Polybrominated biphenyl ethers in breast milk and infant formula from Shanghai, China: Temporal trends, daily intake, and risk assessment

Xiaolan Zhang,1, Kaiqiong Zhang,1, Dan Yang,1, Li Ma,4, Bingli Lei,4, Xinyu Zhang,1, Jing Zhou,1, Xiangming Fang,1, Yingxin Yu,1,2,⁎

1 Institute of Environmental Pollution and Health, School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, PR China
2 Food and Chemical Quality Inspection Institution, Shanghai Institute of Quality Inspection and Technical Research, Shanghai 200233, PR China
3 Shanghai Huangpu Maternity & Infant Health Hospital, Shanghai 200020, PR China

HIGHLIGHTS
- PBDEs in milk decreased from 2006 to 2012 which decreases by half every 4 years.
- No significant differences in PBDE conc. among the different brand infant formulas.
- No correlations between the total PBDE conc. and age, parity and BMI of mothers.
- Breast-fed infants are exposed to much more PBDEs during nursing than formula-fed ones.
- PBDE exposure has no obvious adverse effects on breast- and formula-fed infants.

ABSTRACT
To investigate the temporal trend of polybrominated diphenyl ethers (PBDEs) in breast milk and assess the risks to breast- and formula-fed infants, breast milk and infant formula samples were collected from Shanghai, China. The PBDE concentrations decreased from 14.8 to 4.85 pmol/g lipid weight during 2006–2012, with a rate of decrease by half approximately every four years. Although there were no significant correlations between the total PBDEs in breast milk and age, parity, and pre-pregnant BMI of mothers, there were significant differences between primiparous and multiparous mothers for tri- to hepta-BDEs. PBDEs in breast milk were much higher than those in infant formula (equivalent to 91.9 vs. 5.25 pg/mL). Among the different brand infant formulas, there were no significant differences in their PBDE concentrations. The estimated daily intake of PBDEs by breast- and formula-fed infants suggested that breast-fed infants are exposed to much more PBDEs than formula-fed ones (12.9 vs. 0.72 ng/kg-bw/day). However, the hazard quotient values were much smaller than one, indicating that the ingested PBDEs did not exert obvious adverse effects on both breast- and formula-fed infants considering non-carcinogenic effect endpoint. This is the first report on temporal trend of PBDEs in breast milk from China.

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Abbreviations: BMI, body mass index; BW, body weight; EDI, estimated daily intake; GC, gas chromatograph; HQ, hazard quotient; IRIS, Integrated Risk Information System; LOD, limits of detection; MS, mass spectrometer; PBDEs, polybrominated diphenyl ethers; QA, quality assurance; QC, quality control; RfD, reference dose; SIM, selective ion monitoring.

⁎ Corresponding author. Tel.: +86 21 66137736; fax: +86 21 66136928.
E-mail address: yuyingxin@staff.shu.edu.cn (Y. Yu).
1 The authors contributed equally to this work.
1. Introduction

Polybrominated diphenyl ethers (PBDEs) are one class of commonly used brominated flame retardants. They are generally added to polymers which are widely used in various consumer products, especially in electronic appliances (La Guardia et al., 2006). As a kind of additive flame retardants, they are not chemically bound to the materials, and may release from their products into the environment during the entire life span. Because of their high lipophilicity and stability in the environment, PBDEs are persistent with potential for bioaccumulation in organisms and biomagnification through food chains (Wu et al., 2009; Yu et al., 2012a). Finally, they can enter human bodies by various sources and routes, such as food and air by oral ingestion and inhalation, respectively (Domingo, 2012; Harrad et al., 2010). It is possible that PBDEs might cause neurodevelopmental deficits, immunotoxicity, adverse effects on the reproductive system, and endocrine disruption (Chao et al., 2011; Kim et al., 2013; Main et al., 2007; Martin et al., 2007; Zota et al., 2011). Thus, there is a growing public concern over the PBDE exposure. Technical Penta- and Octa-BDE products have been listed as priority-controlled contaminants by Stockholm Convention on persistent organic pollutants (UNEP/POPS/COP.4/17, 2009).

Breast milk is the natural and ideal food for infants, and contains the optimal composition of nutrients to meet the nutritional needs of the newborn. In addition, breast-feeding has practical advantages for mothers. However, many mothers have no sufficient breast milk, or no milk at all. As an alternative, infant formula, a manufactured food designed and marketed for feeding infants, is usually used. Therefore, monitoring of PBDEs in breast milk and infant formula provides a good marker for the PBDE exposure from the environment and the food chain, especially for mothers and their nursing infants (Chao et al., 2007; Devanathan et al., 2012; Dunn et al., 2010; Thomsen et al., 2010a).

Studies have shown that PBDE concentrations in breast milk in North America are at least 10- and can be 100-times higher than those in Europe and Asia because of production of PBDEs at large amounts and their wide usage in North America (Eslami et al., 2006; Fängström et al., 2008; Schecter et al., 2003; Zhang et al., 2011). Time-trend studies indicated that the PBDE levels in breast milk increased rapidly from 1970s, but appeared to have stabilized or decreased since late 1990s or early 2000s (Lind et al., 2003; Meironytė et al., 1999; Thomsen et al., 2007). This may be attributed to the restriction and ban on the production and usage of low brominated BDEs (technical Penta- and Octa-BDE products) in Europe and some countries, such as USA and Japan. Since March 26, 2014, the production, usage, and trade of tetra- to hepta-BDEs have been prohibited in China.

It has been estimated that human milk is the main exposure source of PBDEs for breast-fed infants (Carrizo et al., 2007). PBDEs in breast milk can pose adverse effects on nursing infants, who are far more sensitive than adults (Chao et al., 2011; Main et al., 2007). Hence, continuous surveillance on PBDE levels in breast milk is necessary to accurately evaluate the environmental impact of PBDEs on human health in China (Zhang et al., 2011). However, there has been no investigation on the temporal trends in PBDE levels in breast milk in China.

Therefore, it is critical to conduct surveys and build a continuous monitoring program for PBDEs to assess the impact on human health. Thus, the aim of the present study was to investigate contamination of PBDEs in breast milk and infant formula, and the temporal trends in the PBDE levels during the period of 2006–2012, as well as the associated risk to breast- and formula-fed infants via PBDE exposure. We also examined if PBDE concentrations in breast milk are associated with age, parity, and pre-pregnant body mass index (BMI) of mothers. To our best knowledge, this is the first report on the temporal trends of PBDEs in breast milk in China.

2. Materials and methods

2.1. Sample collection

To determine the temporal trends of PBDE concentrations, we collected breast milk samples in 2006 (n = 16), 2008 (n = 13), 2010 (n = 21), and 2012 (n = 30). A total of 80 breast milk samples were collected in an infant health hospital in Huangpu (combined to the district of Pudong in 2011), Shanghai. The donors, who came from different districts of the city, were all local residents who have lived in Shanghai for at least 5 years. Before sample collection, all mothers were told about the objective of this study and all of them agreed to participate in the study. Their ages, parities, and pre-pregnant heights and weights were recorded. Breast milk samples were collected from these donors within 5 days after delivery. For each donor, more than 20 mL of milk was collected by manual expression or pumping. The collected samples were stored in brown glass flasks at −20 °C until analysis.

To compare the daily intake of PBDEs for breast-fed infants with formula-fed ones and the associated health risks, infant formula samples were collected from Shanghai markets. The samples covered infant formulas (from cow milk) from nine brands for 1–6 or 11–12 month infants, including Beingmate (n = 5), Dumex (n = 4), Wyeth S-26 (n = 5), Frisolac (n = 5), Meiji (n = 4), Enfapro (n = 5), Nestle (n = 5), Yili (n = 5), and Similac (n = 5), which account for at least 80% of the total consumption amounts in Shanghai markets. A total of 43 samples were collected in 2012 (the production time ranged from 2011 to 2012). The samples were purchased and stored at −20 °C until analysis.

2.2. Analytical methods

The methods used for sample extraction and cleanup were similar to those reported in our previous study (Zhou et al., 2012). In brief, breast milk samples were thawed and then homogenized through sonication. After spiking with the surrogate standards of 13C-PCB141 and 13C-BDE209, potassium oxalate solution (8%, w/w), ethanol, and diethyl ether were added before extraction. Generally, breast milk samples (8–10 mL) were extracted three times with n-hexane. The organic phases were combined and dried. The amounts of PBDEs were collected and concentrated to 50 μL after the internal standard of 13C-PCB208 was added. They were stored at −20 °C until analysis.

For infant formula samples, after adding surrogate standard of 13C-PCB141 and 13C-BDE209 to accurately weighed samples (3 g), the samples were added into 20 mL of acetonitrile. Then, anhydrous sodium sulfate (4 g) was added and the mixtures were vortexed violently for 1 min and centrifuged at 4200 rpm for 5 min. The supernatants were collected and the residues were extracted again. The organic phases were combined and dried. The residues were redissolved in 1 mL of n-hexane, and cleaned up with the same procedure as that used for breast milk.

2.3. Instrumental analysis

The PBDE concentrations were determined using a Hewlett-Packard (HP) 6890N gas chromatograph (GC) coupled to a 5975 mass spectrometer (MS). Negative chemical ionization mode was used. Splitless injection of a 1-μL sample was performed. The temperatures of injector and ion source were set at 280 and 250 °C, respectively. Quantification of
tri- to hepta-BDE congeners was achieved using a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, J & W Scientific, USA). And a DB-5MS capillary column (12 m × 0.25 mm × 0.1 μm, J & W Scientific, USA) was used to quantify BDE209 (the deca-BDE congener). The GC operating conditions for tri- to hepta-BDE congeners were as follows: the oven temperature was programmed from 110 °C (held for 1 min) to 180 °C (held for 1 min) at a rate of 8 °C/min, to 240 °C (held for 5 min) at 2 °C/min, to 280 °C (held for 5 min) at 2 °C/min, to 300 °C (held for 15 min) at 20 °C/min, and finally post run for 10 min. However, the oven temperature of GC for BDE209 analysis was programmed from 110 to 300 °C (held for 10 min) at a rate of 10 °C/min. The selective ion monitoring (SIM) mode was selected, and the ions m/z = 79/81 were monitored for tri- to hepta-BDE congeners, 486.7/488.7 for BDE209, 492/494 for 13C-BDE209, 476/478 for 13C-PCB208, and 372/374 for 13C-PCB141.

2.4. Quality assurance/quality control (QA/QC)

A total of 17 procedural blank was processed to monitor interfering peaks. BDE47 and 209 were observed in some of the blank samples. Replicate samples were randomly selected and the detected values of the PBDE congeners were comparable. Calibration plots for all of the congeners had satisfactory linear regression coefficients (R² > 0.99). The reported concentrations were not corrected against the recovery rates of the surrogate standard 13C-PCB141 (98.2 ± 22.7%) and 13C-BDE209 (75.9 ± 23.1%). The recovery rates of spiked PBDEs in infant formula powders were 74.6 ± 22.6%. For breast milk, the limits of detection (LODs) of tri- to hepta-BDE congeners (except for BDE47) ranged from 0.25 to 0.5 ng/g lw, and 3 ng/g lw for BDE209, which were based on 10-fold standard deviation obtained from the values of six measurements of the PBDE standard solutions (1 μL) with a signal-to-noise ratio of approximately 5–10, and were calculated on the basis of 0.2 g of lipid and 50 μL of volume for each sample, although the lipid content was varied for the samples. For infant formula, the LODs ranged from 16.7 to 50.0 pg/g dw for tri- to hepta-BDE congeners. The LOD was 1.2 ng/g lw in breast milk for BDE47, and 348 pg/g dw in infant formula for BDE209, which was set as three-fold average values of the congener in the blanks.

2.5. Calculations

The estimated daily intake (EDI, ng/kg-bw/day) of PBDEs by infants under 6 months was calculated as follows:

\[
\text{EDI} = \frac{C \times V}{\text{BW}}
\]  

(1)

where C (ng/mL) is the concentration of a PBDE congener in breast milk; V (ml/day) is the consumption rate of breast milk; and BW (kg) is the infant body weight. For formulas, the instructions indicate that 4.5 g of formulas is added to 30 mL of water. The density of breast and formula milk was considered to be 1 g/mL. The estimated daily intake was calculated based on a milk assumption rate of 700 mL per day and an infant body weight of 5 kg (Li et al., 2009).

To assess health risks associated with exposure to PBDEs concerning non-carcinogenic endpoints, hazard quotient (HQ) values were estimated and used. It can be calculated simply by dividing the estimated daily intake by the reference dose (RfD) of PBDEs reported by the Integrated Risk Information System (IRIS) (http://www.epa.gov/iris/) of the USEPA as follows:

\[
\text{HQ} = \frac{f \times \text{EDI}}{\text{RfD}}
\]  

(2)

where f is the transfer factor of unit (which is 0.001 in this case).

According to the risk addition method, the total HQs of all PBDE congeners can be treated as the mathematical sum of the HQ values of single PBDE congener:

\[
\text{THQ} = \text{HQ}_1 + \text{HQ}_2 + \ldots + \text{HQ}_n
\]  

(3)

According to the IRIS report, RfD values of 0.1, 0.1, 0.2, and 7 μg/kg-bw/day were used for BDE47, 99, 153, and 209, respectively. When an estimated HQ value is less than one, it can be assumed that PBDE exposure does not pose a significant non-carcinogenic health risk, whereas a value of more than one suggests potential risks.

2.6. Statistical analysis

The concentration of a PBDE congener was considered to be zero when it was not detectable, and values below the LOD were assigned to be half of the LOD. For BDE209, it was considered to be zero when its concentrations were three times lower than of blank values even if it was detected. The statistical analyses of the data were carried out with SPSS 11.5 for Windows. Relationships between two groups of data were analyzed by independent sample test or bivariate correlations unless otherwise specified. All p-values were two-tailed, and the level of p < 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the participants

The characteristics of the mothers are shown in the Supporting information (Table S1). The lipid contents of breast milk ranged between 0.6% and 5.5%, with a mean of 2.18%. The mean age of the women was 28 years. The parities of the mothers were recorded only for the samples collected during 2008 and 2012, the primiparous and multiparous mothers made up 62.5% and 37.5% of the 64 participants, respectively. The average height and weight of the mothers were 161.7 cm and 63.6 kg, with a mean pre-pregnant body mass index of 24.3 kg/m² for the mothers in 2010–2012.

3.2. Concentrations of PBDEs in breast milk and infant formula

The concentrations of PBDEs in breast milk, which were expressed as ng/g lw (lipid weight) unless otherwise specified, were listed in Table 1. The sum of the concentrations of the 14 PBDE congeners in the breast milk samples varied widely from 0.61 to 25.3 ng/g lw, with a mean of 4.91 ng/g lw for all samples collected during 2006 and 2012. The mean concentration of tri- to hepta-BDEs was 3.27 ng/g lw for all samples. The PBDE concentrations were of the same order of magnitude as the concentrations in breast milk collected in 2007 in the same city (Ma et al., 2012). However, our data were much higher than that reported by Zhang et al. (2011) for tri- to hepta-BDEs in breast milk in Shanghai, which was 1.5 ng/g lw. The difference might be attributed to lower lipid contents in the samples in the present study than those in the report of Zhang et al. (2011) (2.18% vs. 3.3%), which might be due to the different time points of the sample collection. In the present study, the samples were collected within 5 days after delivery. By contrast, Zhang and coauthors might collect samples after 5 days post-delivery, since approximately 100 mL of breast milk was collected from each mother. As observed by Hooper et al. (2007), breastfeeding for 6 months decreases PBDE levels in mothers by 12–18%. A similar situation was noticed by Thomsen et al. (2010a), who reported a monthly decline of PBDE concentrations in breast milk by 1.7–4.7%. In addition, pooled samples used in the study by Zhang et al. (2011) might also influence their results.

To understand the magnitude of PBDE contamination, our data are compared with those reported in other regions. Although it may be
Table 1

<table>
<thead>
<tr>
<th>Infant formula (pg/g dw)</th>
<th>Breast milk (ng/g lw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>BDE17</td>
<td>nd</td>
</tr>
<tr>
<td>BDE28</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>BDE31</td>
<td>nd</td>
</tr>
<tr>
<td>BDE47</td>
<td>8.33</td>
</tr>
<tr>
<td>BDE66</td>
<td>nd</td>
</tr>
<tr>
<td>BDE99</td>
<td>8.33</td>
</tr>
<tr>
<td>BDE100</td>
<td>nd</td>
</tr>
<tr>
<td>BDE153</td>
<td>11.1</td>
</tr>
<tr>
<td>BDE183</td>
<td>nd</td>
</tr>
<tr>
<td>∑3-7PBDE</td>
<td>28.0</td>
</tr>
</tbody>
</table>

DR: detection rate; n: number of samples; nd: not detected.

Concentrations of PBDEs in breast milk and infant formula collected in Shanghai, China.

Breast milk (ng/g lw)

<table>
<thead>
<tr>
<th>Year</th>
<th>Median</th>
<th>Mean</th>
<th>Range</th>
<th>DR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006 (n = 16)</td>
<td>0.14</td>
<td>0.2</td>
<td>0.2</td>
<td>0.22</td>
</tr>
<tr>
<td>2007 (n = 13)</td>
<td>0.14</td>
<td>0.2</td>
<td>0.2</td>
<td>0.22</td>
</tr>
<tr>
<td>2008 (n = 12)</td>
<td>0.14</td>
<td>0.2</td>
<td>0.2</td>
<td>0.22</td>
</tr>
<tr>
<td>2009 (n = 30)</td>
<td>0.14</td>
<td>0.2</td>
<td>0.2</td>
<td>0.22</td>
</tr>
</tbody>
</table>

BDE31 was not detected in infant formulas.

The concentrations of PBDEs in breast milk decreased from 14.8 pmol/g lw in 2006 to 4.85 pmol/g lw in 2012, with the tri- to hepta-BDE concentrations reducing from 11.6 to 3.39 pmol/g lw. A sharp decrease was observed from 2006 to 2008. After that, the decrease in the PBDE concentrations became slight. The data measured by Ma et al. (2012) in the same city were combined with our data for further analysis, similar decreases were still observed, especially for the congeners of BDE28, 153, 183, and 209 (Fig. 1B). The total concentrations of BDE28, 47, 99, 153, 183, and 209 in the two studies for years 2006, 2007, 2008, 2010, and 2012 were 12.8, 6.39, 6.29, 5.59, and 4.2 pmol/g lw, respectively. There were significant differences between year 2006 and the others, and between year 2012 and years 2006 to 2008 (p < 0.05). The concentrations of PBDEs in breast milk in Shanghai seemed to have stabilized since 2007. Although the production, usage, and trade of tetra-
hepta-BDEs were prohibited in China recently, the usage of PBDEs might decrease because of the production of PBDE replacement, such as hexabromocyclododecane. More investigations should be carried out to study the temporal trends of PBDEs in China.

Temporal trend studies, which have been investigated by the monitoring of PBDE levels in breast milk for decades, offered valuable information for human health issues, especially for long-term changes. In a temporal trend study, the concentrations of PBDEs in breast milk in Sweden from early 1970s to 1997 showed a significant increase (Meironyté et al., 1999) and decreased between 1999 and 2001 (Lind et al., 2003). An increase in PBDE levels has been also observed in human samples in Japan, Norway, and North America (Akutsu et al., 2003; Sjödin et al., 2004; Thomsen et al., 2002). However, studies pointed out that the PBDE levels in serum seemed to have stabilized from 1999 to 2003 (Thomsen et al., 2007), and PBDE levels in breast milk were significantly lower in 2007 and 2008 than those in 2000 and 2001 (Chao et al., 2007; Koh et al., 2010), although some studies did not observe the decreases (Shy et al., 2012). The decreases of PBDE levels were probably due to the decreasing amounts of PBDEs released into the environment. Since 1999, technical PBDE products were restricted or banned in many countries. The decrease observed in the present study might also result from the restriction and ban.

In a previous study, the authors used an exponential equation to estimate that PBDE levels doubled every five years (Norén and Meironyté, 2000). We used an exponential equation to estimate the half-lives of PBDEs in breast milk and found that it took approximately four years for the PBDE concentrations to decrease by half ($p = 0.054$). The value was consistent with the reported half-lives of PBDEs in humans, such as BDE47 1.8–3.0 years, BDE99 2.9–5.4 years, and BDE153 6.5–11.7 years (Herbstman et al., 2007). However, it should be noted that the long half-lives of PBDEs do not necessarily result in high concentrations of PBDEs in humans. The decrease of PBDEs in breast milk in Shanghai might reflect the decrease in the PBDE concentrations in the environment. More studies are warranted for the monitoring of PBDE levels in various matrices.

### 3.4. Congener profiles and indicators of PBDEs in breast milk and infant formula

Congener profile analysis can offer valuable information of the sources of PBDEs (Fig. 2). In the present study, BDE209 was the predominant congener in breast milk. However, it was detected only in 54.3% of samples. The next predominant congeners were BDE153 and BDE47, which were detected in 100% and 96% of breast milk samples. The three congeners constituted 61.2% of the total amount of the PBDEs in breast milk collected during 2006–2012. Other abundant congeners included BDE100, 28, 183, and 99, which had high detection rates (76.5%–91.4%). For infant formulas, BDE153 was the predominant congener with a detection rate of 83.7%, followed by BDE47 and 99. The three congeners accounted for 80.7% of the total PBDEs in infant
formulas. Unexpectedly, BDE209 was not detected in all formula samples. The higher level of BDE209 in breast milk might be attributed to the large amount of usage of technical Deca-BDE products, in which BDE209 is the main component. Sudaryanto et al. (2008) assumed that the occurrence of BDE209 in human breast milk was probably due to preferential binding of BDE209 to serum protein rather than lipid matrix. In the present study, the high levels and relatively lower detection rate of BDE209 in breast milk may be an indicator of recent exposure to technical Deca-BDE products rather than mobilization from adipose tissues, because BDE209 is a PBDE congener with a short half-life in humans. High levels of BDE209 were generally observed in the environmental matrices in China (Ni et al., 2013).

BDE153 was the major congener in both breast milk and infant formulas. Recently, many studies, especially those from Asia and Europe, observed that BDE153 was the most common congener in a few human adipose tissue samples, such as breast milk (Fängström et al., 2005; Zhang et al., 2011). BDE153 is a minor component of technical Penta-BDE and Octa-BDE products (La Guardia et al., 2006). Based on our previous studies, the concentrations of BDE153 in food collected from markets and in indoor/outdoor house dust in Shanghai were much lower than those of BDE47 (Yu et al., 2011, 2012b). Food and house dust were generally considered the major sources of PBDEs for humans (Daso et al., 2010; Harrad et al., 2010; Li et al., 2014). Thus, the high concentrations and unique pattern of PBDEs in breast milk, with BDE153 having the higher concentration than BDE47, could possibly be explained by the higher persistence of BDE153 than BDE47 (6.5 vs. 1.8 years estimated from the daily intake and the total body burden under steady state conditions in non-occupationally exposed adult humans) (Geyer et al., 2004). The speculation could also obtain support from the relationship between the average ratio of BDE153 to 47 and the age of the mothers (Fig. S2). There was a positive nonparametric correlations between them ($p = 0.045$). The apparent increase in the predominance of BDE153 in the breast milk samples in Shanghai was suggestive of a shift in the congener profile in human bodies.

Relatively high abundance of BDE47, 99, and 100 was expectable, because these congeners are the main components of technical Penta-BDE products, and were detected in food and dust in Shanghai with high abundance (La Guardia et al., 2006; Yu et al., 2011, 2012b). However, the abundance of BDE28 was unusual, given that its contents in DE-71 and Bromkal 70-5DE are very low (0.25% and 0.1%, respectively) (La Guardia et al., 2006). A study by Zhang et al. (2011) found that BDE28 was a major congener in breast milk samples in China. Similarly to Japan, the abundance of BDE28 in breast milk might be attributed to the past use of peculiar technical Penta-BDE products (Akutsu et al., 2003). The same speculations were also proposed by Qiu et al. (2010) and Sudaryanto et al. (2008), who assumed this peculiar product to be the sources of PBDEs in the air in Taihu Lake and in human milk in Nanjing and Zhoushan in East China. Meanwhile, BDE183, a major congener found in technical Octa-BDE products, was observed as a major congener in the present breast milk samples, which was consistent with the production of commercial Octa-BDE products in China (Sudaryanto et al., 2008).

The composition profiles of PBDE congeners reflected the usage of technical PBDE products in local areas and the fates of PBDEs in the environment. The present results indicated that Shanghai residents were probably exposed to multiple sources of PBDEs from multiple pathways. To determine the sources and routes of PBDEs in humans, BDE209 is generally not a good indicator in breast milk because its low half-life and relatively low detection rate (Fängström et al., 2008). As proposed by Chao et al. (2007), BDE47 was the best predictive indicator for the total PBDE concentrations in breast milk. In the present study, BDE153 and 47 were observed to be the predominant PBDE congeners with high detection rates in the breast milk samples, and there was a significantly positive correlation ($p < 0.001$) between the total PBDE concentrations and the sum concentrations of BDE153 and 47 (Fig. S1). Considering the shift in the congener profile of PBDEs in human bodies, the two congeners might be a more useful indicator for the PBDE contamination in breast milk.

3.5. Influence of age, parity, and pre-pregnant BMI on PBDE concentrations in breast milk

Maternal age is an important parameter that is related to the variation in persistent organic pollutant concentrations in breast milk (Devanathan et al., 2012). Because of the bioaccumulative nature and slow depuration rates of these compounds, their concentrations tend to increase with age (Tanabe and Kunifuji, 2007). In the present study, with the exception of BDE183 ($p = 0.001$), we did not find a significantly age-dependent increase in the total or individual PBDE concentrations in breast milk ($p > 0.05$). The increase in the PBDE levels in breast milk with age has been demonstrated (Haraguchi et al., 2009; Thomsen et al., 2010b). For example, Thomsen et al. (2010b) observed that there was a correlation between the PBDE levels and age, while a study from Bizerte (Tunisia) by Hassine et al. (2012), BDE183, the predominant congener, showed a significantly negative correlation with age, and there was no correlation between the total PBDE concentrations and age. Actually, many studies showed that no age dependency had been found for the total PBDE concentrations in breast milk, although some congeners such as BDE153 showed correlations with age (Dunn et al., 2010; Eslami et al., 2006; Haraguchi et al., 2009; Lacorte and Ikonomou, 2009; Schecter et al., 2003; Zhang et al., 2011). These studies generally attributed the phenomenon to the small size of samples and narrow age range of participants. Nevertheless, no age dependency was found for PBDEs in human adipose tissue samples in Czech Republic with an age range of 17–60 years, and in Brazil with an age range of 40–70 years (Kalantzis et al., 2009; Pulkrobová et al., 2009). The absence of age dependency might be mainly because of continuing exposure; notably, steady-state PBDE concentrations are never achieved in human tissues, and bioaccumulation from the environment has a shorter time span than other persistent organic pollutants (Tanabe and Kunifuji, 2007). Furthermore, certain potential confounders, such as parity and pre-pregnant body mass index, might partly conceal the age correlation.

Parity was recognized as one of the confounders affecting PBDE levels in the different age groups. In the present study, there were significant differences between primiparous and multiparous mothers for BDE28 and tri- to hepta-BDEs ($p < 0.05$). Higher PBDE levels were detected in breast milk from primiparous mothers. For primiparous ($n = 40$) and multiparous mothers ($n = 24$), the concentrations were 2.8 and 2.1 ng/g lw for tri- to hepta-BDEs, and 4.06 and 3.57 ng/g lw for total PBDEs, respectively. The multiparous mothers were generally older than primiparous ones, with their mean ages being 29.8 and 27.1 years, respectively. Similar results were observed in the literature. As reported by Daniels et al. (2010), the mean concentrations of PBDEs were lower in women at the ages of 34 years compared with those at the ages of 25–29 years. Higher PBDE concentrations in younger mothers suggested that younger generations are exposed to higher levels of PBDEs than older ones (Daniels et al., 2010).

BMI may also be a factor affecting the concentrations of persistent organic pollutants in breast milk. No significant correlations were observed between the levels of PBDEs in breast milk and pre-pregnant BMI using Pearson correlation tests. A similar result was found between the body burden of PBDEs and the pre-pregnant BMI of mothers in Shenzhen (Zhang et al., 2012), although BDE47 had significantly higher concentrations in breast milk from mothers with the pre-pregnant BMI at <22.0 kg/m$^2$ than those with the pre-pregnant BMI at $\geq 22.0$ kg/m$^2$ ($1.59$ vs. $0.995$ ng/g lw, $p = 0.041$) (Wang et al., 2008). However, we found that higher PBDE concentrations were generally detected in mothers with medium pre-pregnant BMI. This was not consistent with the result reported by

Daniels et al. (2010), who observed that higher PBDE concentrations in breast milk were found in obese women compared to those with normal BMI. The result in the present study might be attributed to lipophlicity of PBDEs and the distribution of PBDEs among lipid, blood, and breast milk (Berghe et al., 2012).

### 3.6. Infant exposure and risk assessment

We estimated the daily intake of PBDEs by infants below 6 months. It was not difficult because breast milk, infant formula, or their combination were generally the only foods for them. The data were listed in Table 2. The estimated daily intake of total PBDEs on the basis of 95% concentrations was 35.3 ng/kg-bw/day for breast-fed infants. For formula-fed infants, the value was 1.39 ng/kg-bw/day, which was much lower than that for breast-fed ones (Table 2). This was consistent with the observation reported by Carrizo et al. (2007), who found that concentrations of PBDEs in sera from breast-fed children were much higher than those in infant formulas. Among the different infant formulas from the nine brands, there were no significant differences in their PBDE concentrations. BDE47 and 153 might be useful indicators for PBDE contamination in breast milk. There were no significant correlations between the total PBDE concentrations in breast milk and the maternal age, parity, and pre-pregnant BMI. In addition, the estimated daily intake of PBDEs by breast- and formula-fed infants indicated that breast-fed infants would be exposed to much more PBDEs during nursing. However, the hazard quotient values for the two groups of infants were much smaller than 1, suggesting that the health risks associated with ingestion of PBDEs were very low.

###Conflict of interest

The authors declare no competing financial interest.

###Acknowledgment

This research was financially supported by the National Nature Science Foundation of China (Nos. 21277086 and 21277087), the Key Discipline Construction Project of Shanghai Municipal Public Health (No. 12GWZX0401), and Program for Innovative Research Team in University (No. IRT13078).

###Appendix A. Supplementary data

Supplementary data associated with this article can be found in Table S1–2 and Fig. S1–3. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2014.08.034.

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