BROMINATED FLAME RETARDANTS (HBCD,TBBPA AND ΣPBDES) IN MARKET BASKET FOOD SAMPLES OF NORTHERN KYUSHU DISTRICT IN JAPAN

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Abstract
We developed an analytical method for HBCD in food samples using gel permeation chromatography and a mini-column coupled with LC/MS/MS. In this report, to estimate the trend of human exposure to brominated flame retardants (BFRs) via food as well as to describe our validation results using this method, we analyzed two sets of market basket food samples prepared in Fukuoka prefecture in the fiscal years of 2002 and 2005. The estimated dietary intakes of HBCD, TBBPA and ΣPBDEs by an adult were 2.2, 1.1, and 2.3 ng/kg b.w./day, respectively, in F2002, and were 1.4, 0.1, and 1.4 ng/kg b.w./day, respectively, in F2005 when calculated for ND=0. The BFR intake levels by Japanese populate were considered as not so concerned.

Introduction
Polybrominated diphenyl ethers (PBDEs), tetrabrominated bisphenol A (TBBPA), and hexacycrododecane (HBCD) have been revealed as ubiquitous contaminants in human and wild live tissues as well as in other environmental samples. As another noteworthy point, they are closely related to the occurrence of polybrominated dioxins (PBDD/DFs)[1]. For example, the detection of PBDD/Fs in a PBDE formula or in ashes caused from the burning of plastics with PBDE as a flame retardant is well-known as a significant evidence[2]. When we estimate human exposure to these pollutants, the route via food is the most important. In our previous study, we found PBDEs in almost all fish samples collected from the Japanese near coasts and also found a tendency for PBDD/Fs to be detected in fish accompanying with highy brominated diphhenyl ethers such as nona- and deca-brominated diphenylethers. From such a viewpoint, BFR monitoring in food is important in preventing health hazardous effects not only from BFRs themselves but also from PBDD/DFs. According to a domestic trade paper, TBBPA is the mostly used BFR in Japan, with as much as 35,000 tons as a demand in F2004, followed by HBCD(2600 tons) and decabrominated diphenyl ether (2000 tons). In Japan, PBDEs without decabrominated diphenyl ether are self-controlled by the trade, and the value of the domestic demand for DBDE is only officially announced. Basically, there is only limited data on food pollution by BFRs. The best of the convenient ways to estimate the trend of human exposure to BFRs via food is to analyze market basket food samples. However, market basket food samples usually contain too many matrices that make an analysis difficult. Therefore, we have to modify the basic analytical method depending on the characteristic conditions of food—for example, whether it is a fatty food or not, or whether it is chlorophyll-rich or not. The appropriate modification is the key point in determining pollutant concentration with accuracy. In this study, we designed a pre-treatment method for the HBCD analysis for market basket samples, paying attention to the fact that HBCD is a large molecule of MW 641 and has a non-polar structure. For the method validation and the preliminary estimation of BFR intakes, the market basket samples prepared in our laboratory were used

Materials and Methods
Market basket food samples: Thirteen mixed food samples were prepared following the method of the Market Basket Study, alternatively termed the Total Diet Study. More than one hundred food items were chosen from 99 categories of foods that the Fukuoka populace commonly consumes, and the respective amounts of food items composing each food group (from 1 to 13) were determined by referring to the data of the latest national and prefecture survey (2002 and 2005).

Analysis of HBCD: Five grams of an homogenized sample with an addition of $^{13}C_{12}$ -α,β, and γ-HBCD were extracted twice with 20mL of dichloromethane (DCM) using a Polytron®. The extracts were dried over sodium sulfate dehydrate and concentrated. (I) Each residue of groups 1 (rice and its products), 2 (other grains, seeds, potatoes), 3 (sugar and a confectionary) and 4 (oils) was dissolved in 20mL of methanol/water (15:5, v/v) and re-extracted with n-hexane. One-half of each hexane layer was purified by liquid-liquid partition with DMSO. The purified extracts were concentrated and dissolved into 0.2mL of acetone and loaded onto a column of gel permeation chromatography (GPC). (II) Each half residue of groups 6 (fruits), 7 (colored vegetables) and 8 (other vegetables, mushroom, sea weed) was purified by multi-layered column chromatography of 22% H_2SO_4-silica (4g) and 44% H_2SO_4-silica (3g). HBCD was recovered with 10% DCM/n-hexane as an eluate. Each eluate was concentrated and dissolved into 0.2mL of acetone and subjected to GPC. (III) Each residue of the others--groups 6 (beans), 9 (drink, beverage), 10 (fish), 11 (meat and eggs), 12 (milk), and 13 (seasoning)-- was dissolved in 10% DCM/n-hexane and was treated twice with 5mL of sulfuric acid. After centrifuging at 2000 rpm, the upper hexane layer was collected and evaporated. The residue was dissolved in 0.2mL of acetone, and a half of it was subjected to GPC.
HBCD was fractionated in 12 to 14 min after large molecules such as crude fatty acids eluted in 10 to 12 min. The fraction was re-purified with a cartridge mini-column (Varian BOND ELUT-PSA, 500mg) prior to analysis by LC/MS/MS (Table 1). Detection limits of α-and γ-HBCD were 0.02 pg/g wb. That for β-HBCD was 0.01 ng/g wb.

Analysis of PBDEs: Each food group sample (fifty to one hundred grams) except for group 4 (oils) was freeze dried. After being spiked with $^{13}C_{12}$-labelled 2,3,7,8-substituted PBDD/DFs (128-500pg), $^{13}C_{12}$-1-Br-2,3,7,8-TeCDD (50pg) and $^{13}C_{12}$-labelled PBDEs (500-2500pg), it was extracted by accelerated solvent extractor(100 ℃ ,1500psi, 10% DCM/n-hexane) and with sulfuric acid and two kinds of column chromatography with silica-gel activated overnight at 130 ℃ and florisil deactivated with 1% of water. Group 4(oil) sample was directly diluted with n-hexane and then cleaned up as same as the other group. The PBDE fraction was cleaned up by liquid-liquid partition with DMSO. Prior to measurement by HRGC/HRMS (Table 2), $^{13}C_{12}$- 2,2',3,4,4',5',6 -HpBDE was added. Detection limits of tetra-to hepta-isomers were 0.1 pg/g wb.

Analysis of TBBPA: A homogenized sample (5 g) was spiked with ^{13}C-labeled TBBPA (0.5 ng) as a clean-up standard and then extracted with methanol. The methanol extract (ca.50 mL) was defatted by liquid-liquid partition with 20 mL of hexane. Then, to the methanol layer, 120 mL of 5% sodium chloride solution was added and re-extracted with DCM. The extract was concentrated to dryness and then ethylated with diethyl sulfate under an alkaline condition. After that, TBBPA ethylate was extracted with n-hexane and was cleaned up with florisil mini-column chromatography. The purified eluate was concentrated, re-dissolved in 20 μL of nonane with 2.5 ng of chrysene-d_{12} as a syringe spike, and subjected to measurement by HRGC/HRMS (Table 2). The detection limit of TBBPA was 0.01ng/g wb.

Results and Discussion
HBCD are highly lipophilic chemicals and large molecules similar to PBDEs. There are three stereo isomers: α,β,and γ. So far, detecting α,β,and γ separately is difficult with GC/MS. Therefore, replacing GC/MS by LC/MS/MS is likely to be used in the analysis of HBCD. However, there is a basic problem in LC/MS/MS analysis: the suppression of ionization caused by co-eluting the matrix. Good recovery and good reproducibility are required, when developing an analytical method. Therefore, sufficient clean-up to reduce the adverse problem as much as possible is necessary. In this study, we employed GPC and an additional clean-up by mini-column chromatography. With GPC, HBCD and TBBPA eluted separately from a large molecule such as crude fatty acid of fish. In the experiment, using the group 10 sample (fish as the main food) prepared in 2007, we obtained satisfactory recoveries: a mean of 73.4% ranging from 62.2% to 81.9% for α–isomer, a mean of 83.4% ranging from 66.5% to 92.6% for β-isomer, and a mean of 73.4% ranging from 54.8% to 90% for γ –isomer, as well as satisfactory reproducibilities of 12.2%, 11.7% and 15.4% for α,β,and γ–isomers, respectively. And for the other various food group samples, the recoveries of HBCD were

42%~106% for α–isomer, 59%~130% for β-isomer and 53%~124% for γ-isomer, except for 182%~225% for group 9.

HBCD was detected in each group 10 of F 2002 and F 2005 and in group 11 of F 2002. TBBPA was detected in each group 10 of F2002 and F2005. It was also detected in the groups 3, 4, 5 and 11 of F 2002 and in the groups 3 and 11 of F 2005. On the other hand, due to the lower detection limit of each PBDE congener, PBDEs were sensitively detected in the almost samples of F 2002 and F 2005. In group 10 among the 13 food groups of each year set, PBDEs were most abundantly detected. The partition coefficient of n-octanol to water (Log K_{ow}) for TBBPA, HBCD and PBDEs was 4.5~5.3, 7.74 and 6.27 (as DeBDE), respectively. The very frequent detection of HBCD and PBDEs like PCBs in group 10 (fish) seems to be acceptable; in contrast, TBBPA was detected only in each group 10 of F2002 and F2005 but at a very low level, which would be due to the instability of the phenol moiety of TBBTA. TBBPA is reported to be rapidly metabolized biologically to sulfate or gluculonide conjugates in the environment[3]. On the basis of the above data, the daily intakes of HBCD, TBBPA and ΣPBDE were calculated by multiplying each pollutant's concentration by the daily food consumption amount by one person (Table 3). Assuming the average adult body weight as 50 kg, the daily intakes of HBCD, TBBPA and ΣPBDEs were 2.2, 1.1, and 2.3 ng/kg b.w./day, respectively, in F2002, and 1.4, 0.1, and 1.4 ng/kg b.w./day, respectively, in F2005, when calculated for ND=0. The daily intakes of HBCD, TBBPA and ΣPBDEs were 3.1, 1.3, and 2.3 ng/kg b.w./day, respectively, in F2002, and 2.4 ng, 0.3 ng, and 1.4 ng/kg b.w./day, respectively, in F2005 when calculated for ND=1/2xLOD. Only TBBPA varied greatly between F2002 and F2005. The dietary intakes of HBCD in F2002 and F2005 were at the same level as those of ΣPBDEs. In other reports, Swedish people's intake of BFRs was <3ng ng/kg b.w. day for HBCD[4] , and the UK people's intake of these brominated retardants in 2003 and 2004 was <5.9 ng/kg b.w./day for HBCD, <1.6 ng/kg b.w./day for TBBPA, and <5.9 ng/kg b.w./day for ΣPBDEs[5], respectively. The food standards agency of the UK that carried out the above market basket study concluded that the estimated adult dietary intake of HBCD, TBBPA and ΣPBDEs does not raise toxicological concerns. Although there is a report which simulates the increase of Br emission from TV casing waste in the near future, basically not only the available references about the intake data of BFRs, but also the fate of BFRs are too limited; the increase or decrease of emission and deposition into the environment are not completely clear. Therefore, we recommend continuing to collect more data in order to clarify the trend of food pollution by those BFRs and avoid probable human hazardous exposure.

Acknowlegements
This work was supported by a Scientific Research Grant from the Ministry of Health, Welfare, and Labor of Japan.

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Table 1 The LC/MS/MS conditions for HBCD analysis

LC/MS/MS: Waters Quatro Micro API
Column:Inertsil ODS-3 (GL Sciences) 2.1mmi.d. x150m, 5μ
Injector volume.:5 uL
Column temp.:40℃
Flow rate: 0.2mL/min
Moving phase: 10mM ammonium acetate/methanol/acetonitrile (10:55:35)
Monitor Ions: native-HBCD, 641 >79 (Q1) , 639 >79(Q2)
$^{13}C_{12}$-HBCD; 653>79(Q1), 651>79(Q2)
Ionization;ES negative; Ion source temp.,130℃; Capillary energy ; 2.0kV

Table 2 The GC/MS conditions for PBDEs and TBBPA analysis

GC/HRMS: HP6890 (Hewlett Packard) /Autospec Ultima (MicroMass)
Electron energy,38eV; filament current, 750μA; ion source Temp.,270℃; resolution,10000
PBDE analysis: Column: HP-5MS(Agilent) 0.25mmi.d. x15m, film thickness 0.1μm
Injector temp.:260℃
Column temp.:120℃(2min)-20℃/min-200℃-10℃/min-300℃(7.5min)
TBBPA analysis: Column:DB-5 (J&W) 0.25mmi.d. x 30m, film thickness 0.25μm
Injector temp.:280℃
Column temp.:120℃(1min)-20℃/min--300℃(8min)

Table 3 The estimated dietary intakes of HBCD, TBBPA and ΣPBDEs

Food group		Food consumption (g/day) in F2002	Daily intake in F2002						Food consumption (g/day) in F2005	Daily intake in F2005					
			α-HBCD	β-HBCD	γ-HBCD	ΣHBCD	TBBPA	ΣPBDEs		α-HBCD	β-HBCD	γ-HBCD	ΣHBCD	TBBPA	ΣPBDEs
I	(rice)	459	ND	ND	ND	ND	ND	0.11	446.5	ND	ND	ND	ND	ND	0.19
II	(other grains, potatoes,seeds)	226.1	ND	ND	ND	ND	ND	0.37	209.9	ND	ND	ND	ND	ND	0.50
III	(sugar, confectionary)	36.6	ND	ND	ND	ND	0.33	0.17	33.7	ND	ND	ND	ND	0.33	0.67
IV	(oils)	15.2	ND	ND	ND	ND	0.15	1.85	11.5	ND	ND	ND	ND	ND	0.65
V	(legume and its products)	80.4	ND	ND	ND	ND	0.73	0.30	56.4	ND	ND	ND	ND	ND	0.13
VI	(fruits)	130.6	ND	ND	ND	ND	ND	0.00	136.0	ND	ND	ND	ND	ND	2.60
VII	(colored vegetable)	108.3	ND	ND	ND	ND	ND	0.05	96.6	ND	ND	ND	ND	ND	0.10
VIII	(other vegetables)	234.6	ND	ND	ND	ND	ND	0.03	219.0	ND	ND	ND	ND	ND	0.06
IX	(bebariges)	172.2	ND	ND	ND	ND	ND	0.02	504.2	ND	ND	ND	ND	ND	0.00
X	(fish)	100.8	57*	ND	19.7*	76.7*	50.7*	102*	90.7	49.3*	ND	19.7*	49.3*	2.72*	57.8*
XI	(meat and eggs)	157.9	34.5*	ND	ND	34.5*	4.42*	9.17*	133.6	ND	ND	ND	ND	0.67*	4.19*
XII	(milk and its products)	122.5	ND	ND	ND	ND	ND	1.04*	146.8	ND	ND	ND	ND	ND	0.74*
XIII	(seasonings)	38.1	ND	ND	ND	ND	ND	0.06	80.9	ND	ND	ND	ND	ND	0.65
XIV	(water)		-	-	-	-	—	—		-	-	-	-	—	—
Daily intake ng/day at ND=0			91.5	0	19.7	111	56.3	115		49.3	0	19.7	68.4	3.7	68.3
Daily intake ng/day at ND=1/2 xLOD			108.5	9.4	37.5	155	63.9	116		70.1	10.8	39.9	121	13.8	69.3
Daily intake ng/kg,b.w./day at ND=0						2.2	1.1	2.3					1.4	0.1	1.4
Daily intake ng/kg,b.w./day at ND=1/2xLOD						3.1	1.3	2.3					2.4	0.3	1.4

LOD: 0.01ng/g for β-HBCD and TBBPA; 0.02ng/g for α-HBCD and γ-HBCD; 0. 0001ng/g for each PBDE congener (from tetra- to hepta-brominated isomer)

* means an average of two food group samples prepared separately.