Serum PBDEs and age at menarche in adolescent girls: Analysis of the National Health and Nutrition Examination Survey 2003–2004 ☆

Aimin Chen [a,*], Ethan Chung [a], Emily A. DeFranco [b], Susan M. Pinney [a], Kim N. Dietrich [a]

[a] Division of Epidemiology and Biostatistics, Department of Environmental Health, University of Cincinnati College of Medicine, P.O. Box 670056, Cincinnati, OH 45267-0056, USA
[b] Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA

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ABSTRACT

Background: Polybrominated diphenyl ethers (PBDEs), widely used as flame retardants since the 1970s, have exhibited endocrine disruption in experimental studies. Tetra- to hexa-BDE congeners are estrogenic, while hepta-BDE and 6-OH-BDE-47 are antiestrogenic. Most PBDEs also have antiandrogenic activity. It is not clear, however, whether PBDEs affect human reproduction.
Objectives: The analysis was designed to investigate the potential endocrine disruption of PBDEs on the age at menarche in adolescent girls.
Methods: We analyzed the data from a sample of 271 adolescent girls (age 12–19 years) in the National Health and Nutrition Examination Survey (NHANES), 2003–2004. We estimated the associations between individual and total serum BDEs (BDE-28, -47, -99, -100, -153, and -154, lipid adjusted) and mean age at menarche. We also calculated the risk ratios (RRs) and 95% confidence intervals (CI) for menarche prior to age 12 years in relation to PBDE exposure.
Results: The median total serum BDE concentration was 44.7 ng/g lipid. Higher serum PBDE concentrations were associated with slightly earlier ages at menarche. Each natural log unit of total BDEs was related to a change of -0.10 (95% CI: -0.33, 0.13) years of age at menarche and a RR of 1.60 (95% CI: 1.12, 2.28) for experiencing menarche before 12 years of age, after adjustment for potential confounders.
Conclusion: These data suggest high concentrations of serum PBDEs during adolescence are associated with a younger age of menarche.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of brominated flame retardants introduced in the 1970s (CDC, 2009). They have been added to polyurethane foams used in carpet padding and upholstery, acrylonitrile–butadiene–styrene used in computer and appliance casings, and polystyrene, nylon, and other polymers used in adhesives, wire insulation, and casings (CDC, 2009). Because PBDEs are not chemically bound to the polymer matrices, these compounds may enter the environment. PBDEs, like polychlorinated biphenyls (PCBs), have 209 congeners with different bromine placements on the two phenyl rings, and the chemical properties of these congeners may be different. Lower brominated congeners have longer half-lives in the environment and are more likely to bioaccumulate (Watanabe and Sakai, 2003). Humans can be exposed to PBDEs through the environment via air, dust, and food. Infants may be exposed through breast milk intake, and fetal exposure can occur transplacentally (Lorber, 2008). PBDEs are lipophilic and accumulate in human body fat. Over the last three decades, human exposure levels of PBDEs in the U.S. have increased from virtually nonexistent in 1973 to a mean (or median) of 40–60 ng/g serum lipid in 2003–2004 (Schecter et al., 2005; Sjodin et al., 2008). Children may have 2–3 times higher exposure levels than their parents, probably because of significantly more indoor dust ingestion from hand-to-mouth behaviors (Fischer et al., 2006; Toms et al., 2009). However, the health implication of PBDE exposure, especially in children, is largely unexplored. This data gap needs to be closed because children may have relatively higher exposure on a body weight basis compared with adults and some PBDE congeners are persistent, bioaccumulative, and potentially toxic in human beings (Herbstman et al., 2010; Lorber, 2008). Nevertheless, epidemiologic data sources on measured PBDEs in children in relation to health outcomes are scarce.

Of potential toxicities of PBDEs, endocrine disruption is a major concern in children (Costa et al., 2008; Darnerud, 2008). PBDEs have been shown to function as either agonists or antagonists in binding estrogen and thyroid hormone receptors, and as

antagonists in binding androgen and progesterone receptors (Costa et al., 2008; Hamers et al., 2006). Lower brominated BDEs (e.g., up to hexa-BDEs) are estrogenic, while higher brominated BDEs and some hydroxylated PBDEs (e.g., hepta-BDE and 6-OH-BDE-47) are antiestrogenic (Hamers et al., 2006; Meerts et al., 2001). PBDEs and hydroxylated metabolites can also inhibit estradiol sulfotransferase (E2SULT) and increase bioavailability of endogenous estrogens (Hamers et al., 2006, 2008; Kester et al., 2002). Hydroxylated PBDEs may also inhibit aromatase activity that converts testosterone to estradiol (Canton et al., 2008). Most PBDEs act as antiandrogens in experimental studies (Hamers et al., 2006; Stoker et al., 2005). Animal studies have suggested potential perturbation of puberty and reproductive function in PBDE-exposed rodents (Kuriyama et al., 2005; Lilienthal et al., 2006; Stoker et al., 2004; Talsness et al., 2008, 2005; Tseng et al., 2006). Reproductive hormone disruption by PBDEs has not been specifically studied in humans, but a recent observational study suggested a possible longer time to pregnancy (i.e., reduced fecundability) in exposed women (Harley et al., 2010). Because the potential mechanisms of PBDEs on estrogens and androgens are multifaceted and most related animal studies focused on the onset of puberty, we analyzed the National Health and Nutrition Examination Survey (NHANES) 2003–2004 data to investigate any possible endocrine disruption of PBDEs on age at menarche in adolescent girls. We hypothesized that the serum PBDE concentrations may be associated with alteration in age at menarche.

2. Materials and methods

2.1. Study population

The NHANES 2003–2004 is a nationally representative sample of the civilian non-institutionalized populations in the United States. The survey was conducted by the Centers for Disease Control and Prevention (CDC), including a complex, multi-stage, stratified, clustered sample of 10,122 subjects. A random one-third sample of all participants aged 12 or above was drawn for assessment of brominated flame retardants (BFRs), and 2062 serum specimens were tested for BFRs (Sjodin et al., 2008). In this sample, 1942 subjects (including both males and females) had the most abundant PBDE congeners-28, -47, -99, -100, -153, and -154 measured. We further restricted the sample to female adolescents (aged 12–19 years) to examine the association between serum PBDEs and age at menarche. Because the NHANES used probability sampling techniques, the final sample of 271 adolescent girls represented 11,986,088 in the general population. The research was exempted from review at the University of Cincinnati Institutional Review Board.

2.2. PBDE exposure

The serum concentrations of the 6 above-mentioned PBDE congeners were used as the exposure variables for the analysis. In this sample of adolescent girls, a majority of them (254 out of 271) already had reached menarche prior to blood draw for PBDE measurement. Because the PBDE concentrations were similar during adolescence in this cross-sectional study and the lower brominated PBDEs (e.g., BDE-47, -99, -154) have an estimated half-life of 2–3 years in humans (Geyer et al., 2004; Sjodin et al., 2008), the exposure levels in the adolescents were used to approximate the perimenarcheal exposure levels. The PBDE concentrations were assayed in the National Center for Environmental Health at the CDC using methods reported elsewhere (Sjodin et al., 2004, 2008). In the NHANES datasets, those with PBDE concentrations below the limit of detection (LOD) were assigned a value of LOD/2 (ranging from < 1% for BDE-47 to 45% for BDE-154 in this sample). PBDE concentrations were positively skewed, so we used a natural logarithmic (log) transformation to convert the original PBDE congener concentrations and the sum of the six congeners to logarithmic values, which followed or approximated a normal distribution. The sum of the six congeners was used because the individual PBDEs were highly correlated (correlation coefficients of natural log BDE congeners in unweighted sample 0.54–0.93, p all < 0.0001) and it was difficult to differentiate the association from one congener to the other. Further, these congeners are all lower brominated congeners with no more than 6 bromines (BDE-28 has three bromines, BDE-47 has four, BDE-99 and BDE-100 has five, and BDE-153 and BDE-154 has six). In addition to using natural log transformed PBDE concentrations, we also used quartiles of PBDEs in regression models to examine the exposure–response association.

2.3. Age at menarche

Age at menarche was assessed in a reproductive health questionnaire in the NHANES by the question: "How old were you when you had your first menstrual period?" Of the 271 adolescent girls, 17 had not experienced menarche (14 at age 12 years, and 3 at age 13 years). We analyzed the age at menarche as continuous variable in those who already had menarche. Additionally, we categorized the age at menarche as < 12 years and ≥ 12 years (including those who had not experienced menarche but their age at survey was at least 12 years). For secondary analysis, we also examined the percentage of having menarche less than 11 years, an indicator of early menarche used in other epidemiologic investigations (Freedman et al., 2002), to identify the association of PBDE exposure and pubertal development.

2.4. Statistical methods

We analyzed the association between serum PBDEs and age at menarche using both linear and logistic regression models. Because of the complex and multi-stage sampling used in the NHANES, we used SUDAAN 10.0 (Research Triangle Institute, RTP, NC) for the main analysis after preparing data with SAS 9.2 (SAS Institute Inc., Cary, NC). We report SUDAAN-based results in this report unless otherwise stated. We estimated the regression coefficients and 95% confidence intervals (CIs) in the linear model of age at menarche as a function of natural log PBDEs or quartiles of PBDEs among those who already had experienced menarche. Then, in a model with all study subjects, we calculated the risk ratios (RRs) and 95% CIs of PBDEs in relation to experiencing menarche before 12 or 11 years of age using predicted marginals provided by the PROC RLOGIST in SUDAAN. In all regression models, we adjusted for a priori covariates: age (continuous), race and ethnicity (Non-Hispanic Whites, African Americans, Mexican Americans and others), nativity (U.S. born or not), and poverty income ratio (PIR: < 1, 1-, 2-, 3-) (Euling et al., 2008). Higher body mass index (BMI) at prepubertal ages (5–9) years may increase the likelihood of early menarche (Freedman et al., 2002; Kaplowitz, 2008). Because we did not have premenarcheal BMI in this cross-sectional survey, we considered in a secondary analysis additional adjustment for concurrent BMI z-score (determined based on the 2000 CDC growth curve) at the time of PBDE assessment. We estimated the association of age at menarche and the sum of PBDEs as well as individual PBDE congeners in all regression models. All the statistical tests were two sided with a significance level set at 0.05.

3. Results

In the selected sample of adolescent girls, the median of total BDEs was 44.7 ng/g lipid (geometric mean 45.5 ng/g lipid). The BDE-47 was the dominant congener, with a median of 26.2 ng/g lipid (geometric mean 24.1 ng/g lipid), followed by BDE-153, BDE-99, and BDE-100. Table 1 shows the median and range of six major PBDE congeners and total BDEs in the sample by demographic and socioeconomic characteristics. Study subjects with younger age (12–15 years) had PBDE concentrations similar to those with older age (16–19 years). A breakdown of total PBDE concentrations by age also did not show significant variations in the adolescent girls (Fig. 1). Non-Hispanic Whites had slightly lower total BDE concentrations than Mexican Americans and others ($P < 0.05$). Being born in the U.S. and lower family income was associated with higher PBDE concentrations, but the differences were not statistically significant in this sample.

Table 2 shows the mean age of menarche by total BDE concentrations and sociodemographic factors. The mean age of menarche among those who had experienced menarche was 12.1 years (range 9–15 years). In this sample, the younger age group of adolescents (12–15 years old) was more likely to report having menarche earlier, with slightly younger mean age at menarche (11.8 years) compared with the 16–19 year olds (12.4 years). Table 2 also shows the percentage of adolescents experiencing menarche before 12 years or before 11 years. This percentage counted those adolescents who had not reported to experience menarche at the time of the survey as having menarche ≥ 12 years. From the first to the fourth quartile of total BDE concentrations, the adolescents with higher PBDE exposure had a higher percentage of experiencing menarche before 12 years.

Table 1
Serum congener-specific and total BDE concentrations (median [range] in ng/g lipid) in adolescent girls in the NHANES 2003–2004.

|	n	Weighted n	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	ΣBDEs
Total	271	11,986,088	1.1 (0.2–11.8)	26.2 (2.0–392.0)	5.5 (1.3–131.0)	4.3 (0.6–65.5)	6.1 (0.6–127.0)	0.4 (0.2–7.8)	44.7 (6.4–636.5)
Age (years)									
12–15	144	6,101,117	1.2 (0.3–11.8)	26.3 (3.9–392.0)	5.4 (1.6–131.0)	4.4 (0.6–65.5)	7.5 (1.0–127.0)	0.4 (0.3–7.8)	44.7 (9.1–636.5)
16–19	127	5,884,971	1.1 (0.2–9.9)	24.5 (2.0–212.0)	5.4 (1.3–45.9)	3.8 (0.6–42.0)	5.7 (0.6–60.8)	0.4 (0.2–6.7)	43.5 (6.4–334.3)
Race/ethnicity									
Non-Hispanic Whites	75	7,859,303	1.0 (0.3–9.4)	21.8 (2.0–207.0)	4.6 (1.6–48.0)	3.7 (0.6–45.0)	6.0 (0.8–127.0)	0.4 (0.3–4.4)	39.6 (6.4–334.7)
African Americans	96	1,822,428	1.1 (0.3–9.1)	25.2 (3.6–392.0)	5.7 (1.8–131.0)	4.8 (0.6–65.5)	5.7 (1.0–73.2)	0.5 (0.3–7.8)	45.4 (13.2–636.5)
Mexican Americans and others	100	2,304,357	1.6 (0.2–11.8)	33.3 (3.9–212.0)	7.7 (1.3–54.2)	5.9 (0.7–39.5)	7.0 (0.6–39.9)	0.7 (0.2–6.7)	57.2 (8.3–334.3)
U.S. born									
Yes	241	11,239,439	1.1 (0.3–11.8)	26.2 (2.0–392.0)	5.4 (1.6–131.0)	4.3 (0.6–65.5)	6.2 (0.8–127.0)	0.4 (0.3–7.8)	44.7 (6.4–636.5)
No	30	746,649	1.2 (0.2–6.7)	22.3 (2.5–149.0)	7.7 (1.3–45.9)	4.0 (0.7–17.8)	3.0 (0.6–20.1)	0.6 (0.2–6.7)	37.2 (7.8–227.2)
Poverty income ratio									
<1	109	3,436,424	1.3 (0.2–11.8)	31.5 (2.5–259.0)	8.3 (1.3–98.2)	5.8 (0.6–39.5)	7.8 (0.6–46.4)	0.8 (0.2–6.3)	57.3 (7.8–448.4)
1-	57	2,291,075	1.2 (0.3–9.4)	24.1 (3.6–392.0)	5.4 (1.7–131.0)	4.6 (0.6–65.5)	7.6 (1.1–60.8)	0.5 (0.3–7.8)	50.7 (9.3–636.5)
2-	36	1,433,480	1.1 (0.3–3.2)	19.8 (6.5–74.4)	4.2 (1.8–23.9)	3.3 (1.3–15.5)	4.4 (1.0–42.7)	0.4 (0.3–2.0)	31.4 (16.6–136.9)
3-	69	4,825,109	0.8 (0.3–4.6)	22.1 (2.0–131.0)	4.4 (1.6–50.4)	3.2 (0.6–45.0)	5.0 (0.8–127.0)	0.4 (0.3–3.8)	37.9 (6.4–262.8)
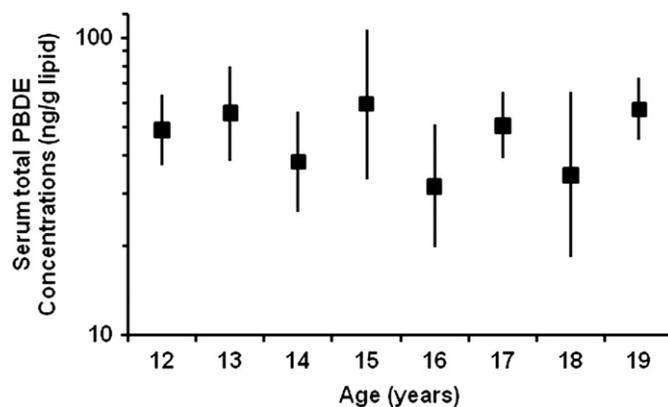
Fig. 1. Serum total PBDE concentrations by age in adolescent girls. The solid squares are geometric means and the lines indicate their 95% confidence intervals.

Table 2
Mean age at menarche (95% confidence interval [CI]) and the percentage of menarche < 12 or < 11 years by PBDE quartiles and other demographic characteristics in adolescent girls in the NHANES 2003–2004.

	Age at menarche[a] Mean (95% CI)	Menarche < 12 years (%)	Menarche < 11 years (%)
Total	12.1 (11.9, 12.3)	24.0	6.0
∑BDE concentrations (ng/g lipid)[†]			
Quartile 1 (< 23.3)	12.4 (12.1, 12.7)	10.0	1.7
Quartile 2 (23.3-)	12.1 (11.6, 12.5)	24.9	7.8
Quartile 3 (44.7-)	12.0 (11.6, 12.3)	24.6	6.3
Quartile 4 (≥ 79.8)	12.1 (11.7, 12.4)	35.8	8.1
Age (years)[‡]			
12–15	11.8 (11.6, 12.0)	30.2	10.1
16–19	12.4 (12.3, 12.6)	17.6	1.7
Race/ethnicity			
Non-Hispanic Whites	12.2 (12.0, 12.3)	21.8	2.5
African Americans	12.1 (11.7, 12.4)	29.0	11.2
Mexican Americans and others	11.9 (11.5, 12.3)	27.7	13.8
U.S. born			
Yes	12.1 (11.9, 12.3)	24.3	5.9
No	12.4 (11.6, 13.2)	19.3	7.9
Poverty income ratio			
< 1	12.3 (12.0, 12.6)	20.8	4.8
1-	12.0 (11.5, 12.5)	24.1	10.0
2-	12.4 (11.9, 12.9)	19.2	2.8
3-	12.0 (11.7, 12.3)	27.6	5.9

[a] Excluding those who had not experienced menarche ($n=17$ in unweighted sample).
[†] $P < 0.05$ for the comparison of the percentage of menarche < 12 years by quartiles.
[‡] $P < 0.05$ for the comparison of mean age at menarche by age groups.

Table 3 shows the regression coefficients and 95% CIs of total and congener-specific BDE concentrations in the linear regression model of age at menarche. For each natural log unit increase in total BDE concentrations, the age at menarche was 0.10 years younger, but it did not reach statistical significance. Compared with the first quartile of total BDEs, the second, third, and fourth quartiles of BDEs all had lower mean age at menarche, with the third quartile being marginally significant. Analysis of individual PBDE congeners yielded slight variation in estimates, but generally lower age at menarche was observed at higher PBDE exposure levels.

In the analysis of dichotomous age at menarche, higher PBDE exposure levels were associated with increased risk of having

Table 3
Association of total BDEs and mean age at menarche in adolescent girls in the NHANES 2003–2004.

PBDE congeners	Independent variable (ng/g lipid)[a]	n	Weighted n	Age at menarche (years) Estimate (95% CI)[b]
$\sum$BDE	Natural log	254	11,384,201	−0.10 (−0.33, 0.13)
	Quartile 1 (< 23.3)	50	2,773,387	Reference
	Quartile 2 (23.3-)	69	2,842,400	−0.32 (−0.81, 0.18)
	Quartile 3 (44.7-)	65	2,791,827	−0.43 (−0.89, 0.04)
	Quartile 4 (≥ 79.8)	70	2,976,585	−0.32 (−0.87, 0.23)
BDE-28	Natural log	254	11,384,201	−0.04 (−0.29, 0.21)
	Quartile 1 (< 0.5)	38	2,264,534	reference
	Quartile 2 (0.5-)	58	2,473,152	−0.08 (−0.57, 0.40)
	Quartile 3 (1.1-)	77	3,454,122	0.05 (−0.31, 0.42)
	Quartile 4 (≥ 1.8)	81	3,192,387	−0.22 (−0.76, 0.32)
BDE-47	Natural log	254	11,384,201	−0.10 (−0.32, 0.12)
	Quartile 1 (< 12.9)	51	2,688,748	Reference
	Quartile 2 (12.9-)	68	2,807,298	−0.35 (−0.79, 0.08)
	Quartile 3 (26.2-)	63	2,917,815	−0.19 (−0.69, 0.31)
	Quartile 4 (≥ 44.1)	72	2,970,339	−0.49 (−0.98, −0.01)[#]
BDE-99	Natural log	254	11,384,201	−0.04 (−0.27, 0.20)
	Quartile 1 (< 2.6)	42	2,469,697	Reference
	Quartile 2 (2.6-)	72	3,099,200	−0.19 (−0.47, 0.09)
	Quartile 3 (5.5-)	70	2,878,160	−0.53 (−0.92, −0.15)[#]
	Quartile 4 (≥ 11.2)	70	2,937,142	−0.30 (−0.82, 0.21)
BDE-100	Natural log	254	11,384,201	−0.12 (−0.32, 0.08)
	Quartile 1 (< 2.3)	45	2,546,390	Reference
	Quartile 2 (2.3-)	67	3,064,596	−0.20 (−0.61, 0.20)
	Quartile 3 (4.3-)	72	2,812,780	−0.64 (−0.97, −0.31)[#]
	Quartile 4 (≥ 8.1)	70	2,960,433	−0.35 (−0.84, 0.13)
BDE-153	Natural log	254	11,384,201	−0.08 (−0.26, 0.10)
	Quartile 1 (< 3.4)	61	2,596,443	Reference
	Quartile 2 (3.4-)	63	3,108,895	0.23 (−0.28, 0.74)
	Quartile 3 (6.1-)	71	2,970,714	−0.21 (−0.78, 0.36)
	Quartile 4 (≥ 11.9)	59	2,708,148	−0.02 (−0.50, 0.45)
BDE-154	Natural log	254	11,384,201	0.00 (−0.29, 0.30)
	Quartiles 1 & 2[c] (< 0.43)	102	5,438,169	Reference
	Quartile 3 (0.43-)	71	2,722,606	−0.41 (−0.79, −0.04)[#]
	Quartile 4 (≥ 1.0)	81	3,223,423	−0.14 (−0. 68, 0.39)

[a] The quartiles of total BDEs and each congener were based on all study subjects (same quartiles used in Table 4).
[b] Adjusted for age, race/ethnicity, nativity, and poverty income ratio.
[c] Quartiles 1 and 2 combined because in the unweighted sample 90 subjects had 0.4 ng/g lipid BDE-154.
[#] $P < 0.05$.

menarche before 12 years of age (Table 4). Each natural log unit of total BDEs had a RR of 1.60 (95% CI: 1.12, 2.28). Compared with the first quartile of total BDEs, the second, third, and fourth quartiles all had higher risk, but only the fourth quartile reached statistically significance. The unadjusted estimates were similar to those in the covariates-adjusted models (data not shown). Each individual PBDE congener was significantly associated with higher risk of experiencing menarche before 12 years in the analysis using continuous PBDE concentrations. Some estimates for quartiles of individual PBDE congener reached statistical significance. For analysis of menarche before 11 years, the RRs were mostly more than unity (i.e., 1) but not statistically significant. Because the percentage of subjects experiencing menarche prior to 11 years was low and the original sample was not large, the estimation of RR for experiencing menarche before 11 years was less precise, with wide confidence intervals.

Because the age range of the adolescent girls in the analysis was 12–19, we further restricted the sample to those with age between 12 and 15 years inclusive to reduce the problem of having PBDE measured too long after menarche. For each natural log unit total BDEs, the regression coefficient was −0.22 (95% CI: −0.43, −0.01) year in the model of continuous age at menarche, and the RR of experiencing menarche < 12 years was 1.84 (95% CI: 1.20, 2.81). By contrast, in adolescent girls at ages 16–19 years, the association was attenuated and non-significant (β=0.02 [95%

CI: −0.29, 0.34] year and RR=1.63 [95% CI: 0.69, 3.84] per log unit total BDEs, respectively).

In the secondary analysis with the inclusion of concurrent sex- and age-specific BMI z-score (continuous variable) as a covariate, the association between PBDE concentrations and age at menarche did not change markedly from the primary analysis. To illustrate, with additional BMI z-score adjustment, each natural log unit total BDEs was associated with −0.16 (95% CI: −0.39, 0.08) year change in mean age at menarche, and with a RR of 1.76 (95% CI: 1.20, 2.60) for having menarche before 12 years of age.

4. Discussion

In this data analysis of adolescent girls in the NHANES 2003–2004, we observed an association between higher serum PBDE concentrations and lower age at menarche. The association was more pronounced in the analysis of categorical age at menarche (i.e., < 12 years vs. ≥ 12 years) that used all available study subjects. The results are from a national cross-sectional survey, rather than a prospective study with measurements of exposure prior to the onset of puberty or menarche. This association needs to be examined in future epidemiologic cohort studies, presumably with exposure levels similar to or higher than the current

Table 4
Association of PBDE congeners and age at menarche in adolescent girls in the NHANES 2003–2004.

PBDE congeners	Independent variable	n	Weighted n	Menarche < 12 years RR (95% CI)[a]	Menarche < 11 years RR (95% CI)[a]
$\sum$BDE	Natural log	271	11,986,088	1.60 (1.12, 2.28)[#]	1.39 (0.59, 3.24)
	Quartile 1	52	2,900,961	reference	reference
	Quartile 2	71	2,990,254	2.30 (0.53, 10.02)	2.88 (0.31, 26.27)
	Quartile 3	73	3,013,957	2.31 (0.70, 7.57)	1.86 (0.16, 21.91)
	Quartile 4	75	3,080,914	3.56 (1.02, 12.49)[#]	2.67 (0.18, 40.63)
BDE-28	Natural log	271	11,986,088	1.29 (1.16, 1.42)[#]	1.34 (0.67, 2.68)
	Quartile 1	40	2,392,108	reference	reference
	Quartile 2	63	2,750,852	5.54 (1.61, 19.26)[#]	5.94 (0.74, 47.59)
	Quartile 3	83	3,563,928	5.68 (2.01, 16.04)[#]	3.29 (0.29, 37.52)
	Quartile 4	85	3,279,198	8.21 (2.75, 24.50)[#]	7.11 (0.67, 76.00)
BDE-47	Natural log	271	11,986,088	1.54 (1.07, 2.23)[#]	1.31 (0.58, 2.97)
	Quartile 1	54	2,942,304	reference	reference
	Quartile 2	72	2,960,837	2.73 (1.07, 6.92)[#]	4.02 (0.36, 44,67)
	Quartile 3	68	3,008,277	1.94 (0.51, 7.42)	2.20 (0.19, 25.96)
	Quartile 4	77	3,074,668	4.17 (1.52, 11.44)[#]	3.56 (0.22, 56.43)
BDE-99	Natural log	271	11,986,088	1.29 (1.03, 1.63)[#]	1.27 (0.64, 2.51)
	Quartile 1	43	2,486,416	reference	reference
	Quartile 2	76	3,377,750	1.52 (0.65, 3.54)	7.30 (0.87, 61.50)
	Quartile 3	76	3,055,977	2.65 (0.83, 8.42)	6.27 (0.45, 87.49)
	Quartile 4	76	3,065,942	3.19 (1.16, 8.74)[#]	7.87 (0.57, 108.47)
BDE-100	Natural log	271	11,986,088	1.46 (1.22, 1.74)[#]	1.51 (0.83, 2.76)
	Quartile 1	47	2,673,964	reference	reference
	Quartile 2	70	3,305,528	6.12 (1.41, 26.69)[#]	1.24 (0.19, 8.11)
	Quartile 3	77	2,908,046	10.95 (3.22, 37.21)[#]	6.51 (0.62, 67.99)
	Quartile 4	77	3,098,549	10.35 (2.74, 39.02)[#]	6.15 (0.36, 106.05)
BDE-153	Natural log	271	11,986,088	1.34 (1.08, 1.66)[#]	1.43 (0.80, 2.55)
	Quartile 1	63	2,731,769	Reference	Reference
	Quartile 2	65	3,147,585	1.01 (0.17, 5.85)	0.72 (0.10, 5.12)
	Quartile 3	74	3,018,716	2.59 (0.89, 7.52)	2.39 (0.47, 12.05)
	Quartile 4	69	3,088,115	1.75 (0.53, 5.73)	1.59, (0.19, 13.51)
BDE-154	Natural log	271	11,986,088	1.23 (1.13, 1.33)[#]	1.51 (1.05, 2.17)[#]
	Quartiles 1 & 2[b]	107	5,806,674	Reference	Reference
	Quartile 3	76	2,807,447	2.98 (1.54, 5.79)[#]	2.47 (0.57, 10.70)
	Quartile 4	88	3,371,966	2.35 (1.36, 4.08)[#]	3.42 (0.50, 23.59)

[a] Adjusted for age, race/ethnicity, nativity, and poverty income ratio.
[b] Quartiles 1 and 2 combined because in the unweighted sample 95 subjects had 0.4 ng/g lipid BDE-154.
[#] $P < 0.05$.

study (e.g., the Breast Cancer and the Environment Research Center cohorts) (Windham et al., 2010).

In peripubertal animals exposed to a commercial PBDE mixture DE-71, decreased weight of androgen-dependent tissues (seminal vesicle and ventral prostate) and delayed preputial separation in males as well as delayed vaginal opening in females were observed (Stoker et al., 2004). Gestational exposure to BDE-99 reduced sperm and spermatid counts in male offspring and altered ultrastructure of ovary mitochondria morphology in female offspring (Kuriyama et al., 2005; Talsness et al., 2005). Another study of gestational exposure to BDE-99 found delayed puberty onset in female offspring but slightly accelerated puberty onset in male offspring with low dose exposure (Lilienthal et al., 2006). PBDE-47 or -99 exposure also reduced ovarian follicle numbers and decreased circulating estradiol concentrations (Lilienthal et al., 2006; Talsness et al., 2008). Postnatal exposure to BDE-209 was also associated with decreased epididymal sperm function in male mice (Tseng et al., 2006). In the experimental studies, it is difficult to determine the estrogenic or antiestrogenic properties of PBDEs using dose regimens that strictly mimic human exposure, which extends from prenatal period, early postnatal period, childhood, to peripuberty but with different doses and combinations of certain PBDE congeners. The findings on puberty in animals were either from peripubertal exposure (postnatal day 22–41) to DE-71 (a PBDE mixture of BDE-47, -99, -100, and few other congeners) (Stoker et al., 2004) or in utero exposure to BDE-47 or -99 at gestational day 6 or 10–18 (Lilienthal et al., 2006; Talsness et al., 2008, 2005). The dose in these animal studies ranged from 60 µg/kg body weight to 60 mg/kg body weight (Lilienthal et al., 2006; Stoker et al., 2004; Talsness et al., 2008, 2005). In contrast, the average adult intake dose of total PBDEs was estimated to be 7.7 ng/kg body weight in the U.S. and the child intake was about 49.3 ng/kg body weight at age 1–5 years, 14.4 ng/kg body weight at age 6–11 years, and 9.1 ng/kg body weight at age 12–19 years (Lorber, 2008).

The differences in timing, congener mixture, and dose of PBDE exposure between experimental animals and humans did not necessarily mean different findings on puberty. However, we observed an earlier age at menarche in adolescent girls associated with high serum PBDE concentrations, which is inconsistent with study findings in female experimental animals. Animal studies and epidemiologic research also differ in the association between PBDE exposure and thyroid hormones, with an inverse association (mostly total T_4) in experimental animals and a positive association (mostly free T_4) in human adults (Chevrier et al., 2010; Kodavanti et al., 2010; Kuriyama et al., 2007; Meeker et al., 2009; Tseng et al., 2008; Turyk et al., 2008; Zhou et al., 2001). Thyroid hormones have certain regulatory roles in female reproduction. Prepubertal hyperthyroidism may advance age at menarche or hypothyroidism may delay the onset of puberty, but the literature

is not consistent (Cassio et al., 2006; Krassas, 2000; Saxena et al., 1964). Because PBDE congeners have various properties in disrupting estrogens, androgens, and thyroid hormones, reproductive health outcomes should be examined in more relevant studies. In humans, these outcomes include onset of puberty, menstrual function, fertility, pregnancy outcomes, and steroid hormonal profiles.

Few studies investigated reproductive endpoints in humans in relation to PBDE exposure. In a small study of organic pollutants in The Netherlands, PBDEs were not associated with age at menarche, but the median total PBDEs were only 8.2 ng/g lipid in the serum samples and the number of girls was merely 9 (Leijs et al., 2008). Three studies analyzed the association of PBDE exposure and menstrual cycle characteristics in adults (Chao et al., 2010, 2007; Harley et al., 2010). The U.S. study found delay in time to pregnancy but no changes in menstrual cycle function (Harley et al., 2010). The two studies in Taiwan were relatively small but observed longer menstrual cycle length and decreased birth weight and length in relation to breast milk total PBDE concentrations (Chao et al., 2010, 2007). One of these two studies retrospectively examined difference in breast milk PBDE concentrations by age at menarche (≤ 12 years or > 12 years) and did not find a significant association (Chao et al., 2010). That study was small ($n=46$) and the breast milk total PBDE concentrations were low (3–4 ng/g lipid) (Chao et al., 2010). Prior studies on environmental exposure to other persistent organic pollutants (POPs) have suggested potential role of PCBs and dichlorodiphenyldichloroethylene (DDE) in advancing age at menarche, however, the evidence is still limited and inconsistent. This study of PBDEs and age at menarche provides additional information regarding POPs and age at menarche, but certainly more research is needed.

This is a relatively large study to explore the association between PBDE exposure and puberty in adolescent girls. However, it has several limitations that need to be considered. First, the cross-sectional design of NHANES did not allow for a prospective observation of association after prior exposure. We used serum PBDE concentrations measured after menarche to approximate the perimenarcheal concentrations. A sensitivity analysis restricting the sample to 12–15 year olds did not change the interpretation, but the association was not evident in 16–19 year olds. It is plausible that the early postmenarcheal PBDE concentrations were closer to that prior to menarche. It may also be that the random error or smaller sample size can explain the differences in results between 12–15 and 16–19 year olds. However, it is unlikely that the exclusion of 17 girls who had not experienced menarche (at ages 12–13 years) produced the association because the categorical analysis took into account all study participants. Second, age at menarche is often the concluding event of the puberty process, and is not sensitive enough to capture possible alterations in growth spurt, breast development, and pubic hair staging. The age at menarche was self-reported by the study subjects (in 16–19 year olds) or family members (in 12–15 year olds) in discrete years and it was not precise enough to document months of the year. Recall of age at menarche after a short interval, however, is usually accurate in epidemiologic surveys (Bean et al., 1979; Koo and Rohan, 1997). Third, we were not able to adjust for hereditary components of age at menarche, e.g., mother's age at menarche or a proxy of genetic markers of pubertal development. Age at menarche has an estimated heritability of 0.5–0.8 (Parent et al., 2003; Treloar and Martin, 1990). But unless there is significant interaction between PBDE exposure and genetic markers of puberty, the association of age at menarche with PBDE exposure would not be markedly affected. Nevertheless, we adjusted for other potential determinants of age at menarche including race, age, nativity, and socioeconomic

status. Although we did not have the prepubertal BMI, a separate model with concurrent BMI did not change the estimates of PBDEs significantly. Because the PBDE body distribution may depend on body size, we additionally attempted to adjust for height, but the estimates of PBDEs did not change markedly. Despite the limitations, the study used currently available data from the NHANES for serum PBDEs, which is still a valuable resource before the emergence of large cohort data that measure these environmental compounds. We were able to analyze the outcome in both continuous and categorical analysis, with adjustment for potential confounds. We described the association for total BDEs as well as individual congeners, and the results can be easily compared with future studies addressing this topic.

In conclusion, in this analysis of NHANES 2003–2004 data, current exposure levels of PBDEs are associated with earlier age at menarche in the U.S. adolescent girls. This association, however, should be verified in prospectively designed studies.

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