LEVELS OF HEXABROMOCYCLODODECANE AND TETRABROMOBISPHENOL-A IN FOODS AND HUMAN MILK FROM CHINA

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Abstract

Tetrabromobisphenol A (TBBPA) and hexabromocyclododecane diastereoisomers (α, β and γ-HBCD) were determined in 24 pooled human milk samples and 48 Chinese Total Diet Study (TDS) samples collected in 2007. Based on ultra performance liquid chromatography-mass spectrometry (UPLC-MS/MS) analysis, levels of TBBPA ranged from <LOD to 5124pg/g lipid weight (lw) in human milk and from <LOD to 2044pg/g lw in TDS samples. The α-HBCD diastereoisomer, which ranged from <LOD to 2776pg/g lw in human milk and from <LOD to 2224pg/g lw in TDS samples, was generally the most abundant isomer comparing with β and γ-HBCD. The average Estimated Daily Intake (EDI) of TBBPA via human milk for nursing infants with a range of 320-37240pg/kg bw/day was 5094pg/kg bodyweight (bw)/day, while that of ∑HBCD was 5837pg/kg bw/day with a range of 670-17320pg/kg bw/day. The medium bound (<LOD=1/2LOD) EDI_{TBBPA} for a "reference" man via animal origin foods was 256pg/kg bw/day and $EDI_{\sum HBCD}$ was 432pg/kg bw/day. Meat and meat products were the main source in the total dietary intake of TBBPA and ∑HBCD.

Introduction

Hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA) are two Brominated flame retardants (BFRs) currently in use. In commercial HBCD, γ-HBCD is the main component, consisting 75%-89% of the total weight (α-HBCD, 10-15%; β-HBCD, 0.5%-12%). The production capacity of TBBPA and HBCD in China are about 18000 and 7500 metric tons in 2007 respectively[1]. Studies indicated that BFRs are bioaccumulative and persistent compounds, thus they should be regarded as persistent organic pollutants (POPs)[2]. It is generally accepted that human exposure to BFRs is mainly through diet. The purpose of this research is to examine the levels of HBCD diastereomers (α, β and γ-HBCD) and TBBPA in two categories of samples: pooled human milk samples collected in 2007 from 12 provinces of China and TDS samples from the fourth Chinese total diet study in 2007, including four animal-origin food groups. Based on the data we obtained, we can estimate the human daily intake of TBBPA and HBCD via human milk and food consumption in China.

Materials and Methods

TDS Sample Collection. The fourth Chinese total diet study was carried out in 2007. Overall study design and experimental methods were similar to those carried out in 1990[3]. The food composite approach was used to study the total diet in 12 provinces representing the average dietary patterns of different geographical areas on the mainland China and covering about 50% of the Chinese population[4]. The 12 provinces are Heilongjiang, Liaoning, Hebei, Henan, Shanxi, Ningxia, Jiangxi, Fujian, Hubei, Sichuan, and Guangxi provinces and Shanghai

City. Average food consumption of a "reference" man was used as the standard food consumption pattern. The food consumption data for the "reference" man was the result of the third Chinese total diet study performed by Chinese CDC in 2000. The survey covers 1080 households and nearly 4000 individuals[5]. "Reference man" was defined as an adult male, 18-45 years of age and 63 kg of body weight, undertaking light physical work.

A total of 662 food items consumed by "reference" man were aggregated into 13 food groups. Four animal-origin food groups of these 13 groups were subjected to HBCDs and TBBPA analysis: (1) eggs and egg products, including chicken and duck eggs, and salted and limed duck eggs; (2) aquatic foods, including fish, shrimp, and oysters; (3) milk and milk products, including cow milk, cow milk powder, yogurt, and sheep milk; (4) meat and meat products, including pork, mutton, beef, chicken, duck, rabbit, pork liver, and swine blood. The samples were collected from local markets, grocery stores, and rural households. In order to achieve more realistic dietary exposure estimates, the foods were prepared and cooked to a "table ready" state according to local cuisine and then blended to form the respective group composites with weights proportional to the average daily consumption of each province.

Human Milk Collection. In 2007, 1237 breast milk samples were collected from 1237 healthy donors living in the same 12 provinces of China as in the TDS. In each province, there are 50 urban donors and 50-60 rural donors according to the living area. The samples were collected within 3-8 weeks after child birth. About 50 mL of breast milk was collected from each milk donor. Individual samples (10mL from each sample) were shaken for homogeneity and pooled either into one rural sample or one urban sample from each province.

Reagents and Chemicals. Standard solution of α, β and γ-HBCD (50 μg/mL in toluene), TBBPA (50 μg/mL in methanol), isotopic internal standard (IS), $^{13}C_{12}$-labeled α, β and γ-HBCD (50 μg/mL in toluene), and $^{13}C_{12}$-labeled TBBPA (50 μg/mL in methanol) were obtained from Cambridge Isotope Laboratories (U.S.).

Sample Preparation and Analysis. The methodology used for the analysis of TBBPA and HBCD was based on Soxhlet extraction, GPC cleanup, and UPLC-MS/MS detection. Briefly, about 20-30 mL of pooled breast milk or 5-30g of composite food sample were freeze-dried. After spiking with IS (10ng of $^{13}C_{12}$-labeledα, β and γ--HBCD and $^{13}C_{12}$-labeled TBBPA) and equilibrating for 5 h, the samples were ground with anhydrous sodium sulfate and extracted in a Soxhlet apparatus with a mixture of n-hexane and acetone (1:1, v/v) for 20-24 h. This extract cleanup was performed by gel permeation chromatography (GPC) followed by a sulfuric acid treatment. GPC cleanup was performed on an AccuPrep MPS GPC cleanup system (J2 Scientific Inc., U.S.) using a low-pressure column (GPC, Bio-Beads S-X3, Bio-Rad, CA, 2cm i.d. × 50 cm). After Soxhlet extraction, the extract was evaporated to dryness and lipid content was determined by gravimetry. Then, the extract was redissolved in ethyl acetate/cyclohexane (1:1 v/v) and injected onto the GPC system. The GPC column was eluted with an ethyl acetate/cyclohexane mixture (1:1, v/v), and the flow rate was 5 mL/min. Fractions were collected from 18 to 30 min, evaporated to dryness, reconstituted in 5mL n-hexane, and then shaken with 1mL concentrated sulfuric acid to degrade the remaining lipid. The final hexane extract was concentrated to dryness and reconstituted to 200 μL using methanol.

UPLC MS/MS detection was performed on a Waters Acquity ultra performance liquid chromatographic system (Waters Company, MA, USA). Chromatographic separations were performed on a UPLC BEH C18 column (1.7μm particle size, 50mm×2.1mm I.D.). A gradient programme was used with the mobile phase, combining solvent A (mixture of methanol and acetonitrile (1:1, v/v)) and solvent B (water) as follows: 50% A (initial-1min), 50–90%A (1–5 min), 90% A (5–8min). The flow rate was 0.2 mL/min while the injection volume was 10μL. The column temperature were maintained at 40℃. MS/MS detection was performed on a Micromass-Quattro Premier triple quadrupole mass detector (Micromass, Manchester, UK). Quantification was performed under electrospray negative ionization (ESI-) mode. The conditions used for the electrospray source were as follows: capillary voltage, 3.0kV; cone voltage, 20V for HBCD and 70V for TBBPA ; source temperature, 120°C; desolvation gas temperature, 350°C; collision energy, 30eV; argon collision gas flow, 0.2mL/min. The Multiple Reaction Monitoring (MRM) mode was used for the determination of the four BFRs. α-, β-, and γ-HBCD were monitored at m/z transitions of 640.7→80.7; $^{13}C^{12}$-α-, β-, and γ-HBCD were monitored at m/z transitions of 652.7→80.7; monitored m/z of TBBPA and $^{13}C^{12}$-TBBPA were 542.6→447.6 and 554.6→459.6, respectively.

Results and Discussion

Levels of TBBPA and HBCDs in Breast Milk. This study is the first to report the levels of TBBPA and HBCDs in human milk in China. As shown in Table 1, TBBPA was found in 80% of the pooled human milk samples with concentrations ranging from <LOD to 5124 pg/g lw. α-HBCD was the predominant diastereoisomer in human milk samples, probably due to selective metabolism or biotransformation process[6,7]. α-HBCD was detected in most of the samples with concentrations ranged from <LOD to 2776 pg/g lw. γ-HBCD was only detected in rural sample of Hebei (462 pg/g lw) and β-HBCD was not detected in any samples. TBBPA is manufactured and used in greater quantity than HBCD in China; however, in 63% of the pooled human milk samples, the concentrations of TBBPA were lower than that of HBCD. This can be explained by the differences in the usage and bioaccumulation potential of the two BFRs. HBCD is an additive BFR, while TBBPA is a reactive BFR. TBBPA is bound chemically to the polymer structure; thus, the leaching/release of TBBPA into the environment is limited. In addition, pharmacokinetics of the two BFRs in the human body is different; TBBPA is rapidly metabolized by the mammalian liver and eliminated into bile[8].

TABLE 1. Concentrations (pg/g lw) of α-HBCD and TBBPA in Human Milk from China.

	α-HBCD		TBBPA	
location	rural	urban	rural	urban
Hebei	2287	1129	4458	1641
Shanxi	433	325	239	482
Liaoning	671	1397	3920	195
Heilongjiang	2776	1543	331	232
Hubei	2427	1220	110	158
Fujian	989	997	ND	ND
Ningxia	ND	361	ND	957
Henan	702	ND	631	ND
Jiangxi	1031	1185	ND	5124
Shanghai	1600	675	1271	394
Guangxi	753	528	172	1134
Sichuan	813	899	313	816

Mean	1209	857	961	933

The concentrations below LOD were treated as 1/2LOD for arithmetic mean. ND, not detected.

Estimated Daily Intake (EDI) via Human Milk. EDI_{TBBPA} and $EDI_{\Sigma HBCD}$ via human milk were calculated on the basis of levels of TBBPA and HBCDs in breast milk and infant ingest data (742 mL/day) and body weight data from the U.S. Environmental Protection Agency[9], assuming that human milk is the only food source for nursing infants (1-6 months old). As the levels of TBBPA or HBCDs in certain samples were below the LOD, <LOD was replaced by 1/2LOD when calculating EDI. The average EDI_{TBBPA} via human milk was 39 730 pg/day for nursing infants (1-6 months old) with a range 2480-290 500pg/day; and the average $EDI_{\Sigma HBCD}$ via human milk was 45 520 pg/day with a range of 5200-135100 pg/day. When assuming a 7.8 kg body weight for a 6-month old boy, the mean EDI of TBBPA and ΣHBCD was 5094 and 5837 pg/kg bw/day, respectively.

Levels of TBBPA and HBCDs in Foods. These results are listed in Table 2. The highest contamination level of TBBPA was found in the aquatic food group followed by the meat/meat products group. The lowest concentrations occurred in the egg/egg products group.

Overall, the ∑HBCD concentrations detected ranged from <LOD to 9208 pg/g lw. α-HBCD was also the predominant diastereoisomer except in the aquatic food composite of Shanghai City. The highest contamination level of HBCD was found aquatic food group. The mean and median levels of α-HBCD in the meat group were both lower than those in the egg group. The lowest concentrations occurred in the milk/milk products group. β-HBCD and γ-HBCD were found in only a few samples. β-HBCD was detected in the aquatic food of Jiangxi (193 pg/g lw). γ-HBCD was detected in the aquatic food of Liaoning and Jiangxi, meat samples from Hubei, and milk from Henan, with levels of 1149, 274, 386, and 628 pg/g lw, respectively. In particular, the highest levels of HBCDs were found in the aquatic food composite of Shanghai City, where the concentrations of α-, β-, and γ-HBCD were 2224, 358, and 6626 pg/g lw, respectively. The pattern of HBCDs in this sample was significantly different from the other animal origin foods. This special HBCD pattern may result from the high level of γ-HBCD in some kinds of fishes. In another study, Xian et al. had reported that high levels of HBCDs (12-330 ng/g lw) were detected in fishes from the Yangtze River Delta, where Shanghai City is located[10]. In that study, 17 freshwater fishes belonging to nine species were examined. α-HBCD was found to be the predominant isomer in most of the fish species and tissues, followed by γ- and β-HBCD. However, γ-HBCD was found to be predominant in the muscle and egg of Mandarin fish (Siniperca chuatsi). The reason for this particular pattern of HBCDs in Mandarin fish is still unknown, and may be the result of differences in metabolism and/or other sources of exposure. In addition, the concentration of TBBPA in the aquatic food composite of Shanghai City is also the highest. High levels of BFRs in aquatic animals may result from high market demand of BFRs in Shanghai. Shanghai, being an industrialized and urbanized area, has a large amount of polymer raw materials, textiles, electronic appliances, and fine petrochemicals, which could be sources of BFRs in the surrounding areas and eventually lead to high level of BFRs in animals and the environment.

TABLE 2. Levels of TBBPA and α-HBCD in TDS Samples (pg/g lw).

location	TBBPA	α-HBCD

	meat	eggs	aquatic food	milk	meat	eggs	aquatic food	milk
Hebei	217	ND	ND	ND	872	1156	ND	437
Shanxi	102	ND	877	323	ND	ND	464	ND
Liaoning	546	349	1046	ND	ND	ND	1213	ND
Heilongjiang	145	267	1283	466	ND	144	315	ND
Hubei	227	692	606	ND	859	315	410	ND
Fujian	209	ND	1682	ND	ND	357	992	ND
Ningxia	184	ND	ND	ND	ND	258	201	ND
Henan	ND[a]	476	ND	848	420	ND	809	853
Jiangxi	ND	ND	ND	ND	ND	570	1037	ND
Shanghai	1386	ND	2044	ND	183	ND	2224	407
Guangxi	ND	368	574	148	137	429	114	ND
Sichuan	ND	ND	541	541	173	ND	914	ND
Mean	263	194	738	211	230	273	727	160

[a] The concentrations below LOD were treated as 1/2LOD for arithmetic mean.

Estimated Daily Intake (EDI) via Foods. Daily dietary intakes of TBBPA and ΣHBCD for the "reference" man (63 kg body weight) were estimated by multiplying the measured concentrations (pg/g wet weight) of TBBPA and ΣHBCD with the daily consumption data (g/day) from the survey mentioned above. Table 3 shows the medium bound EDI (replace <LOD by 1/2LOD) of TBBPA and ΣHBCD.

The percentage contribution from the four food groups varied between provinces. For the medium bound EDI_{TBBPA}, although the contamination level of aquatic food was the highest, the contribution from aquatic food (30%) was less than that from meat/meat products (52%) as its consumption was higher than that of aquatic food in China. For the medium bound $EDI_{\Sigma HBCD}$, with the highest contamination level and relative high consumption, the contribution from the aquatic food group (42%) was greatest, a result from the high levels of HBCDs in aquatic food sample from Shanghai City. If the value of aquatic food of Shanghai City was excluded from the discussion, the contribution from meat and meat products is greatest (44%), followed by aquatic foods (24%), eggs (17%), and milk (15%). In summary, meat and meat products account for a major fraction of daily intake because of high consumption in China.

TABLE 3. Medium Bound EDI (pg/kg bw/day) of TBBPA and ∑HBCD

	TBBPA					∑HBCD				
Location	Meat	Eggs	Milk	Aquatic food	Total	Meat	Eggs	Milk	Aquatic food	Total
Hebei	40	5	11	10	66	199	127	24	31	380
Shanxi	16	3	10	7	34	20	17	42	9	88
Liaoning	211	32	44	14	302	89	70	78	109	346
Heilongjiang	43	35	87	18	183	49	59	48	53	210
Hubei	56	90	1	86	233	310	85	3	110	507
Fujian	127	7	13	471	617	115	62	27	464	668
Ningxia	36	2	6	5	50	60	17	13	16	106

Henan	24	39	14	2	80	204	37	29	9	280
Jiangxi	20	6	0	6	32	53	66	0	43	163
Shanghai	947	12	59	285	1304	239	79	120	1285	1723
Guangxi	27	11	1	23	62	137	22	4	20	182
Sichuan	50	4	50	9	113	250	29	220	27	526
Mean	133	21	25	78	256	144	56	51	181	432

Assessment of Human Exposure to TBBPA and HBCDs. In our study, the average TBBPA and ΣHBCD intake values via human milk for a 6-month-old boy were 5094 and 5837 pg/kg bw/day, which were much higher than that for a "reference" man (256 and 432 pg/kg bw/day). In addition, Meng et al. reported that the median total PBDEs intakes via human milk for nursing infants (0-1 years old) was 6870 pg/kg bw/day for a male and 7370 pg/kg bw/day for a female in China[11]. It is indicated that the body BFRs burden of a nursing infant in China is much higher than that of adult. The possible toxicological impact of such a high daily intake should certainly be investigated.

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