

## Concentrations of brominated flame retardants in Japanese human blood

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### Introduction

Flame retardants are used to prevent combustion in consumer products such as electronics, construction materials, and textiles. Brominated flame retardants (BFRs) are the most important group of flame retardants. BFRs include polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA). PBDEs have become an important commercial substance, with worldwide sales in 2001 of ~70,000 metric tons (Hites 2004). However, the consumption of PBDEs in Japan is decreasing drastically (Watanabe & Sakai 2003), while TBBPA is the most widely used flame retardant worldwide (Hakk & Letcher 2003). In Japan, the market demand for TBBPA in 2001, at 27,300 tons, was the greatest among BFRs (Watanabe & Sakai 2003). The current status of BFR use seems to differ from region to region and from country to country (Watanabe & Sakai 2003). The routes of human exposure to PBDEs are mainly thought to be food consumption (Ohta et al. 2002) and house dust (Wu et al. 2007).

The purpose of the present study was to identify human body exposure to BFRs. We measured the concentrations of PBDEs including 27 PBDE congeners and TBBPA in Japanese blood.

### Materials and Methods

Blood samples were obtained from 16 peoples (S1-S16) in Chiba, Japan. Subjects who participated in this study were healthy. The study was approved by the Congress of Medical Bioethics of Chiba University. All samples were obtained after receipt of written informed consent. The age of the subjects who participated in the study was ranged from twenties to thirties. The blood samples (10 ml) were collected into a blood tube with heparin and stored at -20°C until use.

The concentrations of the following chemicals were measured in blood: PBDEs (BDE-3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, and 209) and TBBPA. The concentration of each chemical was measured by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The measurements of the concentrations of these compounds were outsourced to SRL, Inc. (Tokyo, Japan) or Shimadzu Techno-Research, Inc. (Kyoto, Japan).

Approximately 4 g of maternal blood were used for the preparation of samples to measure PBDEs. Internal standard mixtures ( $^{13}\text{C}_{12}$ -labeled PBDEs and  $^{13}\text{C}_{12}$ -labeled PCBs) were added to maternal blood samples. Saturated ammonium sulfate 1.5 ml and 25% ethanol/hexane 6 ml were added to each human blood sample. Each sample was extracted by liquid-liquid extraction. Each sample was dehydrated with sodium sulfide after it was washed with distilled water 5 ml, was evaporated, and then dried. Weight of lipids in maternal blood was measured, and immediately afterward hexane 4 ml was added to dissolve the lipids. Lysates 1 ml eliminated were used to measure PCBs. Lysates 2 ml eliminated were treated by multilayer silica gel column (anhydrous sodium sulfate 2.0 g, 10% silver nitrate silica gel 1 g, silica gel 0.5 g, 44% sulfuric acid silica gel 3.5 g, silica gel 0.5 g, and anhydrous sodium sulfate 2.0g) with 50 ml of 10% dichloromethane/hexane. The fractions were evaporated and analyzed after the addition of  $^{13}\text{C}_{12}$ -labeled BDE-138.

Approximately 3 g of human blood were used for the preparation of samples to measure TBBPA. Internal standard mixtures ( $^{13}\text{C}_{12}$ -labeled TBBPA) were added to human blood samples. Saturated ammonium sulfate 3 ml, EtOH 0.5 ml, hexane 2 ml, and HCl were added and each sample was shaken. After adding hexane 1 ml twice, dehydration and concentration were performed by washing in  $\text{H}_2\text{O}$ . In measuring the concentrations of PBDEs, gas chromatography was performed using an Agilent 6890 series GC system (Agilent Technologies Inc., Wilmington, DE, USA) equipped with an AutoSpec Ultima (Micromass Ltd., Manchester, UK) mass spectrometer. An ENV-5MS column (0.25 mm inner diameter (i.d.)  $\times$  30 m (Kanto Kagaku)) was used to separate each PBDE congener. Five ml of each final solution were injected into the column using Programmable Temperature Vaporization mode (PTV). The column temperature program was as follows: 60°C for 2.5 min, 20°C  $\text{min}^{-1}$  to 180°C for 5 min, 5  $\text{min}^{-1}$  to 300°C for 12.5 min, and 10  $\text{min}^{-1}$  to 320°C for 5 min. The ionizing current was 700  $\mu\text{A}$ , the ionizing energy was 38 eV, and the accelerating voltage was 8 kV. The resolution of the mass spectrometer was maintained at approximately  $R > 10,000$  (10% valley) throughout, and the analysis was carried out according to selected ion monitoring (SIM).

In measuring the concentrations of TBBPA, gas chromatography was performed using an Agilent 6890 series GC system (Agilent Technologies Inc.) equipped with a MAT 95 XL (Thermolectron, San Jose, CA, USA) mass spectrometer. A DB-5MS column (0.25 mm i.d.  $\times$  15 m (J&W Scientific, Folsom, CA, USA) was used for separation. The column temperature program was as follows: 120°C for 1 min, 5°C  $\text{min}^{-1}$  to 150°C, and 15  $\text{min}^{-1}$  to 300°C for 3 min. The ionizing current was 450  $\mu\text{A}$ , the ionizing energy was 30-40 eV, and the accelerating voltage was 5 kV. The resolution of the mass spectrometer was maintained at approximately  $R > 10,000$  (10% valley) throughout, and the analysis was carried out according to SIM.

The PBDE concentrations on the wet weight basis were calculated by multiplication of PBDE concentrations on lipid weight basis and the lipid weight per gram fresh weight of each sample. Samples below the limit of quantification (LOQ) were set to zero.

All statistical analyses were completed with SPSS version 16.0J (SPSS Inc., Chicago, IL, USA). Correlation among tissues was analyzed by Spearman's rank correlation coefficient.

## Results

### 1. Concentrations of PBDEs

We investigated 27 PBDE congeners in human blood. To compare them with the concentrations of phenolic compounds, which were measured on a wet-weight basis, we also calculated the

concentrations of PBDEs on a wet-weight basis by using their concentrations on lipid weight basis and the lipid weight per gram fresh weight of each sample.

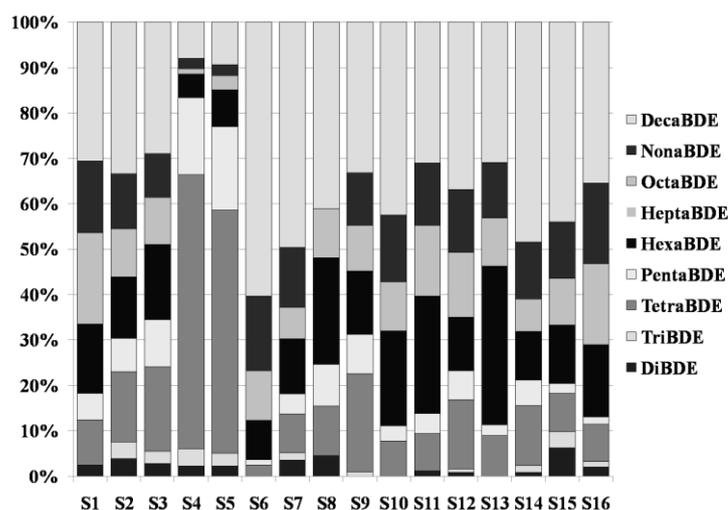
The concentration of total PBDEs (mean  $\pm$  SD; median) was found to be  $25 \pm 23$  pg/g; 18 pg/g in human blood (n = 16). BDE-47 and BDE-209 were the dominant compounds, the concentration of BDE-47 and BDE-209 (mean  $\pm$  SD; median) was  $7.3 \pm 15$  pg/g; 1.8 pg/g and  $7.0 \pm 3.7$  pg/g; 6.5 pg/g, respectively. Among the 27 PBDE congeners investigated, the following 12 were below the LOQ in all samples: BDE-3, 7, 71, 77, 119, 126, 138, 156, 184, 191, 196, and 206.

The concentrations of PBDE congeners were shown in Table 1. Moreover, ratio of the concentrations of PBDE congeners was shown in Figure 1. This result indicated that ratio of concentrations of PBDE congeners was uneven in each sample.

**Table 1. Concentrations of PBDE congeners (pg/g)**

	Mean $\pm$ SD	Median	Range
DiBDE	0.54 $\pm$ 0.62	0.36	<LOQ-2.1
TriBDE	0.54 $\pm$ 0.94	0.17	<LOQ-3.6
TetraBDE	7.6 $\pm$ 16	1.9	0.31-57
PentaBDE	2.6 $\pm$ 4.6	1.1	0.16-16
HexaBDE	3.0 $\pm$ 1.1	2.9	1.1-5.3
HeptaBDE	<LOQ		
OctaBDE	1.9 $\pm$ 0.7	1.9	1.1-3.8
NonaBDE	2.3 $\pm$ 0.87	2.2	1.4-4.9
DecaBDE	7.0 $\pm$ 3.7	6.5	3.6-19
<b>Total PBDE</b>	<b>25 <math>\pm</math> 23</b>	<b>18</b>	<b>11-95</b>

Values below the limit of quantification (LOQ) were set to zero.



**Fig.1 Ratio of PBDE congeners**

## 2. Concentrations of TBBPA

TBBPA was measured in six samples of human blood. TBBPA was detected in 67% of human blood samples; the concentration (mean  $\pm$  SD; median) was  $26 \pm 38$  pg/g; 17 pg/g. There were no significant correlations between the concentrations of PBDEs and TBBPA ( $r = -0.435$ ,  $p = 0.389$ ) in human blood.

## Discussion

In this study, PBDEs were detected in all human blood. Moreover, BDE-47 and BDE-209 were major congeners. In a study on PBDE levels in fish and food products sold in Japan (Ohta et al.2002), 2,2',4,4'-tetra-BDE (BDE-47) was found to be the major congener in fish, spinach, and pork. Among PBDEs, only technical deca-BDE is currently used in Japan (Watanabe & Sakai 2003). Moreover, in house dust and office dust on Japanese report (Suzuki et al.2006), high concentration of BDE-209 was detected. This result suggested that participants of this study were exposed through food consumption and house dust. In addition, ratio of concentrations of PBDE congeners was uneven in each sample. Therefore, it assumed that participants of this study had different sources of exposure from PBDEs.

Of TBBPA, 67% of subjects were exposed. Moreover, exposure to TBBPA was not concerned with exposure to PBDE. Several studies indicate that TBBPA may interfere with thyroid hormone function, possibly by acting as an "endocrine disruptor" (Meerts et al. 2000; Birnbaum & Staskal 2004). In

addition, nephrotoxicity of TBBPA (Fukuda et al. 2004) and induction of liver and kidney lesions by TBBPA (Tada et al. 2006) have been reported. Because of the high demand for TBBPA in Japan (Watanabe & Sakai 2003), we anticipate that Japanese are exposed to TBBPA increasingly.

In conclusion, our result indicated that all subjects exposed to PBDEs and that they might have different sources of exposure to PBDEs in each subject. Moreover, because TBBPA was detected from 67% of subjects, adverse effect of TBBPA was anticipated. However, because of the limited number of samples studied, further investigation is necessary.

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