Comparison of Brominated and Phosphorus Flame Retardants (FR) in Human Breast Milk Samples of Japan Based FR Demands

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Introduction

Polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA) are brominated flame retardants (BFRs) used in polymeric materials for fire safety of textiles, building materials and electronics. BFRs have been identified in various environment samples, including sediment, air and house dust. Moreover, BFRs have been identified in human tissue samples, such as blood and breast milk, because of their bio accumulative nature. We have previously reported the detection of TBBPA in Japanese human milk[1]. Furthermore, in vitro studies have shown that TBBPA disrupts thyroid hormone, estrogen, and immunosuppressive homeostasis, and in vivo studies have demonstrated its effects on endocrine signaling and neurobehavioral activity.

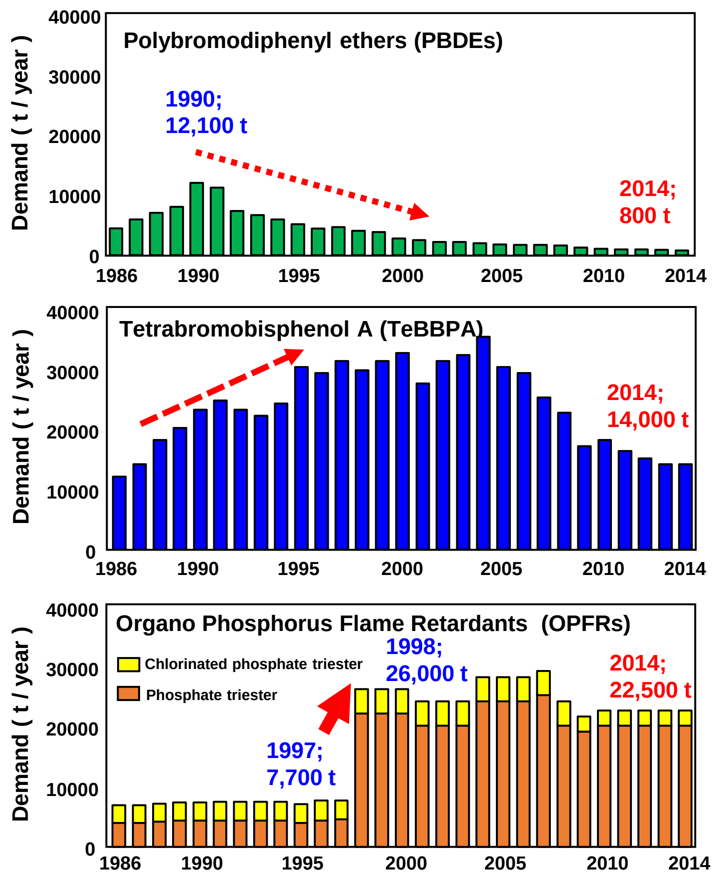

Fig. 1 Changes in demand for major flame retardants in the last three decades

On the other hand, organ phosphorus flame retardants (PFRs) were replaced by BFRs between 1990 to 1998 (Fig. 1). PFRs are widely used as additive flame retardants in polyurethane foams. A large demand among PFRs are triphenyl phosphate (TPP) and tris(dichloroisopropyl) phosphate (TDCIPP). These compounds possess a phosphate backbone. More studies that have investigated the effects about TPP and tricresyl phosphate (TCP). Recent in vitro studies showed that TPP could bind to and activate PPARγ ligands in humans[2]. PFRs has aroused great attention regarding its potential adverse effects. However, long term health effects of PFRs are not known, there is a need for human biomonitoring of PFRs. Because regulation of BFRs as Stockholm Convention on Persistent Organic Pollutants, usage of these PFRs increase rapidly. Based on these things, it is important to assess for human pollution by BFRs and PFRs.

In this paper, BFRs (PBDEs and TBBPA) and PFRs were investigated in breast milk samples of Japan.

Materials and methods

1. Samples

The samples of breast milk were collected from four women (age; 23 – 32 years old) at one week after delivery between December 2012 to June 2013.

2. Materials

PBDEs, ^{13}C-PBDEs, TBBPA, ^{13}C-TeBBPA, PFRs and d-PFRs standards were obtained from Cambridge Isotope Laboratory (MA, USA). BPA and d-BPA standards were purchased from Kanto Chemical (Tokyo, Japan). Oasis HLB cartridges (500 mg, 6 cc) used for purification was purchased from Waters (Tokyo, Japan). The other reagents and solvents were purchased from Wako Pure Chemicals (Osaka, Japan).

3. Experimental method

Breast milk samples (5.0 mL) were spiked with 5.0 ng each of 13C-PBDEs, 13C-TeBBPA, d-BPA and d-PFRs (d-TPP, d-tripropyl phosphate, d-tributyl phosphate, d-triphenyl phosphate, d-tris(2-chloroisopropyl) phosphate, d-TDCIPP) in a glass flask. Next, 25% 2-propanol in formic acid was added, and the samples were sonicated for 5 min in an ultrasonic bath. The samples were then diluted with 50% 2-propanol in water, and after another 5 min of sonication, were purified by solid-phase extraction (SPE). Treated samples were loaded onto the cartridge, and the flasks were rinsed with 25% methanol in water to remove any residual milk, which was also loaded onto the cartridge. These cartridges were then washed with 0.05% 2-propanol in water. After complete drying of the cartridges, the adsorbed matter in the cartridge was eluted with 70% dichloromethane in methanol, and the eluate was gently evaporated to dryness at 45 ˚C under a stream of nitrogen.

For PBDEs and PFRs analysis, an aliquot of extract was cleaned up by partitioning with acetonitrile and n-hexane. Consequently, the partitioning sample was concentrated to 1 mL and separated by chromatography into two fractions on a florisil column chromatography. For TBBPA analysis, an aliquot of extract was ethylation and then separated by florisil chromatography. All purified samples were analyzed by the use of HP6890GC-JEOL JMS700 MS in EI-SIM mode.

Results and discussion

BFRs (Tri to DeBDE and TBBPA) and PFRs levels were compared using breast milk samples from four Japanese mothers (Table 1). PBDE was detected in the range of 13-150 ng/g lipid, and TBBPA was detected in the range of 1.9-34 ng/g lipid. The correlation was not accepted to PBDEs and TBBPA. Total PBDE levels (collected at 2012-2013) in breast milk shows to decrease slightly comparison with our previous data (collected at 2003). On the other hand, PFRs was detected in all samples. Their level was 310-1100 ng/g lipid. In particular, high PBDEs and PFRs levels was observed in No. 4 (150 and 1100 ng/g lipid, respectively). Main compounds are TPP and TCDIPP. Because these compounds have high octanol-water partition coefficient, it was estimated that TPP and TDCIPP bioaccumulated in breast milk. This result suggests that the existence of pollutants by BFRs and PFRs in living environment such as food, drinking water and indoor air etc. Consequently, it is important to investigate about elucidation of exposure sources and risk assessment for the baby.

Acknowledgements

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Table 1 Concentrations (ng/g lipid weight) of brominated and phosphorus flame retardant in human breast milk

	No. 1	No. 2	No. 3	No. 4
Brominated flame retardant (BFR)				
TriBDEs	2.2	1.0	2.7	60
TeBDEs	1.3	0.81	1.3	6.9
PeBDEs	2.4	1.5	1.2	4.8
HxBDEs	11	7.7	8.6	54
HpBDEs	1.6	0.76	1.5	6.6
DeBDE	1.3	1.4	4.4	17
Total PBDEs	**20**	**13**	**20**	**150**
TBBPA	34	1.9	3.2	2.8
Total BFRs	**54**	**15**	**23**	**150**
Phosphorus flame retardant (PFR)				
Tripropyl phosphate	100	170	ND	2.1
Triisopropyl phosphate	15	ND	ND	48
Tributyl phosphate	150	22	ND	58
Tripentyl phosphate	2.4	ND	ND	24
Tri(2-ethylhexyl) phosphate	200	ND	ND	ND
Triphenyl phosphate	76	51	110	320
Tricresyl phosphate	34	27	21	110
Tris (1-chloro-2-propyl) phosphate*	320	250	180	510
Tris (1,3-dichloro-2-propyl) phosphate*	46	ND	ND	66
Total PFRs	**940**	**520**	**310**	**1100**

ND: Not Detected,

*: Chlorinated phosphorus flame retardant, others show phosphorus flame retardant