INFANT EXPOSURE TO POLYBROMINATED DIPHENYL ETHERS (PBDEs) IN BREAST MILK FROM KOREA

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Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants (BFRs) commonly used in commercial products such as electronics and polyurethane foams. PBDEs have three commercial mixtures: pentabromodiphenyl ether (penta-BDE), octabromodiphenyl ether (octa-BDE) and decabromodiphenyl ether (deca-BDE). Among these, penta-BDE showed the highest toxicological effects such as developmental neurotoxicity and thyroid hormone homeostasis in laboratory animals[1,2]. PBDEs were designated as emerging persistent organic pollutants (POPs) by the United Nations Environment Programme (UNEP) in 2009. General populations are exposed to POPs mainly through dietary intake including seafood consumption[3,4,5] and accumulate POPs in the body fat. Unlike adults, infants have additional exposure pathways of POPs such as breast milks from their mothers. Because human breast milk has been used as specimen of human tissues for POP monitoring including PBDEs[6,7], the World Health Organization (WHO) has performed breast milk monitoring programs around the world under the POPs under Stockholm Convention since 2000s. Some studies have reported that breast milk feeding is an important exposure source of the POPs for infants to the total intakes of POPs[8,9]. Human breast milk provides a measure of maternal body burden and offers a unique opportunity to estimate POP exposure to infants during their lactation. The objectives of this study were to determine the concentrations of PBDEs in breast milks sampled from Korean pregnant women and to assess the potential risks to infant through PBDE exposure.

Materials and methods

Pregnant women were recruited from 5 university hospitals located in Seoul, Pyungchon, Ansan and Jeju, Korea during February-December in 2011. Breast milk samples (n=208) were collected from different periods after lactation (after 7, 15, 30 and 90 days) at delivery, which samples were separated in the hospital and stored in polypropylene tube at -70°C until analysis. Participants completed a detailed questionnaire including current or previous pregnancy history, medical history and demographic parameters. Questionnaire survey was conducted before delivery.

The experimental procedures of PBDEs in breast milks were optimized with some modifications from previous studies[5,10]. In brief, breast milk samples (2 mL) were fortified with formic acid and water for protein denaturation, after spiking with ^{13}C-labeled internal standards (MBDE-MXE; Wellington Laboratories, Guelph, ON, Canada). The samples were extracted by solid phase extraction (SPE) using C_{18} SPE cartridges (Waters; Milford, MA, USA), pre-washed with methanol and conditioned with Milli-Q water. The cartridge was rinsed with Milli-Q water and dried. A Sep-Pak Plus NH_2 cartridge (Waters, USA), prewashed with 6 mL of hexane, was attached to the lower end of the C_{18} cartridge. Eight milliliters of hexane were passed through NH_2-C_{18} cartridges and were collected. After removing C_{18} cartridge, 6 mL of 5% DCM in hexane was passed through NH_2 cartridge. The pooled eluants were cleaned up onto a silica gel/florisil SPE cartridge (Waters, USA), using 12 mL of 50% DCM in hexane. The eluants were concentrated and dissolved in 100 μL nonane for instrumental analysis. High-resolution gas chromatography interfaced with a high-resolution mass spectrometer (HRGC/HRMS; JMS 800D, JEOL, Tokyo, Japan) using relative response factors (RRF) of individual BDE congeners. Quantification was performed according to the isotope dilution method using ^{13}C-labeled standards. The HRMS was operated under selected ion monitoring (SIM) using molecular ions of PBDEs. The capillary column used was a DB5-MS (15 m length, 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific, Palo Alto, CA, USA) for the separation of PBDE congeners. All of the labeled internal standards were detected with no interferences. Solvents injected before and after the injection of standards showed negligible contamination or carryover. Procedural blanks were processed similarly to samples; blanks did not contain quantifiable amounts

of the target compounds. Limit of detection (LOD) was calculated as three times the signal to noise ratio, which ranged from 0.1 to 0.5 pg/g for tri- to hepta-BDEs. The concentrations of undetected congeners were treated as square root the respective LOD for each PBDE congener.

Results and discussion

Residue levels of PBDEs in breast milk

PBDEs were detected in all the breast milks from Korea with different detection frequencies (Table 1). Most frequently detected congeners of PBDEs were BDE 47, accounting for 99%. The following detected congeners of PBDEs were BDEs 99 (83%), 100 (57%), 153(42%), 183 (16%), 28 (15%) and 154 (7.2%). BDEs 156, 184 and 191 were not detected in all the samples. The other congeners of PBDEs were detected less than 5% frequencies for PBDEs.

Table 1. Concentrations of PBDEs (ng/g lw) in 208 breast milk collected from Korea, 2011

PBDE congener	Detectable [no. (%)]	Mean	SD	Median	Minimum	Maximum
BDE 28	32 (15)	0.08	0.51	-	ND	5.77
BDE 47	205 (99)	1.03	2.64	0.54	ND	28.2
BDE 99	172 (83)	0.67	2.15	0.29	ND	20.4
BDE 100	119 (57)	0.25	0.63	0.12	ND	5.59
BDE 153	88 (42)	0.57	1.78	-	ND	17.7
BDE 154	15 (7.2)	0.01	0.06	-	ND	0.37
BDE 183	34 (16)	0.07	0.21	-	ND	1.40
ΣPBDEs	208	2.74	6.91	1.46	0.23	68.4

The concentrations of total PBDEs (ΣPBDE; the sum of 19 PBDE congeners) in breast milks ranged from 0.23 to 68.4 ng/g lw (mean ± SD: 2.74 ± 6.91 ng/g lw; median: 1.46 ng/g). Among all the congeners of PBDEs, BDE 47 showed the highest concentration with average 1.03 ng/g lw. The next highest concentrations of PBDEs were BDE 99 (mean ± SD: 0.67 ± 2.15 ng/g lw), BDE 153 (0.57 ± 1.78 ng/g lw), BDE 100 (0.25 ± 0.63 ng/g lw) and BDE 183 (0.07 ± 0.21 ng/g lw). PBDE concentrations in breast milk measured in the present study were within the ranges of those reported for previous studies in some Europe countries, such as Sweden[6], Finland[11], Belgium[12] and Poland[13] and some Asia regions, such as China[14], Taiwan[15] and Japan[16]. PBDE levels in present study were lower to those reported in previous studies from the North America such as the United States[8] and Canada[17] with high consumption of penta-BDE technical mixtures. In Korea, the major component of BFRs is deca-BDE with minor proportions of penta- and octa-BDE technical mixtures. Some studies have reported the occurrence of deca-BDE in abiotic and biotic compartments including human in Korea[5,18].

Accumulation profiles of PBDEs in breast milk

The relative contributions of the PBDE congeners for breast milk from Korea are shown in Figure 1. The predominant congener was BDE 47, which accounted for 45% of the total PBDE concentrations. Other major congeners of PBDEs were BDEs 99, 100, 153 and 183, consistent with previous studies[15,17,19]. Interestingly, the concentrations of BDE 153 were higher than the other congeners of PBDE congeners for the samples detected for BDE 153. Similar results were found for the breast milks from the United States[17], the Faroe Islands[7], China[14,20] and South Africa[9]. The prevalence of BDE 153 relative to BDE 47 seems to be associated with the longer half-life of BDE 153 in human[21] and debromination from highly brominated congener of PBDEs such as deca-BDE[8,16]. The accumulation patterns of PBDEs can be dependent on the human sample matrices such as blood, breast milk and adipose tissue[22]. Human blood reflects the recent exposure[23], while breast milk and adipose tissue reflect long-term exposure. In the same sample set of our study, human blood showed the dominance of BDE 47 relative to BDE 153 in all the samples. This difference in the congener patterns of PBDEs in blood and breast milk may be associated with biotransformation and debromination in human. The higher proportion of BDE 153 to BDE 47 was observed in human adipose tissue from Korea and other studies[18,22]. Further studies are needed to clarify the accumulation profiles of PBDEs among human tissues.

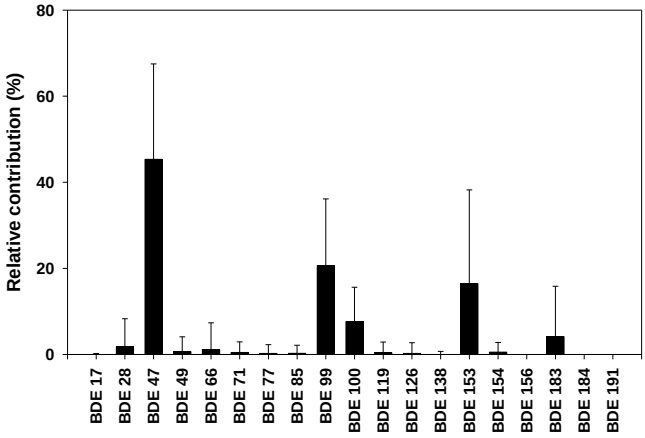

Figure 1. Accumulation profiles of PBDEs in breast milk (n=208) from Korea. Data were normalized to the total concentrations. Error bars represent standard deviations.

Estimated intake of PBDEs through breast milk

The estimated intakes (EI) of PBDE congeners through breast milk with different lactational periods for infants are summarized in Table 2. The EIs of ΣPBDE for < 7 days, 15 days and 30 days were 12.4, 17.5 and 14.8 ng/kg body weight/day, respectively. The EIs of ΣPBDE and individual congeners of PBDEs were the highest at 15 days (17.5 ng/kg bw/day) and were the lowest at < 7 days (12.4 ng/kg bw/day) lactation periods. The EIs of ΣPBDE measured in our study were higher than those measured in PBDE intakes for dust (10.3 ng/kg bw/day) and seafood consumption (1.09 ng/kg bw/day) in Korea, indicating the importance of breast milk for PBDE exposure to Korean infants. Compared to other countries, The EI (average: 14.9 ng/kg bw/day) of ΣPBDE to Korean infants were lower than those reported for Taiwan (20.6 ng/kg bw/day)[26] and China (28.6 ng/kg bw/day) [20]. However, the EI (average: 12.3 ng/kg bw/day) for sum of BDEs 47, 99 and 153 in our study was higher than those of Indonesia (6.0 ng/kg bw/day)[24] and USA (4 ng/kg bw/day) [25].

Table 2. Estimated intake of PBDE with separated lactational periods and compared to oral reference doses (RfD) from US EPA

After birth	< 7 days	15 days	30 days	
	PBDE intake (ng/kg bw/day)	PBDE intake (ng/kg bw/day)	PBDE intake (ng/kg bw/day)	RfD (ng/kg bw/day)
BDE 47	4.46 ± 11.0	6.29 ± 16.9	6.13 ± 10.2	100
BDE 99	2.94 ± 8.52	4.30 ± 15.6	3.35 ± 7.58	100
BDE 153	2.58 ± 10.7	3.73 ± 8.80	3.00 ± 5.83	200
ΣPBDE	12.4 ± 32.6	17.5 ± 42.8	14.8 ± 25.6	

To assess the potential health risks to Korean infants through breast milk feeding, the exposure levels were compared with oral reference doses (RfD) for BDEs 47, 99 and 153, obtained from integrated risk information system (IRIS) proposed by the United States Environmental Protection Agency (US EPA). In our study, the EIs of BDEs 47 (5.57 ng/kg bw/day), 99 (3.54 ng/kg bw/day) and 153 (3.15 ng/kg bw/day) through breast milk feeding in Korea were lower than the RfD for each congeners of PBDEs proposed by US EPA.

PBDE levels with demographic parameters

The concentrations of PBDEs showed age-dependent increase in breast milk (r = 0.176, p = 0.006), suggesting higher exposure to PBDEs with increasing age. The concentrations of PBDEs in breast milk samples did not correlate with different lactation periods (< 7, 15, 30 and 90 days), primi/multi-para conditions and BMI of pregnant women. No relationship was also found between questionnaire parameters such as life style and diets and the concentrations of PBDEs.

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