Temporal Trend of Polybrominated Diphenyl Ethers in Archived Breast Milk Samples from Osaka, Japan

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Introduction

Polybrominated diphenyl ethers (PBDEs) were widely used as flame retardants in consumer products and are now classified as persistent organic pollutants (POPs). PBDEs are lipophilic and they accumulate in the fatty tissue of wildlife and humans. Infant exposure to PBDEs via breastfeeding may have the potential to cause neurodevelopmental disorders and thyroid hormone disruption. Therefore, monitoring of PBDEs in breast milk is important. Our previous study showed that PBDE levels in Japanese pooled breast milk samples had increased between the 1970s and 1990s (Akutsu et al. 2003). The aim of the present study was to provide information on the recent levels and temporal trend of PBDEs in Japanese mother's milk.

Materials and Methods

*Sample*s: Breast milk samples were collected from healthy women living in Osaka, Japan between 2001 and 2006. Informed consent was obtained from each donor. The age of the volunteers (13–31 mothers per sampling year) ranged from 25 to 29 years. Breast milk was collected within a period of 30–90 days after their first childbirth.

Analytical Standards and Chemicals: Native and isotopically labeled standard solutions of PBDEs were purchased from AccuStandard (New Haven, CT, USA) and Wellington Laboratories (Ontario, Canada). In this study, 36 PBDE congeners having three to ten bromine atoms were monitored; tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and decabromo congeners were abbreviated as TrBDE, TeBDE, PeBDE, HxBDE, HpBDE, OcBDE, NoBDE, and DeBDE, respectively. The monitored congeners included eight TrBDEs (#17, #25, #28, #30, #32, #33, #35, and #37); six TeBDEs (#47, #49, #66, #71, #75, and #77); seven PeBDEs (#85, #99, #100, #116, #118, #119, and #126); five HxBDEs (#138, #153, #154, #155, and #166); three HpBDEs (#181, #183, and #190); three OcBDEs (#196, #197, and #203); three NoBDEs (#206, #207, and #208); and one DeBDE (#209). Most of these were representative congeners found in technical penta-, octa-, and decaBDE formulations. The nine isotopically labelled PBDEs ($^{13}C_{12}$-BDE-28, $^{13}C_{12}$-BDE-47, $^{13}C_{12}$-BDE-99, $^{13}C_{12}$-BDE-153, $^{13}C_{12}$-BDE-154, $^{13}C_{12}$-BDE-183, $^{13}C_{12}$-BDE-197, $^{13}C_{12}$-BDE-207, and $^{13}C_{12}$-BDE-209) were used as surrogate internal standards, and $^{13}C_{12}$-BDE-126 was used as a syringe spike. Acetone, acetonitrile, and n-hexane of pesticide analysis grade and 44% sulfuric acid-impregnated silica gel and n-nonane of dioxin-analysis grade were purchased from Wako Pure Chemicals (Osaka, Japan).

Analysis: Lipid was quantitatively extracted from each milk sample with a mixture of potassium oxalate, ethanol, diethyl ether, and n-hexane. The extracted lipid samples were pooled per sampling year by mixing equal amounts of the individual lipid samples, and the pooled samples were maintained at −20°C until clean-up. The pooled milk lipid samples collected from 2001 to 2006 were analyzed. The pooled milk lipid samples were spiked with ^{13}C-labeled PBDEs and were then purified by a gel permeation chromatography (GPC). The collected fraction was evaporated to dryness and

redissolved in n-hexane. The extract was purified with sulfuric acid-impregnated silica gel by using n-hexane as an eluent. The eluate was concentrated, and then spiked with a syringe spike solution. The cleaned extract was analyzed by GC/MS. Quantitation was based on an isotope dilution method by using $^{13}C_{12}$-labeled internal standards. The identification of PBDE congeners was performed by comparing their retention times and isotope ratios with those of authentic calibration standards. The LODs of individual PBDE congeners varied from 0.01 to 0.2 ng/g lipid weight (ng/g lw).

Results and Discussion

Table 1 shows the concentrations of PBDE congeners in pooled breast milk samples collected between 2001 and 2006. A total of 14 congeners of tri- to nona-BDE were detected. Twelve congeners of tri- to octa-BDE (#28/33, #49, #47, #66, #100, #99, #155, #154, #153, #183, and #197) were detected in all the samples. Two congeners of tri- and nona-BDE (#37 and #207) were detected in several samples at low concentration. Concentrations of sum of 14 BDE congeners (∑14 PBDEs) ranged from 1.60 to 2.85 ng/g lw. BDE-47 (32%–57% of the ∑14 PBDEs) was the most abundant congener followed by BDE-153 (12%–27% of the ∑14 PBDEs) in the breast milk samples. The PBDE profiles and levels in the pooled breast milk samples in the present study are almost in good agreement with those reported in other studies on Japanese breast milk (Inoue et al. 2006; Haraguchi et al. 2009). Typical chromatograms of tetra- to hexa-BDE congeners detected in pooled breast milk are shown in Figure 1.

Table 1. Concentrations of PBDEs in breast milk samples from Osaka, 2001–2006

	Concentration (ng/g lw)					
Congener	2001	2002	2003	2004	2005	2006
BDE-28/33	0.19	0.14	0.20	0.09	0.11	0.19
BDE-37	0.01	0.02	0.01	ND	0.01	0.01
BDE-49	0.07	0.07	0.07	0.06	0.08	0.08
BDE-47	0.73	0.71	1.07	0.60	0.53	1.63
BDE-66	0.03	0.03	0.03	0.03	0.02	0.02
BDE-100	0.17	0.18	0.21	0.15	0.16	0.21
BDE-99	0.31	0.16	0.16	0.16	0.07	0.19
BDE-155	0.02	0.03	0.02	0.01	0.03	0.02
BDE-154	0.04	0.05	0.05	0.04	0.05	0.05
BDE-153	0.34	0.37	0.43	0.33	0.45	0.35
BDE-183	0.05	0.07	0.04	0.04	0.04	0.04
BDE-197	0.07	0.12	0.09	0.09	0.13	0.06
BDE-207	0.05	ND	ND	ND	ND	ND
∑14PBDEs	2.08	1.95	2.38	1.60	1.68	2.85

ND: not detected.
Only congeners detected in at least one sample are shown in the table.

Figure 2 shows the time trend of pentaBDE related congeners (sum of 6 congeners) in the pooled breast milk samples, 1973–2006. The concentrations of pentaBDE related congeners increased until the late 1980s, and then almost leveled off. It is not possible to see any temporal trend for decaBDE related congeners in the pooled milk samples due to low levels over the period studied. It is yet not

clear how the concentrations of PBDEs will develop over the future time. Environmental and human monitoring studies should be continued.

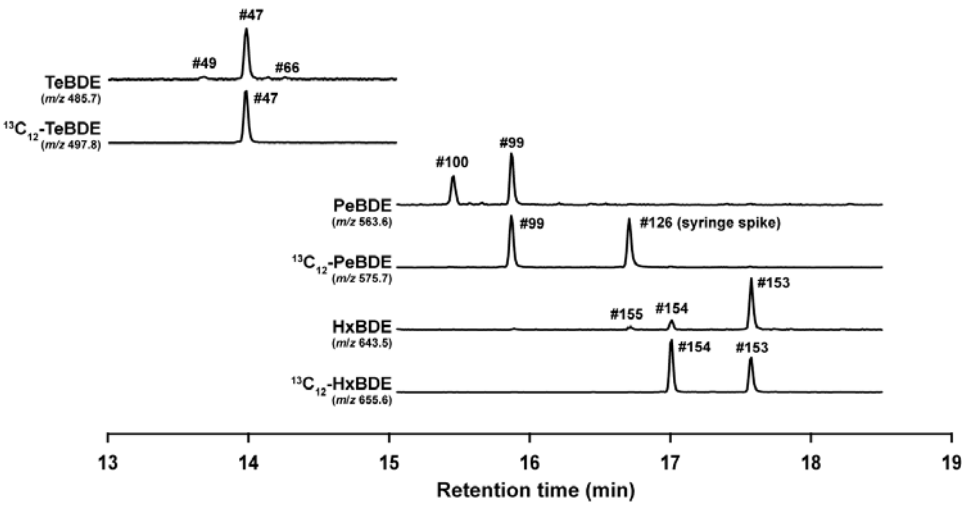

Figure 1. Typical chromatograms of tetra- to hexa-BDEs in breast milk samples. The chromatograms are normalized to the most intense peak in each *m/z* value.

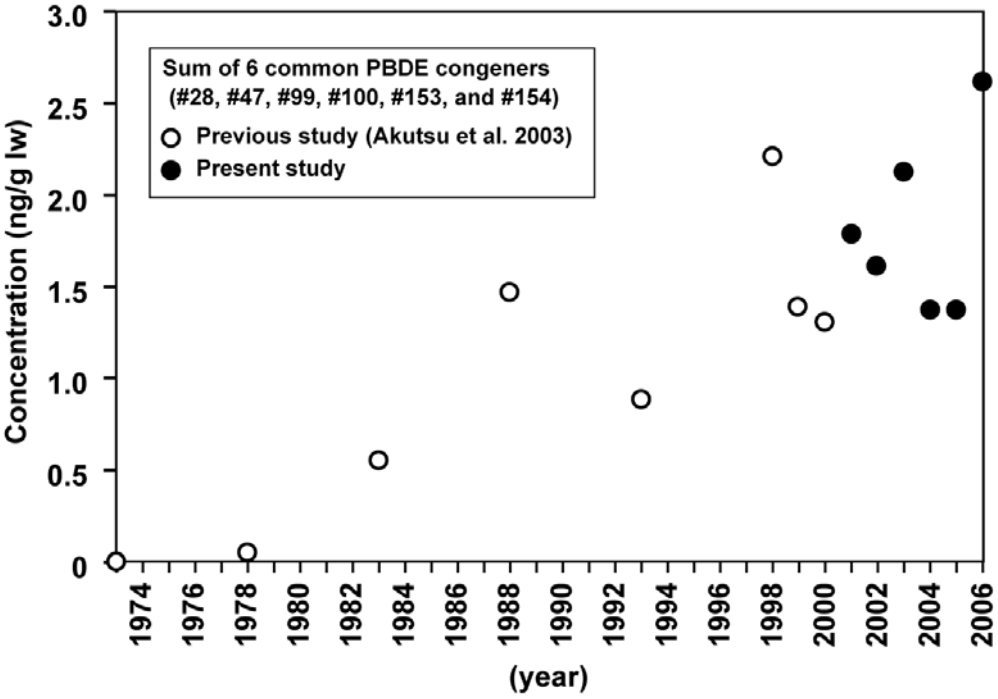

Figure 2. Temporal trend data of sum of pentaBDE related congeners in Japanese breast milk samples collected between 1973 and 2006. The data points obtained from our previous and present studies are indicated by open and solid dots, respectively.

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