limit of this method was 2 pg/g ww. The concentration of DBDPE was lower than that of PBDEs. Analysis of DBDPE in

fish samples has also been carried out in other countries, with values of 35–68 ng/g lw from Chinese fish and ND–3.3 ng/g lw from Canadian fish have been reported[9)10)]. The values obtained in our study are lower than those reported previously.

Table 2 Concentrations of DBDPE, PBDEs, PBBs, and Co-PXBs in fish samples

No.	Fish	Production regions of Japan	DBDPE		PBDEs[8)]	PBBs[8)]	Co-PXBs[8)]
			(pg/g ww)	(ng/g lw)	(ng/g ww)	(pg/g ww)	(ng/g ww)
1	Sea bream-1	Chubu	ND		0.100	0.230	ND
2	Sea bream-2	Chubu	8.08	0.292	0.247	0.813	ND
3	Sea bream-3	Kyushu	ND		0.016	0.105	ND
4	Sea bream-4	Chugoku-Shikoku	ND		0.018	ND	ND
5	Conger eel-1	Chubu	6.38	0.0541	0.818	2.24	ND
6	Conger eel-2	Chugoku-Shikoku	6.62	0.0670	0.406	1.83	ND
7	Flatfish	Chugoku-Shikoku	5.86	0.533	0.044	ND	ND
8	Shrimp	Kyushu	ND		0.033	ND	ND
9	Horse mackerel	Kyushu	ND		0.334	1.43	ND
10	Sand borer	Chugoku-Shikoku	ND		0.095	0.299	ND
11	Mackerel	Kyushu	ND		0.617	1.98	ND
12	Sardine	Kyushu	ND		0.167	0.827	ND

Daily intake of brominated compounds

Market basket samples were collected from 2 regions (A region: Kanto, B region: Kyushu) in Japan. They were analyzed for the estimation of the daily intake of BFRs. Daily intakes of DBDPE, PBDEs, PBBs, and Co-PXBs in each food group are shown in Table 3.

For DBDPE, the total daily intake was estimated as 1.27 ng/day for the A region and 0.190 ng/day for the B region, assuming that ND=0. In the case of 50 kg of body weight (bw), the daily intake was calculated as 0.0254 ng/kg bw/day and 0.0038 ng/kg bw/day, respectively (assuming ND=0). In the case of assuming that ND=1/2 limits of detection (LOD), the daily intake was calculated as 0.0690 and 0.0484 ng/kg bw/day.

For PBDEs, the daily intakes for the A and B regions were estimated to be 82.8 and 96.3 ng/day, respectively. In the case of 50 kg bw, the daily intake was calculated as 1.66 ng/kg bw/day for the A region and 1.93 ng/kg bw/day for the B region (assuming ND=0). In the case of assuming that ND=1/2 LOD, the daily intakes were calculated as 1.71 and 1.98 ng/kg bw/day, respectively. In a recent report, the lowest observed adverse effect level (LOAEL) value suggested as reasonable for compounds or mixtures belonging to the PBDE group were 1 mg/kg bw/day[11)]. Since the calculated value in this study was much less than this LOAEL value, the daily intake level of PBDEs was not considered a serious problem.

For PBBs, the daily intakes of A and B regions were estimated to be 0.393 and 0.119 ng/day, respectively. In the case of 50 kg body weight, the daily intake was calculated as 0.00786 ng/kg bw/day for the A region and 0.00238 ng/kg bw/day for the B region (assuming ND=0). In the case of assuming that ND=1/2 LOD, the daily intakes were calculated as 0.0662 and 0.0598 ng/kg bw/day, respectively. It was suggested that the total daily intake of PBBs should be less than 0.15 μg/kg bw/day by WHO Environmental Health Criteria. Compared with this value, the levels of the PBBs obtained in this study were not considered a serious problem.

Table 3 Daily intakes of DBDPE, PBDEs, and PBBs in each food group

No.	Food group	DBDPE (ng/day)		PBDEs (ng/day)		PBBs (ng/day)	
		A region	B region	A region	B region	A region	B region
1	Rice and rice products	0	0	1.72	9.30	0	0
2	Cereals seeds and potatoes	0	0	3.45	6.40	0	0
3	Sugars and confectioneries	0	0	0.304	1.05	0	0
4	Fats and oils	0.407	0	1.32	11.3	0	0
5	Pulses	0	0	2.33	1.45	0	0
6	Fruits	0	0	0.335	0.591	0	0
7	Green vegetables	0	0	1.62	0.681	0	0
8	Other vegetables and sea weeds	0	0	7.60	2.61	0	0
9	Beverages	0	0	7.99	5.44	0	0
10	Fish and shellfish	0.102	0	40.7	45.4	0.386	0.119
11	Meat and eggs	0.759	0.19	8.08	8.51	0.007	0
12	Milk and dairy products	0	0	1.30	2.65	0	0
13	Other foods (seasoning)	0	0	6.00	0.929	0	0
	Total	1.27	0.19	82.8	96.3	0.393	0.119

Daily intake calculated assuming that ND=0.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor, and Welfare, Japan.

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