POLYBROMINATED DIPHENYL ETHERS IN HUMAN SERUM AND SPERM QUALITY

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Abstract
Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in different types of consumer products. PBDEs are now ubiquitous environmental contaminants. Several studies have indicated that PBDEs may affect male fertility. We present the results of a pilot study on the relationship between human serum PBDEs and sperm quality. Serum and sperm samples from 10 healthy Japanese males aged 18–22 years were obtained in St. Marianna University. The PBDE concentrations in the serum samples were determined using gas chromatography/mass spectrometry. Four PBDE congeners (2,2′,4,4′-tetrabromodiphenyl ether (TeBDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (PeBDE-99), 2,2′,4,4′,6-pentabromodiphenyl ether (PeBDE-100), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (HxBDE-153)) were quantified in all serum samples. The median levels of the individual PBDE congeners were 1.4 ng·g^{-1} lipid weight (lw), TeBDE-47; 0.21 ng·g^{-1} lw, PeBDE-99; 0.24 ng·g^{-1} lw, PeBDE-100; and 0.72 ng·g^{-1} lw, HxBDE-153. These levels are comparable to those found in European countries. Clear inverse correlations were observed between the serum HxBDE-153 concentration and sperm concentration (r = –0.838, p = 0.002) and testis size (r = –0.764, p = 0.01). However, the serum concentrations of the other 3 congeners did not correlate with sperm concentration or testis size. Extensive studies on the relationship between PBDEs and sperm quality are required.

Introduction
Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in the production of common consumer products. PBDEs are now ubiquitous and persistent environmental contaminants, and they have been detected in human tissues. Because PBDEs have some structural similarity to thyroid hormones such as thyroxine (T4), it was speculated that PBDEs may mimic thyroid hormones and may disrupt thyroid homeostasis. Several studies indicate that exposure to PBDEs can reduce circulating levels of T4 in laboratory animals[1] and can cause permanent neurological effects similar to those associated with thyroid hormone deficiencies[2]. In addition, several PBDEs possess weak estrogenic/antiestrogenic activities[3]. The proliferation and differentiation of Sertoli cells and sperm production are regulated by thyroid and sexual hormones. Thus, PBDEs may affect male reproductive health by interfering with thyroid and sexual hormone function. Kuriyama et al. have reported that developmental exposure to a single low dose (60 μg·kg^{-1} body weight) of 2,2′,4,4′,5-pentabromodiphenyl ether (PeBDE-99) decreased sperm counts in male Wistar rats[4]. However, no previous studies have examined the relationship between human PBDE levels and sperm quality. We participated in the international project examining the sperm quality of fertile men and found that the sperm concentration of Japanese men was lower than that of European men[5]. The examination of sperm quality and the estimation of the concentration of chemicals in the serum would be required to reveal the correlation between the sperm quality of Japanese men and their exposure to chemicals. The aim of this pilot study was to measure PBDEs in serum samples from Japanese young males and to examine the relationship between serum PBDE levels and sperm quality.

Materials and Methods
Sample collection: Blood serum and sperm samples were collected monthly from 45 young Japanese males at the Department of Urology, St. Marianna University School of Medicine, in 2003. The men were asked to remain abstinent for at least 48 h before sperm collection. The blood samples were collected in vacuum tubes, and the serum fractions were separated by centrifugation. Serum samples were stored at –80°C until analysis. Of the 45 sample sets, 10 sample sets were randomly chosen for this study. For PBDE analysis, 10 pooled serum

samples (0.5 g × 12 months; total, 6 g per person) were prepared, and each pool was regarded as a representative sample of each set. In addition, 2 brands of commercially pooled human serum ("L-Consera N" and "L-Suitrol I," both purchased from Nissui Pharmaceutical, Tokyo, Japan) were used as in-house reference materials. The mean ± standard deviation (SD) age of the 10 participants was 22 ± 1 years (range, 18–22 years). The mean ± SD abstinence time was 3.1 ± 0.4 days (range, 2.6–3.8 days).

Chemicals: Standard mixture solutions of native PBDEs (BDE-AAP-A-15X) were purchased from AccuStandard (New Haven, CT, USA), and $^{13}C_{12}$-labeled PBDEs (MBDE-MXC) were purchased from Wellington Laboratories (Ontario, Canada). In this study, 29 PBDE congeners having 3 to 7 bromine atoms were monitored. The PBDE numbers are assigned according to the IUPAC PCB nomenclature. Acetone, acetonitrile, and *n*-hexane of pesticide analysis grade; ammonium sulfate of biochemistry grade; and 44% sulfuric acid-impregnated silica gel and *n*-nonane of dioxin analysis grade were purchased from Wako Pure Chemicals (Osaka, Japan). Water was deionized and purified using a Milli-Q cartridge system (Millipore, Bedford, MA, USA).

Sperm analysis: Sperm analyses were performed at the Department of Urology, St. Marianna University School of Medicine, according to the World Health Organization's recommendations as described elsewhere[5].

Serum PBDE measurements: Serum samples were analyzed at Osaka Prefectural Institute of Public Health. The serum sample (6 g) was extracted using ethanol/*n*-hexane (1:3 v/v, 14 mL) in a 50 mL test tube, after adding $^{13}C_{12}$-labeled surrogate standards ($^{13}C_{12}$-2,4,4′-tribromodiphenyl ether ($^{13}C_{12}$-TrBDE-28), $^{13}C_{12}$-2,2′,4,4′-tetrabromodiphenyl ether ($^{13}C_{12}$-TeBDE-47), $^{13}C_{12}$-2,2′,4,4′,5-pentabromodiphenyl ether ($^{13}C_{12}$-PeBDE-99), $^{13}C_{12}$-2,2′,4,4′,5,5′-hexabromodiphenyl ether ($^{13}C_{12}$-HxBDE-153), $^{13}C_{12}$-2,2′,4,4′,5,6′-HxBDE ($^{13}C_{12}$-HxBDE-154), and $^{13}C_{12}$-2,2′,3,4,4′,5′,6-heptabromodiphenyl ether ($^{13}C_{12}$-HpBDE-183); 10 pg for each congener) and 3.6 mL saturated ammonium sulfate solution. The test tube was shaken for 30 min and then centrifuged for 10 min at 3000 rpm. The *n*-hexane phase was collected, and the aqueous phase was re-extracted twice with 12 mL *n*-hexane. The 3 *n*-hexane phases were combined and washed with 12 mL water. After evaporation of the solvent, the lipid content was determined gravimetrically with a semimicro balance (Sartorius RC210P, Goettingen, Germany). The lipid was dissolved in *n*-hexane and was transferred to a column of 44% sulfuric acid-impregnated silica gel (3 g). The column was eluted with 30 mL *n*-hexane, and the eluate was evaporated to 2 mL. The *n*-hexane solution was transferred to a test tube and partitioned with *n*-hexane-saturated acetonitrile (4 mL) 3 times by shaking the test tube for 10 min and then centrifuging for 10 min at 3000 rpm. The acetonitrile phase was combined and then evaporated to dryness. The residue was redissolved in *n*-hexane and was transferred to a microconcentration tube. After addition of the injection standard ($^{13}C_{12}$-3,3′,4,4′,5-PeBDE) and keeper solvent (10 µL *n*-nonane), the extract was finally evaporated to approximately 10 µL under a gentle stream of nitrogen. The serum extract was assayed by a gas chromatography/mass spectrometry (GC/MS) system (Agilent 6890A GC coupled with JEOL JMS-GCmateII, Tokyo, Japan) with a fused silica capillary column (Rtx-1MS, 15 m, 0.25 mm i.d., 0.1 µm; Restek, Bellefonte, PA, USA). For each compound, 2 ions of the molecular ion or fragment ion cluster were monitored. Quantitation was based on the isotope dilution method using $^{13}C_{12}$-labeled internal standards. The PBDE concentrations were adjusted for total serum lipids and are expressed in units of nanogram per gram lipid weight (ng·g^{-1} lw). TeBDE-47, PeBDE-99, PeBDE-100, and HxBDE-153 were of interest because they are dominant in human serum.

Quality assurance and quality control: We validated the serum extraction procedure before beginning sample analysis by analyzing 4 replicate samples of pooled serum fortified with target analytes at 0.04–0.1 ng·g^{-1} serum. The mean percent recovery of 7 representative PBDE congeners (TrBDE-28, TeBDE-47, PeBDE-99, PeBDE-100, HxBDE-153, HxBDE-154, and HpBDE-183) ranged from 91% to 107%, and the relative standard deviation (RSD) ranged from 2% to 10%. The limit of detection (LOD) and limit of quantification (LOQ) were defined as 3 times and 10 times of the SD values obtained from the analysis of the 7 blank samples. However, for congeners that could not be detected in the blanks, values that were 3 times and 10 times of the SD values that were obtained from the analysis of 5 replicates of the lowest calibration standard were used as LOD and LOQ. The LOD values for all the PBDE congeners were below 0.3 ng·g^{-1} lw. In the analysis of 3 split unfortified serum samples, the RSD values for all the detected congeners were below 10%.

Results and Discussion

Of the 29 PBDE congeners monitored, 4 congeners (TeBDE-47, PeBDE-99, PeBDE-100, and HxBDE-153) were mainly detected in human serum samples (Figure 1). The concentrations of the detected PBDE congeners in the serum samples (n = 10) are shown in Table 1. The median levels of the individual PBDE congeners were as follows: BDE-47, 1.4 ng·g^{-1} lw; BDE-99, 0.21 ng·g^{-1} lw; BDE-100, 0.24 ng·g^{-1} lw; and BDE-153, 0.72 ng·g^{-1} lw. The levels of total PBDEs in Japanese human serum samples were almost the same as those reported in European countries but were one order of magnitude lower than those reported in USA[6]. Significant positive correlations were observed between the concentrations of TeBDE-47 and PeBDE-99 (r = 0.988, p < 0.001), TeBDE-47 and PeBDE-100 (r = 0.938, p < 0.001), and between PeBDE-99 and PeBDE-100 (r = 0.915, p < 0.001). In contrast, no significant correlations were observed between the concentration of HxBDE-153 and those of the other 3 congeners (r = 0.306–0.390, p = 0.26–0.39). The absence of a significant correlation between HxBDE-153 and the other dominant 3 congeners (TeBDE-47, PeBDE-99, and PeBDE-100) means that the main sources and/or biological properties of HxBDE-153 were different from those of the other 3 congeners. It has been reported that the technical mixtures of pentaBDE (DE-71 and Bromkal 70-5DE) and octaBDE (DE-79 and Bromkal 79-8DE) both contained HxBDE-153 in the range 5.32–5.44% w/w and 0.15–8.66% w/w, respectively[7]. The congeners TeBDE-47, PeBDE-99, and PeBDE-100 have been found in pentaBDE as the major components, but they have not been found in octaBDE[7]. These 3 congeners and HxBDE-153 have never been found in a technical decaBDE mixture (Saytex 102E and Bromkal 82-0DE)[7]. Therefore, TeBDE-47, PeBDE-99, and PeBDE-100 are mainly sourced from pentaBDE, although HxBDE-153 is sourced from both pentaBDE and octaBDE. In the early 1990s, Japanese manufacturers

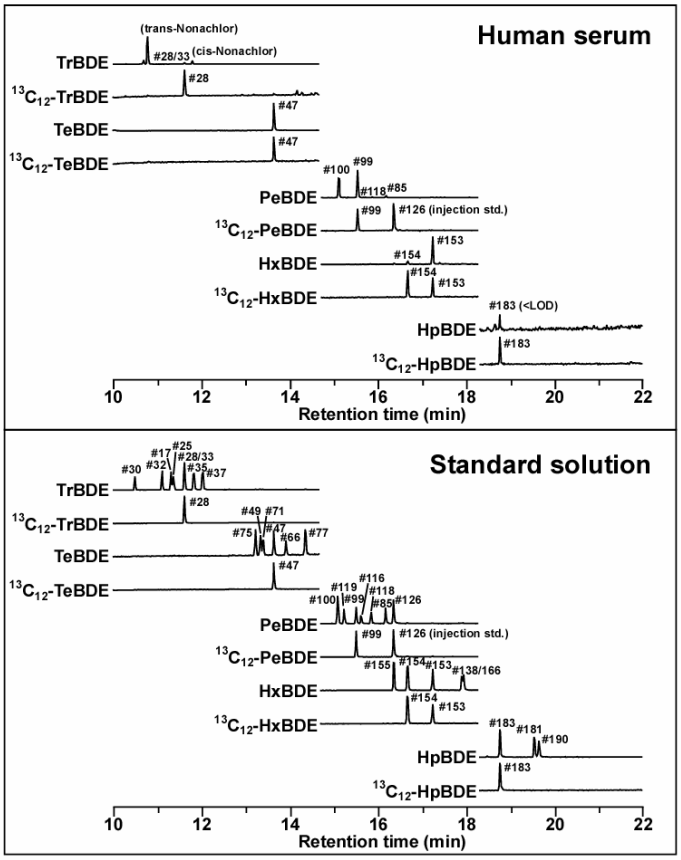

Fig.1 Chromatograms of PBDEs in human serum (participant No.2) and standard solution (1 to 2.5 ng·mL^{-1} each)

Table 1 Concentrations of PBDEs in serum samples from 10 Japanese males (ng·g^{-1} lw)

	Participant No.									
Congener	1	2	3	4	5	6	7	8	9	10
TrBDE-17	tr <0.04	tr <0.05	nd <0.01	nd <0.01	nd <0.02	nd <0.01	nd <0.02	nd <0.01	nd <0.02	nd <0.02
TrBDE-28/33	tr <0.2	0.37	0.16	tr <0.2	0.16	0.24	tr <0.2	0.17	tr <0.2	tr <0.2
TrBDE-37	tr <0.02	tr <0.03	nd <0.01	nd <0.01	nd <0.01	nd <0.01	nd <0.01	nd <0.01	nd <0.01	nd <0.01
TeBDE-49	nd <0.02	nd <0.03	0.09	tr <0.07	tr <0.08	0.07	nd <0.02	0.09	nd <0.03	tr <0.09
TeBDE-47	1.3	5.9	1.5	0.96	1.6	1.8	0.54	2.9	0.93	0.81
TeBDE-66	nd <0.04	nd <0.05	nd <0.04	nd <0.04	nd <0.04	nd <0.04	nd <0.04	tr <0.2	nd <0.05	nd <0.05
PeBDE-100	0.23	0.67	0.24	0.21	0.24	0.40	0.13	0.31	0.21	0.25
PeBDE-99	0.21	1.1	0.21	0.16	0.25	0.21	0.10	0.49	0.15	0.20
PeBDE-118	0.02	0.03	tr <0.02	tr <0.02	0.02	0.03	tr <0.02	tr <0.02	0.03	0.03
PeBDE-85	tr <0.07	tr <0.09	tr <0.07	nd <0.02	tr <0.08	nd <0.02	nd <0.02	tr <0.07	nd <0.03	nd <0.02
HxBDE-155	nd <0.02	tr <0.07	tr <0.05	tr <0.05	nd <0.02	tr <0.06	nd <0.02	nd <0.02	tr <0.07	nd <0.02
HxBDE-154	tr <0.06	0.08	0.05	0.05	tr <0.06	0.06	tr <0.06	tr <0.06	tr <0.07	tr <0.07
HxBDE-153	0.76	0.96	1.1	0.56	0.58	0.68	0.37	0.52	0.91	0.79
HpBDE-183	nd <0.1	nd <0.2	tr <0.4	tr <0.4	tr <0.4	tr <0.4	nd <0.1	nd <0.1	tr <0.5	nd <0.2
Sum of 4 PBDEs*	2.5	8.6	3.1	1.9	2.7	3.1	1.1	4.2	2.2	2.1

Abbreviations: tr, trace; nd, not detected. *Sum of TeBDE-47, PeBDE-100, PeBDE-99, and HxBDE-153.

voluntarily stopped the production and use of pentaBDE because of concern for its potency to accumulate in biota and to produce toxic polybrominated dibenzofurans/dioxins under thermal stresses. However, the production and use of octaBDE were continued in Japan until the early 2000s. There may still be a large number of consumer products that contain octaBDE in the Japanese indoor environment. Thus, with regard to octaBDE components such as HxBDE-153 and HpBDE-183, inhalation and dermal exposure may be important exposure routes for the Japanese people.

Table 2 Sperm concentration and testis size of 10 Japanese males

	Participant No.									
	1	2	3	4	5	6	7	8	9	10
Sperm concentration (million·mL^{-1})*	49	55	38	108	83	74	115	78	25	30
Testis size (mL)	36	36	40	50	46	42	51	54	29	33

*Annual average of monthly data.

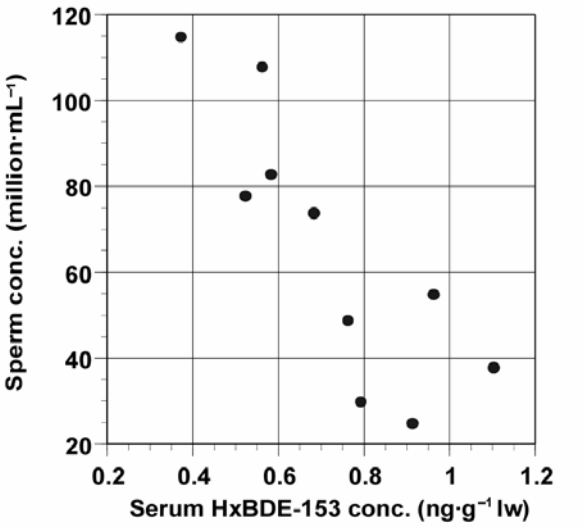

Fig.2 Relationship between serum HxBDE-153 concentration and sperm concentration

The sperm concentration and testis size of the 10 participants are shown in Table 2. The sperm concentration of these participants ranged from 25 to 115 million·mL^{-1}. No participant had a sperm concentration below 20 million·mL^{-1}, a preliminary diagnostic value of male infertility. Clear inverse correlations were observed between serum HxBDE-153 concentration and sperm concentration (r = –0.841, p = 0.002, Fig.2) and testis size (r = –0.764, p = 0.01). However, no significant relationships were observed between the serum concentrations of any of the other congeners and the sperm concentration or testis size. Researchers have hypothesized that endocrine disrupting chemicals with thyroid hormonal or sexual hormonal activities may adversely affect male fertility. The thyroid-disrupting and estrogenic/antiestrogenic activities of PBDEs have been reported in several studies[1, 3]. In addition, considerable evidence is available for the reproductive effects of PBDEs from in vivo studies. Kuriyama et al. have reported that developmental exposure to a single low dose (60 μg·kg^{-1} body weight) of PeBDE-99 decreased sperm counts in male Wistar rats[4]. Although the levels of PBDEs found in our study are relatively low, we observed significant inverse associations between the serum concentration of HxBDE-153 and sperm concentration and testis size; this suggests an association between serum HxBDE-153 concentration and human sperm quality. The lack of a significant relationship among other individual PBDE congeners and sperm parameters may indicate a difference in bioactivity between congeners. The relationship between PBDEs and sperm quality is a complicated problem and needs further study.

Acknowledgments

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