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CONTAMINATION LEVELS OF POLYBROMINATED BISPHENOL A COMPOUNDS IN HUMAN BREAST MILK

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Introduction

Tetrabromobisphenol A (TeBBPA), in which the hydrogens of bisphenol A (BPA) are substituted with bromine, is a common reactive BFR for ABS and epoxy resins due to its low toxicity and cost. However, TeBBPA is likely to contaminate the environment during its production, use, and disposal. In fact, TeBBPA has been detected in river sediment and sludge from wastewater treatment plants. In addition, there have been reports of TeBBPA bioaccumulation in aquatic organisms and marine mammals throughout the food chain. Because introduction of TeBBPA into humans occurs via ingestion of contaminated food, TeBBPA has also been detected in human blood, adipose tissue, and breast milk. Furthermore, in vitro studies have shown that TeBBPA disrupts thyroid hormone, estrogen, and immunosuppressive homeostasis, and in vivo studies have demonstrated its effects on endocrine signaling and neurobehavioral activity. However, there are few reports on the in vivo metabolism of TeBBPA, and in particular, little is known of the average contamination levels in biological samples and toxicity of each debrominated TeBBPA metabolite, such as tribromobisphenol A (TriBBPA), dibromobisphenol A (DiBBPA), and monobromobisphenol A (MoBBPA). Furthermore, because the fully-debrominated TeBBPA product is BPA, the contribution of secondary toxic effects due to BPA need to be considered in addition to the health effects of TeBBPA during such studies.
In the present study, the level of six bisphenol compounds, TeBBPA, BPA, MoBBPA, dibromobisphenol A (2,2'-and 2,6-DiBBPA) and TriBBPA were investigated in breast milk samples, and daily intake in infants was estimated using these data.

Material and methods

1) Sample collection
From December 2012 to June 2013, nineteen breast milk samples were collected from healthy donors living in Osaka. Three samples were collected, at one week, one month, and three months after birth. Approximately 50-mL aliquots of whole milk were stored at -20°C for future analysis. The dietary preferences of each donor were investigated using an index of intake frequency per week of meat, seafood, vegetables, and fruits and dairy products.
2) Analytical methods
Breast milk samples (5.0 mL) were spiked with 5.0 ng each of 13C12-TeBBPA and 2H16-BPA 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.
3) Method of QA/QC for analytical data
TeBBPA and BPA were identified by comparing the retention times and mass spectra with those of the commercial standards, and MoBBPA, DiBBPA and TriBBPA were identified using the synthesized standards. The acceptance criteria were set from -30% to 30% of ratios observed with the commercial and synthesized standards. Concentrations were corrected utilizing the recovery efficiency of their respective internal standards: 2H16-BPA for BPA, MoBBPA and DiBBPA, and 13C12-TeBBPA for TriBBPA and TeBBPA. Samples which had high internal standard recoveries in the range of 60 – 120% were used for data collection. The limits of detections (LODs) and the limits of quantitations (LOQs) were defined as three (S/N=3) and ten times (S/N=10) the noise level, respectively. LOQs of BPA, MoBBPA, DiBBPA, TriBBPA and TeBBPA in breast milk were 0.002, 0.010, 0.010, 0.010, and 0.018 ng/g lipid, respectively.

Result and discussion

BPA and TeBBPA levels were compared using breast milk samples from nineteen Japanese mothers (Fig. 1). Average age was 32 years old (min - max; 19 - 39), weight 53 kg (min - max; 45 - 64) and height 158 cm (min - max; 151 - 162). Three mothers (Sample Nos. 4, 13 and 14) were primiparas, accounting for 16% of the samples. BPA was detected in the range of 1.4 – 380 ng/g lipid (mean: 36 ng/g) and TeBBPA was detected in the range of N. D. - 8.7 ng/g lipid (mean: 1.9 ng/g). Detected BPA levels were approximately 18 times higher than TeBBPA levels. In particular, high BPA levels were observed in four samples (75, 380, 47, and 67 ng/g lipid in Sample Nos. 4, 6, 17, and 18, respectively). The sample with the highest TeBBPA level (8.7 ng/g lipid in Sample No. 13) was approximately 62 times higher than the sample with the lowest TeBBPA level (0.14 ng/g lipid in Sample No. 9), excluding N.D. samples. A questionnaire survey on dietary preferences of the nineteen donors demonstrated that approximately 70% of the mothers consumed meat, seafood, and dairy products five to thirteen times per week, and vegetables and fruits were consumed at every meal. We conclude that there was no correlation between dietary preferences and breast milk BPA-related compound levels in the donor samples.
Table 1 shows the concentrations of BPA, MoBBPA, DiBBPA, TriBBPA, and TeBBPA in the breast milk samples. TriBBPA was detected in all samples except in those from Sample No. 4, with a range of N.D. to 22 ng/g lipid (mean: 5.5 ng/g lipid). Interestingly, the average concentration of TriBBPA was 2.9 times higher than that of TeBBPA. DiBBPA was detected in some breast milk samples at very low levels, while MoBBPA was not detected. TeBBPA is metabolized into TriBBPA in vivo and is rapidly distributed into breast milk, or is metabolized into glucuronide and sulfate conjugates to be excreted into feces or urine (Schauer et al.1). Additionally, a previous study showed that ultraviolet-irradiated water containing TeBBPA, generated debrominated TeBBPA-like TriBBPA. Therefore, we investigated the generation of debrominated TeBBPA using a TeBBPA standard under light-shielding and non-light-shielding conditions, and observed that TeBBPA was not debrominated into TriBBPA via photolysis under either condition (data not shown). The present study was the first to detect significant TriBBPA concentration in breast milk. Previous studies have shown that levels of TeBBPA in breast milk were low,2 suggesting a low risk of causing adverse health effects. However, the present study revealed higher levels of TriBBPA than TeBBPA in human breast milk.

Acknowledgements
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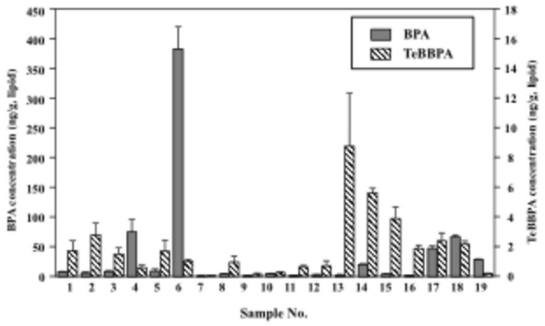

Fig. 1 Concentrations of BPA and TeBBPA in breast milk collected at one week after delivery.
Limits of quantitation of BPA and TeBBPA were 0.002 and 0.018 ng/g lipid, respectively.
Data are mean ± SD of three independent analyses.

Table 1 Concentration of BPA, moBBPA, 2,2'-DiBBPA, 2,6-DiBBPA, TriBBPA and TeBBPA in breast milk collected at one week after birth

Subject No	BPA	MoBBPA[b]	2,2'-DiBBPA[b]	2,6-DiBBPA[b]	TriBBPA[b]	TeBBPA[b]
1	8.4 ± 0.59	<LOQ	<LOQ	<LOQ	1.9 ± 0.24	1.7 ± 0.67
2	6.6 ± 0.53	<LOQ	<LOQ	<LOQ	3.0 ± 0.45	2.8 ± 0.79
3	8.9 ± 1.1	<LOQ	<LOQ	<LOQ	1.5 ± 0.31	1.5 ± 0.41
4[a]	75 ± 21	<LOQ	<LOQ	<LOQ	<LOQ	0.58 ± 0.17
5	9.3 ± 3.8	<LOQ	<LOQ	0.22 ± 0.26	11 ± 0.32	1.7 ± 0.74
6	380 ±37	<LOQ	<LOQ	<LOQ	8.6 ± 0.12	1.0 ± 0.11
7	1.4 ± 0.16	<LOQ	<LOQ	<LOQ	7.9 ± 0.37	<LOQ
8	4.7 ± 0.16	<LOQ	<LOQ	<LOQ	5.5 ± 0.53	0.95 ± 0.42
9	1.7 ± 0.036	<LOQ	<LOQ	<LOQ	12 ± 0.91	0.14 ± 0.087
10	5.4 ± 0.28	<LOQ	<LOQ	<LOQ	5.9 ± 0.70	0.24 ± 0.042
11	1.9 ± 0.066	<LOQ	0.017 ± 0.007	0.093 ± 0.047	1.6 ± 0.26	0.61 ± 0.12
12	3.4 ± 0.064	<LOQ	<LOQ	<LOQ	0.002 ± 0.003	0.70 ± 0.35
13[a]	2.9 ± 0.74	<LOQ	<LOQ	<LOQ	1.3 ± 0.71	8.7 ± 3.5
14[a]	21 ± 1.0	<LOQ	<LOQ	<LOQ	10 ± 1.5	5.6 ± 0.30
15	4.3 ± 0.70	<LOQ	<LOQ	<LOQ	22 ± 1.6	3.9 ± 0.80
16	2.1 ± 0.094	<LOQ	<LOQ	<LOQ	7.7 ± 0.20	1.9 ± 0.23
17	47 ± 3.7	<LOQ	<LOQ	<LOQ	0.016 ± 0.017	2.4 ± 0.53
18	67 ± 3.6	<LOQ	<LOQ	<LOQ	4.2 ± 0.35	2.2 ± 0.22
19	28 ± 1.1	<LOQ	<LOQ	<LOQ	0.24 ± 0.12	0.20 ± 0.021