

LEVELS AND PROFILES OF HEXABROMOCYCLODODECANES IN HUMAN BREAST MILK FROM THE GENERAL POPULATION IN SHENZHEN, CHINA

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Introduction

Hexabromocyclododecanes (HBCDs) are a kind of additive brominated flame retardants (BFRs) which are mainly used in expanded polystyrene foam (EPS) and extruded polystyrene foam (XPS) for insulation and construction, and they are also used in textile application and electric as well as electronic appliances. HBCDs are one kind of highest-volume BFRs used nowadays. In 2001, the world market demand for HBCDs was 16,700 tons, in 2011, annual production of HBCDs is approximately 28,000 tons per year (9,000 to 15,000 tons in China, 13,426 tons in Europe and the US). The commercial HBCDs mixtures mainly consist of three diastereoisomers (α -HBCD, β -HBCD and γ -HBCD)¹.

HBCDs could release into the environment during production, manufacturing, processing, transportation, or disposal of them or products containing them. Due to their environmental persistence, long distance migration and toxicities, they have been added up to Annex A of Stockholm Convention on Persistent Organic Pollutants in 2013, and the amendment will enter into force on 26 November 2014. In recent years, HBCDs have been detected in various samples from environment and organism matrix². However, few studies regarding HBCDs concentrations in human samples have been reported, especially in China.

In the present study, the main objective is to determine the individual levels of HBCD diastereomers (α , β and γ -HBCD) in human breast milk collected in two districts in Shenzhen city. And we hope these data can provide the preliminary scientific data of HBCDs of human body burden for Shenzhen area, furthermore to provide the basis for assessing the relationship between the HBCDs body burden and human health effects in the future.

Materials and methods

Sample Collection. In this research, 118 breast milk samples were collected from participants during 4 to 8 weeks postpartum from Apr.2011 to Aug.2013. The subjects were single delivery primiparas who have lived in Shenzhen for more than 3 years without occupational exposure to HBCDs. Approximately 100mL of milk were collected in the prewashed bottle with Teflon seals. Personal demographic characteristics of nursing mothers were also collected.

Chemical Analysis. The determination of HBCDs from the breast milk samples was based on the method developed and validated elsewhere³. Breast milk samples were freeze-dried, homogenized, and stored at -20°C before extraction. 3g dry weight of breast milk were spiked with 20 μ L of ¹³C₁₂ labeled mixed standard α -, β -, γ - HBCD at 100ng/mL, and then Soxhlet extracted for about 5h with a mixture of n-hexane and acetone (1:1, v/v). The lipid content was determined by gravimetry. And the extract was purified by gel permeation chromatography (GPC) and concentrated sulfuric acid treatment. A 6ml mixture of ethyl acetate/cyclohexane (1:1, v/v) was used to elute the GPC column at 4.8mL/min flow rate. The fractions collected eluate from 8 to 15 minutes were rotated to dryness. After reconstitution in 7ml n-hexane, 2mL 98% concentrated sulfuric acid was used to remove the remaining fat and repeated for 3 times. And the final hexane extract was dried by nitrogen evaporation to nearly dryness, and reconstituted to 100 μ L using methanol before analyzing by HPLC-MS/MS. High-performance liquid chromatographic system coupled with a triple quadrupole mass spectrometer equipped with electrospray ionization source was operated in negative ion mode. Multiple reaction monitoring (MRM) was employed for quantitative measurement.

Statistical Analysis. All data were analyzed using SPSS 17.0 statistical software.

Results and discussion

1 Characteristics of the human milk donors

The personal demographic characteristics of nursing mothers were listed in Table 1. All 118 donors in this study were primiparas lived urban of Shenzhen. The age ranged from 20 to 35 years old with a mean age of 29.18 years old, and their BMIs ranged from 14.53 to 31.96 kg m⁻² with an average of 22.53 kg m⁻², their local residence time in Shenzhen ranged from 3 to 31 years with an average of 7.57 years.

Table 1 Personal demographic characteristics of nursing mothers (n=118)

parameters	Range	$\bar{x}\pm s$
Age(year)	20-35	29.18±2.81
Weight(kg)	41-87	58.00±7.87
Height(cm)	150-172	160.39±4.53
Body mass index(kg m ⁻²)	14.53-31.96	22.53±2.80
Local residence time(year)	3-31	7.57±5.42

2 HBCDs levels and diastereoisomers profiles

The concentrations of α -HBCD, β -HBCD, γ -HBCD and Σ HBCDs as determined in breast milk samples were listed in Table 2. In this study, α -HBCD was found in all 118 breast milk samples, while β -HBCD was found in 18.6% of the samples and γ -HBCD was found in 22% of the samples. The levels of Σ HBCDs in all analyzed samples ranged from 0.58 to 15.08 ng g⁻¹ lipid weight with mean level of 2.41 ng g⁻¹ lipid weight and median level of 1.78 ng g⁻¹ lipid weight. The levels of α -HBCD ranged from 0.58 to 14.27 ng g⁻¹ lipid weight with mean and median levels of 2.34 ng g⁻¹ lipid weight and 1.78 ng g⁻¹ lipid weight, respectively. The concentrations of β -HBCD and γ -HBCD were <LOD-1.30 ng g⁻¹ lipid weight (mean level: 0.02 ng g⁻¹ lipid weight) and <LOD-1.30 ng g⁻¹ lipid weight (mean level: 0.05 ng g⁻¹ lipid weight), median levels of both β -HBCD and γ -HBCD were below detectable limit.

Table 2 Concentrations of HBCDs (ng g⁻¹ lipid weight) in breast milk samples

	Range	Mean	25th percentile	median	75th percentile
α -HBCD	0.58-14.27	2.34	1.29	1.78	2.40
β -HBCD	ND-1.30	0.02	ND	ND	ND
γ -HBCD	ND-1.30	0.05	ND	ND	ND
Σ HBCDs	0.58-15.08	2.41	1.33	1.78	2.40

ND: Not detected.

In all samples, the contributions of α -HBCD ranged from 61.68% to 100% of total HBCDs with average of 97%, whereas β -HBCD comprising 0% to 25.44% (average of 0.9%) of total HBCDs, and γ -HBCD ranged from 0.00% to 37.10% (average of 2%). α -HBCD was the absolutely predominant isomer. This was quite different from most environmental samples and commercial products, but it was similar with many other studies on animal and human samples.

The HBCDs levels in breast milk in present study were higher than the literature data³ collected from 12 provinces of China in 2009. In that study, the HBCDs levels ranged from <LOD to 2.77 ng g⁻¹ lw (mean: 1.03 ng g⁻¹ lw). The present results were also higher than another data⁴ collected from a hospital of Shanghai in 2011, which the HBCDs levels ranged from 0.11 ng g⁻¹ lw to 37.75 ng g⁻¹ lw (median: 1.42 ng g⁻¹ lw). However the results were relatively lower than that in Beijing⁵ in 2013 (range: <LOD-78.28 ng g⁻¹ lw, median: 2.4 ng g⁻¹ lw, mean: 4.29 ng g⁻¹ lw). Compared with some other foreign reports, our results were lower than those in Australia⁶ (range: <LOD-19 ng g⁻¹ lw, mean: 10.2 ng g⁻¹ lw), the UK⁷ (range: 1.0-22.37 ng g⁻¹ lw, median: 3.83 ng g⁻¹ lw) and Spain⁸ (range: 3-188 ng g⁻¹ lw, median: 27 ng g⁻¹ lw) and similar to those in an e-waste recycling site in Vietnam⁹ (range: 1.4-7.6 ng g⁻¹ lw, median: 2.0 ng g⁻¹ lw) and a dumping site in India¹⁰ (range: 1.2-13 ng g⁻¹ lw, mean: 2.2 ng g⁻¹ lw). However, the levels were higher than those in non-dumping site in India¹⁰ (range: <0.05-3.6 ng g⁻¹ lw, mean: 0.36 ng g⁻¹ lw), Japan¹¹ (range: ND-4.0 ng g⁻¹ lw), Philippines¹² (range: 0.13-2.0 ng g⁻¹ lw, median: 0.62 ng g⁻¹ lw), North Russia¹³ (range: ND-1.67 ng g⁻¹ lw), Belgium¹⁴ (range: 1.0-22.37 ng g⁻¹ lw, median: 3.83 ng g⁻¹ lw), Norway¹⁵ (range: ND-31 ng g⁻¹ lw, median: 0.86 ng g⁻¹ lw) and South Africa¹⁶ (range: ND-1.4 ng g⁻¹ lw, median: 0.3 ng g⁻¹ lw). The concentrations of HBCDs in breast milk samples from different regions were listed in Table3. And comparison on median levels of HBCDs of some existing reports from domestic and abroad were shown in figure 1, it indicated that the body burden of HBCDs of mothers lived in Shenzhen was in above average level.

Table 3 Concentrations of HBCDs (ng g⁻¹ lipid weight) in breast milk samples from different regions

Region	Sampling year	Samples	Concentrations (ng g ⁻¹ lipid weight)				Reference
			ΣHBCD	α-HBCD	β-HBCD	γ-HBCD	
Belgium	2006	22*	2.0-6.4	-	-	-	14
Norway	2003-2005	310	ND-31	-	-	-	15
South Africa	2004	14	ND-1.4	-	-	-	16
Ghana	2004	25	ND-18	ND-16	ND	ND-2.2	17
	2009	42	0.010-3.2	0.030-3.2	ND-0.09	ND-0.07	
The UK	-	34	1.04-22.37	-	-	-	7
Australia	1993-2009	12*	ND-19	ND-10	3.6 #	ND-9.2	6
Japan	1973-2006	14	ND-4.0	ND-1.9	ND	ND-2.6	11
Philippine	2004	33	0.13-3.2	0.13-2.0	ND-0.46	ND-1.9	12
12 provinces , China	2007	24*	ND-2.78	ND-2.78	ND	0.46#	3
Shanghai, China	2006-2007	48	0.11-37.75	ND-37.68	ND-2.46	ND-4.92	4
Beijing, China	2011	103	ND-78.28	ND-71.18	ND-0.71	ND-6.49	5
Shenzhen, China	2011-2013	118	0.58-15.08	0.58-14.27	ND-1.30	ND-1.30	This Study

ND: Not detected.

*: Pooled samples.

#: Only detected in one sample.

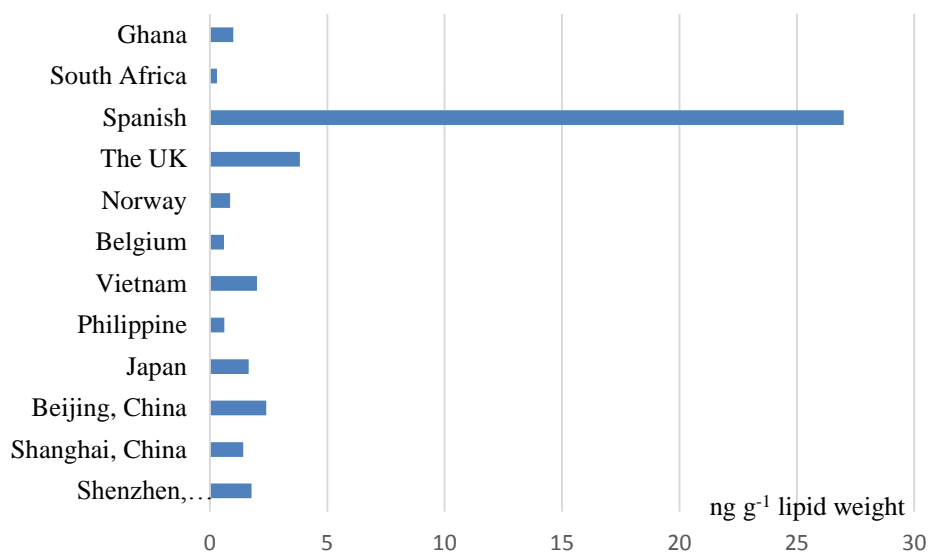


Figure 1 Median levels of HBCDs in some existing reports from domestic and abroad

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