DIETARY INTAKE ESTIMATIONS OF POLYBROMINATED DIPHENYL ETHERS BASED ON A TOTAL DIET STUDY IN OSAKA, JAPAN

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Abstract
This study presents the results of a total diet study (TDS) performed for estimating the dietary intake of polybrominated diphenyl ethers (PBDEs) in Osaka, Japan. The concentrations of 36 PBDEs were measured in 14 TDS food group samples (groups I–XIV). PBDEs were detected only in groups IV (oils and fats), V (legumes and their products), X (fish, shellfish, and their products), and XI (meat and eggs) at the concentrations of 1.8, 0.03, 0.48, and 0.01 $ng \cdot g^{-1}$, respectively. For an average adult, we estimated the lower bound dietary intake of penta- and decaBDEs (sum of tri- and nona- through hexabrominated and decabrominated congeners, respectively) to be 46 and 21 $ng \cdot d^{-1}$, respectively (assuming ND = 0). PentaBDE constituents were dominant in groups V, X, and XI. In contrast, we observed a high proportion of DeBDE-209 in group IV. To confirm the presence of DeBDE-209 in vegetable oils, we performed an additional analysis using 18 vegetable oil samples; of these, 7 contained DeBDE-209 at the ppb level. Further studies are required to reveal the pathways of oil contamination and to examine the formation of toxic polybrominated dibenzo-*p*-dioxins/dibenzofurans (PBDDs/PBDFs) from PBDEs under specific cooking conditions.

Introduction
Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in a variety of consumer products. PBDEs are persistent and lipophilic in nature, and thus they accumulate in the food chain. Fish and other fatty foods have been recognized as sources of human contamination with PBDEs. We have reported the PBDE levels detected in marine fish[1] and dietary supplements[2] from samples collected in Japan. Other researchers have also reported the PBDE levels in a variety of Japanese food items[3–5]. However, human exposure to PBDEs has not been sufficiently documented through a total diet study (TDS) in Japan. This study presents the results of a local-scale TDS performed for estimating the dietary intake of PBDEs. Further, the results of an additional analysis of 18 vegetable oil samples has been discussed.

Materials and Methods
Sample collection: A total of 125 food samples were purchased from 2 supermarkets in Osaka in 2006. TDS samples were prepared based on the official food classification and consumption data obtained from the National Nutrition Survey, which was conducted by the Ministry of Health and Welfare of Japan from 2001 to 2003. These food samples were cooked or prepared for consumption in a typical manner and blended to form 14 food group composites. These food groups were designated as groups I–XIV as shown in Table 1. In addition, 18 bottled vegetable oil samples obtained from rapeseed, corn, safflower, sesame, olive, and soybean were purchased from 2 supermarkets in Osaka in 2006 (Table 2).

Chemicals: Standard solutions of PBDEs were purchased from AccuStandard (New Haven, CT, USA) and Wellington Laboratories (Ontario, Canada). In this study, 36 PBDE congeners having 3–10 bromine atoms were monitored. PBDE numbers were assigned according to the IUPAC PCB nomenclature. Acetone, acetonitrile, and *n*-hexane of pesticide analysis grade and 44% sulfuric acid-impregnated silica gel and *n*-nonane of dioxin analysis grade were purchased from Wako Pure Chemicals (Osaka, Japan).

PBDE measurements: The TDS sample was digested with 1 $mol \cdot L^{-1}$ aqueous KOH for 2 h at room temperature after adding $^{13}C_{12}$-labeled surrogate standards. Subsequently, alkaline hydrolysate was extracted

twice with *n*-hexane. The extract was purified with sulfuric acid-impregnated silica gel by using *n*-hexane as an eluent. The eluate was concentrated and then spiked with a $^{13}C_{12}$-labeled injection standard. The additional vegetable oil samples were diluted with *n*-hexane after adding $^{13}C_{12}$-labeled surrogate standards and partitioned 3 times with *n*-hexane-saturated acetonitrile. The acetonitrile phase was combined and evaporated to dryness. The residue was treated with the sulfuric acid-impregnated silica gel and then spiked with the $^{13}C_{12}$-labeled injection standard. The cleaned extract was assayed with a gas chromatography/mass spectrometry (GC/MS) system. Quantitation was based on an isotope dilution method by using $^{13}C_{12}$-labeled internal standards. The mean percent recovery of most PBDEs ranged from 80% to 110%. The limit of detection (LOD) for all the PBDE congeners ranged from 0.01 to 0.1 ng·g^{-1}.

Results and Discussion

Example chromatograms of the standard solution and TDS samples are shown in Fig. 1. The PBDE concentrations in the TDS samples are shown in Fig. 2A. PBDEs were detected only in food groups IV (oils and fats), V (legumes and their products), X (fish, shellfish, and their products), and XI (meat and eggs) at the concentrations of 1.8, 0.03, 0.48, and 0.01 ng·g^{-1}, respectively. Fig. 2B reveals the lower bound intake of PBDEs (assuming ND = 0). For an average adult, we estimated the lower bound dietary intakes of pentaBDE (sum of 26 PBDEs: #17, #25, #28, #30, #32, #33, #35, #37, #47, #49, #66, #71, #75, #77, #85, #99, #100, #116, #118, #119, #126, #138, #153, #154, #155, and #166) and decaBDE (sum of 4 PBDEs: #206, #207, #208, and #209) to be 46 and 21 ng·d^{-1}, respectively, and the middle- (ND = 1/2 LOD) and upper bound intakes (ND = LOD) to be 330 and 610 ng·d^{-1} for pentaBDE and 310 and 600 ng·d^{-1} for decaBDE, respectively.

Table 1. Information of 14 food groups of the total diet study in Osaka, 2006

Group No.	Food composition	Food variety (No. of food items)	Lipid content (%)	Daily intake per capita (g·d^{-1})
I	Rice and rice products	2	0.28	334
II	Grains, seeds, and tubers	15	2.9	179
III	Sugar and confectioneries	6	20	34.2
IV	Oils and fats	4	93	11.4
V	Legumes and their products	6	8.2	53.8
VI	Fruits	11	0.16	123
VII	Brightly colored vegetables	13	0.29	100
VIII	Other vegetables, mushrooms, and seaweeds	13	0.28	184
IX	Beverages	7	0.01	577
X	Fish, shellfish, and their products	23	7.7	89.4
XI	Meat and eggs	8	18	124
XII	Milk and dairy products	5	6.7	157
XIII	Seasonings and other processed foods	11	8.8	91.3
XIV	Drinking water	1	0	250

Daily intake was estimated for an average adult consumer in Osaka based on the reports of National Nutrition Survey, 2001 to 2003.

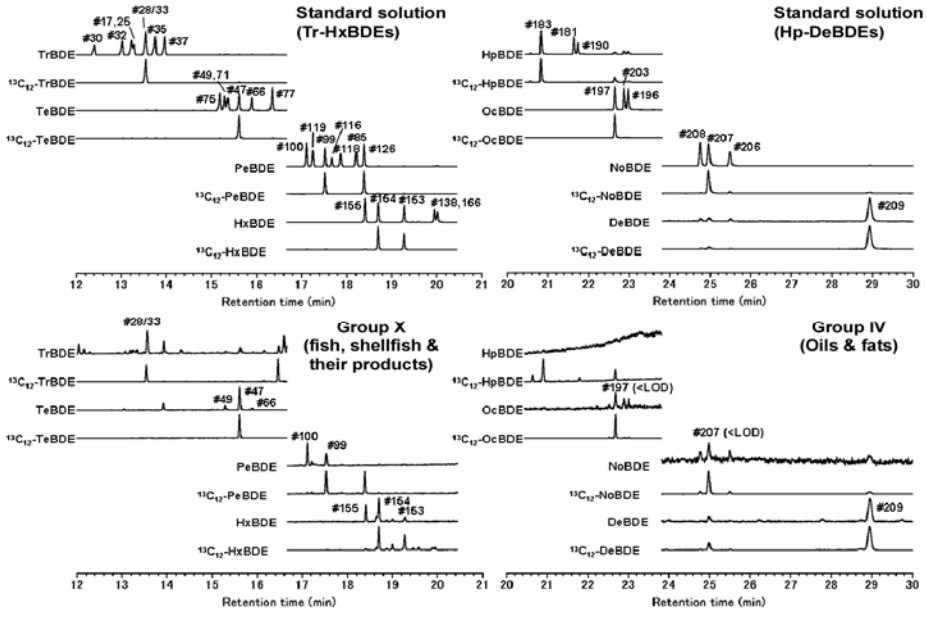

Figure 1. Example chromatograms of standard solution and food samples

Comparison data of the estimated dietary intake of PBDEs in different countries are shown in Table. 3. The lower bound PBDE dietary intake values estimated in this study were almost comparable to

those reported in the UK (91 ng·d^{-1})[6], USA (88 ng·d^{-1})[7], Spain (82 ng·d^{-1})[8], Japan (68 ng·d^{-1})[4], Sweden (51 ng·d^{-1}) (middle bound)[9], and Belgium (23 ng·d^{-1})[10]. Assuming that a typical Japanese adult weighs merely 50 kg, the lower, middle, and upper bound intakes were estimated to be 0.0009, 0.007, and 0.012 μg·kg^{-1}·d^{-1} for pentaBDE and 0.0004, 0.006, and 0.012 μg·kg^{-1}·d^{-1} for decaBDE, respectively. These values were 2–5 orders of magnitude lower than the reference doses of penta- and decaBDE (2 and 10 μg·kg^{-1}·d^{-1}, respectively), both of which were

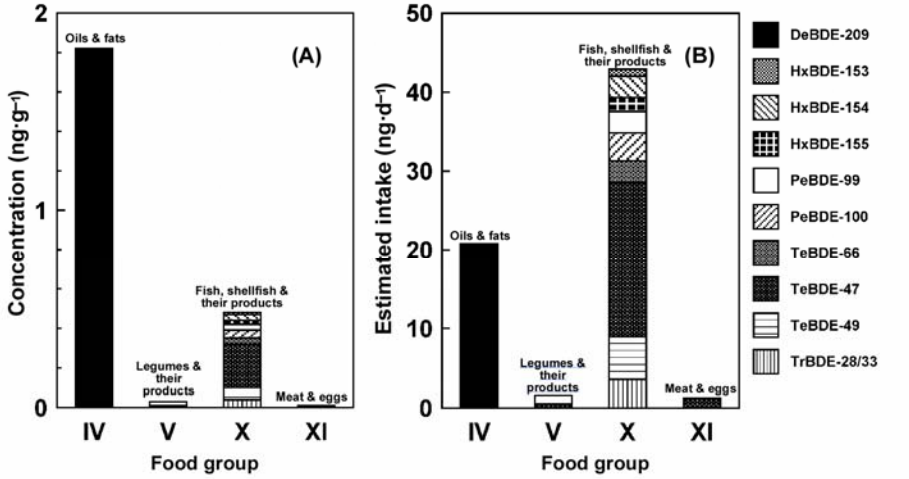

Fig. 2 Concentrations of PBDEs in foods (A) and estimated daily intakes of PBDEs from foods (B)

Table 2. Information of vegetable oil samples

No.	Oil composition	Country of origin	Bottle material	JAS certified
1	Rapeseed oil	NA	PET	No
2	Rapeseed oil	NA	PET	Yes
3	Rapeseed oil	NA	PE, EVOH	Yes
4	Rapeseed oil	Japan	PET	No
5	Rapeseed oil	Japan	Glass	No
6	Rapeseed oil	Japan	Glass	No
7	Brended oil (rapeseed, soybean)	NA	PE, EVOH	Yes
8	Brended oil (rapeseed, soybean)	NA	PE, EVOH	Yes
9	Corn oil	NA	PET	Yes
10	Corn oil	NA	PET	No
11	Corn oil	NA	PE, EVOH	No
12	Safflower oil	NA	PET	Yes
13	Safflower oil	NA	PET	Yes
14	Sesame oil	NA	Glass	No
15	Sesame oil	NA	Glass	Yes
16	Brended oil (perilla, sesame)	NA	Glass	No
17	Olive oil	Spain	Glass	No
18	Brended oil (unspecified)	NA	PET	No

Abbreviation: NA, not available; PET, polyethylene terephthalate; PE, polyethylene; EVOH; ethylene-vinyl alchol copolymer; JAS, Japanese Agricultural Standard

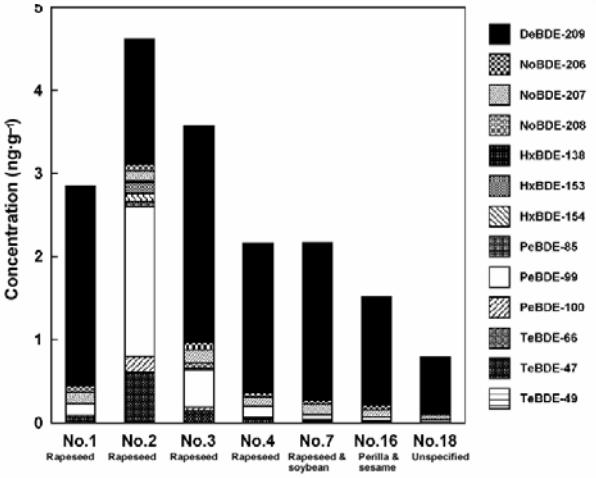

Fig 3. Concentrations of PBDEs in vegetable oil samples

Table 3. Comparison of estimated dietary intake of PBDEs in defferent countries

Country	Daily intake per capita (ng·d^{-1})*			Sampling year	Target congeners	Reference
	Lowerbound	Middlebound	Upperbound			
UK	91	–	–	1999–2000	47, 99, 100, 153, 154	Harrad et al., 2004
USA	88**	–	–	2003–2004	17, 28, 47, 66, 77, 85, 99, 100, 138, 153, 154, 183, and 209	Schecter et al., 2006
Spain	82	97	–	2000	Tetra- through octabrominated congeners	Bocio et al., 2003
Japan	–	94	–	1995	47, 99, 100, and 153	Wada et al., 2005
Japan	68	–	–	–	47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, and 183	Ashizuka et al., 2004
Sweden	–	51	–	1999	47, 99, 100, 153, 154	Darnerud et al., 2001
Belgium	23	35	48	2005	28, 47, 99, 100, 153, 154, and 183	Voorspoels et al., 2007
Japan	46	330	610	2006	PentaBDE (17, 25, 28, 30, 32, 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153, 154, 155, and 166)	This study
Japan	21	310	600	2006	DecaBDE (206, 207, 208, and 209)	This study

*Lower, middle, and upper bound intakes were estimated by assuming the nondetect values as zero, one half of the detection limit, and the detection limit, respectively. **The intakes were estimated for 70 kg males aged 20–39 years.

proposed by the US Environmental Protection Agency. These results suggested that the dietary exposure to PBDEs was not serious in Japan as well as in the other reported countries.

PentaBDE constituents such as TeBDE-47 and PeBDE-99 were dominant in food groups V, X, and XI. In contrast, a high proportion of DeBDE-209, a major constituent of decaBDE, was observed in the group IV food samples, which mainly consisted of vegetable oils (Fig. 2A). To confirm the presence of DeBDE-209 in vegetable oils, we performed an additional analysis using individual oil samples obtained from rapeseed, corn, safflower, sesame, olive, and soybean. We observed that 7 out of the 18 oil samples contained DeBDE-209 as a major or secondary dominant congener at approximately the ppb level (0.7–2.6 $ng \cdot g^{-1}$, Fig. 3). These results partially explained the reason for the high proportion of DeBDE-209 found in the group IV food samples. Sample No. 2 was the most contaminated rapeseed oil, and it contained TeBDE-47, PeBDE-99, and DeBDE-209 at the concentrations of 0.59, 1.8, and 1.5 $ng \cdot g^{-1}$, respectively. The results indicated that this vegetable oil sample was contaminated with both decaBDE and pentaBDE. The contamination may have occurred during the oil manufacturing processes. Another possible pathway of contamination involved the absorption and adsorption of PBDEs by the original farm plants during their growth processes. Mueller et al. reported that both radish (*Raphanus sativus L.*) and summer squash (*Cucurbita pepo L.*) absorbed pentaBDE from contaminated soil in a model experiment[11]. Thus, farm plants probably absorb a part of the PBDEs from contaminated soil. Hale et al. reported that 11 biosolid fertilizer (recycled sewage sludge) samples that were collected from different regions in the US all contained high concentrations of penta- and decaBDE (1100–2290 and 84.8–4890 $ng \cdot g^{-1}$ dry weight, respectively)[12]. The land application of biosolids may increase PBDE levels in farm plants and their products. However, the relationship between PBDE levels in plants and those in soils has not been sufficiently documented. In addition, it is known that considerable amounts of polybrominated dibenzo-p-dioxins/dibenzofurans (PBDDs/PBDFs) can be formed from PBDEs under thermal stress conditions[13]. Further studies are required to reveal the pathways of oil contamination and to examine the formation of toxic PBDDs/PBDFs from PBDEs in heated vegetable oils under specific cooking conditions.

Acknowledgments

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