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Polybrominated Diphenyl Ethers and Hexabromocyclododecanes in Japanese Human Adipose Tissues

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Abstract—Residue levels of brominated flame retardants (BFRs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) were measured in the Japanese human adipose tissues collected during 2003–2004 from Ehime prefecture and Kanto region of Japan. Concentrations of PBDEs (1.5–27 ng/g lipid wt) and HBCDs (0.85–39 ng/g lipid wt) in adipose tissues were 1–2 orders of magnitude lower than those of organochlorines (OCs). However, observed PBDE levels in this study were relatively higher than those in Japanese human adipose tissues reported previously in the samples collected during the year 2000, while OC levels were comparable to those in specimens reported by our group in the samples collected during 1999. No age-dependent accumulation of PBDEs and HBCDs was observed, while OC levels except chlordane compounds increased with age. These results indicate recent human exposure to PBDEs and HBCDs in Japan. This is the first report on HBCDs in Japanese human tissue. Among PBDE congeners accumulated in Japanese adipose tissues, BDE-153 was dominant, but this trend was different from those in human milk (BDE-47) and blood (BDE-209) reported previously in Japan. α-HBCD was predominant among the three isomers, which is consistent with the other reports on high trophic animals. These results imply some inferences and explain the congener-specific kinetics in human bodies. The significant positive correlations between PBDEs and HBCDs observed in Japanese adipose tissues indicate similar exposure routes of these contaminants for Japanese citizens.

Keywords: brominated flame retardants, hexabromocyclododecanes (HBCDs), liquid chromatography-tandem mass spectrometry (LC-MS-MS), human adipose tissue

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), which are in use as brominated flame retardants (BFRs), have been detected in a wide range of animal species as a result of their bioaccumulative nature (Rahman *et al.*, 2001; Covaci *et al.*, 2003, 2006; Law *et al.*, 2003, 2006; Hites, 2004; Kajiwara *et al.*, 2004; Ueno *et al.*, 2004, 2006; Isobe *et al.*, 2007; Ramu *et al.*, 2007), as in the case of persistent organochlorines (OCs). Human exposure to PBDEs and HBCDs is of great concern due to their persistence and toxicity. In Japan, technical tetra- and octa-BDE products were used until 1990 and 1999, respectively, and technical deca-BDE is in use even now (Watanabe and Sakai, 2003). To assess the human health risk, investigations on residue levels of PBDEs in Japanese human blood and breast milk have been recently conducted (Akutsu *et al.*, 2003; Takasuga *et al.*, 2004; Sudaryanto *et al.*, 2005; Eslami *et al.*, 2006; Inoue *et al.*, 2006). To our knowledge, however, only limited data on PBDEs in adipose tissue and no information on HBCDs in Japanese are available. In this study, 14 PBDE congeners and 3 HBCD isomers were determined in Japanese human adipose tissues to reveal their contamination status, exposure patterns and sex- and age-dependent accumulation.

MATERIALS AND METHODS

Samples

The present study was approved by the Ethics Committee of the Ehime and Keio University Institutional Review Boards. Informed consent was obtained from all the donor's families before sample collection. Adipose tissue (mesenteric fat) were collected from 28 donors (male; $n = 18$, female; $n = 10$) at autopsy during 2003–2004. All the samples were stored in the Environmental Specimen Bank (*es*-BANK), Ehime University (Tanabe, 2006) at –20°C until analysis.

Chemical analysis

Analysis of PBDEs and HBCDs was performed following the previously reported method with some modifications (Ueno *et al.*, 2004; Tomy *et al.*, 2005; Isobe *et al.*, 2007). Briefly, 2–4 g (wet wt) of the adipose sample was ground with anhydrous sodium sulfate and Soxhlet extracted with diethyl ether/hexane (75:25, v/v) for 7–8 h. An aliquot of the extract, after spiking with 5 ng of ^{13}C-labeled PBDEs ($^{13}C_{12}$-BDE-3, 15, 28, 47, 99, 153, 154, 183, 197, 207, 209) and 10 ng of ^{13}C-labeled HBCDs ($^{13}C_{12}$-α-, β-, and γ-HBCD), was loaded to a gel permeation chromatography (GPC, Bio-Beads S-X3, Bio-Rad, CA, 2 cm i.d. × 50 cm) column for lipid removal. The GPC fraction containing organohalogens was concentrated and subjected to activated silica gel column (Wakogel DX, 4 g, Wako Pure Chemicals, Tokyo) for clean-up and fractionation. PBDEs were eluted with 80 ml of dichloromethane/hexane (5:95, v/v) followed by the elution of HBCDs with 100 ml of dichloromethane/hexane mixture (25:75, v/v) from the silica gel column. Five nanograms of $^{13}C_{12}$-BDE-139 was spiked as an internal standard to

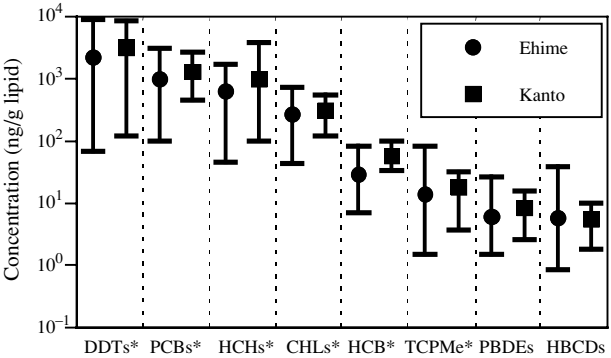

Fig. 1. Concentrations of PCBs, DDTs, CHLs, HCHs, HCB, PBDEs, and HBCDs in human adipose tissue collected from Japan. *Kunisue *et al*., 2007.

the fraction containing PBDEs and subjected to GC-MS analysis. The HBCDs fraction was evaporated and spiked with 10 ng of deuterized HBCDs (α-, β-, and γ-HBCD-d_{18}) prior to LC-MS-MS analysis. The diastereoisomer-specific analysis of HBCDs was performed based on the reported analytical method by Tomy *et al*. (2004). Identification and quantification were carried out using an Alliance 2795 (Waters, Tokyo) liquid chromatograph equipped with a Quattro Micro API (Waters, Tokyo) triple quadrupole mass spectrometer. LC separation of all the three isomers (α-, β-, and γ-) of HBCDs was achieved with an Extend-C18 column (2.1 mm i.d. × 150 mm, 5 μm, Agilent, Tokyo). The mobile phase consisted of water/acetonitrile/methanol (20:30:50) at 0.2 ml/min initially for 2 min and gradually changed to acetonitrile/methanol (30:70) for 5 min and kept for 6 min. The MS-MS analysis in negative mode of electrospray ionization (ESI) was performed in multiple reaction monitoring mode (MRM). Quantification of native HBCDs was obtained using Mass Lynx 4.0 (Waters, Tokyo) software from the mean value of the response at two MRM transitions (i.e., *m/z* 640.6 > 81, *m/z* 642.6 > 81) corrected against the response of $^{13}C_{12}$-HBCDs (i.e., *m/z* 652.6 > 81 MRM transition). Performance of the instrument and effect of matrices in sample extracts were evaluated by responses of α-, β-, and γ-HBCD-d_{18} (i.e., *m/z* 658.6 > 81 MRM transition). Concentrations of analytes were expressed as ng/g lipid weight unless otherwise stated.

RESULTS AND DISCUSSION

Contamination status

Concentrations of organohalogen compounds analyzed in this study are shown in Fig. 1. PBDEs and HBCDs were detected in all the human adipose tissue samples analyzed in this study. Concentrations of PBDEs (1.5–27 ng/g lipid wt) and HBCDs (0.85–39 ng/g lipid wt) in adipose tissues were 1–2 orders of

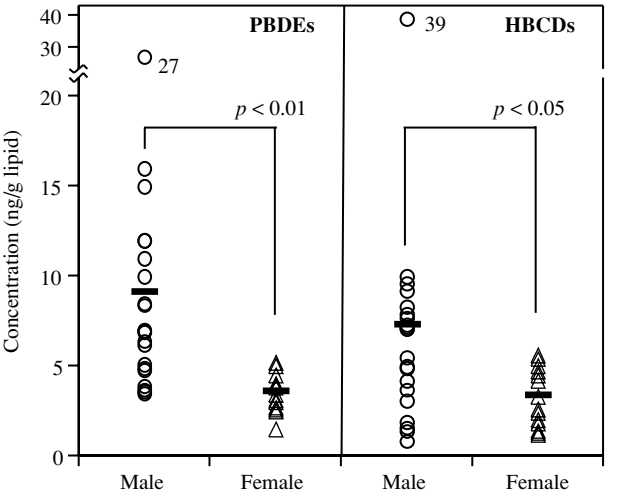

Fig. 2. Gender difference of PBDEs and HBCDs in human adipose tissue.

magnitude lower than those of organochlorines (OCs). Observed PBDE levels in this study were relatively higher than those in Japanese human adipose tissues reported previously in the samples collected during the year 2000, while OC levels were comparable to those in specimens reported by our group in the samples collected during 1999. This implies that Japanese people have been recently exposed to relatively high levels of PBDEs. This study reports contamination by HBCDs in Japanese for the first time. HBCD levels were higher in adipose tissue than those reported in blood and breast milk, indicating the bioaccumulative nature of this compound. This might be reflecting the higher consumption of HBCDs in Japan compared to North America.

Gender difference

When concentrations of BFRs in adipose tissues of males and females were compared, both PBDE and HBCD levels in males were significantly higher than those in females. The possible reason for this sex dependent difference is breast-feeding in female (Fig. 2). In an investigation on PBDEs in Japanese human milk, however, no significant difference in PBDE levels was observed between primiparas and multiparas (Eslami *et al.*, 2006), indicating BFRs elimination via breast-feeding could be less than exposure. Although we could not obtain information on the food habits and occupational histories of donors, greater food intake and occupational exposure of males compared to female are the other possibilities. Statistically significant positive correlation between PBDEs and HBCDs and relatively high concentrations might suggest occupational exposure of males to PBDEs.

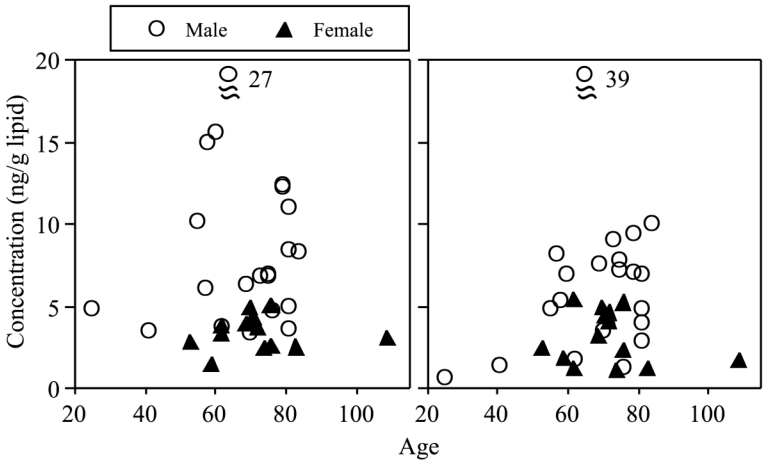

Fig. 3. Age-dependent accumulation of PBDEs and HBCDs in human adipose tissue.

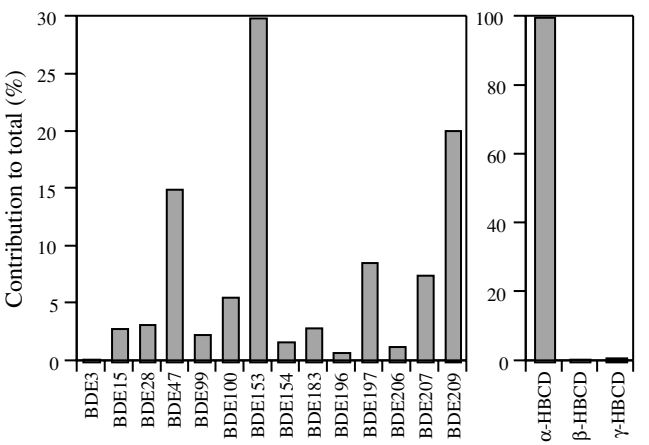

Fig. 4. Isomeric composition of PBDEs and HBCDs in human adipose tissue.

Age-dependent accumulation

No age-dependent accumulation of PBDEs and HBCDs was observed (Fig. 3), while OC levels such as PCBs and DDTs increased with age. Eslami *et al.* (2006) also reported that PBDE levels in Japanese human milk did not increase with age. No age-dependent accumulation of PBDEs was observed in human adipose tissue in USA (She *et al.*, 2002; Johnson-Restrepo *et al.*, 2005), Belgium (Covaci *et al.*, 2002; Naert *et al.*, 2006), and Spain (Fernandez *et al.*, 2007), while

no information is available on HBCDs so far. In Japan, technical deca-BDE and HBCDs are still in use, indicating that human exposures to these compounds are continuing and so BFRs do not show an increasing trend with age at present.

Relative isomer composition

Figure 4 shows relative isomeric composition of PBDEs and HBCDs detected in Japanese adipose tissue. Among PBDE congeners analyzed in this study, BDE-153 was the dominant, followed by BDE-47 and BDE-209. On the other hand, it was reported that higher levels of BDE-47 than BDE-153 were detected in Japanese breast milk and BDE-209 was the dominant isomer in Japanese blood (Inoue *et al.*, 2006). Biotransformation and accumulation kinetic properties in the body could vary for each PBDE congener, implying toxicological risk would be different among tissues and organs. Among three isomers of HBCD analyzed, α-HBCD occupied more than 95% of total HBCDs. Other studies on the isomeric composition of HBCDs in higher trophic animals also reported the predominance of α-HBCD (Budakowski and Tomy, 2003; Tomy *et al.*, 2004; Law *et al.*, 2006). This can be ascribed to isomer-specific biomagnification of α-HBCD in the upper trophic level and also low bioavailability of γ-HBCD.

CONCLUSIONS

PBDEs and HBCDs were detected in all the Japanese adipose tissue samples analyzed in this study, indicating ubiquitous contamination by BFRs in Japan could be confirmed. This is the first report on HBCDs accumulation in Japanese. No age-dependent accumulation of PBDEs and HBCDs was observed, probably reflecting recent and continuous exposure to BFRs. Both PBDEs and HBCDs levels were higher in male than those in female, indicating specific exposure (e.g. occupational) to male is suggested. Different PBDE congener pattern among adipose tissue, breast milk and blood was observed. Therefore, toxic risk of PBDEs could be different among tissues and organs. α-HBCD was the dominant isomer among three HBCD isomers. This is consistent with the previous studies that concluded bioaccumulation and persistence of α-HBCD. To reveal exposure route and toxicological risk of BFRs, widespread monitoring survey and comprehensive risk assessment are warranted.

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