Contamination level of organo phosphorus flame retardants (OPFRs) in human breast milk of Japan

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Introduction

Organo phosphorus flame retardants (OPFRs, Fig. 1) is used for a synthetic resin used in an OA apparatus or a life article for the purpose of flame retardant widely. In recent years, these OPFRs came to be frequently used as a replacement of polybrominated diphenyl ethers (PBDEs) regulated in Stockholm convention and Act on the Evaluation of Chemical Substances and Regulation of their Manufacture, etc. in Japan. The demand increases rapidly from about 1998, and 21,500 tons is used in 2016 (Fig. 2). Although some OPFRs such as tris (2,3-dibromopropyl) phosphate (TDCIPP) using for a household articles for textiles, a bedding, a curtain and a floor carpet regulated by a law in our country, there is no regulation about other OPFRs. On the other hand, TDCIPP and tris (2-ethyl hexyl) phosphate are prohibited in Notrth America (Washington state etc.) and EU.

There are many reports about the toxicity of OPFRs. Triphenyl phosphate (TPP) have anti-androgenic activity from *in vitro* test[1]. TDCIPP show endocrine disruption of the thyroid hormone by antiandrogenic action in an *in vitro* test[2]. We concerned the adverse effects on ecosystem including human. In particular, the biological defense of fetus and infant is weak, their health effects are more likely to be affected by environmental pollutants. Althoght there are several reports about human contamination by OPFRs, a few investigation of health effect to infant via the breast milk conduct until now.

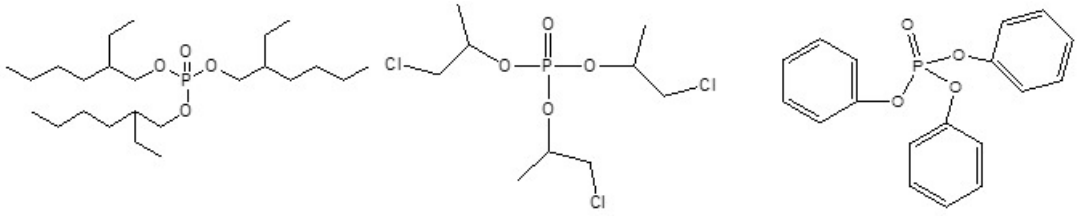

Fig. 1 A type of phosphorus flame retardant structures

Based on the above background, we examined the contamination level of OPFRs in the breast milk for the purpose of investigating the health effect on infants in this study. Moreover, we tried to clear exposure souces by OPFRs.

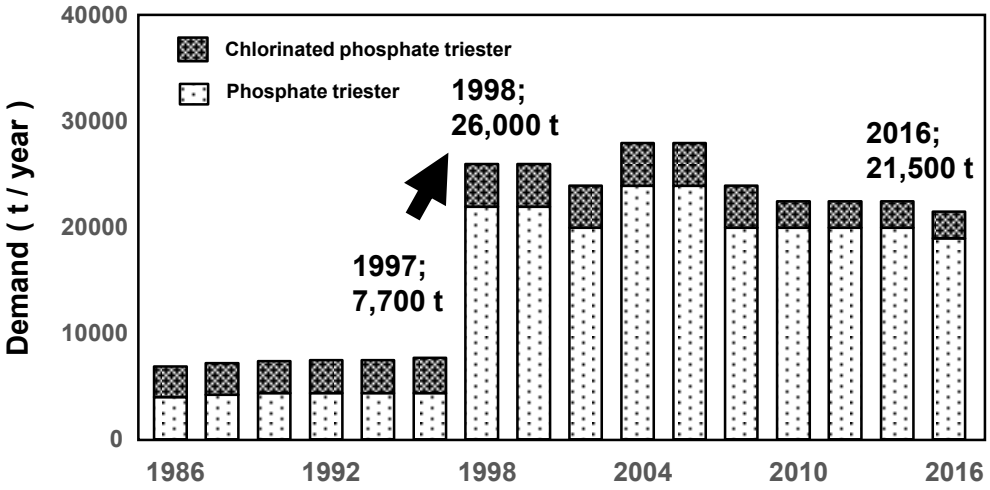

Fig. 2 Changes in demand for organo phosphorus flame retardants in the last three decades

Materials and methods

1. Samples

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

An atmospheric sample: Washed and bundled wool (3 g, acrylic : wool = 3 : 2) was left under indoor environment for four days.

2. Materials

PFRs and d-PFRs standards were obtained from Cambridge Isotope Laboratory (MA, USA). 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

Analytical procedure of breast milk: Breast milk samples (5.0 mL) were spiked with 5.0 ng of d-PFRs (d-TPP, d-tripropyl phosphate, d-tributyl 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. 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. The second purified fraction was analyzed by the use of HP6890GC-JEOL JMS700 MS in EI-SIM mode.

A lipid content of breast milk: After addtion of saturated oxalic acid sodium (4 mL), 2 mL of breast milk was shaken with 15 mL of ethanol, 20 mL of diethyl ether and 20 mL of *n*-hexane. After separation of the organic phase, the aqueous phase was extracted with 20 mL of diethyl ether. The conbination of the first and second extracts was washed twice with 10 mL of 5% saline solution, followed by washing twice with 10 mL of water. After evaporating to dryness over anhydrous sodium sulfate, the washed extract was concentrated and the remaining solvent was completely evaporated. The lipid content of breast milk was calculated on the basis of the gained lipid weight.

Analytical procedure of atmospheric sample: The 4 days left wool sampler was added d-PFR, and extracted with toluene under reflux for 3 hrs. The extract was concentrated to 1 mL and separated by chromatography into two fractions on a florisil column chromatography. The second purified fraction was analyzed by the use of HP6890GC-JEOL JMS700 MS in EI-SIM mode.

We conducted the preliminary study that we confirmed corelation a wool sampler and an air pump sampler with XAD-2 resin for OPFRs. The four day left wool sampler was equivalent to about 10 m^3 of the air pump sampler.

Results and discussion

Table 1 shows contamination levels of three mother's milk and three indoor air. OPFRs were detected in the range of 130 to 310 ng/g lipid. In particular, TPP, Tricresyl phosphate and tris (1-chloro-2-propyl) phosphate were high contamination level. OPFRs are classified into three type as aliphatic, aromatic and halogenated aliphatic phosphate tirester. The aromatic and halogenated aliphatic triester were detected in breast milk. However, the aliphatic OPFRs were low level in human breast milk.

On the other hand, OPFRs were detected in all indoor air samples. The indoor air level was in ranged of 2800 to 5900 ng/4 days. The main component was aliphatic tris (2-ethylhexyl) phosphate, and this level showed 46 to 71% of the whole level. In addition, tricresyl phosphate and tris (1-chloro-2-propyl) phosphate were detected in indoor air samples.

We compared indoor air and breast milk, although it was not the indoor air sampling in the house of the mother. Tris (1-chloro-2-propyl) phosphate detected in both of indoor air and milk samples. However, the composition ratio of tributyl phosphate, tris (2-ethylhexyl) phosphate, TPP and Tris (1,3-dichloro-2-propyl) phosphate in indoor air was greatly different from breast milk.

We estimated that some OPFRs in breast milk were bioaccumulate via indoor air. Consequently, it is important to investigate about elucidation of exposure sources and risk assessment for human including infant.

Table 1 Concentrations of PFRs in breast milk and indoor air

	Breast milk (ng/g, lipid)			Air (ng/4 days)		
	A	B	C	a	b	c
Tripropyl phosphate	n.d.	12	n.d.	n.d.	n.d.	n.d.
Triisopropyl phosphate	0.88	n.d.	n.d.	n.d.	n.d.	n.d.
Tributyl phosphate	0.088	3.7	n.d.	190	68	55
Tripentyl phosphate	n.d.	n.d.	n.d.	0.63	0.33	0.56
Tris (2-ethylhexyl) phosphate	0.38	0.27	n.d.	2100	1300	4200
Triphenyl phosphate	93	99	110	16	29	11
Tricresyl phosphate	32	32	22	510	370	1400
Tris (1-chloro-2-propyl) phosphate	n.d.	86	180	220	800	130
Tris (1,3-dichloro-2-propyl) phosphate	n.d.	2.6	n.d.	93	200	77
Total PFRs	**130**	**240**	**310**	**3100**	**2800**	**5900**

References

1. Fang H. et al., *Chem Res Toxicol*,16, 1338-1358,2003

2. Meeker JD. et al., *Environ Health Perspect*, 118, 318-323, 2010