Dietary Exposure Assessment of Chinese Population to Legacy and Novel Brominated Flame Retardants: Results of a Chinese Total Diet Study and National Human Milk survey

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Introduction

Tetrabromobisphenol-A (TBBPA), hexabromocyclododecane (HBCD) and polybrominated diphenyl ethers (PBDEs) are the three Brominated flame retardants (BFRs) mainly used in China, which have been used since the 1970s and are called "legacy BFRs". With the restriction of the legacy BFRs, NBFRs, such as decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy)-ethane (BTBPE) and so on, are produced and used in increasing amounts in China. Studies indicated that BFRs are bioaccumulative and persistent compounds, thus they should be regarded as persistent organic pollutants (POPs), some BFRs including penta-BDE, octa-BDE and HBCD had been added to the POPs list by Stockholm Convention. It is generally accepted that human exposure to BFRs is mainly through diet. The purpose of this research is to examine the levels of legacy and novel BFRs in two categories of samples: pooled human milk samples collected in 2011 from 16 provinces of China and TDS samples from the fifth Chinese total diet study and national human milk survey conducted in 2011. The TDS samples including four animal-origin food groups. Based on the data we obtained, we can estimate the human daily intake of BFRs via human milk and food consumption in China.

Materials and methods

TDS Sample Collection. The fifth Chinese total diet study was carried out in 2011. Overall study design and experimental methods were similar to those carried out in 2007 [1]. The food composite approach was used to study the total diet in 20 provinces representing the average dietary patterns of different geographical areas on the mainland China. The 20 provinces are Heilongjiang, Liaoning, Hebei, Shanxi, Ningxia, Henan, Shanghai, Fujian, Jiangxi, Guangxi, Sichuan, Beijing, Jilin, Neimenggu, Qinghai, Jiangsu, Zhejiang, Hunan, Hubei and Guangdong. In each province, three sampling sites including one urban site and two rural sites which were randomly selected, and then 30 households were randomly sampled from each site to conduct the food consumption survey by 24-h dietary recall over 3 day for each householder, enabling recording of individual food consumption data. All food items were aggregated into various food groups, then, the average food consumption was calculated to present the pattern of food consumption of a standard Chinese male adult (18-45y, 63kg body weight). Food samples were collected from local food markets, grocery stores or rural households in each sampling site, and then prepared using local practice. The composite of each food group was made by blending the prepared food with weights proportional to the average daily consumption in each province. These provincial composites were shipped to our lab and frozen at -20℃ until analysis.

Food items consumed by "reference" man were aggregated into 12 food groups per province. Four animal-origin food groups of these 12 groups were subjected to BFRs analysis: (1) eggs and egg products; (2) aquatic foods; (3) milk and milk products; (4) meat and meat products. The samples were collected from local markets, grocery stores, and rural households.

Human Milk Collection. In 2011, breast milk samples were collected from healthy donors living in the same 16 provinces of China as in the TDS (hunman milk samples were not collected in Beijing, Hunan, Jiangsu, Sichuan). In each province, there are 50 urban donors and 50 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 the BFRs, isotopic internal standard (IS), $^{13}C_{12}$-labeled BFRs were all obtained from Cambridge Isotope Laboratories (U.S.).

Sample Preparation and Analysis. The methodology used for the analysis of TBBPA and HBCD has been described in our former study [2,3]. An Agilent 5977A MS linked to a 7890B GC was used for BDE-209 and novel BFRs analysis. TBBPA and HBCD isomers were separated and quantified using an Agilent 1290 UHPLC coupled to a 6490 triple quadrupole MS.

Results and discussion

BFRs in Breast Milk. As shown in Table 1, in the 29 pooled human milk samples, contamination levels of the three legacy BFRs were still relatively high, however, in the novel BFRs, DBDPE showed high contamination, which is second only to HBCD, but higher than any other novel BFRs, TBBPA and BDE-209. In China, DBDPE has been produced since 2005, with a production volume of approximately 25,000 t per year. DBDPE has outperformed other BFRs and become the primary produced and used BFR at present. Thus, the relatively high level of DBDPE in the present study might be due to the high production volume and usage. Compared to other studies, it is obvious that leves of the three legacy BFRs levels in our study were higher than those in most of the studies conducted in other countries, indicate that the Chinese population is continuously exposed to relatively high levels of legacy BFRs. As for the novel BFRs, since data is limited, a comparison is difficult[4].

Table 1. Contamination level and EDI via human milk

BFRs	Contamination level (ng/g lw)				EDI via human milk (ng/kg bw/day)			
	Mean	SD	Median	Range	Mean	SD	Median	Range
TBBPA	7.58	17	1.21	0.174-66.8	39.2	88	5.57	0.823-374
HBCD	10.1	15	6.83	1.02-81.1	51.7	82	26.3	6.349-454
BDE-209	0.799	0.56	0.556	0.281-1.957	3.65	2.2	2.77	1.26-9.61
PBT	0.102	0.09	0.086	0.026-0.517	0.437	0.13	0.392	0.219-0.67
DPTE	0.039	0.03	0.033	Nd-0.179	0.17	0.09	0.176	0-0.42
HBB	0.067	0.1	0.033	Nd-0.5	0.342	0.6	0.176	0-3.18
BTBPE	0.129	0.17	0.08	Nd-0.922	0.514	0.39	0.409	0-1.96
DBDPE	8.06	5.5	5.8	2.45-21.8	37	23	27.9	15.6-112

Estimated Daily Intake (EDI) via Human Milk. EDI via human milk were calculated on the basis of levels of BFRs in breast milk and infant ingest data (750 mL/day) and body weight data from Chinese Dietary reference intakes (Edition 2013). <LOD was replaced by zero when calculating EDI. The results were listed in TABLE 1. Obviously, EDI of TBBPA, HBCD, BDE-209 and DBDPE were far higher than other BFRs[5].

Levels of BFRs in Foods. The Levels BFRs in food samples were listed in TABLE 2. In food sample, DBDPE levels were several order of magnitude higher than other novel BFRs. Compared to the three legacy, DBDPE levels were also higher but in the same order. The results strengthened our conclusion that DBDPE has outperformed other BFRs and become the primary produced and used BFR at present [4,5].

Table 2 Levles of BFRs in animal origin food composites from China (ng/g lw)

		Aquatic food	Meat	Egg	Milk
TBBPA	DF	18/20	19/20	10/20	19/20
	Mean±SD	3.05[a]±4.26	1.78±2.67	3.12±7.54	5.76±11.8
	Median	1.27	0.56	0.085	1.49
	Range	nd-13.5	nd-10.9	nd-33.1	nd-52
HBCD[b]	DF	19/20	20/20	18/20	19/20
	Mean±SD	4.29±5.79	2.52±3.32	2.23±2.45	1.98±1.32
	Median	2.55	1.26	1.46	1.82
	Range	nd-25.6	0.373-14.9	nd-10.4	nd-5.29
BDE-209	DF	20/20	19/20	20/20	19/20
	Mean±SD	1.46±1.00	1.77±3.27	2.17±1.06	0.671±0.78
	Median	1.11	0.768	2.07	0.457
	Range	0.446-4.7	nd-14.9	0.589-4.73	nd-3.64
PBT	DF	20/20	20/20	20/20	18/20
	Mean±SD	0.134±0.1	0.065±0.03	0.054±0.03	0.094±0.13
	Median	0.094	0.056	0.055	0.062
	Range	0.03-0.373	0.026-0.156	0.008-0.103	Nd-0.542
DPTE	DF	20/20	9/20	11/20	7/20
	Mean±SD	0.251±0.42	0.018±0.02	0.02±0.02	0.015±0.02
	Median	0.119	Nd	0.025	Nd
	Range	0.03-1.59	Nd-0.081	Nd-0.057	Nd-0.083
HBB	DF	19/20	18/20	12/20	11/20
	Mean±SD	0.25±0.47	0.031±0.03	0.019±0.02	0.016±0.02
	Median	0.119	0.027	0.021	0.009
	Range	nd-2.18	Nd-0.162	Nd-0.076	Nd-0.09
BTBPE	DF	12/20	7/20	14/20	5/20
	Mean±SD	0.152±0.43	0.018±0.03	0.305±0.89	0.053±0.12
	Median	0.077	Nd	0.085	Nd
	Range	nd-1.95	Nd-0.067	Nd-4.08	Nd-0.475
DBDPE	DF	16/20	16/20	18/20	19/20
	Mean±SD	12.4±15.8	6.52±6.31	8.15±5.54	8.02±6.94
	Median	7.94	5.34	6.85	6.44
	Range	nd-69.8	Nd-23.4	Nd-19.6	Nd-27.6

Estimated Daily Intake (EDI) via Foods. Daily dietary intakes of BFRs for the “reference” man (63 kg body weight) were estimated by multiplying the measured concentrations (pg/g wet weight) of the BFRs with the daily consumption data (g/day) from the survey mentioned above. Table 3 shows the lower bound EDI (replace <LOD by 0) of the BFRs. Large differences in food consumption patterns among provinces were found, which was resulted from differences in geographical conditions, culture, food habits and economic levels between regions in China.

Contribution percentages from the four food groups varied between provinces, whereas in the mean EDI, the contribution from meat group is always the greatest , indicating that although contamination levels in meat and meat product were not the highest among the four food groups, the contributions from meat and meat product were still higher than those from the other food groups, because the consumption of meat and meat product was evidently higher than that of other animal-original foods in China. [4,5].

Table 3. Lower Bound EDI (pg/kg bw/day) of BFRs

BFRs	EDI via food (ng/kg bw/day)				BFRs	EDI via food (ng/kg bw/day)			
	Mean	SD	Median	Range		Mean	SD	Median	Range
TBBPA	1340	1530	936	53-5710	DPTE	43.1	87	14.2	0.4-383
HBCD	1510	1680	863	278-6940	HBB	38.9	59	19	2.1-249
BDE-209	960	1100	707	122-5520	BTBPE	36.5	69	14.1	0-315
PBT	46.4	31	39.6	7.4-131	DBDPE	4600	4100	3380	641-18200

Risk assessment. The margin of exposure (MOE) approach was recommended by European Food Safety Authority (EFSA) for evaluating potential health risks from dietary intake of BFRs. MOE was calculated by comparing the BMDL10 to the EDI of each BFR. However, EU only suggested BMDL for TBBPA, HBCD and BDE-209. All these three BFRs demonstrated large MOEs for both dietary exposure and human milk exposure, indicating that it is unlikely that the current dietary exposure of the general population to TBBPA, HBCD and BDE-209 would pose a health risk. However, in this study, the average BFRs intake values via human milk for a 6-month-old boy were far higher than that for a "reference" man. It is indicated that the body BFRs burden of a nursing infant in China is much higher than that of adult (Fig 1)[4,5]. The possible toxicological impact of such a high daily intake should certainly be investigated.

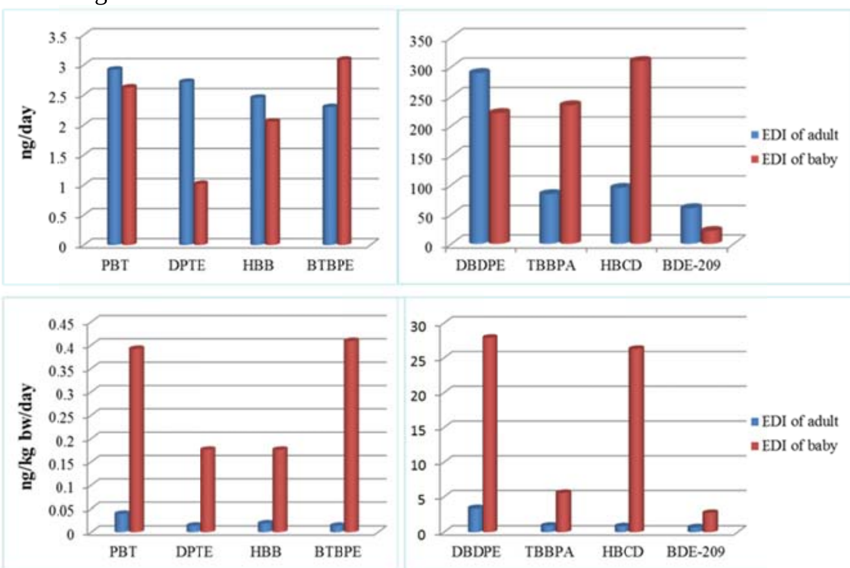

Fig. 1. Comparison of EDI between adult and nursing infant

Temporal Trends of TBBPA and HBCD in the TDS Compared with our former study[1], the fourth TDS which conducted in 2007, in which TBBPA and HBCD were measured at the first time, the comparison showed that the contaminant levels of BFRs increase evidently between 2007 and 2011. In the fourth TDS, the mean level of TBBPA and HBCD in pooled human milk were only 947 and 1033 pg/g lw, which was 4 to 7 fold lower than this TDS. For TBBPA and HBCD in food, the lower bound intakes of TBBPA and HBCD in former TDS were 232 and 256 pg/kg bw/day, respectively. Which was also lower than the EDI in this study (Fig 2). In detail, when the EDIs of TBBPA and HBCD via food consumption in the same 12 provinces from TDS 2007 and 2011 were compared (Fig .3), an increase in the EDIs of both TBBPA and HBCD were also observed from 2007 to 2011. For HBCD, rising EDIs were observed in most provinces, except in Shanghai and Hubei; however, the high EDIHBCD value in Shanghai in TDS 2007 was exceptional because of the extremely high HBCD levels in aquatic foods. For TBBPA, the EDIs in some provinces, such as Shanxi, Jiangxi, Sichuan and Guangxi, jumped sharply by 20 to 80 times from 2007 to 2011, whereas in other provinces, only a small increase or small decrease were observed. In summary, higher levels and EDIs of TBBPA and HBCD in TDS 2011 suggested a rapid increase in demand for these two BFRs between 2007 and 2011. In China, the legislative focus is still firmly on PBDEs, while TBBPA has received little attention until now; especially after the restriction of PBDEs and the EU official approval of the use of TBBPA as a safe FR, the production and application of TBBPA obviously increased. Thus, we predict that TBBPA contamination levels in China will continue to increase in the future. HBCD was listed as a POP in 2013; the TDS in the present study was conducted in 2011, and thus HBCD was still in use when we performed the study. Therefore, we conclude that the production and application of HBCD also continued to increase between 2007 and 2011, resulting in higher levels of HBCD in TDS 2011. In addition, although HBCD has been phased out in many fields in China since 2016, the use of HBCD in building materials is still permitted. That is, the production and application of HBCD continues in China, and the contamination levels of HBCD may also continue to increase in the future [4,5].

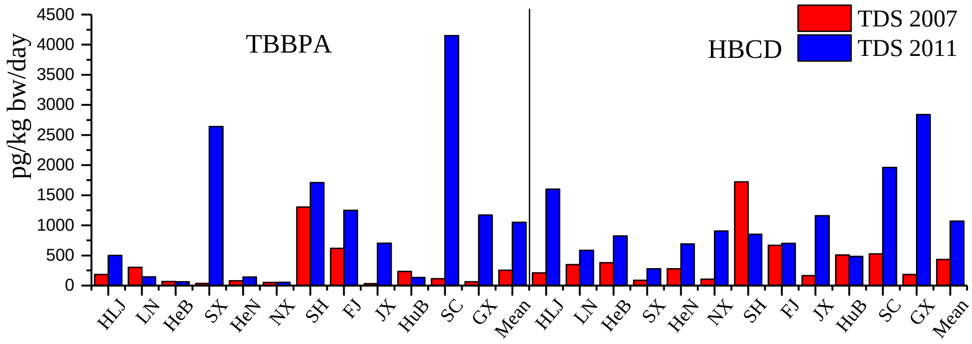

Fig.2. Comparison of EDIs of TBBPA and HBCD via food consumption

A Comparison between legacy and novel BFRs The mean and median levels of the three legacy BFRs, TBBPA, HBCD and BDE-209, were still far higher than those of the novel BFRs (except for DBDPE). Particularly, DPDPE showed high contamination levels in both human milk and food samples. Levels of DBDPE were not only several orders of magnitude higher than those of other novel BFRs, but also similar and higher than that of the legacy BFRs, indicating that the production and application of DBDPE has outperformed most of the currently used BFRs, resulting in high contamination level in human milk and food (Fig 3,4)[4,5]. The contamination, human exposure and health effect of DBDPE should be deeply investigated in later study.

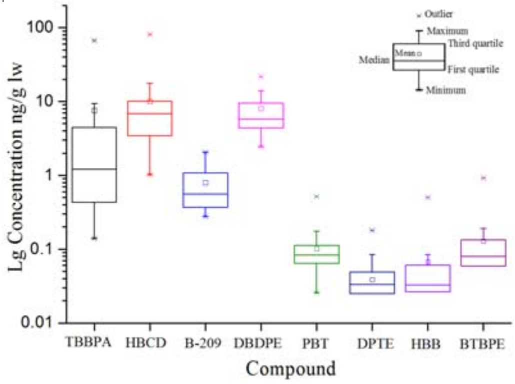

Fig. 3. A comparison between legacy and novel BFRs in the national human milk survey in 2011.

Fig. 4. A comparison between legacy and novel BFRs in EDI via food

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References

1. Shi ZX, Wu YN, Li JG., Zhao YF, Feng JF. (2009) *Environmental Science & Technology*, **43(12)** 4314-4319.
2. Shi, ZX, Wang YF, Niu PY, Wang JD, Sun ZW, Zhang SH, Wu YN. (2013) *Journal of Separation Science*, **36** 3402–3410.
3. Gao L, Li J, Wu YD, Yu MH, Chen T, Shi ZX, Zhou XQ, Sun ZW (2016) *Analytical and Bioanalytical Chemistry*, **408(27)** 7835–7844.
4. Shi ZX, Zhang L, Li JG, Zhao YF, Sun ZW, Zhou XQ, Wu YN. (2016) *Environment International*, **96** 82-90
5. Shi ZX, Zhang L, , Zhao YF, Sun ZW, Zhou XQ, Li JG, Wu YN. (2017) *Science of the Total Environment*, **599-600** 237-245