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Polybrominated Diphenyl Ethers in Human Serum and Sperm Quality

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Abstract: Polybrominated diphenyl ethers (PBDEs) are widely used flame retardants; currently, they are identified as ubiquitous environmental contaminants. Several studies indicate that PBDEs might affect male fertility. We present the results of a pilot study on the relationship between human serum PBDEs and sperm quality. The PBDE levels in Japan are comparable to those found in European countries. Strong inverse correlations were observed between the serum concentration of 2,2′,4,4′,5,5′-hexabromodiphenyl ether and sperm concentration (r = –0.841, p = 0.002) and testis size (r = –0.764, p = 0.01). Extensive studies on the relationship between PBDEs and sperm quality are required.

Keywords: polybrominated diphenyl ethers; flame retardants; human serum; sperm.

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Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in the production of common consumer products such as electronics, furniture, and textiles. PBDEs are currently recognized as environmental pollutants of global concern because their levels in the environment and in humans have increased markedly over the past several decades (Meironyté et al., 1999; Ikonomou et al., 2002; Akutsu et al., 2003). Since PBDEs are somewhat structurally similar to thyroid hormones such as thyroxine (T4), it was speculated that PBDEs might mimic thyroid hormones and disrupt thyroid homeostasis. Several studies indicate that exposure to PBDEs can decrease the circulating levels of T4 in laboratory animals (Fowles et al., 1994; Zhou et al., 2002) and can cause permanent neurological effects similar to those associated with thyroid hormone deficiencies (Eriksson et al., 2001; Viberg et al., 2004). In addition, several PBDEs possess weak estrogenic/antiestrogenic activities (Meerts et al., 2001). The proliferation and differentiation of Sertoli cells and sperm production are regulated by thyroid and sex hormones. Thus, PBDEs might affect male reproductive health by interfering with the thyroid- and sex-hormone functions. Kuriyama et al. (2005) have reported that developmental exposure to a single low dose (60 μg/kg body weight) of 2,2′,4,4′,5-pentabromodiphenyl ether (PeBDE-99) decreased the sperm count in male Wistar rats. However, no previous studies have examined the relationship between human PBDE levels and sperm quality.

We participated in an international project examining the sperm quality of fertile males and found that the sperm concentration of Japanese males was lower than that of European males (Iwamoto et al., 2006). The examination of sperm quality and an estimation of the concentration of chemicals in the serum would be required to reveal the correlation between chemical exposure and the sperm quality in Japanese males. The aim of this pilot study was to measure PBDEs in serum samples from young Japanese males and to examine the relationship between serum PBDE levels and sperm quality.

MATERIALS AND METHODS

This study was performed in accordance with the protocols which were approved by the ethical committees of the St. Marianna University School of Medicine and Osaka Prefectural Institute of Public Health. Written informed consent was obtained from all study participants. Blood serum and sperm samples were collected on a monthly basis in the year 2003 from 45 young Japanese males at the Department of Urology, St. Marianna University School of Medicine. The participants were instructed to abstain from ejaculation for at least 48 h prior to sperm collection. The blood samples were collected in vacuum tubes, and the serum fractions were separated by centrifugation. The serum samples were stored at –80°C until analysis. Of the 45 sample sets, 10 were randomly selected 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. The mean ± standard deviation (SD) of the age of the 10 participants was 22 ± 1 years (range, 18–22 years). The mean ± SD abstinence period was 3.1 ± 0.4 days (range, 2.6–3.8 days). In addition, 2 brands of commercially pooled

human serum ("L-Consera N" and "L-Suitrol I," Nissui Pharmaceutical, Tokyo, Japan) were used as in-house reference materials.

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 with 3 to 7 bromine atoms were monitored. The PBDE numbers are assigned according to the International Union of Pure and Applied Chemistry nomenclature for polychlorinated biphenyls. Acetone, acetonitrile, and *n*-hexane of pesticide analysis grade; ammonium sulfate of biochemistry grade; 44% sulfuric acid-impregnated silica gel; and *n*-nonane of dioxin analysis grade were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was deionized and purified using a Milli-Q cartridge system (Millipore, Bedford, MA, USA).

Sperm analyses were performed at the Department of Urology, St. Marianna University School of Medicine, according to World Health Organization criteria as described elsewhere (World Health Organization, 1999; Iwamoto et al., 2006).

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 using a semimicro balance (Sartorius RC210P, Goettingen, Germany). The lipid was dissolved in *n*-hexane and 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 transferred to a microconcentration tube. After addition of the injection standard ($^{13}C_{12}$-3,3′,4,4′,5-PeBDE ($^{13}C_{12}$-PeBDE-126)) 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) using a fused silica capillary column (Rtx-1MS, 15 m length, 0.25 mm i.d., 0.1 μm film thickness; 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 were expressed in units of nanogram per gram lipid weight (ng/g lw). TeBDE-47, PeBDE-99, PeBDE-100, and HxBDE-153 were of interest because they are dominant in human serum.

We validated the serum extraction procedure prior to beginning sample analysis by analyzing 4 replicate samples of pooled serum fortified with target analytes at 0.04–0.1 ng/g 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 the SD values obtained from the analysis of the 7 procedural blank samples (6 g of water), respectively. However, for congeners that could not be detected in the blanks, the values that were 3 times and 10 times the SD values 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 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: TeBDE-47, 1.4 ng/g lw; PeBDE-99, 0.21 ng/g lw; PeBDE-100, 0.24 ng/g lw; and HxBDE-153, 0.72 ng/g lw. The levels of total PBDEs in Japanese human serum samples were almost the same as those reported in European countries but were 1 order of magnitude lower than those reported in USA (Hites, 2004). 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 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 3 dominant congeners (TeBDE-47, PeBDE-99, and PeBDE-100) implies 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 (La Guardia et al., 2006). 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 (La Guardia et al., 2006).

These 3 congeners and HxBDE-153 have never been found in a technical decaBDE mixture (Saytex 102E and Bromkal 82-0DE) (La Guardia et al., 2006). 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 voluntarily stopped the production and use of pentaBDE because its potency to accumulate in the biota and produce toxic polybrominated dibenzofurans/dioxins under thermal stresses was a cause of concern. However, the production and use of octaBDE were continued in Japan until 2002 (Ministry of the Environment, Japan, 2006). Therefore, many consumer products containing octaBDE in the Japanese indoor environment might continue to exist. Thus, with regard to octaBDE components such as HxBDE-153 and HpBDE-183, inhalation and dermal exposure might be important exposure routes for the Japanese people. Geyer et al. (2004) have predicted elimination half-lives of PBDEs in the human adipose tissue; the predicted half-lives of individual congeners in an adult male were as follows: TeBDE-47, 1.9 years; PeBDE-99, 3.5 years; PeBDE-100, 2.4 years; and HxBDE-153, 7.8 years. It is expected that the half-lives of Te–HxBDEs increase with the number of bromine atoms per molecule, and the half-life of HxBDE-153 is much longer than those of other dominant congeners detected in human serum. Further research is needed to examine the difference between the elimination half-lives and toxicity of individual PBDE congeners in animals and humans.

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. No participant had a sperm concentration below 20 million/mL, the minimum fertility standard established by the World Health Organization (World Health Organization, 1999). Strong inverse correlations were observed between the serum HxBDE-153 concentration and sperm concentration (r = –0.841, p = 0.002; Figure 2) and testis size (r = –0.764, p = 0.01). However, no significant relationships were observed between the serum concentrations of the other congeners and the sperm concentration (r ranged from –0.187 to –0.099, p = 0.605–0.786) or testis size (r ranged from –0.216 to –0.054, p = 0.548–0.883). Researchers have hypothesized that endocrine-disrupting chemicals with thyroid-hormonal or sex-hormonal activities might adversely affect male fertility. The thyroid-disrupting and estrogenic/antiestrogenic activities of PBDEs have been reported in several studies (Meerts et al., 2001; Zhou et al., 2002). In addition, considerable evidence regarding the reproductive effects of PBDEs is available from *in vivo* studies. Kuriyama et al. (2005) have reported that developmental exposure to a single low dose (60 μg/kg body weight) of PeBDE-99 decreased the sperm count in male Wistar rats. 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 the sperm concentration and testis size; this suggests an association between the serum HxBDE-153 concentration and human sperm quality. The lack of a significant relationship among other individual PBDE congeners and sperm parameters might indicate a difference in bioactivity between the congeners. The relationship between PBDEs and sperm quality is a

complicated problem and needs further study.

Acknowledgments We thank the participants who donated their blood and semen samples. This study was supported by grants from the Ministry of the Environment and the Ministry of the Health, Labor and Welfare, Japan.

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Figure legends

Figure 1 Chromatograms of PBDEs in human serum (participant No.2) and standard solution (1 to 2.5 ng/mL each)

Figure 2 Relationship between the serum HxBDE-153 concentration and sperm concentration

Table 1 Concentrations of PBDEs in serum samples from 10 Japanese males (ng/g 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[a]	2.5	8.6	3.1	1.9	2.7	3.1	1.1	4.2	2.2	2.1

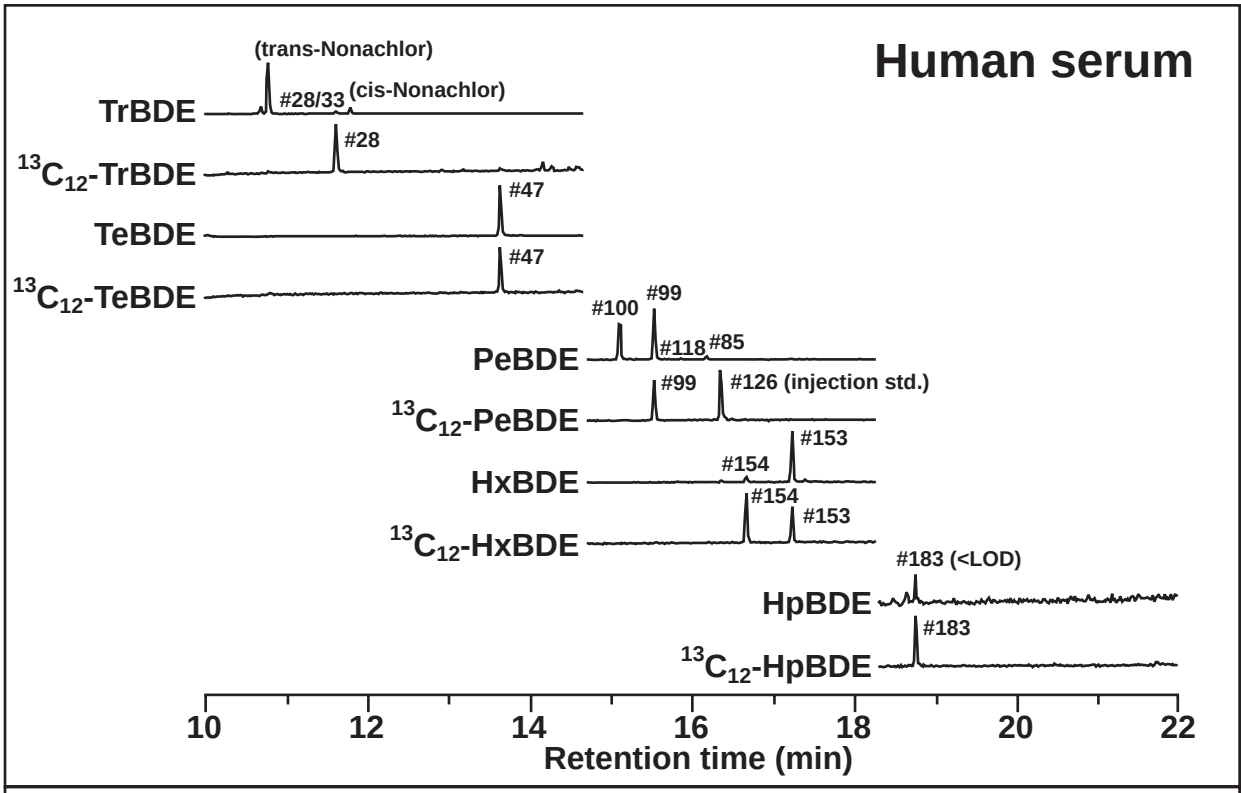
Abbreviations: tr, trace; nd, not detected. [a]Sum of TeBDE-47, PeBDE-100, PeBDE-99, and HxBDE-153.

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)[a]	49	55	38	108	83	74	115	78	25	30
Testis size (mL)[b]	36	36	40	50	46	42	51	54	29	33

[a]Annual average of monthly data. [b]Total of right and left testes.

Fig.1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(cis-Nonachlor)
TrBDE
#28
$^{13}C_{12}$-TrBDE
#47
TeBDE
#47
$^{13}C_{12}$-TeBDE
#100
#99
#118
#85
PeBDE
#99
#126 (injection std.)
$^{13}C_{12}$-PeBDE
#153
#154
HxBDE
#154
#153
$^{13}C_{12}$-HxBDE
#183 (<LOD)
HpBDE
#183
$^{13}C_{12}$-HpBDE
10
12
14
16
18
20
22
Retention time (min)

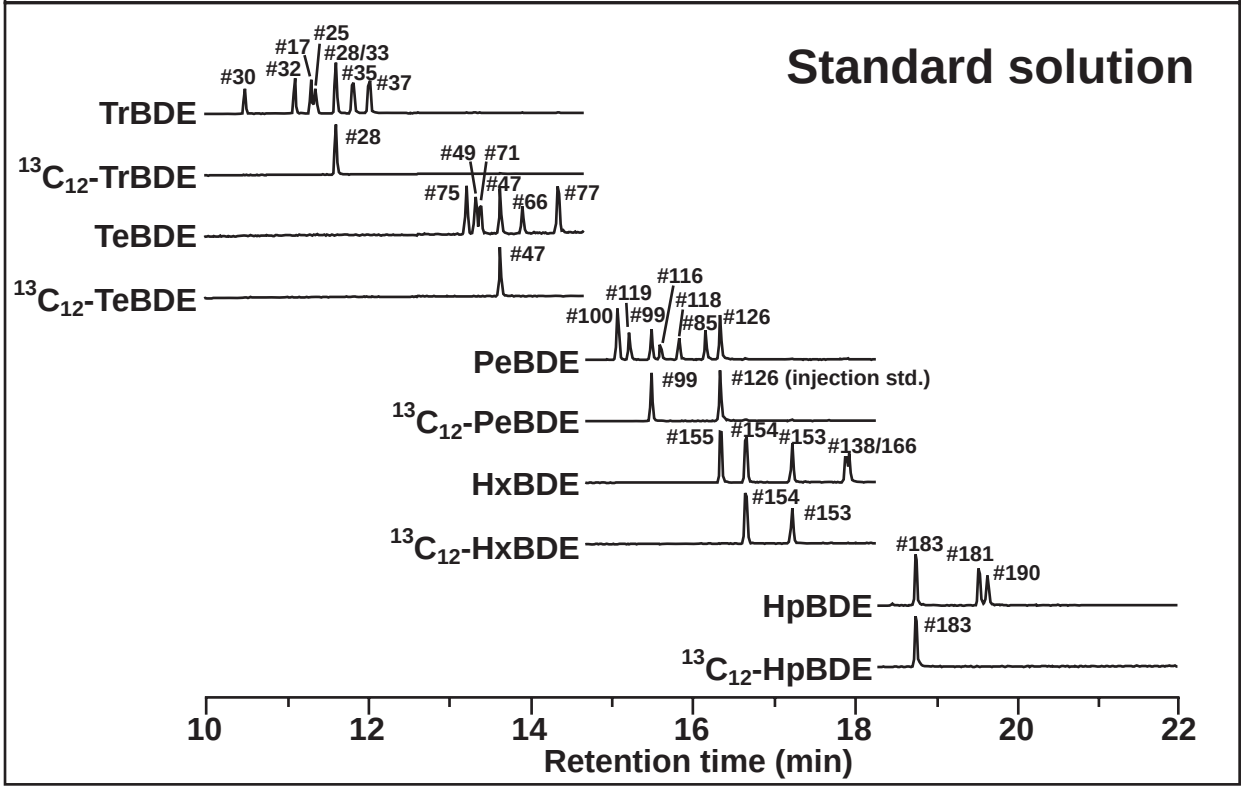
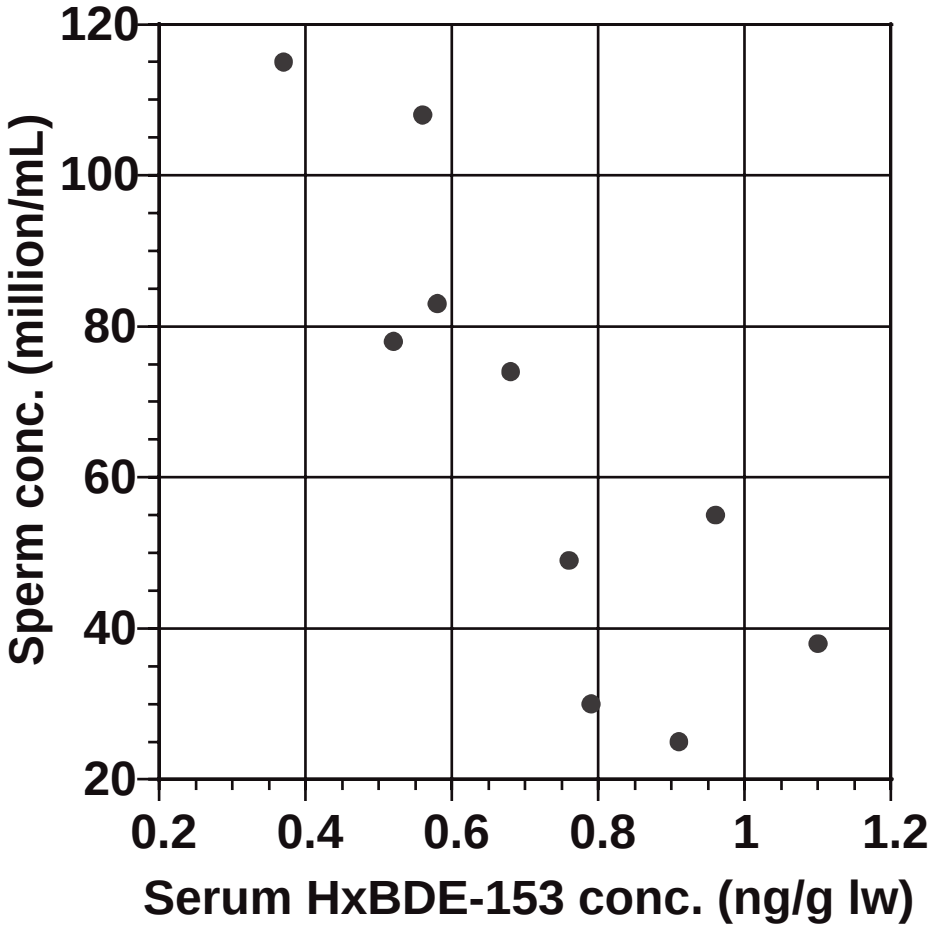

Standard solution
#30
#32
#17
#25
#28/33
#35
#37
TrBDE
#28
#49
#71
#47
#75
#66
#77
$^{13}C_{12}$-TrBDE
TeBDE
#47
$^{13}C_{12}$-TeBDE
#100
#119
#99
#116
#118
#85
#126
PeBDE
#99
#126 (injection std.)
$^{13}C_{12}$-PeBDE
#155
#154
#153
#138/166
HxBDE
#154
#153
$^{13}C_{12}$-HxBDE
#183
#181
#190
HpBDE
#183
$^{13}C_{12}$-HpBDE
10
12
14
16
18
20
22
Retention time (min)

Fig.2


120
100
80
60
40
20
Sperm conc. (million/mL)
0.2
0.4
0.6
0.8
1
1.2
Serum HxBDE-153 conc. (ng/g lw)