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Prenatal polybrominated diphenyl ethers exposure and anogenital distance in boys from a Shanghai birth cohort

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ABSTRACT

Background: Polybrominated diphenyl ethers (PBDEs) are major brominated flame retardant (BFR) chemicals with endocrine-disrupting properties. One small-scale study on humans has suggested that prenatal exposure to PBDEs is adversely related to anogenital distance (AGD) a sensitive marker for prenatal androgen exposure. The aim of the present study was to examine the associations between prenatal exposure to PBDEs and AGD among boys 0–4 years of age in a cohort study.

Methods: In the Shanghai-Minhang Birth Cohort Study (S-MBCS), nine PBDE congeners were measured in cord plasma of 192 male infants. We measured anopenile distance (AGD_{AP}) and anoscrotal distance (AGD_{AS}) at birth, 6 months, 12 months, and 48 months of age. A total of 190 boys with neonatal concentrations of PBDEs (ng/g lipid) who had at-least one AGD measurement were included in our study. Information on potential confounding variables were collected through in-person interviews. Multiple linear regression models and generalized estimating equation (GEE) models were used to evaluate the associations between prenatal PBDEs concentrations and AGD.

Results: Among the nine congeners, BDE-47 had the highest detection rate (83.68%) and the highest median concentration (0.18 ng/g lipid). Boys who had neonatal concentration of BDE-47 or Σ_4PBDEs (sum of BDE-47, BDE-99, BDE-100, and BDE-153) in the higher quartile generally had shorter AGD_{AP} and AGD_{AS} than those in the first quartile. Significant inverse associations were found between AGD_{AS} and fourth quartile BDE-47 levels among boys 12 months and 48 months of age ($\beta = -5.57$, 95% confidence interval (CI): −9.89, −1.25 for 12 month of age; $\beta = -4.32$, 95% CI: −8.18, −0.46 for 48 month of age). Inverse associations were also observed between AGD_{AS} and fourth quartile Σ_4PBDEs levels among boys 12 months of age ($\beta = -5.13$, 95% CI: −9.89, −1.25). In GEE models, similar patterns of association were also observed between BDE-47 and AGD_{AS}.

Conclusions: Our findings provide preliminary evidence that prenatal exposure to BDE-47 and Σ_4PBDEs, even at low environmental levels, may be associated with shorter AGD in boys. This data suggest that prenatal exposure to PBDEs may have adverse effects on male reproductive development. Further studies should be conducted to validate these results.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are major brominated flame retardant (BFR) chemicals that have been widely used in plastics, textiles, electronic circuitry and other consumer materials since the 1970s (Shi et al., 2018). There are three commercial mixtures of PBDEs: penta-BDE, octa-BDE, and deca-BDE. Penta-BDE (comprising congeners BDE-47, -99, -100, -153) has been used primarily in textiles as an additive in polyurethane foams and can produce toxic effects at the lowest concentrations among the three commercial mixtures (Darnerud et al., 2001; Hale et al., 2002). Although Penta-BDE was phased out from the European (since 1998) and North American (since 2004) marketplace,

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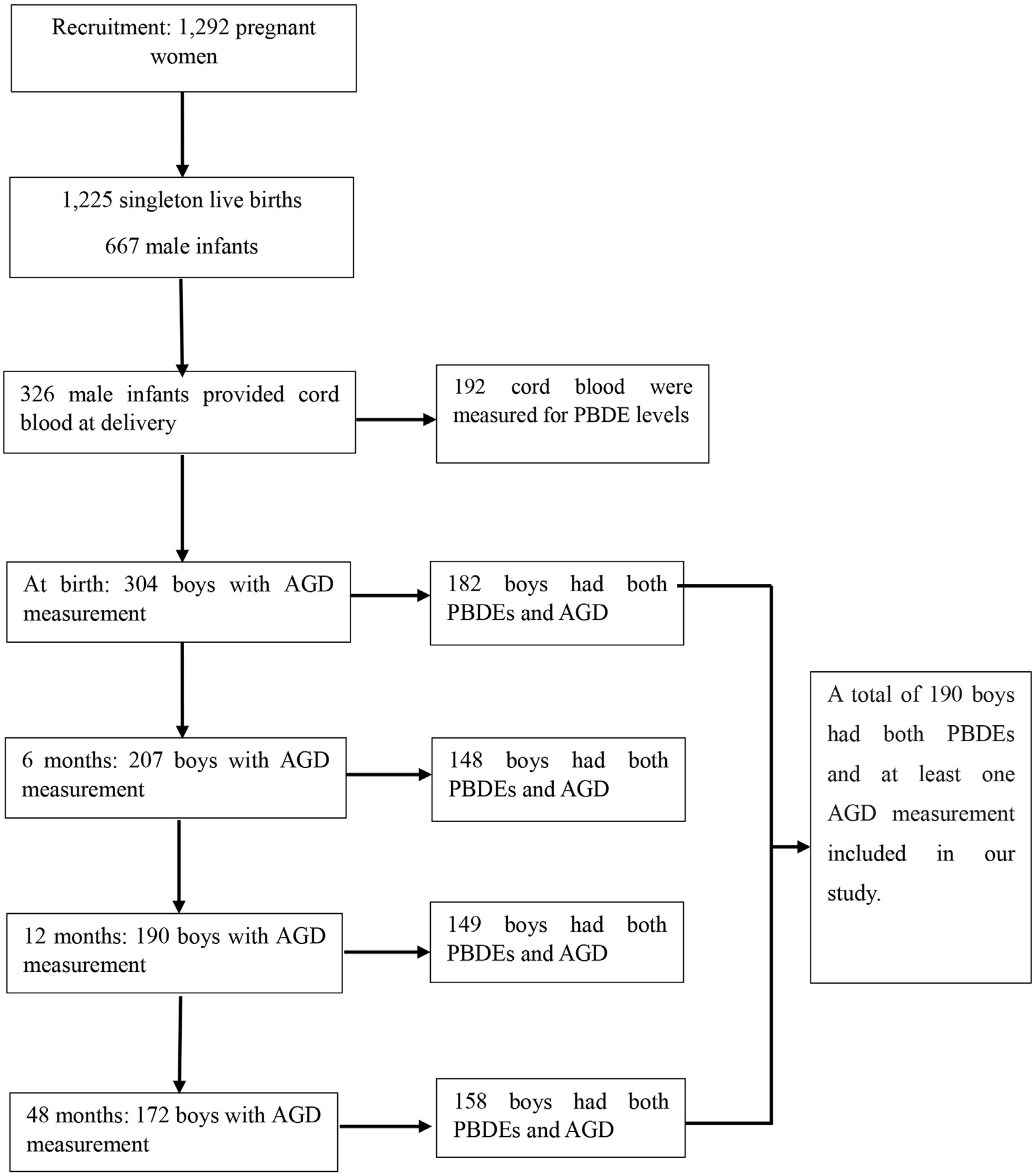

Fig. 1. Study population of the present study from the Shanghai-Minhang Birth Cohort Study.

there are no regulations limiting the production and use of penta-BDE in mainland China (Ding et al., 2015). PBDEs are not chemically bound to plastic or textiles. Therefore, they may leach out as dust particles from the surface of their product applications into the environment (de Wit, 2002). In addition, because of long biological half-lives (2–12 years) and limited degradation in the environment, like other persistent organic pollutants (POPs) (Sjodin et al., 2004; Tiwari et al., 2018), PBDEs are ubiquitous in the environment and highly detectable in humans. Humans continue to be exposed to PBDEs via ingestion, dermal absorption, and inhalation (Abdallah and Harrad, 2014; Zhao et al., 2018). Biomonitoring data show that PBDEs can be found not only in human tissue, including blood, breast milk, and lipid-rich tissues (Frederiksen et al., 2009), but also in cord serum and placenta. This suggests that PBDEs can cross the placental barrier and the developing fetus can be exposed (Alaee and Wenning, 2002; Frederiksen et al., 2009; Hites, 2004).

Studies have shown that PBDEs have endocrine-disrupting properties (Khalil et al., 2017). In-vivo and in-vitro studies have demonstrated that several PBDE congeners have anti-androgenic properties or weak estrogenic activities (Hamers et al., 2008; Yang et al., 2011). Animal studies have shown that perinatal or postnatal exposure to PBDEs can lead to significant changes in testes transcriptome and the weight of reproductive organs; and damage sperm motility and motion (Czerska et al., 2013; Khalil et al., 2017). In human studies, prenatal PBDEs exposure has been reported to be associated with earlier timing of puberty (Harley et al., 2017), alteration of sex hormones in adolescent boys (Eskenazi et al., 2017), and abnormal migration of testes in the male fetus (Goodyer et al., 2017).

Anogenital distance (AGD), the distance from the anus to the genital tubercle, can be used as a sensitive marker for prenatal androgen action in animals (Hsieh et al., 2008; Palanza et al., 1995) and has been added to the U.S. EPA testing guidelines as an endpoint for reproductive toxicity (Gallavan et al., 1999). AGD in newborns may reflect the degree of intrauterine hormonal alternation following exposure to hormonal disruptors (Agramunt and Carreras, 2011). Consequently, measurement of AGD has been used to evaluate the effects of prenatal exposure to exogenous chemicals with potential endocrine disrupting activity (Thankamony et al., 2016). Moreover, shorter anogenital distance has been associated with adverse reproductive outcomes, including undescended testis (Jain and Singal, 2013), hypospadias (Singal et al., 2016), poor sperm quality (Mendiola et al., 2011), reduced testosterone levels (Eisenberg et al., 2012), and diminished fertility (Mendiola et al., 2015). To date, only one human study which was limited by small size (n = 27), has reported an association between prenatal BDE-99/BDE-153 exposure and shorter anogenital index (AGI = AGD/subjects' weight [mm/kg]) at 18 months of age in males (Garcia-Villarino et al., 2018).

In the present prospective study, we investigated the associations between prenatal exposure to PBDEs and AGD among boys 0–4 years of age based on a birth cohort in Shanghai, China.

2. Materials and methods

2.1. Study participants and design

Pregnant women who visited Minhang Maternal and Child Health Hospital for their first prenatal care at 12–16 weeks of gestation were enrolled in the Shanghai-Minhang Birth Cohort Study (S-MBCS) from April to December 2012 (Tian et al., 2018). Women who were native Chinese and residents of Shanghai; had no hospital-diagnosed major chronic disease of the liver, kidney, or other organs; were willing to complete scheduled interviews during pregnancy and after delivery; and intended to give birth in the study hospital were invited to participate in the study. A total of 1292 pregnant women were recruited in the cohort.

At delivery, a total of 1225 live singletons were born, among whom 667 (54.4%) were boys. Cord blood samples were collected at delivery from 326 boys, among whom we measured AGD in 304, 207, 190, and 172 of boys at birth, 6 months, 12 months, and 48 months of age, respectively, through home visits. PBDEs concentrations were measured in 192 cord blood samples of boys. The conditions for measurement of cord blood samples were: completely delivery information, sufficient cord plasma volume analyzed for PBDEs (≥1 ml), and at-least one home visit at 12 or 48 months of age. Finally, boys (n = 190) who had both PBDEs concentrations in cord plasma and had received at-least one AGD measurement were included in our study, as shown in Fig. 1. Information related to maternal lifestyles, social demographic characteristics, diet, and medical history was collected during recruitment and during home visits using structured questionnaires. The baby's birth date, gender, and gestational age were extracted from the medical records.

The study protocol was approved by the research ethics committees of Shanghai Institute of Planned Parenthood Research. Both the women and their husbands gave informed consents before enrollment in the study and at postnatal follow-up visits for their children's participation.

2.2. Prenatal PBDEs measurements

Cord blood samples were collected from an umbilical vein immediately after delivery. All samples were frozen and stored at −80 °C until shipment on dry ice to the Center for Disease Control and Prevention in Hubei Province for testing of PBDEs.

The protocol for analysis of nine congeners (BDE-28, -47, -66, -85, -99, -100, -153, -154, and -183) has been described elsewhere and this protocol was used in this study with minor modification (Li et al., 2008; Zhang et al., 2011). First, 1 ml of serum and 2 ng of internal standard solution (13C12-BDE-28, -47, -99, -100, -153, -154, and -183) were added to a 15 mL glass tube and then mixed with a 2 mL × 4% water solution of phosphoric acid. After ultrasound extraction, the sample was cleaned using two SPE cartridge (Agilent Bond Elut Plexa and Agilent Bond Elut-AL-N). Finally, the eluate was evaporated to about 100 μL with a stream of nitrogen. The 13C12-labeled injection standard (13C12-BDE-77, -138) was added as the recovery standard prior to instrument analysis.

The identification and quantification were performed by high resolution gas chromatography - high resolution mass spectrometry (HRGC/HRMS, DFS, Thermo Fisher, USA) with a capillary DB-5ms column (15 m, 0.25 mm i.d., 0.1 μm film thickness; Agilent, USA). Samples were injected using the splitless method at 270 °C and the column oven temperature program was set at an initial temperature of 120 °C (held for 2 min), ramped at 15 °C/min to 230 °C, ramped at 5 °C/min to 270 °C, and finally ramped at 9 °C/min to the final temperature of 325 °C, where it was held for 2 min. The mass spectrometer was operated in electron impact (EI) mode. The source temperature was 260 °C and the resolution was ≥6000. The mass spectrometer was operated in selected ion monitoring (SIM) mode. Samples were quantified by the isotope dilution method.

2.3. Quantification and quality control

All glassware was rinsed with dichloromethane (DCM) and hexane prior to use. For every batch of eleven samples, a procedural blank was performed. To be considered significant, the quantity of detected congeners in a sample had to be more than three times the quantity detected in the procedural blank. The concentrations of the detected congeners were corrected by subtracting the procedural blank concentration. The recovery levels of internal standard were all in the range of 50–120% for all samples. The laboratory performance was validated by successful participation in inter-laboratory comparison studies of PBDEs by the Norwegian Institute of Public Health.

PBDEs concentrations are reported on a serum lipid basis (nanograms per gram lipid). Total serum lipid concentrations were determined based on the measurement of total cholesterol and triglycerides (Phillips et al., 1989). The limits of detection (LOD) for nine PBDE congeners ranged from 0.03 to 0.6 pg/ml. Specifically, the LOD was 0.06 pg/ml for BDE-85, 0.6 pg/ml for BDE-183, and 0.03 pg/ml for others.

2.4. Measurements of AGD in boys

Physical examinations of the boys were conducted within 3 days after delivery, and at the ages of 6 months ± 2 weeks, 12 months ± 2 weeks, and 48 months ± 2 weeks. The body weight, length, and external genitalia of the boys were measured during the physical examinations (Xia et al., 2018). At all examinations, AGD_{AP} (from the center of the anus to anterior base of the penis, where the penile tissue meets the pubic bone) and AGD_{AS} (from the center of the anus to the posterior base of the scrotum, where the skin changes from rugate to smooth) were measured by four trained examiners. The examiners were unware of the PBDEs levels in the subjects. The detailed methods and procedures to measure AGD have been described elsewhere (Salazar-Martinez et al., 2004; Swan et al., 2005, 2015) (Fig. 2). Briefly, each infant was placed on his back on a flat surface with his hips relaxed outward and pulled back towards his shoulders by his mother or an assistant. The examiners stood in front of the infant and made independent measurements of AGD_{AP} and AGD_{AS} using the same digital caliper. To evaluate the inter-examiner variability, two examiners took independent measurements on 15 male infants on the same day using the same method; no statistically significant difference was noted, as described previously (Xia et al., 2018).

2.5. Data analysis

We described the distributions of the demographic characteristics of

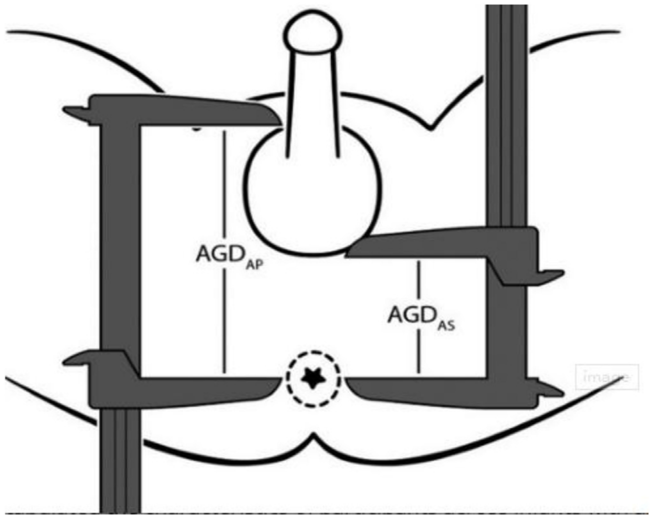

Fig. 2. Schematic diagram of anatomical landmarks to measure both AGD in boys (Adapted from [Salazar-Martinez et al., 2004; Swan et al., 2005]).

the boys and their mothers using counts and percentages for categorical variables and mean (± standard deviation, SD) for continuous variables. Detection rates of the nine congeners were tabulated. Five congeners (BDE-28, -47, -99, -100, and -153) that had detection rates greater than or close to 50% were included in further analysis. In addition, the sum of penta-BDE (Σ_4PBDEs) (comprising congeners BDE-47, -99, -100, and -153) was calculated and included in further analyses. To examine the associations between PBDEs and AGD, congeners with detection rate > 75% (BDE-47, Σ_4PBDEs) were categorized according to the quartiles with a reference group of the lowest quartile. Those with detection rates < 75% (BDE-28, -99, -100, and -153) were categorized as below LOD (reference group), LOD to the median of the detected measures, and above the median. We used multiple liner regression models to examine the associations between prenatal PBDEs exposure and AGD. Potential confounding variables were identified *a priori* based on their relationship with AGD. These included maternal age at conception (years), gestational weeks at birth, maternal education (middle school or below, high school, and college or above), parity (0 or ≥ 1), maternal pre-pregnancy body mass index (kg/m^2), and child age at physical examination (days). Besides, AGD is known to be correlated with body weight-for-length (Sathyanarayana et al., 2015). Therefore, we adjusted for birth weight in analysis at birth, and for weight-for-length z-scores (WLZs), calculated using World Health Organization (WHO) standard growth curves (WHO, 2009), in analysis at 6 months, 12 months, and 48 months of age (Swan et al., 2015).

2.6. Sensitivity analysis

To take the repeated measurements of AGD into consideration, we used generalized estimating equation (GEE) model to examine the associations between prenatal PBDEs exposure and AGD. Besides, we reran the main analyses in boys who were examined at each of the 4 age-stages. In addition, although PBDEs concentrations standardized by lipid content are widely used in PBDEs relevant analysis, the most appropriate method for adjustment is unclear (Hoffman et al., 2016; O'Brien et al., 2016; Schisterman et al., 2005). Thus, the analysis was repeated with PBDEs concentrations expressed on a volume basis (pg/ml), with total serum lipids as a covariate. Considering that co-exposures of other Endocrine Disrupting Chemicals (EDCs) may produce confounding effects, maternal urine BPA concentration (Sun et al., 2018) was added as a covariate to examine whether the effect of PBDEs exposure would change. Finally, we repeated the analyses in mothers who were nulliparous, between 18 and 35 years old, or had normal pre-pregnancy body mass index (18.5 < BMI < 24 kg/m^2) to reduce any concern about the effects of these confoundering variables on the study results.

The regression coefficients and the corresponding confidence intervals (95% CI) were reported using an alpha (α) level of 0.05. All analyses were conducted with SAS 9.4 (SAS Institute Inc, Cary, NC, USA). Centile curves were created using l ms Chart-Maker Light version 2.3 (T. Cole, H. Pan, Medical Research Council, London, UK).

3. Results

3.1. Participants' characteristics

Among the 190 mothers in the study, approximately half (51.58%) were 25–29 years old and more than one-third (36.17%) reported a monthly per capita household income greater than 8000 CNY. In addition, two-thirds (69.52%) had a normal weight before conception and the clear majority (91%) had graduated from high school or above. Moreover, the majority of women (83.07%) were nulliparous and more than half (59.47%) of the participants reported that they were not exposed to passive smoking.

The mean (± SD) gestational age of infants was 39.62 (± 1.18) weeks. The mean (± SD) weights of boys at birth, at 6 months, 12 months, and 48 months of age were 3.52 (± 0.42) kg, 8.78 (± 0.95) kg, 10.76 (± 1.09) kg, and 18.28 (± 2.47) kg, respectively. All AGD measures were normally distributed. The means (± SD) of AGD$_{AP}$ at birth, 6 months, 12 months, and 48 months of age were 41.07 (± 3.87) mm, 65.71 (± 9.64) mm, 69.33 (± 10.6) mm, and 96.22 (± 10.07) mm, respectively; the corresponding means (± SD) for AGD$_{AS}$ were 15.11 (± 3.68) mm, 27.15 (± 10.35) mm, 30.15 (± 9.46) mm, and 45.05 (± 8.37) mm, respectively (Table S1). Fig. 3 shows the trend and distribution of the measurements of AGD$_{AP}$ and AGD$_{AS}$ in boys superimposed on smoothed centile lines. AGD increased rapidly in early months of life, consistent with the results of the Cambridge Baby Growth Study (Thankamony et al., 2009). The maternal and child characteristics of those included mother-infant pairs were compatible with the characteristics of the excluded pairs (Table S1).

The study only described the distribution across maternal characteristics on Σ_4PBDEs or BDE-47 due to low detection rates for most other congeners. Σ_4PBDEs or BDE-47 was significantly higher among boys whose mothers were multiparous or older at conception (Table S2).

3.2. PBDEs concentrations

The cord plasma levels of nine PBDE congeners in male infants are summarized in Table 1. BDE-47 was detected in the majority (> 80%) of the cord blood samples. The detection rates of BDE-28, BDE-99, BDE-100, and BDE-153 were close to or above 50%. The BDE-47 concentration was the highest (median = 0.18 ng/g lipid), followed by BDE-99 (0.08 ng/g lipid), BDE-28 (0.05 ng/g lipid), and BDE-100 (0.02 ng/g lipid). Other congeners (BDE-66, -85, -154, and -183) had a low detection rate of 20%–30%, thus their concentrations were not applicable to be presented. The five major congeners (i.e., BDE-47, BDE-28, BDE-99, BDE-100, and BDE-153) were moderately correlated (r = 0.16–0.51, $p < 0.05$).

3.3. Prenatal PBDEs exposure in relation to AGD in boys

Table 2 presents the associations between prenatal exposure to PBDEs and AGD at birth (n = 182), 6 months of age (n = 148), 12 months of age (n = 149), and 48 months of age (n = 158) after adjustment for potential confounding variables. For BDE-47, boys in the higher quartiles generally had shorter AGD$_{AP}$ and AGD$_{AS}$ than those in the first quartile. However, significant inverse associations were found only between BDE-47 and AGD$_{AS}$ at 12 and 48 months of age

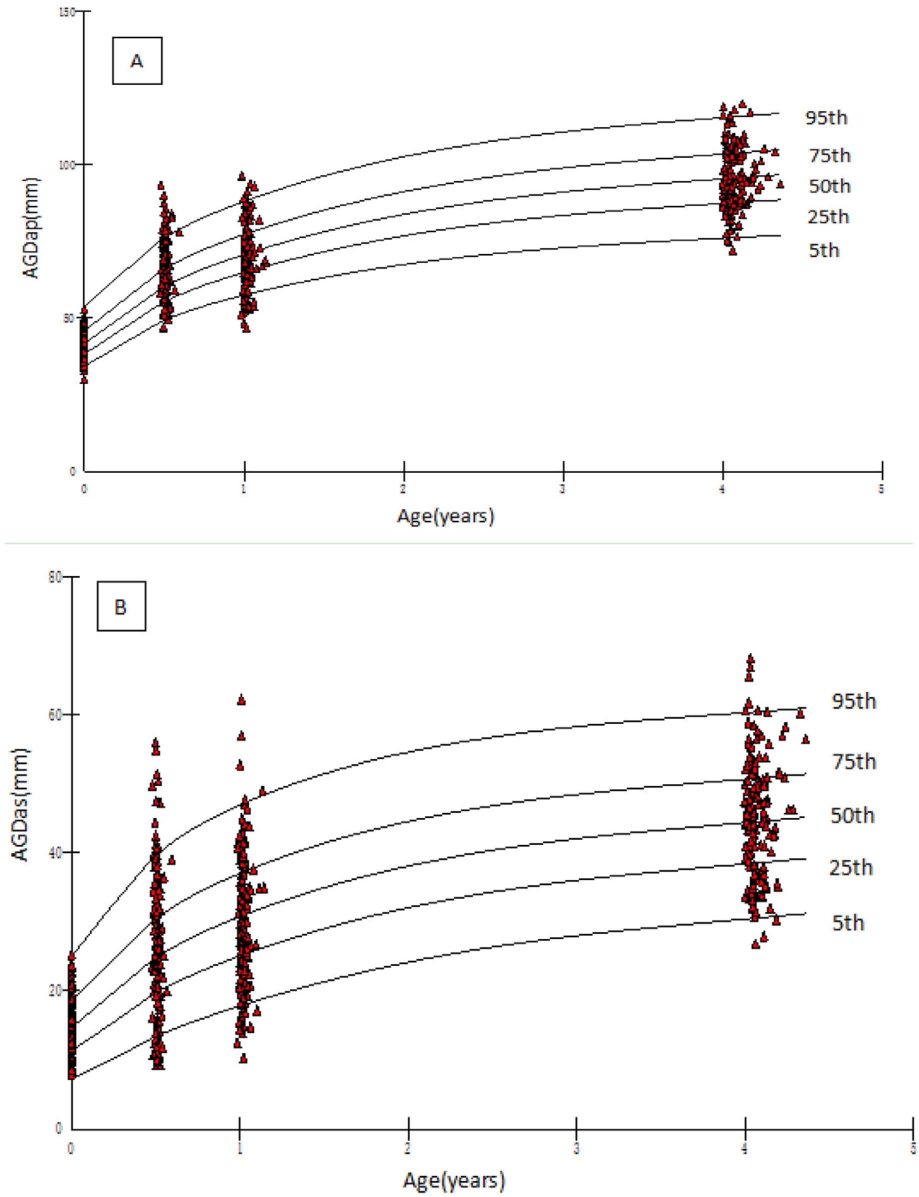

Fig. 3. Centile curves and data plots for AGD_{AP} (A) and AGD_{AS} (B) up to 4 years of age. Data po.

Table 1

Detection rate and percentile of neonatal PBDEs levels (ng/g lipid) measured in cord plasma, Shanghai-Minhang Birth Cohort Study (N = 190).

PBDE CONGENERS	LOD (pg/ml)	> LOD (n (%))	GM(GSD)	Percentiles				
				5th	25th	50th	75th	95th
BDE-28	0.03	115 (60.53)	0.05 (3.97)	< LOD	< LOD	0.05	0.17	0.43
BDE-47	0.03	159 (83.68)	0.14 (4.3)	< LOD	0.06	0.18	0.37	1.12
BDE-66	0.03	41 (21.58)	_	< LOD	< LOD	< LOD	< LOD	0.16
BDE-85	0.06	58 (30.53)	_	< LOD	< LOD	< LOD	0.04	0.18
BDE-99	0.03	118 (62.11)	0.06 (5.04)	< LOD	< LOD	0.08	0.20	0.91
BDE-100	0.03	97 (51.05)	0.04 (2.86)	< LOD	< LOD	0.02	0.08	0.25
BDE-153	0.03	89 (46.84)	_	< LOD	< LOD	< LOD	0.11	0.34
BDE-154	0.03	40 (21.05)	_	< LOD	< LOD	< LOD	< LOD	0.17
BDE-183	0.60	48 (25.26)	_	< LOD	< LOD	< LOD	0.20	1.12
Σ_4PBDEs	–	186 (97.89)	0.39 (3.16)	0.05	0.20	0.38	0.72	2.01

BDE - brominated diphenyl ether, < LOD - below the limit of detection, PBDE - polybrominated diphenyl ether.
GM - geometric mean, GSD - geometric standard deviation.
Σ_4PBDEs is the sum of BDE-47, BDE-99, BDE-100, and BDE-153.
— Detection rates of BDE-66, BDE-85, BDE-153, BDE-154, and BDE-183 were blew 50%, thus these were, not calculated for GM.

Table 2
Association (95%CI) between prenatal PBDEs exposure and Anogenital Distance (AGD) at birth, 6 months, 12 months, and 48 months in boys (ng/g lipid).

PBDEs	AGD_{AP}				AGD_{AS}			
	at birth (mm)[a] N = 182	6 months of age (mm)[b] N = 148	12 months of age (mm)[b] N = 149	48 months of age (mm)[b] N = 158	at birth (mm)[a] N = 182	6 months of age (mm)[b] N = 148	12 months of age (mm)[b] N = 149	48 months of age (mm)[b] N = 158
BDE-47								
1st quartile	ref	ref	ref	ref	ref	ref	ref	ref
2nd quartile	−0.13 (−1.72, 1.47)	2.69 (−2.25, 7.63)	−0.55 (−5.58, 4.47)	0.01 (−4.34, 4.35)	−0.34 (−1.87, 1.19)	1.44 (−3.74, 6.63)	−3.02 (−7.44, 1.40)	−3.28 (−7.11, 0.56)
3rd quartile	0.67 (−0.92, 2.26)	−1.20 (−6.23, 3.82)	0.72 (−4.24, 5.67)	1.04 (−3.42, 5.49)	0.79 (−0.73, 2.32)	−5.20 (−10.48, 0.08)	1.24 (−3.11, 5.60)	−1.27 (−5.20, 2.66)
4th quartile	−0.02 (−1.61, 1.57)	2.10 (−2.67, 6.87)	−3.51 (−8.46, 1.45)	−1.43 (−5.81, 2.95)	−0.77 (−2.30, 0.75)	0.22 (−4.78, 5.21)	**−5.57 (−9.89, −1.25)***	**−4.32 (−8.18, −0.46)***
Σ_4PBDEs								
1st quartile	ref	ref	ref	ref	ref	ref	ref	ref
2nd quartile	1.07 (−0.51, 2.66)	1.86 (−3.27, 7.00)	−2.33 (−7.32, 2.66)	1.32 (−3.13, 5.77)	0.19 (−1.35, 1.73)	−0.22 (−5.71, 5.27)	−2.04 (−6.52, 2.45)	−2.20 (−6.18, 1.79)
3rd quartile	0.02 (−1.55, 1.59)	**−0.67 (−5.44, 4.10)**	−2.21 (−7.07, 2.64)	0.80 (−3.61, 5.20)	−0.19 (−1.72, 1.34)	−0.04 (−5.15, 5.07)	−0.58 (−4.94, 3.78)	−1.84 (−5.78, 2.11)
4th quartile	0.31 (−1.31, 1.93)	1.82 (−3.36, 7.01)	−4.22 (−9.45, 1.02)	−0.85 (−5.35, 3.66)	−0.47 (−2.04, 1.11)	0.54 (−5.04, 6.11)	**−5.13 (−9.79, −0.48)***	−2.68 (−6.71, 1.36)
BDE-28								
< LOD	ref	ref	ref	ref	ref	ref	ref	ref
LOD-median	−0.28 (−1.62, 1.06)	1.19 (−2.88, 5.26)	−0.51 (−4.71, 3.70)	**−4.31 (−7.89, −0.73)***	**1.41 (0.13, 2.69)***	2.77 (−1.57, 7.10)	−0.31 (−4.12, 3.51)	−1.58 (−4.86, 1.69)
> median	0.15 (−1.21, 1.51)	2.63 (−1.76, 7.01)	−2.17 (−6.61, 2.26)	−1.85 (−5.52, 1.82)	0.21 (−1.09, 1.5)	2.15 (−2.50, 6.81)	−2.44 (−6.46, 1.58)	−1.29 (−4.62, 2.05)
BDE-99								
< LOD	ref	ref	ref	ref	ref	ref	ref	ref
LOD-median	−0.15 (−1.51, 1.2)	−2.18 (−6.45, 2.09)	−1.86 (−6.34, 2.62)	1.68 (−2.00, 5.35)	0.42 (−0.89, 1.73)	−0.56 (−5.11, 4.00)	1.55 (−2.62, 5.71)	2.20 (−1.10, 5.50)
> median	−0.63 (−2.00, 0.74)	−0.41 (−4.63, 3.82)	−1.72 (−6.46, 3.02)	2.13 (−1.60, 5.85)	−0.14 (−1.47, 1.18)	−0.41 (−4.95, 4.13)	0.88 (−3.45, 5.21)	1.36 (−1.98, 4.70)
BDE-100								
< LOD	ref	ref	ref	ref	ref	ref	ref	ref
LOD-median	0.14 (−1.23, 1.51)	2.72 (−1.42, 6.85)	−0.66 (−5.03, 3.72)	1.79 (−1.98, 5.55)	0.11 (−1.22, 1.44)	3.88 (−0.58, 8.33)	−0.90 (−4.85, 3.05)	0.10 (−3.27, 3.46)
> median	−0.80 (−2.19, 0.58)	2.06 (−2.13, 6.24)	−0.78 (−5.25, 3.7)	−1.93 (−5.75, 1.89)	−0.67 (−2.01, 0.67)	0.05 (−4.37, 4.47)	−0.89 (−5.01, 3.22)	−2.98 (−6.38, 0.41)
BDE-153								
< LOD	ref	ref	ref	ref	ref	ref	ref	ref
LOD-median	0.04 (−1.36, 1.44)	1.28 (−2.93,5.48)	−1.08 (−5.46, 3.30)	**4.18 (0.48, 7.88)***	0.52 (−0.83, 1.87)	0.05 (−4.49, 4.59)	0.19 (−3.79, 4.18)	−1.10 (−4.46, 2.26)
> median	0.15 (−1.29, 1.59)	**4.42 (0.14, 8.69)***	−1.26 (−5.84, 3.33)	0.97 (−2.91, 4.84)	0.64 (−0.74, 2.03)	4.16 (−0.40, 8.72)	−1.47 (−5.63, 2.68)	−1.36 (−4.87, 2.14)

< LOD - below the limit of detection.

* There are statistically significant differences compared with the reference group, $p < 0.05$ weeks.

[a] Adjusted for maternal body mass index (BMI) before conception, maternal age of conception, maternal education, birth weight, parity, and gestational.

[b] Adjusted for maternal body mass index (BMI) before conception, maternal age of conception, maternal education, parity, gestational weeks, children's age, and individual weight-for-length z-scores (WLZs).

(β = −5.57, 95% CI: −9.89, −1.25 for 12 month of age; β = −4.32, 95% CI: −8.18, −0.46 for 48 month of age; Table 2). Similar patterns were observed in Σ_4PBDEs (the sum of BDEs 47, 99, 100, and 153) at 6, 12, and 48 months of age, with statistically significant reduction for AGD_{AS} at 12 months of age (β = −5.13, 95% CI: −9.79, −0.48; Table 2).

Neither BDE-99 nor BDE-100 was significantly associated with AGD_{AP} or AGD_{AS}. Boys who were exposed to prenatal BDE-28 at the mid-range level (LOD to the median) had shorter AGD_{AP} than boys with undetected levels at 48 months of age (β = −4.31, 95% CI: −7.89, −0.73; Table 2). Conversely, compared with the reference group, boys with the highest levels of BDE-153 (> median) had longer AGD_{AP} at 6 months of age (β = 4.42, 95% CI: 0.14, 8.69; Table 2), while those with mid-range level of BDE-153 had longer AGD_{AP} at 48 months of age (β = 4.18, 95% CI: 0.48, 7.88; Table 2).

3.4. Sensitivity analyses

Generalized estimating equation (GEE) models were fitted to examine the overall associations between PBDEs and AGD. Only BDE-47 and Σ_4PBDEs were include in the analysis since a statistically significant interaction between time and PBDEs exposure were found for other congeners. BDE-47 and Σ_4PBDEs exposure in higher quartiles was associated with shorter AGD_{AP} and AGD_{AS} compared with the first quartile. The difference was statistically significant for AGD_{AS} in the highest quartile for BDE-47 (β = −2.27, 95% CI: −4.09, −0.45; Table 3).

A stronger association between BDE-47 and AGD was observed when the analysis restricted to boys who received physical examinations at all of the time-points. Boys who were exposed to prenatal BDE-47 at the highest levels had a shorter AGD_{AP} at 48 months of age (β = −5.94, 95% CI: −11.17, −0.71; Table 4) and a shorter AGD_{AS} at birth (β = −2.30, 95% CI: −4.3, −0.31; Table 4). Significant reduction in AGD_{AS} was also observed in boys in the third quartile of Σ_4PBDEs exposure, at 48 months of age (β = −5.29, 95% CI: −10.12, −0.46; Table 4). When using BDE-47 concentration expressed on a volume basis (pg/ml) with total serum lipids as a covariate, the results were similar to the results when using lipid adjusted concentrations. A similar pattern was observed for Σ_4PBDEs but the associations were attenuated and none of them was statistically significant (Table S3). The associations did not change substantially when maternal urine BPA concentration was added as one of the covariates (Table S4).

We also repeated analyses for 3 sub-groups: mothers who were nulliparous, mothers who were between 18 and 35 years old, and mothers with normal maternal pre-pregnancy body mass index (18.5 < BMI < 24 kg/m^2). Similar patterns were observed for these subgroups as the patterns noted above (AGD_{AP} and AGD_{AS}) (Tables S5–S7).

Table 3
Association (95%CI) between prenatal BDE-47 and Σ_4PBDE exposure and AGD in boys using generalized estimating equations (ng/g lipid).

PBDEs	AGD_{AP} (mm)	AGD_{AS} (mm)
BDE-47		
1st quartile	ref	ref
2nd quartile	0.15 (−1.8, 2.11)	−1.11 (−3.29, 1.08)
3rd quartile	0.65 (−1.47, 2.78)	−0.62 (−2.68, 1.45)
4th quartile	−1.18 (−3.36, 0.99)	**−2.27(−4.09, −0.45)***
Σ_4PBDEs		
1st quartile	ref	ref
2nd quartile	0.67 (−1.53, 2.88)	−0.54 (−2.8, 1.72)
3rd quartile	−0.51 (−2.45, 1.43)	−0.48 (−2.55, 1.59)
4th quartile	−0.73 (−2.96, 1.5)	−1.1 (−3.15, 0.96)

[#] Adjusted for maternal body mass index (BMI) before conception, maternal age of conception, maternal education, parity, gestational weeks, and individual weight-for-length z-scores (WLZs).
*There are statistically significant differences compared with the reference group, $p < 0.05$.

4. Discussion

This study provided the preliminary epidemiological evidence that prenatal exposure to BDE-47 and Σ_4PBDEs (sum of BDEs 47, 99, 100, and 153), measured from cord plasma at delivery, were associated with shorter AGD_{AS} among boys at 12 months of age. For BDE-47, the effects of shorter AGD_{AS} persisted in boys of 48 months of age. The findings were strengthened when similar associations were observed through various analytic strategies.

BDE-47, consistent with most other studies (Bi et al., 2006; Foster et al., 2011), was the most abundant PBDEs congener in this study. However, the concentration of BDE-47 in this study was much lower than that were reported in Canada and United States. BDE-47 in Canada was two orders of magnitude higher than the BDE-47 in this study (Foster et al., 2011) and Herbstman et al. reported the median concentration of BDE-47 was 11.2 ng/g lipid in cord blood in United States (Herbstman et al., 2010). On the other hand, the median concentration of BDE-47 in this study was just 2% of the value reported in the US. As for all existing PBDE-related studies conducted in China, the level of BDE-47 in this study was also significantly lower than reported in studies conducted in areas that were in close proximity to e-waste and BFR manufactories (Chen et al., 2018; Wu et al., 2010). On the other hand, the level of BDE-47 in this study was close to that reported in studies in areas without obvious PBDEs pollution, including in Chaonan of Shan Tou (Wu et al., 2010), Taiwan (Lin et al., 2011), and Guangzhou (Bi et al., 2006) (Table S8).

Previous studies have linked prenatal PBDEs exposure to adverse birth outcomes such as low birth weight (Zhao et al., 2017), reductions in placental size (Zhao et al., 2018), smaller waist circumference at 4–8 years of age (Vuong et al., 2016), longer gestational age (Chen et al., 2018), and greater head circumference (Robledo et al., 2015). In addition, most epidemiological studies have demonstrated the association between PBDEs exposure and behavioral development of the offspring (Vuong et al., 2018). Only one human study (INMA project) evaluated the association between AGI at 18 months of age and prenatal exposure to BDE-28, BDE-99, and BDE-153 in males (Garcia-Villarino et al., 2018), and found inverse associations between AGI and BDE-99 and BDE-153. These results were not confirmed by our study. The levels of PBDEs in INMA study were measured in maternal serum collected during the first trimester rather than in cord plasma, and the geometric mean of PBDEs was higher than that in our study (BDE-99: 0.39 vs 0.06 ng/g lipid; BDE-28: 0.16 vs 0.05 ng/g lipid). The relatively low exposure level in our study may make it difficult to explore the potential association. In addition, the differences in time of AGD measurements also made the comparison between the INMA study and our study difficult.

To date, there have a plethora of human studies focusing on the effect of prenatal EDCs exposure on AGD in boys. Decreased AGD has been reported in male infants whose mothers had been prenatally exposed to phthalates or BPA (Mammadov et al., 2018; Miao et al., 2011; Sun et al., 2018; Swan et al., 2005, 2015) while positive association has been reported between AGD and prenatal exposure to phthalates in the MIREC study (Arbuckle et al., 2018). A cross-sectional assessment also found significant reduction between anal position index (defined as the ratio of AGD to the distance between the coccyx and the scrotum) and prenatal exposure to dichlorodiphenyldichloroethylene (DDE) in male infants (Torres-Sanchez et al., 2008). Besides, several studies have reported that no significant associations were observed between AGD and prenatal exposure to phthalates or DDE (Huang et al., 2009; Jensen et al., 2016; Longnecker et al., 2007). Considering that other EDCs co-exposures might produce confounding effects, and adverse association between prenatal BPA exposure and AGD in boys was observed in our previous study (Sun et al., 2018), prenatal BPA was adjusted for in this

Table 4

Association (95%CI) between prenatal PBDEs exposure and Anogenital Distance (AGD) in boys who have been followed over four age stages (n = 105) (ng/g lipid).

PBDEs	AGD_{AP}				AGD_{AS}			
	at birth (mm)[a]	6 months of age (mm)[b]	12 months of age (mm)[b]	48 months of age (mm)[b]	at birth (mm)[a]	6 months of age (mm)[b]	12 months of age (mm)[b]	48 months of age (mm)[b]
BDE-47								
1st quartile	ref	ref	ref	ref	ref	ref	ref	ref
2nd quartile	0.15 (−1.99, 2.28)	4.64 (−1.30, 10.58)	−1.69 (−7.52, 4.13)	−3.91 (−9.27, 1.44)	**−2.19 (−4.27, −0.10*)**	1.23 (−4.81, 7.28)	−3.85 (−9.31, 1.62)	**−5.69 (−10.49, −0.88)***
3rd quartile	1.06 (−1.05, 3.18)	−1.72 (−7.50, 4.07)	1.39 (−4.37, 7.16)	0.54 (−4.87, 5.96)	0.59 (−1.47, 2.66)	−3.84 (−9.72, 2.04)	0.20 (−5.20, 5.60)	−1.71 (−6.57, 3.15)
4th quartile	−0.69 (−2.73, 1.35)	1.49 (−4.12, 7.10)	−4.74 (−10.56, 1.07)	**−5.94 (−11.17, −0.71)***	**−2.30 (−4.30, −0.31)***	−0.16 (−5.87, 5.55)	**−6.01 (−11.39, −0.63)***	**−6.83 (−11.52, −2.14)***
Σ_4PBDE								
1st quartile	ref	ref	ref	ref	ref	ref	ref	ref
2nd quartile	1.18 (−0.90, 3.26)	0.77 (−5.16, 6.70)	−2.42 (−8.29, 3.45)	−0.26 (−5.69, 5.16)	−0.11 (−2.24, 2.02)	−1.40 (−7.35, 4.56)	−5.26 (−10.67, 0.15)	−3.57 (−8.44, 1.30)
3rd quartile	−0.00 (−2.08, 2.07)	−0.41 (−6.17, 5.35)	−1.47 (−7.11, 4.18)	−1.62 (−7.00, 3.76)	−0.25 (−2.38, 1.88)	−0.23 (−6.02, 5.56)	−0.66 (−5.86, 4.55)	**−5.29 (−10.12, −0.46)***
4th quartile	0.04 (−2.05, 2.13)	1.72 (−4.15, 7.60)	−5.39 (−11.32, 0.53)	−3.85 (−9.34, 1.65)	−1.19 (−3.33, 0.96)	1.45 (−4.46, 7.35)	**−6.95 (−12.37, −1.54)***	−3.75 (−8.69, 1.18)
BDE-28								
< LOD	ref	ref	ref	ref	ref	ref	ref	ref
LOD-median	0.24 (−1.55, 2.02)	2.2 (−2.72, 7.13)	2.77 (−2.4, 7.93)	**−6.04 (−10.55, −1.54)***	**2.16 (0.39, 3.94)***	4.66 (−0.22, 9.54)	2.98 (−1.93, 7.89)	−1.51 (−5.74, 2.73)
> median	0.34 (−1.43, 2.11)	1.87 (−3.05, 6.79)	−1.37 (−6.21, 3.48)	−2.82 (−7.21, 1.57)	0.41 (−1.34, 2.16)	2.94 (−1.94, 7.81)	−0.27 (−4.90, 4.36)	−1.61 (−5.74, 2.53)
BDE-99								
< LOD	ref	ref	ref	ref	ref	ref	ref	ref
LOD-median	−1.12 (−2.89, 0.66)	−2.85 (−7.77, 2.07)	−1.11 (−6.26, 4.04)	1.81 (−2.80, 6.41)	0.36 (−1.47, 2.19)	−2.40 (−7.38, 2.57)	2.76 (−2.14, 7.66)	1.79 (−2.41, 5.99)
> median	−1.32 (−3.14, 0.50)	−0.06 (−5.04, 4.93)	−3.30 (−8.73, 2.12)	1.90 (−2.88, 6.68)	−0.57 (−2.45, 1.31)	−0.49 (−5.53, 4.55)	−0.16 (−5.30, 4.98)	1.18 (−3.18, 5.53)
BDE-100								
< LOD	ref	ref	ref	ref	ref	ref	ref	ref
LOD-median	−0.64 (−2.44, 1.16)	2.66 (−2.38, 7.69)	−2.84 (−8.06, 2.37)	1.34 (−3.25, 5.93)	0.37 (−1.49, 2.23)	2.92 (−2.15, 8.00)	−1.28 (−6.21, 3.65)	1.43 (−2.71, 5.57)
> median	−1.27 (−3.13, 0.58)	2.40 (−2.71, 7.50)	−0.76 (−5.97, 4.45)	**−4.98 (−9.66, −0.30)***	−0.53 (−2.44, 1.38)	0.65 (−4.49, 5.80)	−2.64 (−7.60, 2.32)	**−5.07 (−9.29, −0.85)***
BDE-153								
< LOD	ref	ref	ref	ref	ref	ref	ref	ref
LOD-median	−0.17 (−2.05, 1.72)	1.27 (−3.98, 6.52)	−0.21 (−5.72, 5.31)	4.34 (−0.37, 9.06)	−0.14 (−2.06, 1.78)	−0.34 (−5.64, 4.96)	1.51 (−3.69, 6.72)	0.28 (−4.07, 4.64)
> median	−0.19 (−2.07, 1.69)	4.54 (−0.57, 9.64)	1.20 (−4.12, 6.52)	−1.24 (−5.96, 3.48)	0.98 (−0.93, 2.90)	3.99 (−1.16, 9.14)	−0.63 (−5.67, 4.42)	−2.58 (−6.94, 1.79)

< LOD - below the limit of detection.

*There are statistically significant differences compared with the reference group, $p < 0.05$.

[a] Adjusted for maternal body mass index (BMI) before conception, maternal age of conception, maternal education, birth weight, parity, and gestational weeks.

[b] Adjusted for maternal body mass index (BMI) before conception, maternal age of conception, maternal education, parity, gestational weeks, children's age, and individual weight-for-length z-scores (WLZs).

study but the results did not change remarkably. We could not adjusted for other EDCs due to limited data, so further investigation is needed.

PBDEs have been reported to be anti-androgenic in in-vitro and in-vivo assays (Hamers et al., 2006; Stoker et al., 2005). Some studies have demonstrated that BDE-47 exposure could down-regulate the steroidogenic proteins (3β-HSD and StAR) (Lardone et al., 2011; Maris et al., 2015; Stickels et al., 2015), inhibit testosterone synthesis in the Leydig cells, and ultimately decrease the level of androgen (Zhang et al., 2017). Penta-BDE could also exhibit anti-androgenic activities with inhibition of AR binding (Kodavanti et al., 2010). Since decreased prenatal androgen levels are associated with shortened AGD (Moore et al., 2001), one possible mechanism underlying the adverse effects of PBDEs on AGD is the anti-androgenic effect. Besides, BDE-47 was found to be associated with estrogen receptor (ER) agonistic responses (Hamers et al., 2006; Liu et al., 2011). Minor alleles that located in the coding region of ERα could regulate estrogen signaling during male genital development - this associated with shorter AGD (Sathyanarayana et al., 2012). Hence, BDE-47 may also interfere with the development of AGD through the estrogen signaling pathway.

This study has several advantages. First, the study was of a prospective design with AGD measurements at birth, 6 months of age, 12 months of age, and 48 months of age, which ensured temporality and eliminated recall bias. The repeated measurements of AGD during early childhood also made it possible to reveal the longitudinal effects of prenatal PBDEs exposure. Second, the study examined PBDEs concentrations in umbilical cord plasma, which could be a better indicator of the exposure of the fetus and provided a better means of directly measuring prenatal exposure to PBDEs in newborns (Herbstman et al., 2007).

Several limitations of this study should be acknowledged. First, some higher-brominated compounds, particularly BDE-209, were not measured in this study. BDE-209 is thermally unstable and has high boiling points, thus its degradation of which during GC–MS analysis was inevitable (Wang et al., 2018). Consequently, this study did not assess BDE-209 and evaluate its potential reproductive developmental toxicity. Second, because PBDEs with half-lives in humans range from 2 to 12 years, it could be expected that the neonatal body burden was retained in young children (Eskenazi et al., 2013). The related data were unavailable to disentangle the effect of postnatal PBDEs exposure from the effect of prenatal PBDEs exposure on AGD. To fully understand the impact of prenatal PBDEs exposure on AGD, future investigations should assess both prenatal and postnatal exposures. Third, only one measurement for each AGD variant at each time point was conducted and the intra-rater reliability was not assessed. However, the reliability coefficient increased only slightly when the average of 2 or 3 repeated measurements was used (Papadopoulou et al., 2013; Priskorn et al., 2018). Intra-rater measurement errors of AGD at each follow-up visit might be more likely to cause non-differential misclassification and bias the association towards null result. Fourth, a clear dose-responsive pattern of the effects was not observed in out study. But low-dose effects and non-monotonic responses have been proposed and are remarkably common in studies of hormones and EDCs (Vandenberg et al., 2012). In addition, a meta-analysis showed that the dose-relationship between PBDEs exposures and TH levels probably did not fit a simple mono-phasic curve, but possibly fitted a u-shaped curve (Zhao et al., 2015). The underlying mechanisms of the effects on AGD await further verification. Lastly, although the consistent associations across different ages contradict type I error to some extent, the small sample size (n = 190) might limit our ability to detect a significant relationship.

5. Conclusion

Our findings provide preliminary evidence that prenatal PBDEs exposure, especially to BDE-47, may have adverse effects on reproductive organ development in boys, as indicated by shorter AGD, a sensitive marker to assess reproductive toxicity. Further studies should be conducted to validate the results.

Conflicts of interest

There is no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.01.008.

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