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Polybrominated diphenyl ether exposure and reproductive hormones in North American men

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Abstract

Background—Polybrominated diphenyl ethers (PBDEs) are flame retardant chemicals that are persistent organic pollutants. Animal experiments and some human studies indicate that PBDEs may adversely affect male reproductive function.

Objectives—To assess the association between PBDE exposure and reproductive hormones (RHs) in a North American male adult cohort.

Methods—From 2010–11, we collected three serum samples from 27 healthy adult men. We assessed associations between PBDEs and RHs using mixed effect regression models.

Results—PBDEs were inversely associated with inhibin-B. In older men, increased concentrations of BDE-47 and BDE-100 were significantly associated with a decrease in inhibin-B, and an increase in follicular stimulating hormone (FSH).

Conclusions—These findings suggest PBDE exposure may affect RHs in older men. We did not measure other parameters of male reproductive function and therefore these results are preliminary.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Transparency document

The Transparency document associated with this article can be found in the online version.

Keywords

Environmental health; Flame retardants; Male reproductive health; Persistent organic pollutants; Polybrominated diphenyl ethers (PBDEs)

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are additive (i.e. not chemically bound) flame retardant chemicals. Pentabro-modiphenyl ether (PentaBDE) is a chemical mixture that predominately contains the PBDE congeners BDE-28, −47, −99, −100, and −153. It was used as an additive flame retardant in furniture containing polyurethane foam in the US from the 1970s. Of the worldwide production, 95% of the PentaBDE produced was used in North America, where concentrations in the general population are significantly higher than in Europeans and Asians [1]. Due to its persistence, ability to bioaccumulate, and potential adverse health effects, U.S. chemical manufacturers voluntarily withdrew PentaBDE from production in 2004. However, older products containing PentaBDE (e.g. furniture) remain in use and continue to contribute to exposure in indoor microenvironments: e.g., homes, offices, and vehicles [2]. As PentaBDEs are commonly found in US food products [3], diet is an additional source of human exposure [4].

Structurally similar to polychlorinated biphenyls (PCBs), PBDEs or their hydroxyl metabolites may activate or antagonize the estrogen and/or androgen receptor, which is associated with reproductive effects [5–7]. However, animal studies to date have been inconsistent regarding the effects of PBDEs on reproductive endpoints. In rats, there is some evidence that exposure to PentaBDEs leads to a adverse effects on reproductive endpoints such as decreased seminal vesicle and prostate organ weight [6,7], decreased testis and epididymis organ weight [8], decreased daily sperm production [8], and an increase in deformed sperm [7]. However, there are two studies that have investigated the effects of PBDEs in rats and reported no decrease on testis organ weight [9] or decrease in testicular function [10].

Human studies have found associations between PBDE exposure and male reproductive hormones. However the direction of associations are inconsistent; see Supplemental Material, Table S1. In humans, studies have reported associations between PBDEs and decreased sperm concentration [11], decreased sperm motility [12], and cryptorchidism [13]. A few studies have linked PBDE exposure in females with potentially adverse effects on reproduction such as decreased age at menarche [14], decreased fecundability [15], and decreased IVF success rates [16].

Our study uses repeated serum measures to assess the association between PBDE exposure and reproductive hormones (RHs) and associated binding proteins (BPs) in a longitudinal cohort of healthy, adult men. Our primary aim is to examine the association between PBDEs and total testosterone (Total T), free testosterone (Free T), inhibin-B, luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, sex hormone binding globulin (SHBG), inhibin-B/FSH ratio, and the free androgen index (FAI).

2. Methods

2.1. Study design and population

Characteristics and descriptions of the entire FlaRE (Flame Retardant Exposure Study) population are presented elsewhere [17]. Briefly, participants had to be healthy, non-smoking, adult office workers planning to reside in the Boston metropolitan area from 2010 to 2011. The recruited population included 52 men and women, but the current analysis is restricted to the subset of 27 men.

We collected three rounds of non-fasting blood samples at approximately six-month intervals. Twenty-six males participated in Round 1 and one additional male participant was recruited in Round 2 (total of 76 samples). Four serum samples were missing for the following reasons: too little serum collected (n = 1), unable to conduct venipuncture (n = 2), and loss to follow-up (n = 1). We used questionnaires to collect information about demographics, general health, prior diagnosis of reproductive disease and the use of medications that can affect testosterone levels: Testosterone, Methadone, Megestrol, Ketoconazole, Spironalactone, and DHEA-sulfate. We obtained informed consent prior to participation and the Boston University Medical Center Institutional Review Board approved the study protocol. The involvement of the Centers for Disease Control and Prevention (CDC) did not constitute engagement in human subjects research.

2.2. Blood samples

A trained phlebotomist collected 30 mL of blood from participants during each sampling round. Samples were collected at various times of day at the convenience of the participants; time of day was recorded. The CDC analyzed serum samples for 11 PBDE congeners (BDE-17, BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209) using established methods [18]. Samples were randomized and analyzed with quality control (QC) (n = 3) and blank samples (n = 3) in each batch of 24 unknowns. The coefficient of variation (CV) of included QC samples was less than 10%. Serum samples were also analyzed at the CDC for total triglycerides (GPO-PAP) and total cholesterol (CHOD-PAP) using text kits from Roche Diagnostics Corp. (Indianapolis, IN). Final determinations were made on a Hitachi Modular P Chemistry Analyzer (Tokyo, Japan). The total lipids concentration was calculated by summation of the individual lipid components [19].

2.3. Hormone analysis

Hormones and binding proteins were analyzed at the Steroid Hormone Research Laboratory at Boston Medical Center, Boston, MA. We analyzed Total T by LC–MS (AB Sciex QTRAP® 5500 System) with a sensitivity of 1 ng/dL, an intra-assay CV of 2%, and inter-assay CV of 7%. We calculated Free T using formula [20]. SHBG and LH levels were measured using a two-site immunofluorometric assay (DELFIA-Wallac, Inc., Turku, Finland). The inter-assay CVs for SHBG were 8.3%, 7.9%, and 10.9%, and intra-assay CVs 7.3%, 7.1% and 8.7%, respectively, in the low, medium, and high pools and the analytical sensitivity of the assays was 0.5 nmol/L. We measured FSH and prolactin using time-resolved fluoroimmunoassay (DELFIA-Wallac, Inc. Turku, Finland) performed on a Wallac-

Victor 1420 Multilabel Counter (Perkin Elmer, Waltham, MA.). We measured Inhibin-B using the Inhibin B Gen II ELISA kit (Beckman-Coulter, Brea, CA).

2.4. Statistical analysis

Continuous population characteristic variables, PBDEs, and RHs are presented with measures of central tendency and the minimum and maximum values by each sampling round. In summary statistics, PBDEs were presented standardized to serum lipids (ng/g lipids) for comparability with previous studies. We substituted ½ LOD for PBDE measurements below the LOD. ΣPBDE was the sum of the PBDE congeners detected >50%: BDE-28, −47, −99, −100, and −153. We used Spearman correlation coefficients to determine the amount of correlation between PBDE congeners. The inhibin B/FSH ratio was calculated as inhibin B (pg/mL)/FSH (IU/L). We calculated the FAI using the formula (total T/SHBG) × 100. We assessed normality of continuous variables using histograms and Shapiro-Wilks tests. All statistical analyses were conducted using SAS statistical software version 9.3 (SAS Institute, Cary, NC, USA) and statistical significance is reported at the 0.05 level.

We used a general linear model for repeated measures with a random intercept to assess the association between the PBDE congeners and RHs. Dependent variables that were log-normally distributed were transformed for regression analysis. We added the following covariates to form our regression models: sampling round (indicator variable − Round 1, 2, or 3), total lipids (mg/dL), age (years), and body mass index (BMI, mg/kg^2). The analysis for the relationship between PBDE congeners and RHs in Table 2 are presented as: Model A, adjusted for round only; Model B, adjusted for round and serum lipids; Model C, adjusted for round, lipids, age, and BMI; Model D, adjusted for round, lipids, and BMI in men under 40 years old; Model E, adjusted for round, lipids, and BMI in men 40 years and older; and Model F, adjusted for round and using a lipid-standardized (ng/g lipid) PBDE exposure metric. Table 3 presents regression analysis for the relationship between PBDE congeners and RHs adjusted for round, lipids, age and BMI. As a sensitivity analysis, we also included time of day of blood sampling as a covariate. To identify the temporal sequence of the exposure and outcome, we used linear regression to assess the association between PBDEs concentrations in Round 1 and RH levels at following sampling rounds, Round 2 or Round 3.

Influential points were identified in a scatterplot of RHs × PBDE. We exponentiated the beta-coefficient to calculate a percent change in hormone level per unit change in PBDE (ng/g serum) for equations with a log-transformed dependent variable. The regression coefficients in the tables have not been transformed to show percent change.

Based on a priori expectation, age, serum lipids, and BMI were evaluated as potential confounders. Confounding was assessed using a change of >10% or greater in the beta-coefficient as a guide. To assess effect measure modification (EMM), we examined regression models with a cross product of PBDE concentrations and covariate (treating age and BMI as continuous variables). We also examined EMM in stratified analysis. We dichotomized our cohort at above and below 40 years because research indicates male fertility becomes clinically reduced around 40 years of age [21]. For BMI, we dichotomized our cohort by the following: normal (BMI < 25 kg/m^2), and overweight/obese (≥25 kg/m^2).

We estimated intraclass correlation coefficients (ICCs) to assess the stability of serum RHs in men [22] using a general linear model with a random intercept. Stability was classified as "poor" (ICC = 0–0.39), "moderate" (ICC = 0.4–0.59), "good" (ICC = 0.6–0.79), or "excellent" (ICC ≥ 0.80).

3. Results

3.1. Study population

We collected 76 serum samples from 27 male participants from 2010 to 2011. Twenty-three men were white, two were Hispanic/Latino, and two were Asian. Participation rate by sampling round was: 92% (24/26) in Round 1, 100% (27/27) in Round 2, and 93% (25/27) in Round 3. All men reported to be in good to excellent health, 100% had a college degree, and the mean age was 41 years old. Fourteen men were considered normal weight (BMI <25 kg/m^2), 12 men were overweight (BMI between 25 and 30 kg/m^2), and one was obese (BMI > 30 kg/m^2). Two participants reported taking a medication that may affect testosterone levels during the study period and one participant reported a history of prostate cancer. Regression analyses were not affected when these men were excluded (not shown).

3.2. Serum PBDE levels

We measured 11 PBDE congeners and nine reproductive tests (hormones and binding proteins) in serum samples. Table 1 presents the round-specific GMs, GSDs, and range for the major PentaBDE congeners as well as other information by sampling round. GM concentrations of ΣPBDEs by sampling round were 25.5 ng/g lipid in Round 1, 25.5 ng/g lipid in Round 2, and 21.1 ng/g lipid in Round 3. BDE-47, BDE-99, and BDE-100 were highly correlated, $r > 0.94$, $p < 0.001$. BDE-153 was not as strongly correlated (r between 0.40 and 0.56, p-values between 0.048 and 0.004) with BDE-47, BDE-99, and BDE-100; see Supplemental material, Table S2. Detection rates for BDE-17, BDE-66, BDE-85, BDE-154, BDE-183, and BDE-209 were low and not further analyzed; see Supplemental Material, Table S3 for detection frequencies for all PBDE congeners. The limits of detection of main PBDE congeners ranged from 0.2 to 0.8 ng/g lipid.

3.3. Serum RHs

As shown in Table 1, RHs and binding proteins were predominantly within normal ranges. Our round-specific free T ranges were generally within the normal range of 50–200 pg/mL for healthy adult males: Round 1 (59.7–167.1), Round 2 (50.4–197.5), and Round 3 (34.6–149.1) pg/mL. Our cohort GM inhibin-B/FSH ratios by round were 63.0, 64.1, and 65.8, respectively. They were higher than those reported in a normal population (median = 48) and comparable to a proven fertile male population (median = 70) [23].

3.4. Relationships between PBDEs and RHs

Table 2 presents the results from linear mixed-effects models using serum PBDEs to predict the RHs: inhibin-B, FSH, and the inhibin-B/FSH ratio. BDE-47 was significantly and inversely associated with inhibin-B, after adjustment for lipids, age, BMI, and sampling round (Model C). These inverse relationships persisted in the crude models (Model A), models adjusted for lipids only (Model B), models using a lipid standardized exposure

metric (Model E), and in cross-sectional analysis (data not shown). In a sensitivity analysis, we found that the PBDE serum concentrations in Round 1 significantly predicted a decrease in serum inhibin-B in Round 3 (not shown). While the negative association between inhibin-B and PBDE congeners was present in analysis of the entire cohort (Model A, B, C, F), stratified analysis revealed the inverse relationship was mostly attributed to men over 40 in our cohort (Model D, E). We did not find that age or BMI were confounding the association we report between PBDEs and the RHs. The beta-coefficients from the lipid-only regression models (Model B) and models adjusted for lipids, age, and BMI (Model C) were similar, e.g. less than 10% change in beta-coefficient. This was also true in models that adjusted for age and BMI separately (not shown).

We found significant evidence of effect measure modification by age in the relationship between PBDEs and FSH, ($p = 0.004$). Table 2 (Model D, E) and Supplemental Fig. S1 present results stratified by age group: <40 years old (14 men, 40 serum samples), ≥40 years old (13 men, 36 serum samples). Among younger men, for every one-unit increase in BDE-100 (ng/g serum) there was a 10% (95% CI = 0.82–120) IU/L decrease in FSH (Table 2, Model D). Among older men for every one-unit increase in BDE-100 (ng/g serum) we estimated a 74% (95% CI = 3.3–1600) IU/L increase in FSH (Table 2, Model E). BDE-47 presented a similar pattern to BDE-100, and BDE-153 had a significant and positive relationship with FSH among older men, but the relationship in younger men was imprecise and appeared null. Among younger men, BDE-99 was inversely associated with FSH, and among older men, this inverse association was attenuated and appeared null.

We also observed effect measure modification by age in the relationship between PBDEs and the inhibin-B/FSH ratio (Table 2). This ratio is a diagnostic tool used in idiopathic male infertility, where a decreased ratio is associated with decreased sperm counts and fertility rates [23]. We found that BDE-47 and BDE-100 exposure was associated with a decrease in the inhibin-B/FSH ratio among older men but an increase in the inhibin-B/FSH ratio among younger men. However, the strength of this pattern differed by congener and some associations were weak and imprecise. For associations between PBDEs and the other RHs we found no evidence of effect modification by age. We did not find any evidence for effect measure modification between PBDEs and any of the RHs by BMI (not shown).

Table 3 presents the results from our linear mixed-effects models using serum PBDEs to predict the other RH and associated binding proteins: Total T, Free T, LH, FAI, Prolactin, and SHBG. We did not observe any important associations between PBDEs and the following: Total T, Free T, prolactin, and SHBG. There was a positive association between BDE-47 and BDE-99 and LH; after removal of a single potentially influential data point, the positive relationship was almost completely absent (not shown). We did find a weak and imprecise inverse association between the lower brominated PBDE congeners and FAI.

3.5. Intraclass correlation coefficients of RHs

Table 4 presents the ICCs for the RHs. Inhibin-B, FSH, SHBG, and the inhibin-B/FSH ratio were highly stable at assessing an individual's status over the one-year study period. LH and FAI had good stability. Total T and prolactin had moderate stability. Free T had poor stability (e.g. a high degree of intra-individual variability). The results for Total and Free T

are expected, as these hormones exhibit diurnal variability, where levels taken in morning are typically higher than those in the afternoon [24]. Our non-fasting serum samples were collected at various times during the day based on the convenience of the participant. Blood collection time of day was an inverse predictor of Total and Free T (not shown), i.e., levels of these hormones decreased toward the end of the day. Inclusion of time of time of day slightly increased ICCs for these hormones. However, inclusion of blood collection time of day did not have any important impacts on our effect estimates for associations of PBDEs with Total or Free T (not shown).

4. Discussion

4.1. PBDEs and RHs (FSH and inhibin-B)

We found that exposure to BDE-47 was associated with decreased inhibin-B in healthy adult men living in the Boston area, especially in men forty years and older. In adult men, the Sertoli cells produce inhibin-B and serum levels of the hormone are strongly and positively correlated with testicular volume and sperm counts [25]. A recent European study of 299 men also reported inverse associations between BDE-47 and inhibin-B, estradiol, T, FAI and one marker of sperm DNA damage [26]. Our finding of decreased inhibin-B is consistent with one study reporting that PBDE exposure was associated with decreased sperm concentration [11]. However, our findings are inconsistent with three other previous human studies that reported a positive association between PBDEs and inhibin-B [27–29]. Meeker et al. and Johnson et al. found a positive association between summed PentaBDEs (BDE-47, BDE-99, BDE-100) in house dust and inhibin-B, SHBG, and estradiol in adult men seeking infertility treatment [27,28]; see supplemental material, Table S1.

FSH and inhibin-B are tightly regulated via negative feedback in the hypothalamic-pituitary-testicular axis. Sub-fertile or infertile adult men typically have low inhibin-B, in combination with high FSH levels, resulting in a low inhibin-B/FSH ratio [23]. Furthermore, the inhibin-B/FSH ratio is a serum marker for seminiferous tubule health and Sertoli cell viability. Thus, men will have decreased inhibin-B levels, a decreased inhibin-B/FSH ratio, and decreased sperm concentrations after undergoing chemotherapy [30]. Among older men in our cohort, we found that increased exposure to BDE-47 and BDE-100 was linked to a significant decrease in inhibin-B, significant increase in FSH, and a non-significant decrease in the inhibin-B/FSH ratio. A recent toxicological study in mice reported exposure to BDE-47 impaired spermatogenesis, possibly driven by an increase apoptosis of germ cells in the seminiferous tubules [31]. Interestingly, within the younger men of our cohort, we found that exposure to BDE-47 and BDE-99 were associated with significant decreases in FSH and significant increases in the inhibin-B/FSH ratio (Table 2). While hormone analysis would be used in combination with other reproductive function tests, clinical evaluations, and semen analysis in the determination of fertility status [32], we have evidence that PBDEs may be disrupting the hypothalamo-pituitary-testicular axis; this relationship may also differ dependent upon age. The effect measure modification by age in the relationship between PBDEs/RHs in men has not been reported in previous studies.

4.2. PBDEs and testosterone

We did not find any important associations between PBDEs and Total or Free T, similar to several other studies that evaluated this relationship [10,33]. This differs from some human studies that have found associations between PBDEs and T [26,27,29,34].

In our study, it is possible methodological issues affected our ability to detect relationships between PBDEs and T measurements. First, T measurements had low ICCs in our cohort; this is expected when using non-timed, non-fasting serum samples. Low ICCs indicate there is a high degree of intra-individual variability in the serum T measurement, which can decrease the precision of effect estimates. Part of this variability arises from normal diurnal variation in T measurements (higher in the morning), which is independent of PBDE exposure. However, addition of blood sampling time into regression models did not have important impacts on our PBDE/T effect estimates. Second, our small sample size may have limited our ability to detect an association between PBDEs and T measurements. Third, it is possible differences between the FlaRE study population and those previously studied have led to divergent results. Using other reproductive endpoints, including in females, studies have shown that PBDEs possibly have adverse effects on fecundability in animals [6,8,31,35,36] and humans [15]. Nevertheless, we do not have evidence that PBDE congeners affected circulating T levels in the men of our cohort.

We did find a small, non-significant, inverse association between the PBDE congeners, BDE-47 and BDE-99, and FAI. While our results of a decrease in FAI are have been reported elsewhere [28], it is unclear how valid FAI is as a marker for reproductive function in men [37]. FAI was historically used as a measure for free testosterone; it has since been determined to be a poor predictor of bioavailable testosterone in men [38].

4.3. PBDEs and serum lipids

We used multiple methods to account for serum lipids (e.g. crude, adjustment, standardization) in our regression models. This has been a source of debate when studying the health effects of lipophilic compounds in non-fasting serum samples [39]. As expected, serum lipids were positively correlated with serum PBDE levels in our cohort [17]. Research has also shown serum lipid levels are correlated with hormone levels in men [40]. While the causal structure between PBDEs, RHs and serum lipids is unknown, in our primary models (Models A, B, C, D, E) we adjusted for serum lipids as a covariate, instead of using a standardized PBDE exposure metric. This allowed us to assess the independent effects of serum lipids and PBDE concentrations. This also allowed us to theoretically remove the possibility of reverse causation through serum lipids by controlling for the covariate, removing a potential back-door pathway.

Based on simulations constructed by Gaskins et al., it is possible we are observing positive confounding by serum lipids in the PBDE/RH relationship [41]. This would lead to a crude association that is biased away from the null, and a standardized model that is biased toward the null, which is precisely what we report. In these simulations, the lipid-adjusted model correctly accounts for serum lipids effect on RHs and presents limited bias for the relationship between the lipophilic exposures and health outcomes [39,41].

4.4. Strengths and limitations

Our study is the first to use repeated serum measures to assess the association between PBDE exposure and male RHs. Our prospective study can more clearly specify the temporal sequence of the exposure and outcome than a cross-sectional study, leading to a decreased likelihood of reverse causation. Furthermore, our study design allowed us to assess the stability of PBDE and RH measures in serum, and we report that serum PBDEs [17] and some RHs are highly stable in our cohort.

Our study was limited by a small sample size. However, we had an excellent retention rate, >92%, so differential loss to follow-up was not likely to introduce bias into our analysis. Our cohort was predominately white, highly educated men living in Boston (USA); this cohort is not representative of the US general population. It is possible there may be other exposures that could confound the relationships we report or contribute to a mixtures effect. An important limitation of this work is the lack of other male reproductive endpoints (e.g. semen analysis) evaluated in conjunction with the hormonal measurements. We believe that a follow-up study on this endpoint would be appropriate. There is also the possibility of chance associations based on the number of congeners and RHs tested. However our main conclusions, regarding the lower brominated PBDEs and the RHs (inhibin-B, FSH, and inhibin-B/FSH ratio) had a consistency in direction of effect.

5. Conclusions

In conclusion, our results suggest that environmental exposure to PBDEs is inversely associated with inhibin-B serum levels, a marker of spermatogenesis, in older men. Additionally, among older men we found PBDE exposure was associated with increases in serum FSH, and a decrease in the inhibin-B/FSH ratio. However, this was a small study and it is important that these results are replicated in a larger study. Future prospective studies would provide important information to further understand how PBDEs and their metabolites may affect reproductive hormone levels and possibly testicular function in healthy adult men.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BDE	brominated diphenyl ether
BP	binding proteins

CV	coefficient of variation
EMM	effect measure modification
FAI	free androgen index
FlaRE	flame retardant exposure study
FSH	follicular stimulating hormone
ICC	intraclass correlation coefficients
LH	luteinizing hormone
PBDEs	polybrominated diphenyl ethers
RH	reproductive hormones
SHBG	sex hormone binging globulin
T	testosterone.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.reprotox.2016.04.009.

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Table 1

Summary statistics for demographic characteristics, repeated serum samples of selected PBDEs, and reproductive function tests in adult men, by sampling round (27 men, 76 samples).

Characteristic	Round 1 (24 samples)			Round 2 (27 samples)			Round 3 (25 samples)		
	GM	(GSD)	Range	GM	(GSD)	Range	GM	(GSD)	Range
Demographics									
Age (years)[a]	41.0	(13.8)	25–66	41.3	(13.3)	25–66	41.8	(12.6)	26–67
BMI (mg/kg^2)[a]	25.3	(3.6)	20–38	25.3	(3.5)	20–38	25.1	(3.6)	20–38
Total Lipids[a]	644.8	(118)	448–864	630.4	(123)	396–912	632.2	(124)	328–843
PBDEs (ng/g lipid)									
BDE−28[b]	0.51	(2.5)	0.15–2.8	0.62	(2.5)	0.15–5.1	0.5	(2.3)	0.15–3.6
BDE−47[b]	9.4	(3.0)	1.7–151	9.9	(2.8)	1.4–149.0	8.2	(2.5)	1.8–98.9
BDE−99[b]	1.9	(3.0)	0.25–43.5	1.9	(3.0)	0.25–34.1	1.6	(2.5)	0.30–20.1
BDE−100[b]	1.8	(4.0)	0.20–42.4	2	(3.5)	0.25–44.1	1.5	(3.0)	0.30–35.2
BDE−153[b]	8.6	(3.1)	1.6–96.7	8.6	(3.1)	1.7–94.7	6.8	(2.8)	1.5–55.2
∑PBDEs[b]	25.5	(2.8)	5.8–294.0	25.5	(2.6)	6.55–293.5	21.1	(2.3)	5.85–211.1
Reproductive Tests[c]									
Total T (ng/dl)[a]	627.6	(254)	310–1182	633.2	(200)	285.4–1031	470.1	(159)	199.4–795.4
Free T (pg/ml)[a]	109.1	(34.2)	59.7–167.1	109.6	(36.8)	50.4–197.5	83.8	(29.5)	34.6–149.1
Inhibin-B (pg/mL)[a]	189.8	(67.2)	16.6–320.9	183.7	(75.8)	18.5–423.3	190.1	(83.6)	17.2–441.1
FSH (IU/L)	3.12	(1.73)	1.40–17.6	3.21	(1.76)	1.32–19.6	3.29	(1.81)	1.41–19.6
LH (U/L)	4.5	(1.58)	2.12–12.57	3.93	(1.53)	1.97–13.9	4.39	(1.60)	1.82–13.7
Prolactin (ng/mL)	4.25	(1.45)	2.05–7.28	4.97	(1.49)	2.39–9.34	5.15	(1.43)	2.40–9.82
SHBG (nmol/L)	42.7	(1.60)	14.0–83.8	45.3	(1.59)	12.7–92.7	40.5	(1.56)	12.2–76.4
Inhibin-B/FSH	63.0	(52.6)	0.94–188	64.1	(56.8)	0.95–258	65.8	(57.1)	0.88–238
FAI	1360	(104)	738–3850	1330	(149)	685–3180	1090	(151)	520–2100

Abbreviations: BMI: body mass index, FAI: free androgen index, FSH: follicular stimulating hormone, GM: geometric mean, GSD: geometric standard deviation, LH: luteinizing hormone, SHBG: sex hormone binding globulin, T: testosterone.

[a]Means and standard deviations presented.

[b] Detection rates presented in Supplemental Table, S2.

[c] Normal Reference Range for adult males in good health: testosterone = 300–1000 ng/dL, free testosterone = 50–200 pg/mL inhibin-B = elevated if above 399 pg/mL, FSH = 0.60–9.98 IU/L, LH = 1.0–8.4 U/L, SHBG = 12.9–61.7 nmol/L.

Table 2

Beta-coefficients, 95% confidence intervals and p-values from general linear models with a random intercept predicting reproductive hormones using PBDE exposure metrics over one-year (27 men, 76 serum samples). Models are: crude (Model A), adjusted for serum lipids (Model B), adjusted for serum lipids, age, and BMI (Model C), adjusted for serum lipids and BMI in men under 40 years old (Model D), adjusted for serum lipids and BMI in men 40 years and older (Model E), crude with a lipid standardized exposure metric (Model F).

Hormone	BDE-47 β (95% CI)	*p*	BDE-99 β (95% CI)	*p*	BDE-100 β (95% CI)	*p*	BDE-153 β (95% CI)	*p*
Inhibin-B (pg/mL)[a]								
Model A	−0.18 (−0.30, −0.068)	0.002	−0.49 (−0.90, −0.077)	0.021	−0.43 (−0.78, −0.086)	0.016	−0.15 (−0.32, 0.031)	0.105
Model B	−0.15 (−0.27, −0.004)	0.011	−0.43 (−0.83, −0.030)	0.036	−0.33 (−0.68, 0.013)	0.059	−0.092 (−0.26, 0.078)	0.283
Model C	−0.14 (−0.27, −0.020)	0.024	−0.39 (−0.82, 0.034)	0.071	−0.31 (−0.67, 0.055)	0.094	−0.094 (−0.27, 0.079)	0.281
Model D[b]	−0.0029 (−0.30, 0.30)	0.984	0.024 (−1.9, 1.9)	0.979	0.043 (−0.47, 0.56)	0.863	−0.043 (−0.23, 0.15)	0.638
Model E[c]	−0.17 (−0.31, −0.030)	0.020	−0.38 (−0.82, 0.067)	0.092	−0.60 (−1.1, −0.088)	0.024	−0.24 (−0.62, 0.13)	0.194
Model F[d]	−1.1 (−1.9, −0.35)	0.005	−2.7 (−5.2, −0.064)	0.045	−3.0 (−5.5, −0.61)	0.015	−0.91 (−2.1, 0.31)	0.140
FSH (IU/L)[e]								
Model A	0.066 (−0.052, 0.65)	0.820	−0.40 (−2.1, 1.2)	0.615	0.016 (−1.9, 1.9)	0.986	0. 027 (−0.68, 1.2)	0.574
Model B	−0.060 (−0.064, 0.53)	0.850	−0.46 (−2.1, 1.1)	0.564	−0.62 (−2.6, 1.3)	0.524	−0.050 (−1.1, 0.96)	0.928
Model C	−0.12 (−0.71, 0.47)	0.681	−0.61 (−2.2, 0.99)	0.447	−0.70 (−2.6, 1.2)	0.469	−0. 01 (−1.0, 0.98)	0.980
Model D[b]	−1.5 (92.7, −0.21)	0.025	−8.0 (−15, −0.84)	0.030	−2.3 (−4.8, 0.20)	0.069	−0.39 (−1.5, 0.70)	0.463
Model E[c]	0.36 (0.04, 1.1)	0.030	−0.28 (−1.9, 1.3)	0.719	4.3 (1.2, 7.4)	0.008	2.3 (0.11, 4.4)	0.040
Model F[d]	0.047 (−3.5, 3.6)	0.979	−2.9 (−12, 6.7)	0.547	1.4 (−13, 16)	0.843	2.4 (−5.1, 9.9)	0.518
Inhibin-B/FSH[e]								
Model A	−0.86 (−2.0, 0.20)	0.109	−1.1 (−4.0, 1.8)	0.446	−2.2 (−5.7, 1.2)	0.195	−1.2 (−2.9, 0.56)	0.181
Model B	−0.45 (−1.5, 0.56)	0.374	−0.82 (−3.6, 2.9)	0.553	−0.47 (−3.9, 2.9)	0.782	−0.26 (−2.1, 1.5)	0.768
Model C	−0.33 (−1.4, 0.70)	0.524	−0.56 (−3.3, 2.2)	0.685	−0. 27 (−3.7, 3.2)	0.874	−0.32 (−2.1, 1.5)	0.721
Model D[b]	1.6 (0.006, 3.2)	0.043	8.8 (0.008, 18)	0.049	3.4 (0.38, 6.5)	0.020	0.41 (−1.0, 1.8)	0.561
Model E[c]	−1.3 (−2.8, 0.28)	0.102	−1.4 (−5.1, 2.3)	0.445	−11 (−17 to −4.6)	0.002	−3.9 (−8.8, 0.92)	0.106
Model F[d]	−3.7 (−10, 2.7)	0.245	−4.0 (−21, 13)	0.639	−19 (−44, 6.8)	0.146	−94 (−230, 43)	0.174

Abbreviations: β (Beta-coefficient), FSH (follicular stimulating hormone), p (p-value), RH (reproductive hormone).

Model A: $Y_{ij} = \beta o + \beta 1\text{PBDE}_{ij} + \beta 2\text{Round}_1 + \beta 3\text{Round}_2 + b_i + \epsilon_{ij}$

Model B: $Y_{ij} = \beta o + \beta 1\text{PBDE}_{ij} + \beta 2\text{Round}_1 + \beta 3\text{Round}_2 + \beta 4\text{LIPID}_{ij} + b_i + \epsilon_{ij}$.

Model C: $Y_{ij} = \beta o + \beta 1\text{PBDE}_{ij} + \beta 2\text{Round}_1 + \beta 3\text{Round}_2 + \beta 4\text{LIPID}_{ij} + \beta 5\text{Age}_{ij} + \beta 6\text{BMI}_{ij} b_i + \varepsilon_{ij}$.

Model D: (Men under 40 y.o.) $Y_{ij} = \beta o + \beta 1\text{PBDE}_{ij} + \beta 2\text{Round}_1 + \beta 3\text{Round}_2 + \beta 4\text{LIPID}_{ij} + \beta 5\text{BMI}_{ij} b_i + \varepsilon_{ij}$.

Model E: (Men over 40 y.o.) $Y_{ij} = \beta o + \beta 1\text{PBDE}_{ij} + \beta 2\text{Round}_1 + \beta 3\text{Round}_2 + \beta 4\text{LIPID}_{ij} + \beta 5\text{BMI}_{ij} b_i + \varepsilon_{ij}$.

Model F: $Y_{ij} = \beta o + \beta 1\text{PBDE}(\mu g/g\text{Lipid})_{ij} + \beta 2\text{Round}_1 + \beta 3\text{Round}_2 + b_i + \varepsilon_{ij}$.

Definitions: Y_{ij} represents the RH level of the ith participant at the jth sampling round. βo is the fixed effect intercept, $\beta 1$ is the effect on the RH of a one-unit change in PBDE level [PBDE_{ij}(ng/g serum)], b_i is the random intercept of the i_{th} individual, and ε_{ij} is the random error

[a] Dependent variable was not transformed by the natural log. Beta-coefficient yields the absolute change in dependent variable per unit change in PBDE predictor (pg/g serum).

[b] Men under 40 (14 men, 40 serum samples).

[c] Men 40 years and older (13 men, 36 serum samples).

[d] Independent variable, PBDEs, was standardized to lipids, e.g. µg/g lipid.

[e] Dependent variable was transformed by the natural log.

Table 3

Beta-coefficients, 95% confidence intervals and p-values from general linear models with a random intercept predicting reproductive hormones using PBDE exposure metrics over one-year (27 men, 76 serum samples).

Hormone	BDE-47 β (95% CI)	*p*	BDE-99β (95% CI)	*p*	BDE-100β (95% CI)	*p*	BDE-153β (95% CI)	*p*
Total T (ng/dL)[a]	0.063 (−0.3, 0.4)	0.715	0.022 (−1, 1)	0.974	0.19 (−0.8, 1)	0.673	0.25 (−0.1, 0.7)	0.207
Free T (pg/mL)[a]	−0.22 (−0.1, 0.04)	0.318	−0.12 (−0.4, 0.1)	0.162	−0.032 (−0.2, 0.1)	0.641	0.040 (−0.03, 0.1)	0.246
LH (U/L)[b]	0.82 (0.7, 2)	0.046	2.8 (−0.2, 6)	0.062	1.7 (−0.5, 4)	0.132	0.28 (−1, 1)	0.602
FAI[b]	−0.49 (−1.2, 0.2)	0.149	−1.9 (−4, 1)	0.138	−1.1 (−3, 1)	0.238	0.08 (−1, 1)	0.849
Prolactin (ng/mL)[b]	−0.50 (−1 to 1)	0.875	0.70 (−2, 3)	0.608	−0.19 (−2, 2)	0.828	−0.50 (−1, 0.3)	0.219
SHBG (nmol/L)[b]	0.50 (−0.2, 1)	0.135	0.69 (−1, 3)	0.501	0.70 (−2, 3)	0.142	0.47 (−0.4, 1)	0.301

Abbreviations: β (Beta-coefficient), CI (confidence interval), FAI (free androgen index), FSH (follicular stimulating hormone), LH (luteinizing hormone), *p* (p-value), SHBG (sex hormone binding globulin), T (testosterone).

Regression Model: $Y_{ij} = \beta o + \beta 1 \text{PBDE}_{ij} + \beta 2 \text{Round}_1 + \beta 3 \text{Round}_2 + \beta 4 \text{LIPID}_{ij} + \beta 5 \text{Age}_{ij} + \beta 6 \text{BMI}_{ij}\, b_i + \varepsilon_{ij}$.

Definitions: Y_{ij} represents the RH level of the ith participant at the jth sampling round. βo is the fixed effect intercept, $\beta 1$ is the effect on the RH of a one-unit change in PBDE level [PBDE_{ij} (ng/g serum)], b_i is the random intercept of the i_{th} individual, and ε_{ij} is the random error.

[a] Dependent variable was not transformed by the natural log. Beta-coefficient yields the absolute change in dependent variable per unit change in PBDE predictor (pg/g serum).

[b] Dependent variable was transformed by the natural log. Exp(beta-coefficient) yields the multiplicative change in dependent variable per unit change in PBDE predictor(ng/g serum).

Table 4

Intraclass correlation coefficients of repeated serum measures of reproductive hormones in men (27 men, 76 serum samples).

Hormones	ICC[a]	ICC[b]
Total T (ng/dl)	0.47	0.50
Free T (pg/ml)	0.38	0.41
Inhibin-B (pg/mL)	0.85	0.89
FSH (IU/L)	0.98	0.98
LH (U/L)	0.71	0.71
Prolactin (ng/mL)	0.54	0.49
SHBG (nmol/L)	0.91	0.92
Inhibin-B/FSH ratio	0.93	0.93
FAI	0.65	0.66

Abbreviations: FAI: free androgen index, FSH: follicular stimulating hormone, ICC: intraclass correlation coefficient, LH: luteinizing hormone, SHBG: sex hormone binding globulin, T: testosterone.

Definitions: Y_{ij} represents the RH level of the ith participant at the jth sampling round. $\beta 0$ is the fixed effect intercept, $\beta 1$ is the effect on the RH of a unit change in blood collection time, b_i is the random intercept of the i_{th} individual, and ε_{ij} is the random error.

[a] Model: $Y_{ij} = \beta 0 + b_i + \varepsilon_{ij}$.

[b] Model: $Y_{ij} = \beta 0 + \beta 1$ Blood collection time$_{ij} + b_i + \varepsilon_i$.