ASSESSMENT OF HUMAN EXPOSURE TO ORGANOPHOSPHORUS FLAME RETARDANTS (OPFRS) IN JAPAN

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Introduction

Organophosphorus 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 the 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. Although some OPFRs such as tris (1,3-dichloroisopropyl) phosphate (TDCIPP) using for a household articles for textile, bedding, a curtain and a floor carpet regulated by 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 North America (Washington state, etc.) and the EU.

There are many reports about the toxicity of OPFRs. Triphenyl phosphate (TPP) has 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 the ecosystem including humans. In particular, the biological defense of fetus and infant is weak, their health effects are more likely to be affected by environmental pollutants. Although there are several reports about human contamination by OPFRs, a few investigations of health effect to the infant via the breast milk conduct until now.

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

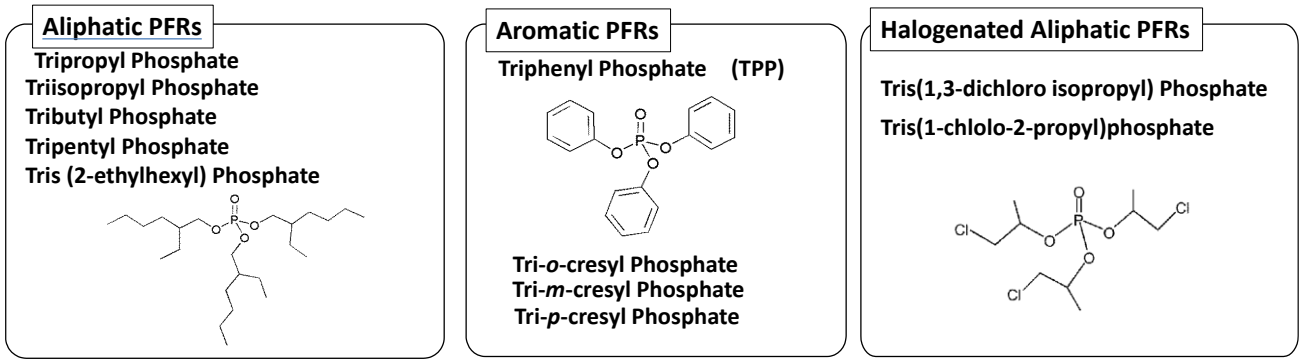

Fig. 1 Structures of organo phosphorus flame retardants

Materials and methods

1. Samples

A breast milk sample: The samples of breast milk were collected from fourteen women (age; 26-40 years old) at one month after delivery between April 2018 to September 2018 (Table 1).

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

A diet sample: The samples of the diet were collected from above fourteen women through breast-feeding.

Table 1 Lists of breast milk sample

Sample No	Age	Primipara /Para	Lipid content (%)
A	37	Para	2.6
B	26	Para	4.2
C	36	Para	2.9
D	31	Para	2.4
E	30	Para	2.6
F	36	Para	4.4
G	33	Primipara	5.0
H	32	Para	4.6
I	31	Para	3.6
J	40	Para	6.9
K	32	Primipara	3.1
L	26	Para	4.9
M	29	Para	4.4
N	30	Para	2.7

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 were 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 the 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 the 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.

Lipid content of breast milk: After the addition 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 combination 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 correlation a wool sampler and an air pump sampler with XAD-2 resin for OPFRs. The four days left wool sampler was equivalent to about 10 m^3 of the air pump sampler.

Analytical procedure of diet sample: The diet was weighed with a wet base, and then was freeze-dried. The freeze-dried sample was fined with the food processor, and extracted with acetone/toluene under reflux for 3 hrs. The extract was analysis with the described above manners.

Results and discussion

OPFRs were detected in the range of 100 to 4400 ng/g lipid. In particular, tricresyl phosphate, tris (2-ethylhexyl) phosphate (TEHP), tris (2-butoxyethyl) phosphate (TBEP) and tris (1-chloro-2-propyl) phosphate were high contamination level (Fig. 2). OPFRs are classified into three types as aliphatic, aromatic and halogenated aliphatic phosphate triester. The aliphatic triester, such as TEHP and TBEP, were detected in breast milk as main components. On the other hand, OPFRs were also detected in indoor air and diet samples (Fig. 3 and 4). The indoor air and diet samples were detected in the range of 65 to 1500 ng/sample and 61 to 200 ng/g, respectively. Tris (isopropyl) phosphate and tris (propyl) phosphate Compare to breast milk, the aliphatic isopropyl and propyl phosphate dominated in indoor air. However, the composition ratio of diet is similar to breast milk. The diet sample was a large ratio of tris (2-ethylhexyl) phosphate and tri cresyl phosphate. Consequently, it was estimated that OPFRs in human breast milk exposed by diets.

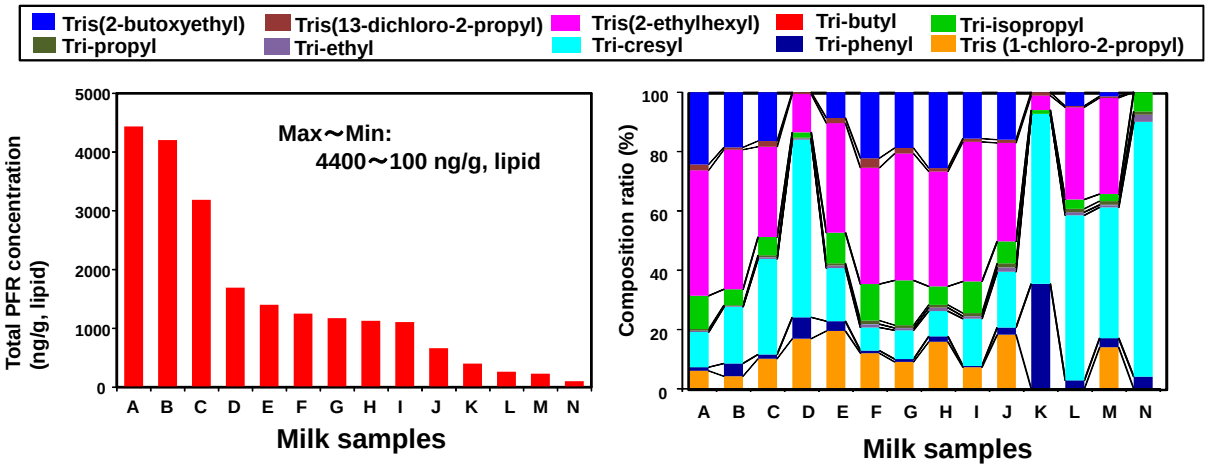

Fig. 2 PFRs contamination levels and composition ratio in breast milk

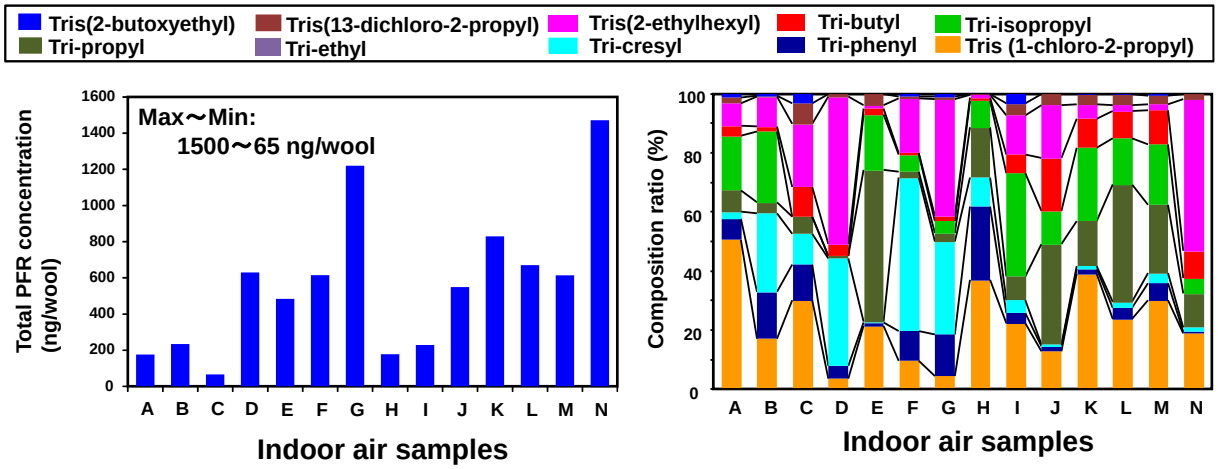

Fig. 3 PFRs contamination levels and composition ratio in indoor air

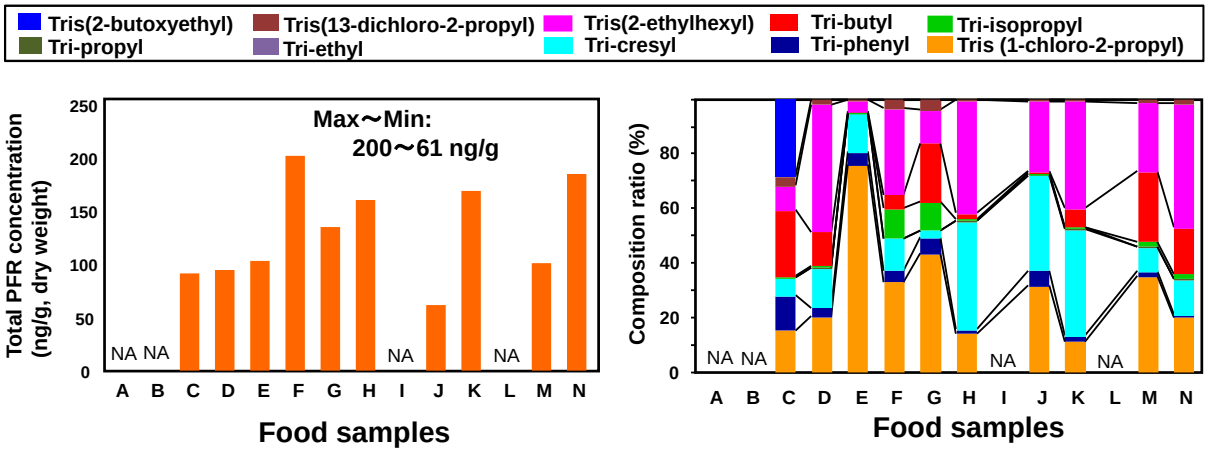

Fig. 4 PFRs contamination levels and composition ratio in diet

Conclusions

OPFRs were detected by all breast milk, indoor air, and diet samples. The range was 10 to 4400 ng/g lipid in breast milk. The main components in breast milk samples are tris (2-ethylhexyl) phosphate and tri cresyl phosphate. In regard to indoor air samples, the main OPFRs were detected the aliphatic phosphorus triesters. The composition ratio of indoor air samples is different from breast milk. On the other hand, the composition ratio of diet is similar to breast milk. Consequently, it was estimated that some OPFRs in breast milk were bioaccumulation through the diet.

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