Associations between PBDEs exposure from house dust and human semen quality at an e-waste areas in South China—A pilot study

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HIGHLIGHTS

- The quality of semen from e-waste area was lower than that in control area.
- BDE28,47 and 153 level in semen was positively associated with that in dust.
- The semen quality was negatively correlated with dust PBDEs level.
- House dust PBDEs might have adverse effects on male fertility.

GRAPHICAL ABSTRACT
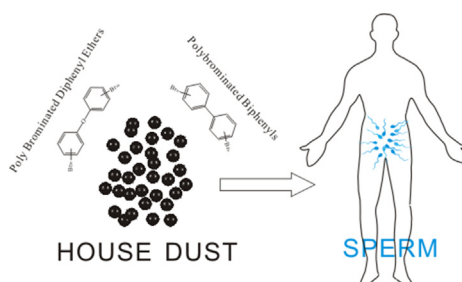
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ABSTRACT

Previous studies have confirmed that house dust is one of the main sources of polybrominated diphenyl ethers (PBDEs) exposure, and also indicated that PBDEs might affect human semen quality. The aim of this study was to explore the association between PBDEs concentration in house dust and the semen quality of male resident. Results showed that the semen qualities of the residents living around the e-waste dismantling workshops for a long time (3—17years) at the e-waste areas in South China significantly decreased, and the DNA damage of sperms were aggravated. The adjusted correlation analysed by multiple linear regression model showed that the sperm concentration and count both had negative correlation with BDE47 level in semen ($\beta = -0.295$, 95%CI: $-0.553\text{~-}0.036$; $\beta = -0.400$, 95%CI: $-0.708\text{~-}0.092$, respectively). In addition, the sperm progressive motility [(A+B)%] and sperm viability both had negative correlation with BDE100 level in dust ($\beta = -0.360$, 95%CI: $-0.680\text{~-}0.040$; $\beta = -0.114$, 95% CI: $-0.203\text{~-}0.025$, respectively). And there were significant linear positive correlation between PBDE congener (e.g. BDE28, 47, 153) concentrations in dust and in paired semen samples ($r_s = 0.367-0.547$, $p < 0.05$). This study suggested that exposure to PBDEs from house dust might have adverse effects on

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human semen quality. But the results need to be confirmed in further studies with a large-scale sampling, and find out more direct and convincing evidence.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), which have been used widely in producing a variety of consumer products such as plastics, electronics, construction and textile as additive of flame retardants, are a groups of ubiquitous organic pollutants. PBDEs are known as persistent organic pollutants (POPs) with the characteristics of environmental persistence, long distance transmission, biological accumulation and toxic effects on organisms and human (Hauser et al., 2005).

People generally spend more than 80% of their time indoor, thus great emphasis is placed on PBDEs contamination in the indoor environment (Jones-Otazo et al., 2005; Betts, 2008). On global comparison, PBDE levels in house dust differed by two to three orders of magnitude (Kim et al., 2016). Much higher levels of PBDEs are found in the US/Canada vs. Europe. In turn, UK PBDEs levels are much higher than in other European countries (Wilford et al., 2005; D'Hollander et al., 2010; de Boer et al., 2016). Levels of PBDEs in house dust at e-waste areas in China are quite high compared with other areas in the world (Wang et al., 2007; Jiang et al., 2014; Yu et al., 2016). Moreover, BDE209 was the dominant congener in the majority of house dust samples (Harrad and Abdallah, 2011; Sahlstrom et al., 2015; Korcz et al., 2017). Compared with BDE209, penta- and octa-BDEs have higher bioaccessibility and longer half-life, thus all of these congeners may greatly impair human health (Bramwell et al., 2016).

Studies conducted over the past decades show that indoor dust and diet are the two main source of PBDEs (Frederiksen et al., 2009; Johnson-Restrepo and Kannan, 2009; Bramwell et al., 2016). Despite the high proportion of total exposure being from diet, neither study found correlation between PBDEs in duplicate diet and internal dose (Fromme et al., 2009; Bramwell et al., 2016). Nevertheless, internal exposure dose of PBDE congeners generally correlated strongly with the levels of PBDE in house dust (Bramwell et al., 2016). For example, PBDE levels in human serum and human breast milk had significant positive correlations with PBDE concentrations in house dust (Karlsson et al., 2007; Wu et al., 2007). For the concentrations of PBDEs, PCBs, and OCPs in serum of residents in an e-waste dismantling region (Guiyu, South China), PBDEs typically accounted for 46% of the total organohalogen chemicals in serum samples, but only 8.7% in the serum samples collected from nearby non e-waste region (Frederiksen et al., 2009).

It is known that PBDEs are endocrine disruptors and may affect male reproduction (Yang et al., 2009; Eskenazi et al., 2017). Previous studies observed that BDE153 level in human serum was negatively correlated with the concentration of sperm ($r = -0.841$, $p = 0.002$) and the size of testis ($r = -0.764$, $p = 0.01$) (Akutsu et al., 2008). Studies also found that semen mobility was negatively related to BDE-47, BDE-100 and $\sum$BDE, and thyroxin levels were negatively associated to serum BDE-47,BDE-99, and $\sum$BDE (Abdelouahab et al., 2011). Furthermore, the concentrations of total PBDEs varied from 15.8 to 86.8 pg g^{-1} ww (median = 31.3 pg g^{-1} ww) in semen samples (n = 101) collected from e-waste dismantling region (Taizhou, East China), and this was the first time PBDEs were detected in human semen (Liu et al., 2012). However, studies about the effect of PBDEs in dust on human reproductive health, especially on male semen quality, are still insufficient.

In our previous studies, high concentrations of PBDEs were found in house dust from Longtang town, which was one of the largest e-waste dismantling areas in China (Wang et al., 2010; Zheng et al., 2015). Because of the primitive e-waste recycling processes, such as open burning and acid processing, there were large numbers of polluted compounds (including PBDEs) released into the environment. The objective of this exploratory study was to investigate the relationship between external exposure of PBDEs in house dust from Longtang town and the semen quality of the residents who have lived around the e-waste dismantling workshops for a long time (3–17years) without working in e-waste dismantling facilities. The semen samples of control group without any known occupational exposure were selected from the semen bank of hospital, and were also assessed. The concentration of PBDEs in indoor dust and in paired semen of the residents, and the quality of sperm samples were analysed. The associations between them was discussed as well.

2. Materials and methods

2.1. Recruitment of study participants

The area of investigation (Longtang town) is located in the rural area of Qingyuan (South China), where electrical and electronic waste (e-waste, such as television sets, computers, and electric fans, etc) dismantling industry has existed for decades, and there are no other industrial activities nearby.

Between October 2015 and July 2016, males between 18 and 50 years of age were recruited to participate in this study. These males were recruited from e-waste dismantling areas at Longtang town, and were also long-time residents (3–17 years) in this town. Excluding those who had reproductive health problems such as epididymitis, vasectomy, varicocele, orchitis, vesiculitis and thyroid disorder, a total of 32 adult men were recruited. So they were convenience samples. Meanwhile, corresponding house dust samples were collected from their home.

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Jinan University, and all participants gave informed consent prior to enrollment.

2.2. Questionnaire

An interview-administered questionnaire was conducted at the time of recruitment by study staff under the guidance of the medical staff of the First Affiliated Hospital of Jinan University. Questionnaire was used to obtain detailed information of participants, which covered age, residence time, abstinence days, occupational history, smoking and drinking, workplace, as well as health information. Participants' height and weight were measured and body mass index (BMI) was calculated as weight (kg)/height (m^2).

2.3. Sample collection

House dust samples (n = 32) were obtained from the floors, furniture, and windowsills. They were collected from October 2015 to July 2016 using woolen brushes that were pre-cleaned with 70%

ethyl alcohol, and wrapped in aluminum foil before use. All of the samples from a particular home were combined in one pooled sample, so that there was a single sample for each participant. After being transported to the laboratory, all samples were sieved through a stainless steel sieve (150 μm) to remove debris and kept at −20 °C until further analysis.

Semen samples (n = 32) of observation group was collected by masturbation into a sterile plastic specimen cup at the Qingyuan People's Hospital. Subjects were instructed to abstain from ejaculation for at least 48 h prior to producing the semen sample. The sample was liquefied for at least 20 min, but no longer than 1 h prior to performing a semen analysis. The semen samples of control group (n = 25) were selected from the semen bank of the First Affiliated Hospital of Jinan University, and the semen donors came from Qingyuan city which was not the e-waste recycling area. We just got two parameters of demographic characteristics for control group, i.e. age and abstinence time.

2.4. Analysis of PBDE congeners

The preparation and analysis method of house dust sample has been described in details elsewhere (Wang et al., 2010). Briefly, about 0.5 g samples were spiked with BDE77, BDE 181 (AccuStandard, Inc., New Haven, CT) and ^{13}C-BDE209 (Cambridge Isotope Laboratories, Inc., Tewksbury, MA), then extracted by soxhlet with acetone and hexane (1:1, v: v) for 48 h. The extracts were purified by acid silica (44%), further spiked with internal standards (BDE118, 128 and 67) (AccuStandard, Inc., New Haven, CT), and finally concentrated to 200 μL.

The extraction and analysis procedure of PBDE congeners and PBB153 in semen samples was the same as the serum method, which has been published elsewhere (Hovander et al., 2000; Liu et al., 2012). The surrogate standards were 4′-F-BDE67 and ^{13}C-BDE209, while the internal standards spiked to the sample, as well as the analytic method of GC-MS, were the same as analysis of house dust. Briefly, the samples spiked with surrogate standards were added hydrochloric acid and isopropanol in order to denaturize the proteins and release the target compounds. The analytes were subsequently extracted with hexane/methyl t-butyl ether (MTBE; 1:1) more than three times. The combined organic phases were washed with a 1% KCl solution, followed by evaporation to dryness for gravimetric determination of extracted lipid content. After re-dissolved in hexane, the analytes were further cleanup on an acid silica column, then eluted with 80 mL of hexane. Finally, the analytes were concentrated to 20 μL after the addition of internal standards, and analysed by GC-MS.

The analysis of PBDEs was performed by Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass spectrometer (Agilent Technologies, Santa Clara CA) in electron capture negative ionization mode (GC-ECNI-MS). BDE 28, 47, 99, 100, 153, 154, 183, 209 and PBB153 (polybrominated biphenyls congener) were separated with a DB-5HT (15 m × 0.25 mm i. d., 0.10 μm film thickness) capillary column. The GC temperature program was initiated at 110 °C, held for 5.0 min, then ramped at 20 °C·min^{-1} to 200 °C (held for 4.5 min), and finally ramped at 10 °C·min^{-1} to 310 °C (held for 15.0 min). Ion fragments m/z 79 and 81 were monitored for these compounds except for BDE209 and ^{13}C-BDE209 for which m/z 486.7 and 488.7, m/z 494.6 and 496.6 were recorded, respectively.

2.5. QA/QC

The procedure for washing glassware referred to the supporting information of reference paper (Whitehead et al., 2015). The quality control was performed by regular analysis of procedural blanks and solvent blanks (two in each batch of 10 samples).

Limit of detection (LOD) of semen samples were established at 0.71–1.41 pg g^{-1} wet weight (ww) for BDE 28, 47, 99, 100, 153 183 and PBB153, respectively, and at 3.11 pg g^{-1} ww for BDE209. The recoveries of surrogate standards were 87.2 ± 9.6% for 4′-F-BDE67, and 64.3 ± 13.5% for ^{13}C-BDE209. The LOD of analysis method was defined as 3 times the noise level, and the calculations of semen samples were based on 1.5 g semen weight although many samples were less than that. The average lipid contents of semen samples of observation group and control group were 0.38% and 0.33%, respectively.

The LOD of these congeners in dust samples ranged from 0.07 to 1.3 ng g^{-1} based on 0.5 g of sample. Average recoveries for surrogates spiked in samples were 97.2 ± 8.9% for BDE77, 81.3 ± 9.2% for BDE181, and 72.3 ± 10.5% for ^{13}C-BDE209.

2.6. Analysis of semen quality and sperm DNA damage

Semen parameters, including sperm concentration, count, progressive motility and viability, was examined at the Qingyuan People's Hospital using computer-aided sperm analysis (CASA; version WLJY-9000, Beijing Weili New Century Technology Development Co., Ltd., China) according to the manual of World Health Organization (WHO, 5th edition).

Comet assay was used to detect the DNA damage of sperm by the alkaline single cell gel electrophoresis technique (SCGE). Briefly, after removing the coverglass, slides were immersed in a cold lysing solution (2.5 M NaCl, 100 mM EDTA disodium salt, 10 mM Tris, pH 10, 1% sodium lauryl sarcosine and 1% Triton X-100, 40 mM dithiothreitol). After 1 h cold lysis, slides were transferred to a solution for enzyme treatment (2.5 M NaCl, 5 mM Tris, pH 7.4, 0.05% sodium lauryl sarcosine) with 10 μg mL^{-1} RNase A. The slides were incubated at 37 °C for 4 h, then were transferred to the solution for enzyme treatment containing 1 mg mL^{-1} DNase-free proteinase K (Amresco, Solon, OH) and incubated at 37 °C for 1.5 h.

TUNEL assay (TdT-mediated dUTP Nick-End Labeling) was used to analyze the apoptosis of sperm cells. The amount of DNA fragmentation was determined using a commercially available kit (TUNEL Bright Green Apoptosis Detection Kit, Vazyme, A112-02, Vazyme Biotech Co.,Ltd, Nanjing, China). Briefly, the spermatozoa sample was fixed in 4% paraformaldehyd for 10 min, and washed two times with PBS. The samples were incubated at room temperature with 2 mg mL^{-1} proteinase K (diluted with PBS by 1:100) and Buffer Equilibration (diluted with water by 1:5) for 5 min and 25 min, respectively. They were incubated in dark at 37 °C for 60 min with TdT incubation buffer (each 50 μL buffer containing 34 μL ddH$_2$O, 10 μL 5 × Equilibration Buffer, 5 μL FITC-12-dUTP Labeling Mix and 1 μL Recombinant TdT Enzyme), and finally dyed with 2 μg mL^{-1} DAPI for 5 min. The fluorescence intensity was observed under a fluorescence microscope analysis (OLYMPUS CKX41, U-CTR30-2).

2.7. Statistical analysis

Concentrations of PBDE congeners in house dust in this study were not normally distributed (Shapiro-wilk test, p < 0.05), and the data were reported by using the geometric mean (GM) and geometric standard deviation (GSD). The non-parametric tests including the Mann-Whitney U test were used to examine the discrepancies between the parameters in semen samples of observation group and control group. The correlations of linear regression models were examined by the Pearson correlation coefficients (r_s). The multiple linear regression model (MLR) were

used to explore the associations between the semen quality parameters and PBDE congener levels in dust or in semen. All tests were performed using the statistical package SPSS 20 (SPSS, Inc.). For statistical analysis, to avoid large bias, only the congeners detected in more than 50% of semen or dust samples were investigated. The level of significance was set at $p < 0.05$ (two tailed). Congeners concentrations <LOD were substituted with a value of 1/2 LOD for calculating total concentrations of PBDEs and linear regression analysis.

3. Results

3.1. Characteristics of the study participants

The demographic characteristics of the observation group are shown in Table 1. The average age of participants was 38.7 ± 9 years, and their height and weight were in a common range with a mean BMI of 23.5 ± 2.9 kg m^{-2}. According to Mann-Whitney U test, there were no significant difference in age and abstinence time between the observation group and control group.

All men from the observation group were residents living around the e-waste dismantling workshops for a long time (3–17years). They didn't work in the e-waste dismantling facility. Nevertheless, they were associated with transportation and trade of the electronic waste, and some of valuable recycled electronic waste such as waste electric cable were always stored in their houses. Therefore, the levels of PBDEs in their indoor environment might be higher than that of the other dwellings.

3.2. PBDE congeners level in house dust

Table 2 shows the concentrations of PBDE congeners and PBB153 in house dust in the area of investigation (Longtang town, South China) from October 2015 to July 2016. The geometric mean of PBDE congener concentrations (excluding BDE209) in house dust varied from 0.212 to 331 ng g^{-1}. Compared with the other two typical e-waste dismantling areas in China, these PBDE congener

Table 1
Characteristics of the study participants.

Variables	Mean ± SD	Range
Observation group (n = 32)		
Age (years)	38.7 ± 9	20–50
		Number of subjects (%)
	20–29	28.1%
	30–39	21.9%
	40–44	21.9%
	45–50	28.1%
Working age (years)	7.3 ± 3.9	3–17
Body mass index (BMI)	23.5 ± 2.9	18.6–30.1
Abstinence time (days)	11.2 ± 13.1	2–60
		Number of subjects (%)
	2–6	53.1%
	7	25.0%
	>7	21.9%
Smoking status		Number of subjects (%)
	Yes	65.6%
	No	34.4%
Alcohol use		Number of subjects (%)
	Yes	59.4%
	No	40.6%
Control group (n = 25) [a]		
Age (years)	36 ± 6 ($p = 0.054$)[b]	24–46
Abstinence time, (days)	4.8 ± 2.7 ($p = 0.103$)[b]	2–15

[a] The semen samples of control group were selected from the semen bank.
[b] The p-value presented the discrepancies between the variables of observation group and control group.

Table 2
PBDEs and PBB153 concentrations (ng/g) in dust from October 2015 to July 2016 (n = 32).

comps.	DF (%)[a]	GM[b]	GSD[b]	Min	25th	50th	75th	Max	Median
BDE 28	100%	1.58	2.07	0.221	0.986	1.46	2.40	9.72	1.45
BDE 47	100%	11.7	18.7	2.12	4.81	12.0	23.9	91.6	12.0
BDE 100	100%	4.35	6.57	1.03	1.85	4.35	7.97	33.2	4.35
BDE 99	100%	21.9	33.6	3.52	10.8	22.7	42.3	172	22.7
BDE 154	100%	4.80	7.26	0.212	2.68	5.45	8.69	37.4	5.45
BDE 153	100%	16.4	29.6	3.08	7.90	14.6	34.2	136	14.6
BDE183	100%	33.5	65.3	5.75	19.2	30.4	76.4	331	30.4
BDE 209	100%	2576	3046	587	1439	2654	5545	11413	2654
∑PBDEs		2722	3092	647	1500	3051	5915	11562	3051
PBB153	87.5%	2.18	5.07	nd	1.55	3.40	7.64	19.5	3.40

[a] DF: Detection frequency.
[b] GM = geometric mean, GSD = geometric standard deviation. nd: not detected.

levels in residents house dust in this study were similar as that in Taizhou (Jiang et al., 2014), but much lower than that in Guiyu (Zheng et al., 2015) (Table S1). Moreover, BDE209 levels in this study (587–11413 ng g^{-1}) was much lower than that in the two e-waste dismantling areas (597–323 919 ng g^{-1} in Taizhou, and 1770–232 000 ng g^{-1} in Guiyu). One of the reasons might be that all the dust samples in this study were from residents house, and the other two studies might include dust samples from workshops. But the detailed reasons need to be further studied. Still, the ubiquitous compounds in residential house dust have considerable concentrations in these e-waste dismantling areas.

3.3. PBDE congeners level in human semen

The concentrations of PBDE congeners and PBB153 in semen and their detection rates are presented in Table 3, and data (detection frequency (DF) < 50%) in observation group was excluded from the statistical analysis. The mean value of BDE28, BDE47, BDE153 and PBB153 in observation group were 5.02 ± 4.99, 6.75 ± 5.61, 7.36 ± 6.62 and 2.96 ± 3.50 pg g^{-1} ww, respectively. Results showed that the level of BDE28, BDE 47 and BDE153 in semen samples of observation group was higher than that of control group (p < 0.05). The result was also similar with previous reports that BDE28, BDE47 and BDE153 were 4.85 ± 1.63, 6.18 ± 2.30 and 1.68 ± 1.19 (pg·g^{-1} ww) respectively in semen samples acquired in another e-waste area in East China (Liu et al., 2012).

3.4. Semen quality and sperm DNA damage

The semen quality of study populations is presented in Table 4. Compared with the control group, there were significant differences of the semen quality showed in the observation group, including total sperm count, sperm progressive motility [(A+B)%] and total motile spermatozoa [(A+B+C)%]. The average mean of sperm concentration of both observation group and control group was lower than that of average healthy man in china, whose value in different age groups (20–60 years) was 66.8-73.3 $\times 10^6$ sperm·mL^{-1} (n = 998) (Zhu et al., 2011). According to the WHO criteria, the qualified rate of sperm quality in the observation group was lower than that of control group (Table S2). The results showed that semen quality parameters (i.e. total sperm count and motility) of the observation group were significantly lower than that of the control group.

In comet assay, the tail DNA%, which was a measure of the proportion of the total DNA that is present in the tail (McAuliffe et al., 2014), were (57.88 ± 6.08)% in the observation group and (33.55 ± 6.99)% in the control group. Meanwhile, the Olive tail moment values were (12.15 ± 2.52)% and (5.14 ± 4.86)%,

Table 3
Summary statistics for PBDE congener and PBB153 levels in semen (pg/g ww) [a]

	DF (%)	Observation group (n = 32)			DF (%)	Control group (n = 25)			LOD	P
		Mean ± SD	Range	median		Mean ± SD	Range	median		value[b]
BDE28	68.8%	5.02 ± 4.99	nd-18.78	3.623	28.00%	1.62 ± 2.15	nd-7.97	nd	0.94	**0.002**[**]
BDE47	81.3%	6.75 ± 5.61	nd-19.31	5.863	20.00%	1.32 ± 2.63	nd-10.81	nd	0.45	**< 0.001**[**]
BDE153	78.1%	7.36 ± 6.62	nd-31.12	6.104	28.00%	3.62 ± 5.92	nd-19.84	nd	1.25	**0.005**[**]
PBB153	59.4%	2.96 ± 3.50	nd-16.12	1.307	48.00%	3.48 ± 4.21	nd-15.29	nd	1.34	0.899

[a] Data of DF < 50% was excluded from the statistical analysis. DF: detection frequency. nd: not detected.
[b] [**]$p < 0.01$ indicate significant differences in observation group compared with control group.

Table 4
Summary statistics for semen parameters and sperm quality.

			Observation group (n = 32)			Control group (n = 25)			p-value[b]
			Mean ± SD	Range	median	Mean ± SD	Range	median	
Semen parameters and sperm quality	Semen volume	(mL)	2.31 ± 1.43	0.5—5.9	2.1	2.3 ± 0.6	1.4—3.8	2.2	0.322
	Sperm concentration	(10⁶ sperm/mL)	50.4 ± 36.4	0.6—147.1	47.7	42.3 ± 20.1	8.55—83.8	44.95	0.597
	Total sperm count	(10⁶ sperm/ejection)	150.3 ± 176.3	1.1—652.2	61.9	165.9 ± 81.5	47.3—316	159	**0.040**[*]
	Sperm progressive motility [(A+B)%]	(%)	34.8 ± 19.4	4.6—80	29.4	54.0 ± 19.0	16.0—87.0	53	**0.001**[**]
	Total motile spermatozoa [(A+B+C)%]	(%)	51.7 ± 27.9	3.3—97.1	54.3	76.1 ± 17.1	33.0—98.0	79	**0.002**[**]
	Sperm viability (%)	(%)	74.5 ± 14.5	45—99	75.0	78.8 ± 13.0	51.0—97.0	81	0.322
Comet assay[a]	Comet tail DNA%	(%)	57.88 ± 6.08	44.45—66.0	58.46	33.55 ± 6.99	20.42—49.47	34.11	**<0.001**[**]
	Oliver tail moment values		12.3 ± 2.58	7.97—12.3	17.56	5.14 ± 4.86	1.24—3.71	20.4	**<0.001**[**]
TUNEL assay[a]	Apoptosis rate	(%)	32 ± 19	9—74	25	20 ± 8	5—41	22	**0.037**[*]

[a] Only 25 samples of the observation group were conducted with Comet assay and TUNEL assay. Data reported as mean (SD: standard deviation).
[b] [*]$p < 0.05$ and [**]$p < 0.01$ indicate significant differences in observation group compared with control group.

respectively (Table 2). Both of these two indexes of comet assay showed significant differences ($p < 0.01$) between the observation group and control group. The sperm apoptosis rate of the observation group and the control group detected by TUNEL were $(32 \pm 19)\%$ and $(20 \pm 8)\%$ respectively with a significant difference between them ($p = 0.037$).

3.5. Association between PBDE congener levels in house dust and paired semen samples

We calculated the correlations among PBDE congeners levels in house dust and in paired semen samples, and there was significant linear positive correlation ($r_s = 0.500$, $p = 0.004$) of BDE28 in these two sample groups (Fig. 1a). It was the same for BDE47 ($r_s = 0.547$, $p = 0.001$) (Fig. 1b) and for BDE153 ($r_s = 0.367$, $p = 0.039$) (Fig. 1c). However, no noticeable relations were observed between other PBDE congener and PBB153 levels in dust and in paired semen. One reason is the detection rates of PBDE congener in most of the semen samples were too low to evaluate the associations with the other PBDE congener concentrations in dust and in paired semen.

3.6. Association between PBDE congener levels and semen quality parameters

We explored the statistical correlations between PBDE congener concentrations in dust and semen quality parameters by using linear regression models (Table S3). Additionally, we explored the statistical correlations between PBDEs congener concentrations in semen and semen quality parameters, as well as the relationships between semen PBDEs congener concentrations and demographic characteristics (Table S4). Results showed that PBDE congener concentrations in dust (e.g. BDE47, 100, 99,154,153 and 183) and in

semen (e.g. BDE47) both showed negative correlations with semen parameters, including sperm concentration, count, motility and viability.

Therefore, we further used the multiple linear regression model (MLR) to establish the relationship between the semen quality parameters and the PBDE concentration in semen and in dust respectively, and the model was adjusted by the age, BMI, abstinence time, smoking and alcohol use, etc. The results of the multiple linear regression are shown in Table 5. The adjusted correlation analysis showed that the sperm concentration had significantly negative correlation with BDE47 in semen ($\beta = -0.295$, 95%CI: -0.553~-0.036), and the total sperm count also had significantly negative correlation with BDE47 in semen ($\beta = -0.400$, 95%CI: -0.708~-0.092). In addition, the sperm progressive motility [(A+B)%] and sperm viability both had significantly negative correlation with BDE100 in dust ($\beta = -0.360$, 95% CI: -0.680~-0.040; and $\beta = -0.114$, 95% CI: -0.203~-0.025), respectively. However, no other significant association was found between other semen quality parameters and PBDE congener levels in semen or in dust.

4. Discussion

To our best knowledge, this was the first study to explore the association between the PBDE congener levels in house dust and male semen quality. Our adjusted results by MLR model demonstrated that BDE47 level in semen was inversely associated with a suggestive dose-related decrease in sperm concentration and count, and the same as the relationships between the levels of BDE100 in dust and sperm motility and viability. Moreover, there was positive correlation between BDE 47 levels in house dust and in paired semen samples. Thus, these results suggested the dust
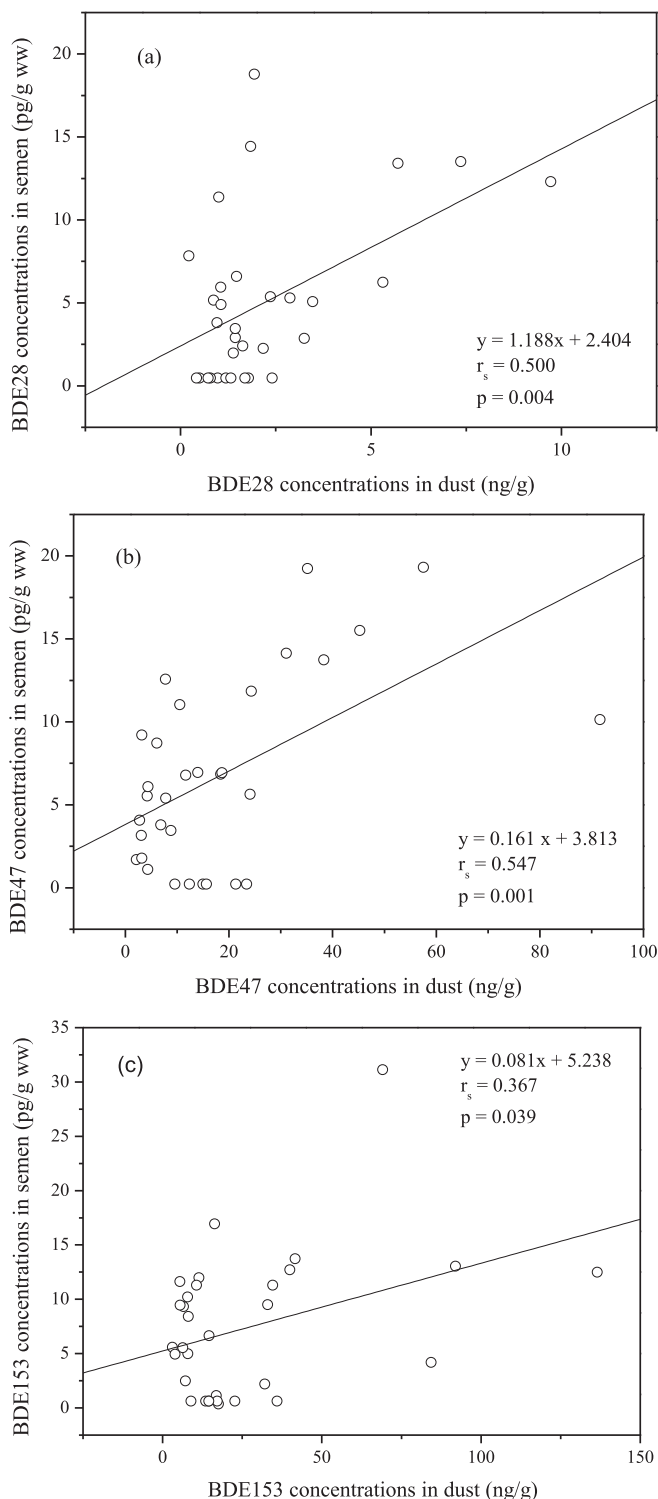
Fig. 1. (a)–(c) Relationship between BDE28, BDE47 and BDE153 concentrations in dust and in paired semen of observation group (n = 32).

BDE47 might have adverse effects on sperm concentration and count. And it was the same for dust BDE100 on the sperm motility and viability.

In clinical study, approximately 15% of male infertility have sperm quality within reference ranges showed by routine semen analysis (Agarwal et al., 2005; Lavranos et al., 2013). Thus, sperm DNA damage, such as sperm DNA strand breaks and sperm DNA

fragmentation, is complementary to reflect the fertilization ability of sperm and the function, and is assessed by Comet assay (Lavranos et al., 2013; Takeda et al., 2015). This study showed that the degree of sperm DNA damage in the observation group was higher than that in the control group. It indicated that the observation group have higher probability of infertility (Ribas-Maynou et al., 2013; Fernandez-Encinas et al., 2016).

Bramwell (Bramwell et al., 2016) concluded that despite the high proportion of total exposure being from diet, correlations between PBDE concentrations in diet and body burden were not apparent, however, internal dose of Penta-BDE congeners generally correlated strongly with dust. So he further concluded that compared to dietary exposures, dust ingestion constitutes an important pathway of exposure to PBDEs. The estimated daily intake of average adult via indoor dust both in e-waste area and the urban area of South China were much higher than those via other indoor pathways such as air inhalation (Wang et al., 2010). By summarizing the PBDEs levels in house dust, Yu et al. (2016) concluded that high levels of PBDEs in indoor dust observed in China proved that indoor contamination was a vital exposure pathway for humans, and it should be highlighted. Therefore, the risk of house dust intake contaminated by PBDEs to human reproductive health should not be ignored, as PBDEs are endocrine disruptors (Meeker et al., 2009; Johnson et al., 2013).

Over the past decade, there were many studies that emphasize the importance of the dust ingestion of organic pollutants, especially PBDEs. (Bi et al., 2007; Harrad et al., 2010; de Boer et al., 2016). Some previous studies showed that PBDEs concentrations in dust and in indoor air were positively correlated (Wilford et al., 2005; Allen et al., 2008; Wei et al., 2016). Therefore, since they live in e-waste areas for a long time, the accumulation of PBDEs from indoor environment may have adverse effects on male residents' semen quality and sperm viability. Besides, it may also lead to exacerbation of DNA damage and aggravation of apoptosis of human sperm. Investigators have also demonstrated that there was significant relationship between residential dust and paired serum concentrations of several predominant PBDE congeners, such as BDE 47, 99, 100, and 153 (Johnson et al., 2010; Stapleton et al., 2012). The PBDE levels in paired samples of human serum and semen were also correlated (Liu et al., 2012). These studies suggested that dust ingestion might affect the quality of semen and may be an important cause of male infertility. Accordingly, whether there is a strong correlation of PBDE congener concentrations between house dust and paired semen quality needs further research.

Even the results show certain reliable and clear information, this study was an exploratory research, and there were many limitations. Firstly, we just recruited 32 participants living around the e-waste dismantling workshops at e-waste area, and the semen sampling scale was not enough. Secondly, the participants in this study were older, as we had not recruited enough young people to participate in this study. Thirdly, the blood sample was neither collected to analyze the PBDE congeners in serum, nor to detect the hormone, such as the testosterone which are closely related to the production and quality of semen. Finally, in the analysis process of PBDE congeners levels in semen, lower detection rate occurred for observation group due to the semen volume were relatively small (many samples less than 1.5 mL). In order to increase the semen volume and to make the samples more representative, further study should collect semen samples regularly for a long period. Hence, the results need to be detailed in a further study at a larger sampling scale and with more direct evidence.

5. Conclusions

In this study, concentrations of PBDE congeners were measured

Table 5
Multiple linear regression coefficients (95% CI) for significant (p < 0.05) associations between semen parameters and PBDE congener concentrations in semen or in dust [a]

Semen parameters	β	95%CI	p-Value	PBDE congener concentrations
Sperm concentration	−0.295	(−0.553,−0.036)	0.027	BDE47 in semen
Total sperm count	−0.400	(−0.708,−0.092)	0.013	BDE47 in semen
Sperm progressive motility [(A+B)%]	−0.360	(−0.680,−0.040)	0.029	BDE100 in dust
Sperm viability	−0.114	(−0.203,−0.025)	0.014	BDE100 in dust

[a] Adjusted for age (years), abstinence time (days), body mass index (BMI), smoking and alcohol use. The values of semen parameters and PBDE congener concentrations in semen and in dust were all log-transformed.

in house dust and in semen of residents in an e-waste dismantling region (Qingyuan, South China). The semen quality of populations from the area was obviously lower than that of the control group from sperm bank. There were significant linear positive correlations between PBDE congener concentrations in dust and in paired semen samples (e.g. BDE28, 47, 153). And the adjusted result of multiple linear regression model showed that the semen quality parameters were negatively correlated with PBDE congener levels in semen samples (i.e. BDE47) and in house dust (i.e. BDE100). These results demonstrated that PBDE congener accumulated from indoor environment might have a complicated adverse impact on human semen quality. However, further comprehensive studies at large sample size are urgently warranted to verify the results.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.01.150.

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