BROMINATED FLAME RETARDANTS IN FOOD SAMPLES COLLECTED FROM THE PHILIPPINES

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Introduction

Environmental levels of brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), have been continuously increasing in the past decade[1,2]. PBDE concentrations are still comparatively lower than those of other environmental pollutants of similar chemical characteristics such as polychlorinated biphenyls (PCBs). However, rising body burdens of endocrine-disrupting chemicals (including PBDEs) may pose a potential public health threat.

Exposure to BFRs can occur from a variety of routes, including consumption of BFR contaminated food, ingestion or inhalation of BFR from contaminated dust, and inhalation of BFR contaminated air[3,4]. Overall, PBDE levels in food are higher in the United States than in European and Asian countries[5,4]. Taking into account that information concerning the occurrence of BFRs in foodstuffs and the dietary intake of these pollutants is scarce, studies to determine BFR exposure through the diet by the general population of a number of countries are clearly necessary.

Due to the lipophilic nature of these man-made chemicals, BFRs are mainly found in lipid-rich food of animal origin, such as meat, fish and dairy products, which are a part of our daily diet. The importance of the different routes of human exposure to BFRs (diet and inhalation/ingestion) is not completely understood at present in Asian developing countries, and opinions about the contribution of the dietary intake vary among publications. The present study determined the concentrations of BFRs including PBDEs and HBCDs in food samples from the Philippines to understand the human exposure routes of BFRs.

Materials and methods

Sample collection

Samples of the same 24 individual food types (market basket study) were collected at two local markets (Payatas and Malate) in the Philippines between 2008 and 2009 (for a total of 70). The samples were shipped frozen to the CMES, research laboratory in Japan and stored at −20°C in the Environmental Specimen Bank (*es*-Bank) of Ehime University[6] until analysis. The samples included composite meat samples *n*=26 [ground chicken (*n*=6), chicken liver (*n*=2), ground beef (*n*=6), beef liver (*n*=2), ground pork (*n*=8), pork liver (*n*=2)], canned meat samples *n*=12 [beef (*n*=3), beef and buffalo (*n*=3), beef and pork (*n*=5), beef and turkey (*n*=1)], vegetable-based foods *n*=11 [margarine (*n*=3), palm oil (*n*=1), coconut oil (*n*=6), coconut oil packed (*n*=1)], dairy products *n*=13 [milk from the Philippines (*n*=3), milk from Switzerland (*n*=2), milk from New Zealand (*n*=2), milk from Thailand (*n*=1), cheese from the New Zealand (*n*=1), cheese from the Philippines (*n*=4)], and eggs *n*=8.

Chemical analysis

Analysis of PBDEs and HBCDs were performed according to the method described elsewhere[7], with slight modifications. Briefly, 10-20 g of food samples were homogenized and freeze-dried, and 5 ng of internal standards of PBDEs ($^{13}C_{12}$ – labeled BDE-3, -15, -28, -47, -99, -100, -153, - 154, -183, -197, -207 and -209), and 10 ng of internal standards of HBCDs ($^{13}C_{12}$-labeled α-, β- and γ-HBCD) were spiked as surrogates and then extracted using high-speed solvent extractor (SE-100; Mitsubishi Chemical Analytech Co., Ltd., Japan) at a flow rate of 10 mL/min with a mixture of acetone and hexane (1:1 v/v) at 35° C for 30 min. The extract was then

subjected to gel permeation chromatography (GPC; Bio-Beads S-X3, Bio-Rad Laboratories, CA, 2 cm i.d. and 50 cm length) for lipid removal and eluted with mixture of 50% hexane/dichloromethane (1:1). The GPC fraction containing BFRs were concentrated and passed through 4 g of activated silica gel (Wakogel DX, Wako Pure Chemical Industries Ltd., Japan). The first fraction of 5% dichloromethane in hexane contained PBDEs, while the second fraction of 25% dichloromethane in hexane contained HBCDs. Identification and quantification of PBDEs were done using gas chromatography combined with mass spectrometry (GC-MS), while HBCD (α- β- and γ-) isomers were identified and quantified using liquid chromatography combined with tandem mass spectrometry (LC-MS-MS) in a multiple reaction monitoring (MRM) mode. Procedural blanks were analyzed simultaneously with every batch of seven samples to check for interferences or contamination from solvent and glassware. The concentrations of BFRs were expressed in pg/g wet weight basis unless otherwise specified.

Results and discussion

Contamination status of PBDEs

An over view of measured BFR levels in the food items are given in Table 1. Total BFR levels were calculated as the sum of the reported congeners using zero for those not detected. The most heavily PBDE contaminated food was dairy and egg samples with total concentration of 260 pg /g ww and 3575 pg/g ww. Following dairy and egg, vegetable based and composite meats were next contaminated items, with 948 and 368 pg/g ww, respectively. High total PBDE concentration in dairy and egg was driven largely by BDE-209 (511), BDE-207 (220) and BDE-206 (155 pg/g ww). Vegetable based samples 948 pg/g ww consisted primarily of BDE-209 and BDE-47 with respective concentrations of 425, and 93 pg/g ww. The greatest contributors to composite meat were from BDE-209 with respective concentrations of 125 pg/g ww. BDE-209 was most prevalent PBDE congener, detected in almost all the samples analyzed. The findings from this part of study confirm that some of the Philippines food remains contaminated with PBDEs.

Table 1. Mean and range concentrations (pg/g wet weight) of brominated flame retardants in the survey items.

	Vegetable based	Dairy and egg	Composite meat	Canned meat
Number of samples	11	21	26	12
Lipid (%)	95 (83- 99)	15 (2.0- 51)	22 (13- 44)	7.8 (6.2- 8.6)
Compounds				
PBDEs				
BDE-28	ND	ND	ND	0.84 (0.32- 2.1)
BDE-47	93 (ND- 241)	15 (ND- 28)	47 (ND- 63)	6.1 (4.2- 8.2)
BDE-99	ND	ND	83 ND- 109)	6.6 (3.0- 12)
BDE-100	ND	ND	ND	1.8 (0.78- 3.1)
BDE-153	ND	ND	ND	4.6 (ND- 4.6)
BDE-154	ND	ND	ND	1.7 (ND- 1.7)
BDE-183	ND	ND	57 (ND- 80)	13 (ND- 13)
BDE-196	4 (ND- 4.0)	7.1 (ND - 12)	ND	ND
BDE-197	ND	ND	ND	1.6 (ND- 3.2)
BDE-206	18 (ND- 18)	155 (ND- 180)	ND	0.18 (0.11- 0.27)
BDE-207	18 (ND- 18)	220 (ND- 220)	ND	0.37 (ND- 0.42)
BDE-208	ND	120 (ND- 120)	ND	ND
BDE-209	425 (13- 745)	511 (10- 3030)	125 (ND- 150)	45 (ND- 89)
Total PBDEs (mono to deca)	948 (33- 1376)	1028 (12- 3575)	368 (ND- 374)	82 (13- 135)
HBCDs				
α-HBCD	37 (ND- 41)	176 (ND- 680)	95 (ND- 157)	7.1 (2.0- 12)
β-HBCD	19 (ND- 19)	ND	ND	ND
γ-HBCD	32 (ND- 32)	ND	33 (ND- 33)	4.5 (ND- 4.5)
Total HBCDs	88 (ND- 84)	176 (ND- 680)	128 (ND- 190)	12 (2.0- 8.8)

ND = not detected

Lowest detected levels were found in canned meat samples. BDE-209 was detected at higher levels in all the products, especially dairy and egg (3575 pg/g ww) and coconut oil (1376 pg/g ww). The reason for this elevation is unknown, but may result from manufacturing and production introducing contaminants possibly from packaging material (for oil). The improved analytic methods used for BDE-209 leads us to believe the results are valid, but contamination of some egg outliers may have produced a typically high value for the composite samples. Further studies will include analysis of individual egg samples. The high levels of nona-BDE congeners; BDE-206 and BDE-207 with respective concentration of 155 and 220 pg/g ww, in egg was possibly from BDE-209 debromination during sample preparation/experiment.

Compared with PBDE levels in composite meat samples in Spain[8], Belgium[3], Japan[9], and USA[5], PBDE levels were higher in the Philippines. As with meat, we found considerable variation between samples of the vegetables based and dairy and egg. Meat is the major source of PBDEs in the Philippines diet, followed by egg and dairy products, unlike some other countries where fish intake predominates[8,9]. Like other countries, the sample size needs to be increased and the samples need to be representative of the diet(s) of the country. Until this is done, uncertainty in estimates of food levels will exist, and as a result, intake estimates will be somewhat imprecise.

While this study expands the database on PBDEs in food, more research still needs to be conducted to determine representative levels of PBDEs for individual foods as well as the effect of food packaging on PBDE levels. Much variation was observed between individual food type samples in this study, which lends uncertainty both to estimations of overall PBDE contamination of a food type as well as the potential dietary intake of PBDEs. Further research needs to be done to estimate average levels of contamination of particular food types as well as to assess differences in PBDE contamination of food grown or produced in different regions of the country or world. A program of representative sampling of foods, especially foods of animal origin, would provide information to help ensure that our food supply is not contaminated with these biologically active compounds.

Contamination status of HBCDs

HBCDs were present at the highest concentrations in egg (176 pg/g ww), followed by composite meat (128 pg/g ww), and vegetable based (88 pg/g ww) samples. HBCD concentration was below LOD in margarine, and some milk products. HBCD was detected in 8 of 26 composite meat, 7 of 11 vegetable based, 15 of 21 dairy and egg samples. A Norwegian study performed an analysis of HBCDs in fish, meat, and dairy products and a dietary intake calculation[10]. For meat, the Norwegian value of 21 pg/g ww in pork was much lower than the value of 190 pg/g ww measured in chicken in the present study. HBCD was not detected in most of the food samples.

Dietary intake of BFRs

For the intake calculations of the present study, the average theoretical daily consumption of each food category or individual food (g) was multiplied with the corresponding concentrations (pg/g ww), which resulted in a total daily intake (ng/day). The data used to estimate the dietary exposure to BFRs might slightly overestimate the actual dietary exposure because most analyses were done on fresh unprocessed foods, which were not boiled, cooked, baked or fried prior to analysis (except the canned samples). Cooking processes have been shown to lead to losses of PCBs and other OCs in trout, via the loss of fat. Other processes that occur during food preparation can also reduce the pollutant load of foods, such as volatilization and extraction into the cooking oil. Several studies reported reductions of PCBs and depending on the applied cooking process, these reductions varied between 15% and 65%[4]. Not much data is currently available on the loss of BFRs following food preparation processes, but it can be assumed that, based on the similar physical properties to PCBs, similar losses will also occur following cooking.

Total average dietary intakes of PBDE and HBCD in the present study were estimated between 3.7 and 0.83 ng/day (data not shown). The dietary intake of PBDEs by Spanish adults was reported to be 1.1 ng/kg/day[11]. The dietary intake of PBDEs by US adults, 1.1 ng/kg/day[12]. A Belgium duplicate diet study of university students was conducted as part of a larger study to determine which factors influence serum concentrations of PBDEs[13]. Estimated average dietary exposure for tri-BDE through hepta-BDE congeners was 10 ng/day, whereas median estimated dietary exposure for BDE-209 was 95 ng/day[13]. Furthermore, food items that were included in the various studies were not always the same, as some authors only analyzed meat[14], while others also included

cereals, fruits, vegetables and beverages[15]. However, contributions of these types of food to the total PBDE intake were rather low, not affecting the total estimated intake to a great extent (<20%).

Estimated Norwegian dietary intake of HBCD was 0.33 ng/kg/day, largely from oily fish[10]. A recently published study measured levels of HBCD in 165 duplicate diet samples from Belgium[16]. The estimated dietary intake of HBCD ranged from 1.2 to 20 ng/day and averaged 7.2 ng/day, with the bulk of HBCD detected in food being γ-HBCD. The estimated dietary intake for HBCD in the present study was 0.83 ng/day, which is lesser than the average intake in the Belgian study, but higher than those of American (0.50 ng/kg/day) and Norwegian (0.33 ng/kg/day) studies. Unlike the Norwegian dietary intake of HBCD largely from oily fish[10], the greatest contributions to intake in this study were derived from meat and egg. Estimated dietary intake of HBCDs in this study was comparable to estimates from recent studies published by European research groups.

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