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In utero exposure to polychlorinated biphenyls (PCBs) and age at menarche in a contemporary cohort of British girls

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Environmental Health and Epidemiology

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Abstract

In utero exposure to polychlorinated biphenyls (PCBs) and age at menarche in a contemporary cohort of British girls

By Heather Christine Freiman

Polychlorinated biphenyls (PCBs) are persistent organic chemicals widely used as coolants in electronics until the 1980s. Despite being banned for decades, PCBs are frequently detected in human serum and have demonstrated endocrine modulating effects. The authors conducted a nested case-control study, with data from the Avon Longitudinal Study of Parents and Children (ALSPAC) in the United Kingdom, to examine the association between *in utero* PCB exposure and age at menarche. Cases were chosen from girls reporting menarche before 11.5 years (n=218) and controls were a random sample of the remaining girls not reporting menarche before 11.5 years (n=230). Maternal serum samples were collected during pregnancy (1991-1992); these samples were analyzed for PCB congeners using solid phase extraction followed by gas chromatography isotope dilution high resolution mass spectrometry. Multivariate logistic regression was used to assess the association between maternal serum PCB concentration during pregnancy (proxy for *in utero* PCB exposure) and odds of earlier age at menarche in daughters.

Most samples had detectable levels of all PCB congeners, and the most prevalent congeners were PCBs 118, 138-158, 153, 170, and 180 (total median serum concentration 184.10 ng/g lipid). The total PCB concentration varied slightly, but not significantly, with maternal and child characteristics. For analyses, PCB congeners were grouped by proposed endocrine action and homolog classification in addition to total PCB level. Logistic regression models adjusted for maternal age at menarche, pre-pregnancy BMI, age at delivery, and parity. All PCB groups, analyzed as continuous variables, were associated with decreased odds of earlier age at menarche (e.g. adjusted odds ratio [aOR] Total PCBs = 0.68, 95% confidence interval 0.34, 1.34). When the PCB groups were analyzed as categorical variables, the association with earlier age at menarche was less consistent. All 95% confidence intervals for the ORs included the null value of 1.0.

Although most ALSPAC study participants had detectable PCB levels, PCB exposure was not significantly associated with age at menarche in offspring; these findings suggest that *in utero* PCB exposure is not associated with earlier age at menarche.

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Background

Early Menarche

Puberty marks the beginning of sexual maturation in humans and is a key period of growth and development. The major physical hallmarks of pubertal development (i.e. secondary sexual characteristics) in females are thelarche (onset of breast development), pubarche (onset of pubic hair), and menarche (first menses). Although these developmental stages are largely under genetic and hormonal control, growing research suggests that environmental and lifestyle factors (e.g. nutrition) also influence timing. Over the past few centuries, age at menarche has decreased, and there is a trend towards earlier sexual development among girls in the United Kingdom (1). Currently, age at menarche in European countries is cited as between 12 and 13 years (2, 3). Within the Avon Longitudinal Study of Parents and Children (ALSPAC) in the United Kingdom, an on-going prospective study, the median age at menarche was 12.93 years (95% CI: 12.89, 12.98), with sixty-percent of the girls achieving menarche by age 13 (1). An analysis of National Health and Nutrition Examination Study (NHANES) data shows that mean age at menarche among study participants in the United States decreased from 13.3 years among individuals born before 1920 to 12.4 years among individuals born in the early 1980s (4). Earlier puberty and menarche are of concern because they have been related to later health outcomes such as breast cancer, asthma, depression, and earlier onset of sexual behavior (3, 5-7).

The biological mechanism for timing of menarche in humans is not well understood, but menarche often occurs after pubarche (the traditional start of puberty) (8). The major endocrine feedback loop implicated in pubertal events, and menstrual function in women, is the hypothalamic-pituitary-gonadal axis (HPG axis) (8). The HPG axis involves gonadotropin releasing hormone (GnRH) release from the hypothalamus to stimulate

hormone release from the anterior pituitary (follicle-stimulating hormone [FSH] and lutenizing hormone [LH]) (8). These hormones also interact with estrogens (17β-estradiol [E2]) produced by, and released from, the ovaries (8). Maturation of the ovaries and ovarian function depend on FSH and LH, and cyclic E2 levels are necessary for ovulation (8). While this is the main system, and hormones, that triggers and maintains pubertal development and growth, many other systems and hormones have a role (9).

Pubarche is initiated by a change in the frequency of GnRH release from the hypothalamus which activates the HPG axis (8). The higher GnRH levels trigger release of FSH and LH from the anterior pituitary, and E2 levels also increase after the onset of puberty (8). Although menstruation is often associated with ovulation, menarche usually occurs before ovulation starts; therefore, it is unclear which of the hormonal factors, and the order of their action, are most critical for menarche (8). Despite this uncertainty, alteration of HPG axis function and associated hormones can affect timing of menarche and puberty (10).

Much of menarche timing is under genetic control, and studies find that girls whose mothers had earlier age at menarche are also likely to have earlier age at menarche (3). Additionally, genes associated with estrogen have been linked to age at menarche (e.g. ESR1, CYP19A1, SHBG-gene) (3). Race and ethnicity are associated with different ages at menarche and puberty (11-12). A longitudinal study of pre-pubertal girls in the U.S. by Biro et al. (11) found that black girls achieved menarche at a slightly younger age (mean onset = 12.0 years) than white girls (mean onset = 12.6 years). Cross-sectional studies also find that black girls experience menarche a few months before white girls (4, 13-14). These consistent differences in age at menarche among racial/ethnic groups may have a genetic origin or could be due to particular lifestyle/cultural factors. Some studies suggest that first born children have an earlier age at menarche/puberty (15).

Child factors relating to age at menarche include body mass index (BMI: weight in kilograms/height in meters^2) and weight from 7-9 years, race/ethnicity, birth order and birth weight. Several studies find that girls with higher BMI between 7 and 9 years of age (before puberty) have menarche and puberty at an earlier age than their peers with lower BMI (16). A certain amount of adipose tissue is necessary for menarche to occur; therefore, it is not surprising that higher BMI would be positively associated with earlier age at menarche (2). It is still unclear if this relationship is causal; additional studies indicate that tempo of growth in the first few years of life is responsible for both higher childhood BMI and age at menarche (2, 17-19). Many factors could be interacting in these relationships such as nutrition, general activity levels, and hormones. Leptin levels (associated with adiposity) have been found to be associated with both puberty onset and body size, suggesting that these relationships may stem from a common causal factor (2).

Although much about the mechanism for menarche remains unclear, alteration of HPG axis function is likely to affect age at menarche and puberty. Therefore, exposure to endocrine modulating environmental chemicals may affect the timing of menarche and puberty (20). It is also biologically plausible that exposures to these chemicals during gestation may affect later reproductive function and development, as demonstrated in animal studies (20). Critical time periods for fetal development of the neural and reproductive systems occur early in gestation (weeks 4-12) and disruption of these processes may influence later function (20).

Endocrine modulating chemicals are human-made substances that alter normal hormone function (e.g. by mimicking or blocking endogenous hormone action), and there is growing concern about the long term effects of exposure to low doses of these chemicals present in the environment. Although the presence of these chemicals in the environment has been recognized for decades, knowledge about the endocrine modulating properties of

chemicals is constantly being revised. Many chemicals have been identified via "natural" animal studies, which are then followed-up with short term, high-dose animal studies. Even though many biological pathways are conserved across species, these animal models are not a direct surrogate for human outcomes following exposure, especially to long-term low doses like those found in environmental exposures (20). Chemicals may interact, bioaccumulate, sequester in body organs, and have differential effects depending on at what point in an individual's life they are exposed. Previous studies have found that exposure to lead, pesticides, and dioxin near puberty affect progression through pubertal stages and may alter age at menarche (21). Rodents given E2 *in utero* often undergo first estrus earlier, suggesting that exposure to estrogen-mimicking chemicals during the gestational period could have a similar effect (20). Additionally, some studies find that combinations of low levels of weak estrogenic chemicals have an additive effect on overall estrogenicity (22).

Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are a class of commercially manufactured persistent organo-chlorines that have been shown to affect the endocrine and reproductive systems in both human and animal studies. PCBs (e.g. Aroclor) were widely used as coolants and heat transfer agents in electronic equipment until the 1980s (23-24). Even though PCB production in the U.S. ended in 1979, PCBs remain in the environment and are still detected in human and animal tissue (23-24). The Stockholm Convention on Persistent Organic Pollutants placed a world-wide ban on PCBs in 2001; however, older products may still contain PCBs (24). Recent NHANES data confirms the ubiquity of PCB exposure among the general U.S. population; of 38 PCB congeners measured, almost everyone tested had at least 34 detectable in serum samples (24). The half-life of congeners varies, but is on the order of months to years, and the persistence of PCBs is one of the reasons people are

concerned about them (24). Individuals can be exposed to PCBs through inhalation, ingestion, and dermal absorption; PCBs also cross the placental barrier, which may result in fetal exposure (25). In the U.S., the estimated dietary intake of PCBs for an average adult was less than 0.001 μg/kg/day in 1991 (23). Individuals are mainly exposed to PCBs via their diet, and they are most often detected in fatty fish, dairy products, and meat (23-24).

Commercial products were formed of mixtures of congeners; approximately 209 congeners exist and about 130 congeners were used in commercial mixtures (23). Around 37 congeners have been found to be biologically relevant (23). Different congeners of PCBs have different biological effects, which may be predicted by PCB structure. All PCBs have a two phenyl (benzene) ring structure, but they vary in the number and placement of chlorine atoms, and can have 1-10 chlorines attached to the carbon atoms on the benzene rings (Appendix Figure 1) (23-24). The conformation of the ring structure changes with the degree, and location, of chlorine substitution (23). PCBs also metabolize to a hydroxylated form (OH-PCBs), and it is still unclear whether endocrine modulating actions are due to the parent compounds (PCBs) or their metabolites (OH-PCBs) (26-28).

While *in utero* PCB exposure seems to primarily affect immune function, reproductive effects have been found following *in utero* exposure in human and animal studies (24, 29). PCBs, and other chlorinated compounds, have also been linked to reproductive difficulties (e.g. altered sex ratio, increased risk of miscarriage or having a stillborn infant) (24). PCBs with a restricted conformation are more likely to have estrogenic actions (26). Research indicates that estrogen receptor (ER) affinity is most associated with PCBs that have multiple chlorines in the ortho positions (rigid planar conformation similar to E2) and PCBs with chlorines at the ortho and para positions (29). Additionally, PCBs without chlorines at the vicinal para-meta positions (i.e. para and meta chlorines that are adjacent to one another) often undergo hydroxylation, where a hydroxyl

group replaces one of the chlorine molecules (29). As mentioned previously, several studies suggest that these hydroxylated PCBs (OH-PCBs) have a stronger affinity for the ER than do their parent PCBs (27, 29). Wolff (30) initially proposed that the following PCBs have estrogenic activity: 31, 70, 44, 49, 52, 101, 174, 177, 187 and 201, and more recent studies have confirmed PCBs that act at the ER. deCastro et al. (29) screened 34 PCB congeners for estrogenic activity, and found thirteen that were weakly estrogenic (PCBs 17, 18, 30, 44, 49, 66, 74, 82, 99, 103, 110, 128, 179). Layton et al. (27) found that OH-PCBs had a much higher affinity for the ER than did individual PCB congeners and Aroclor mixtures when tested with a yeast assay. Pliskova et al. (31) examined PCBs found in human serum, using a cell assay, and they found that PCBs 28, 52, 66, and 74 activated the ER. PCBs 99 and 105 showed weak activation of the ER. This study also found that certain PCBs decreased E2 activity at the ER: PCBs 138, 153, 170, 180, 187, 194, 199, and 203; PCBs 199, 203, and 153 showed the most inhibitory activity (31). Bonefeld-Jørgensen et al. (32) found that PCBs 138, 153, and 180 blocked action of E2 at the ER.

PCBs with a planar or co-planar ring conformation (single ortho substituted chlorine, or no ortho substituted chlorines) can exhibit dioxin-like effects (23). These PCBs have a similar conformation to 2,3,7,8-tetrachlorodibenzodioxin (TCDD), and can act through the aryl hydrocarbon receptor (AhR). PCBs with dioxin-like actions are given toxic equivalency factors (TEF) by the World Health Organization (WHO); these PCBs include: 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189 (33). Of these PCBs, those with the highest TEFs (most potent dioxin-like action) are PCBs 126 and 169 (33). To get an overall measure of dioxin-like activity in a sample, the TEFs are multiplied by the concentration of their respective congener and then summed to get a toxic equivalency quotient (TEQ) (33). The AhR is a transcription factor, and its activation upregulates gene expression; many of its associated genes are involved in metabolism of compounds (34). Through the AhR dioxin,

and dioxin-like compounds, can antagonize ER signaling, leading to an overall anti-estrogenic effect of the chemicals (34). However, a recent study suggests that the interaction between the AhR and ER is more complex, and that some ER functions may depend on a substrate of the AhR (34).

Studies find that PCBs cross the placental barrier in humans, and rat dams exposed to a non-ortho substituted PCB (PCB-126) during pregnancy birthed offspring with altered pubertal timing (35-36). Human studies examining the association between PCBs and the timing of puberty and menarche are contradictory; this lack of consistency may be due to analysis of PCB congeners as a group rather than consideration of PCBs with similar characteristics separately (21). Some studies have found that *in utero* exposure to PCBs has no effect on the age at menarche (37-39); however, the study by Gladen et al. (38) found a non-significant trend towards earlier maturation among the highest exposed girls. A cross-sectional study by Denham et al. (40) found that girls with current higher levels of estrogenic PCB congeners were more likely to have reached menarche at the time of the study than girls with lower exposure. PCBs also alter thyroid hormone levels, which could impact age at menarche (24, 41). Experimental studies find that some PCBs bind to a critical thyroxine (a thyroid hormone) transport protein, and observational studies found a correlation between higher PCB levels and lower thyroid hormone levels (24). PCB exposure has also been associated with factors that relate to earlier age at menarche. Some studies found that children with *in utero* PCB exposure had lower birth weight and may have slowed prenatal growth (24-25, 41).

Introduction

Puberty marks the beginning of sexual maturation in humans and is a key period of growth and development. The major physical hallmarks of pubertal development (i.e. secondary sexual characteristics) in females are thelarche (onset of breast development), pubarche (onset of pubic hair), and menarche (first menses). Although these developmental stages are largely under genetic and hormonal control, growing research suggests that environmental and lifestyle factors (e.g. nutrition) also influence timing. Over the past few centuries, age at menarche has decreased, and there is a trend towards earlier sexual development among girls in the United Kingdom (1). While improved nutrition and certain biological factors are known to be associated with timing of menarche (e.g. BMI, maternal age at menarche, race or ethnicity, birth order, and birth weight), it is also possible that environmental chemicals contribute to earlier menarche (5, 17, 37, 42). Of particular interest are chemicals believed to have endocrine modulating effects and to which individuals are exposed at low levels throughout their life, such as persistent organic pollutants. Endocrine modulating chemicals are human-made substances that alter normal hormone function (e.g. by mimicking or blocking endogenous hormone action), and there is growing concern about the long term effects of exposure to low doses of these chemicals present in the environment.

Even though the biological mechanism for timing of menarche in humans is not well understood, the hormone cascade of the hypothalamic-pituitary-gonadal (HPG) axis is integral (10). Therefore environmental chemicals that alter HPG axis function, or its associated hormones, may affect the timing of menarche and puberty. It is also biologically plausible that *in utero* exposures can affect later reproductive function. Maternal exposures during development of the fetal neural and reproductive systems (weeks 4-12 of gestation) could influence their later function. Additionally, animal studies demonstrate that

gestational exposures affect later reproductive function. Earlier puberty and menarche are of concern because they have been related to later health outcomes such as breast cancer, asthma, depression, rheumatoid arthritis, earlier onset of sexual behavior, and increased risk taking behavior (3, 5-7).

Polychlorinated biphenyls (PCBs) are a class of commercially manufactured persistent organo-chlorines that have been shown to affect the endocrine and reproductive systems in both human and animal studies. PCBs were widely used as coolants in electronic equipment until the 1980s; even though production in the U.S. ended in 1979, PCBs remain in the environment and are still detected in human and animal tissue. Individuals can be exposed to PCBs through inhalation, ingestion, and dermal absorption; PCBs also cross the placental barrier, which may result in fetal exposure (25).

PCBs act through various mechanisms; exposure modulates thyroid hormones and estrogen (25) and reproductive system development in rodents (36). Human studies on the effect of *in utero* PCB exposure on the timing of puberty and menarche are contradictory. Most studies suggest that *in utero* exposure is associated with earlier menarche or that there is no association (21). A cross-sectional study found that girls with current exposure to higher levels of estrogenic PCB congeners were more likely to have reached menarche at the time of the study than girls with lower exposure (40). The lack of consistency in previous study findings may be due to analysis of PCB congeners as a group rather than consideration of PCBs with similar biological characteristics separately (21). Recent technologic advances allow individual congeners to be measured easily within a sample, enabling specific comparisons between congener exposure and outcome of interest. Here, a nested case-control study was conducted with data from a current prospective cohort in the United Kingdom (the Avon Longitudinal Study of Parents and Children [ALSPAC]) to examine the association between *in utero* exposure to PCBs and age at menarche. In

addition to total PCB exposure, PCB congeners were grouped based on their proposed mechanism of action and structural similarities. It was expected that girls exposed to higher concentrations of estrogenic PCBs *in utero* would be more likely to have an earlier age at menarche compared to girls exposed to lower concentrations of estrogenic PCBs.

Materials and Methods

The Avon Longitudinal Study of Parents and Children (ALSPAC) is an ongoing prospective cohort study administered by Bristol University in Great Britain (43). The ALSPAC study was designed to evaluate the relationship between genotype and environmental exposures on long-term development and health (43). This population comprises approximately 14,000 women living in Avon (UK) who were pregnant in 1990-1992, and had an expected delivery date between April 1, 1991 and December 31, 1992, and their children (1, 43). At various points after enrollment, women completed questionnaires to obtain demographic information (43). Biological samples were collected from the women at multiple points during pregnancy and after child birth (43). At delivery, and at later ages, children underwent physical examinations and filled out questionnaires/had questionnaires completed about them (43). Beginning at age 8, children completed 'Growing and Changing' questionnaires about growth and puberty (15, 17). Further details on recruitment and study methods have been published previously (1, 43-44).

Girls completed 'Growing and Changing' questionnaires between 8 and 17 years of age (1999-2008) that collected self-reported information about Tanner Stages, menarche status, and age at menarche (1). Cases, girls with earlier age at menarche, and controls were a random sample of girls who did not report earlier age at menarche selected using previously reported methods (15). Briefly, of 14,610 live births, cases and controls were selected from single births (n=11,820) of females (n=5,756). Girls who completed at least two puberty staging questionnaires between 8 and 13 years of age were eligible (15).

These girls were ordered according to age at menarche and a cut-off of 11.5 years was set for earlier menarche (15). Among girls reporting menarche before 11.5 years (n=338, potential cases), 240 (71%) had prenatal maternal serum samples (15). A random sample (n=394) of girls reporting menarche at or after 11.5 years (potential controls) was chosen; 282 (71.6%) of these girls had a prenatal maternal serum sample (15). Integrity of the maternal serum samples was assessed: 90.8% (n=218) of the cases and 81.6% (n=230) of the controls had useable samples. These serum samples were collected from mothers during pregnancy, and if multiple samples were available, the earliest one was used. Since PCBs are persistent with relatively long half-lives, it is reasonable to assume that serum levels are fairly stable throughout pregnancy (24). For the current study, cases were selected from female offspring who reported menarche before 11.5 years of age and for whom maternal PCB levels were available. Controls were a random sample of girls who reported menarche at or after 11.5 years of age who had maternal PCB levels (Figure 1).

Maternal serum samples were removed from storage facilities at the University of Bristol and sent to the National Center for Environmental Health (NCEH) at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA (15, 45). Serum samples were analyzed for PCB congeners using solid phase extraction followed by gas chromatography isotope dilution high resolution mass spectrometry (45); methodology previously published by Sjödin et al. (46). Two pairs of congeners (138-158 and 196-203) could not be distinguished from each other, therefore each pair was considered as a single congener, with a single concentration, in all analyses. All data analyses were conducted using SAS software Version 9.2 of the SAS System for Windows XP (47). All PCB congeners measured in maternal serum are listed in Appendix Table 1.

When PCB congeners were detected in at least fifty-percent of the samples, levels below the level of detection (LOD) were imputed using $LOD/\sqrt{2}$, followed by a distribution

analysis of the resulting estimates (15). When more than fifty-percent of the values were below the LOD, the levels were input as zero rather than imputed. The association between age at menarche and lipid-adjusted PCB congeners were assessed by summation using the following groupings: 1) estrogenic action; 2) anti-estrogenic action; 3) dioxin-like status (mono-ortho substituted); 4) structural similarity (homologs based on chlorine number); 5) the four most prevalent congeners generally found in serum; 6) the five most prevalent congeners in the samples; and 7) total PCBs (Table 2). The distributions of the lipid adjusted PCB congeners and summary variables were skewed, so the values were transformed using the natural logarithm to approximate normality when the exposure was continuous. Congeners were examined as continuous variables (lipid adjusted concentrations that were log-transformed) and also as categorical variables (lipid adjusted concentrations divided into tertiles).

A literature search identified potential covariates related to age at menarche. Factors associated with onset of menarche are: child's BMI between 7-9 years (<13.6 [underweight], 13.6-18.4 [normal], 18.41-20.59 [overweight] and ≥ 20.6 [obese]) (48), child's birth order (first born, second born or later), child's size at birth/growth in the first 18 months, maternal age at menarche, maternal pre-pregnancy BMI (<18.5 [underweight], 18.5–24.9 [normal], 25–29.9 [overweight], and ≥30 [obese]), maternal age at delivery, maternal education (certificate of secondary education [CSE]/none, vocational, O-level, A-level, degree), maternal social class (lower, middle, upper), child's ethnic background (white, non-white), child's nutrition and activity levels, and environmental exposures. Size for gestational age and preterm birth are also believed to be associated with PCB exposure (25). Social class was previously categorized based on the United Kingdom's 1991 Office of Population Censuses and Surveys (49). Upper class comprised classes I (professional occupations) and II (managerial and technical occupations), middle class was classes IIINM

(non-manual skilled occupations) and IIIM (manual skilled occupations), and lower class was classes IV (partly skilled occupations) and V (unskilled occupations) (15). Certain covariates were not considered in the primary analyses because they may be on the causal pathway between *in utero* PCB exposure and age at menarche: child's BMI at age 8, gestational age at birth and birth weight. The effect of these covariates was examined in secondary analyses. Covariates assessed as potential effect measure modifiers with the PCB exposure variables were child's birth order (maternal parity) and maternal age at menarche.

Confounding was considered by three main methods. First, a directed acyclic graph (DAG) was created to examine the relationships between the potential confounders, *in utero* PCB exposure, and earlier age at menarche (Appendix Figures 2a-c). Next, univariate analyses were conducted to examine the association between the exposure and outcome (separately) and each potential confounder (Appendix Tables 3a-b). Binary logistic regression was used to examine the association between earlier age at menarche (outcome) and each potential confounder (Appendix Table 3a). The magnitude of the OR and its 95% confidence interval (95% CI) was used to determine whether there was an association, p-values for the significance of the model itself were also examined. Variables were further considered for confounding if the OR was non-null (null value = 1.0), if the 95% CI did not include 1.0, or if the p-value for the model was less than 0.30. Linear regression was used to examine the relationship between PCB levels and each potential confounder among the controls (girls without early menarche). PCB levels were estimated with summary variables grouped by proposed PCB action (Table 2); the lipid adjusted concentrations were transformed with the natural logarithm to approximate normality. Additionally, multivariate logistic regression models were used to determine if the effect estimate changed when multiple covariates were included in the model.

Variables associated with earlier age of menarche and PCB levels were considered to be potential confounders and were included in multivariate logistic models to examine the association between maternal serum PCB concentration during pregnancy and earlier age at menarche among daughters. All potential confounders were considered in a multivariate logistic regression model and backwards elimination was used to further consider confounders and effect measure modifiers. Terms other than the main exposure (PCB group) were removed from the model if there was not a change in the OR when they were removed from the model (<10% change); their significance in the model was also considered (inclusion criterion, $p<0.30$). Human subject protection was assessed and approved by the ALSPAC Law and Ethics Committee, the Local Research Ethics Committees, and CDC Institutional Review Board.

Results

Within the cohort, most mothers were of higher social class and were likely to have completed some higher education; however, information on social class was missing for approximately 20% of the cases and controls (Table 1). Mothers of cases often had an earlier age at menarche: 28.9% reported menarche between 9 and 11 years of age, compared to 13.5% of controls ($p= 0.0004$, Table 1). Mothers of cases were also more likely to have higher pre-pregnancy BMI: 19.3% were overweight and 8.7% were obese compared to 8.7% and 5.2% of the controls, respectively ($p=0.00015$, Table 1). Cases were more frequently the first born child than controls (50.46% of cases and 42.17% of controls, $p=0.048$, Table 1). Overall, there was little racial/ethnic diversity among the individuals in this sample; however there were more non-white girls among the cases (5.5% compared to 1.3% among the controls, $p=0.0156$, Table 1). Cases were more likely to have higher BMI at 8 years of age compared to controls (20.6% were overweight and 18.8% obese, compared to 10.4% and 7.0% of controls respectively, $p<0.0001$); however, data on BMI at 8 years of

age was missing for over 20% of both the cases and controls. There was no significant difference in birth weight (p=0.2077) or gestational age at birth (p=0.4678) between cases and controls (Table 1). Approximately 49% of the controls had achieved menarche by the time of selection (median 12.42 years); the median age at menarche among cases was 11.08 years.

The most prevalent congeners in the sample were PCBs 118, 138-158, 153, 170 and 180 (Table 3) with a median concentration of 184.10 ng/g lipid (IQR 139.60-244.10 ng/g lipid); these same congeners are also major components of the Most Common PCB and Anti-Estrogenic PCB groups (Table 2, 3). Median concentrations of lipid adjusted PCB groups varied slightly for cases and controls, with controls having higher median concentrations for all of the PCB groups except for the Estrogenic PCBs (Table 3). Total lipid adjusted PCB values were also examined for white women aged 18-55 years old within the 2003-2004 National Health and Nutrition Examination Survey (NHANES)**;** the geometric mean total lipid adjusted PCB level among these women was 106.8 ng/g lipid (95% CI 94.52, 120.68) (50-52). Levels of total PCBs were higher for the ALSPAC study population than for NHANES (Figure 2).

The median total PCB concentration among study participants was 309.58 ng/g lipid (IQR 241.30-394.20 ng/g lipid), which varied slightly, but not significantly, with maternal and child characteristics (Table 4). Total PCB levels increased as maternal age at delivery increased and as mother's highest education increased (Table 4). PCB levels decreased as maternal pre-pregnancy BMI increased, as child's BMI at 8 years increased and as gestational age at delivery and birth weight increased (Table 4). Children with a non-white ethnic background had a higher total PCB level than did white children (Table 4); however, because of the small number of non-white girls, race was not considered further.

The other mother and child characteristics considered did not have a clear relationship with median total PCB concentrations.

In both the unadjusted and adjusted analyses there were no significant differences in association between maternal serum PCB concentrations during gestation (as a continuous variable or as tertiles) and earlier, compared to not earlier, age at menarche among daughters (Tables 5a, b). Multivariate analyses adjusted for maternal age at menarche, maternal age at delivery, maternal pre-pregnancy BMI and child birth order (parity) as potential confounders. Maternal social class and education variables were excluded from the final models based on backwards elimination examining the change in estimated OR. Overall, the effect estimates for the adjusted models were similar to the effect estimate of their respective unadjusted model (Tables 5a, b). The magnitude and direction of the effect estimates varied slightly between the continuous (Table 5a) and categorical (Table 5b) consideration of PCBs.

All PCB groups analyzed as continuous variables were associated with decreased odds of earlier age at menarche, ORs for the adjusted models were towards the null compared to the unadjusted models, and all 95% CIs include the null value of 1.0 (Table 5a). Anti-estrogenic PCBs (aOR 0.65, 95% CI 0.34, 1.23) and higher chlorinated PCBs (aOR 0.63, 95% CI 0.33, 1.22) were associated with the greatest decrease in odds of earlier age at menarche (Table 5a). Among the PCB homologs grouped by chlorine number, the odds of earlier age at menarche decreased as amount of chlorination increased (Table 5a). When the PCB groups were analyzed as tertile categorical variables, for all groups, the high compared to low tertile was associated with decreased odds of earlier age at menarche (Table 5b). Associations between gestational PCB exposure and odds of earlier menarche varied by PCB group when comparing the middle to low tertiles: total PCBs, estrogenic PCBs, common PCBs, most prevalent PCBs in sample, and 5 and 6 chlorine homologs were

associated with increased odds of earlier age at menarche (Table 5b). The high and low tertiles included a wide range of PCB concentrations for all groups, compared to middle tertile concentrations (Table 5b). All 95% CIs for the adjusted and unadjusted tertile exposure models included the null value of 1.0 (Table 5b).

A secondary multivariate analysis was conducted that included child characteristics (BMI at 8 years, birth weight, gestational age at birth) potentially on the causal pathway from *in utero* PCB exposure to age at menarche (Table 6). The ORs for the models including the additional child characteristics (Model 2, Table 6) varied slightly, but not significantly, from those models excluding the child characteristics (Model 1, Table 6). The direction of the change in OR estimate for Model 2 compared to Model 1 in most PCB groups was down and away from the null (Table 6).

Discussion

We assessed the relationship between *in utero* PCB exposure and age at menarche using data from the ALSPAC cohort. The majority of participants in our study had detectable serum PCB concentrations for most congeners; however, *in utero* PCB exposure, estimated by maternal serum PCB levels during pregnancy, was not significantly associated with earlier age at menarche. Our study suggests that higher levels of PCBs, measured continuously, are slightly, but not significantly, associated with lower odds of earlier menarche. The association between maternal serum PCB levels and daughter's age at menarche was consistently below the null, which is suggestive of a true effect. Yet, when PCB exposure was examined by tertiles, some PCB groups were associated with increased odds of earlier age at menarche comparing the middle to low tertile. This finding suggests that the association between PCB exposure and odds of earlier age at menarche may not be linear. However, this study does not have enough power to determine if these findings occurred by chance.

In general, the factors we found to be associated with earlier age at menarche in our study are similar to those described in previous studies. The lack of ethnic diversity in our sample limits our ability to address differences in either age at menarche or PCB exposure based on ethnic background. The reasons for the association between 8 year BMI and age at menarche are still not clear, but our results were similar to other studies finding that children with higher 8 year BMI were more likely to have earlier onset of menarche. Lower birth weight and birth at a younger gestational age have been linked to higher levels of PCB exposure, and our findings are suggestive of this link as well. Children who are smaller for gestational age often undergo rapid catch-up growth after birth, and it has been hypothesized that this catch-up growth is linked to 8 year BMI and in turn age at menarche (2, 18). Therefore, it is possible that these factors are all on the causal pathway between *in utero* PCB exposure and age at menarche. When child characteristics were included in models with PCB exposure as a continuous variable, slight changes in effect estimate were noted, which suggests that if these variables are not on the causal path, they should be included in a model to get an unbiased estimate of the OR.

The main factors associated with higher PCB levels in the study population may have a biological explanation. Study participants with higher socioeconomic status indicators had higher PCB levels, which could be related to dietary factors (e.g. fatty fish consumption). Positive association with maternal age could be due to accumulation of PCBs over a lifespan. Higher pre-pregnancy BMI and higher BMI at 8 years of age were associated with lower serum PCB concentrations; while these finding were unexpected, a literature search confirmed that higher BMI has been associated with lower PCB levels in other studies (53-56).

While we did not find a clear significant relationship between *in utero* PCB exposure and age at menarche, the association is biologically plausible. Fetal development marks the

period of growth when all major organs and body systems, including the endocrine and reproductive systems, are being created and programmed; therefore, exposures that disrupt normal processes during this period can affect later development. The exact critical periods for exposures are not well known for all chemicals, and it is possible that slight variations in levels at a specific time could have a major effect that could not be detected in our study. Animal studies find that PCBs cross the placental barrier, interfere with the endocrine system, and estrogenic PCBs stimulate the ER; estrogenic PCBs also activate the ER in binding strength screening assays (25-26, 29, 31, 57-58). Anti-estrogenic PCBs antagonize the effects of ER binding (31), and dioxin-like PCBs may activate the aryl hydrocarbon receptor (AhR) and exert anti-estrogenic action (34, 59). Recent studies indicate that the AhR and its related factors can modulate ER activity which suggests a complex relationship between PCBs that act at these receptors and the overall biologic effect (34). Additionally, PCBs have been found to modulate thyroid hormones, which are critical for fetal development (60) and later reproductive function (61).

Those models in our study that found the greatest decreased odds of earlier age at menarche involved anti-estrogenic PCBs and higher chlorinated PCBs. This finding suggests that *in utero* exposure to these PCB groups may be associated with later age at menarche; however, this hypothesis cannot be addressed in the current study. While the ORs for estrogenic PCBs were still below the null in most models, they showed the least decrease in odds of earlier age at menarche, which also could be related to a biological mechanism of action. Based on the different effect estimates for the continuous and categorical (tertile) categorization of PCB groups in the models, it is unclear what form the relationship between PCBs and age at menarche takes. The change in effect estimate within each group when examined as tertiles suggests that higher, compared to lower, levels of *in utero* PCBs

are not associated with odds of earlier age at menarche. However, levels closer to the median, compared to lower levels, may be associated with earlier age at menarche.

A major limitation cited in previous studies was the inability to separate out specific congeners and group them by proposed endocrine action. A major strength of our study was the inclusion of thirty five individual PCB congeners in the serum analysis and the ability to group them by different proposed endocrine actions. Therefore, it was unexpected that the direction of effect (OR) in our study was fairly consistent among PCB groups with different proposed endocrine action. Additionally it appears that there may be a trend between age at menarche and amount of PCB chlorination: as homologs increased in number of chlorines, the odds of earlier age at menarche decreased. A study by Pliskova et al. (31) found that lower chlorinated PCBs were more often estrogenic and that higher chlorinated PCBs had anti-estrogenic effects. These findings suggest that the demonstrated endocrine modulating function of PCBs may not only be through endocrine receptor binding.

PCB concentrations among study participants may not have been high enough to discern a noticeable effect. Overall, a higher proportion of PCBs with proposed anti-estrogenic action were detected in our samples. Therefore, it is also possible that the effect of the anti-estrogenic PCBs outweighed/masked the effect of estrogenic PCBs when present in the same individual, leading to delayed menarche. Even though we were unable to detect a significant association between environmental levels of PCBs and age at menarche, the suggestion of an association is still important. Studies have found that combinations of low-level estrogenic compounds can have an additive effect above what was expected (22, 62). A more significant effect may be found if other weak estrogenic chemicals are assessed along with estrogenic PCBs in a future analysis. A different study design would be needed to examine the relationship between anti-estrogenic PCBs and later age at menarche;

however, our study suggests that anti-estrogenic (and other) PCBs are not associated with earlier age at menarche. When PCB groups were examined as tertile categorical variables there was a slight association between lower levels of anti-estrogenic and dioxin-like PCBs, and earlier age at menarche; this also suggests that higher concentrations of certain PCBs may delay age at menarche.

Limitations of this study include small sample size, lack of complete menarche information for controls at the time of selection, single measures of PCB levels, and missing values for several covariates. The small sample size decreases our ability to detect a true difference, and missing covariate information led to even smaller sample sizes used to create the adjusted models. In addition to not having complete menarche information on all controls, there was not a large difference in the age at menarche between cases (median = 11.08 years) and those controls with menarche at the time of selection (median = 12.42 years). This small difference, and the fact that both of these ages are within the "normal" menarche range, decreases our ability to determine whether PCB exposure is associated with earlier menarche. We could not adequately examine the effect of ethnic background on age at menarche because of the small sample of non-white girls, even though race/ethnicity has been shown to be associated with age at menarche (4, 11-14) and was related to PCB levels in our study.

There were also limitations to our data analysis: the chance of Type I error was increased because of multiple comparisons, and the power of the study may not have been great enough to detect a small association between PCB levels and age at menarche due to the relatively small sample size. Study strengths include the overall size and representativeness of the underlying cohort, prospective data collection, quantification of more than thirty individual PCB congeners, creation of biologically meaningful PCB congener groups, and inclusion of maternal and child characteristics associated with

menarche. Even though the individuals for this study were selected from a large, fairly representative cohort, the results may not be broadly applicable. Most of the women had higher education and were in the middle and upper social classes. Individuals with these characteristics may have other factors that protect against exposure or attenuate the relationship between exposure and outcome. Although our study did not detect any significant associations, the use of multiple PCB groupings – some with varied measures of effect – may suggest a template for future studies.

Conclusions and Future Recommendations

Within the ALSPAC cohort, we compared *in utero* PCB exposure, estimated by maternal PCB exposure during pregnancy, among girls with and without earlier age at menarche. PCB concentrations varied by maternal and child characteristics, but did not vary greatly by case status. In this study, *in utero* PCB exposure was not significantly associated with earlier age at menarche; however, the findings suggest that higher levels of PCBs are slightly, but not significantly, associated with lower odds of earlier age at menarche. These findings, and those of previous studies, suggest that *in utero* PCB exposure may be associated with later age at menarche; however, this association cannot be addressed with the current study design.

The findings from this study provide several avenues for future research. Since the results suggest that PCB exposure is associated with later onset of menarche, a study conducted using a larger sample and an *a priori* hypothesis of later menarche may be able to detect a significant association. In addition, it appears that the extent of chlorination of PCB congeners may be more associated with age at menarche than PCBs grouped by endocrine action. Past studies that did not detect an effect of PCB exposure on age at menarche may not have found one because congeners were not studied in the most meaningful way.

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Tables and Figures

Table 1.
Characteristics of study population.

Characteristic	Early Menarche[a] (N = 218) N	%	Not early menarche[b] (N = 230) N	%	p-value for difference
Mother's highest education					0.3371
CSE/none	31	14.2	26	11.3	
Vocational	17	7.8	15	6.5	
O-Level	67	30.7	73	31.7	
A-Level	61	28.0	66	28.7	
Degree	34	15.6	39	17.0	
Missing	8	3.7	11	4.8	
Mother's social class					0.9241
Lower	18	8.3	19	8.3	
Middle	75	34.4	84	36.5	
Upper	79	36.2	88	38.3	
Missing	46	21.1	39	17.0	
Mother's age at menarche					0.0004
9-11 years	63	28.9	30	13.0	
12-14 years	119	54.6	153	66.5	
≥15 years	12	5.5	15	6.5	
Missing	24	11.0	32	13.9	
Mother's pre-pregnancy BMI					0.0015
<18.5	7	3.2	11	4.8	
18.5 - 24.99	129	59.2	162	70.4	
25-29.99	42	19.3	20	8.7	
≥30	19	8.7	12	5.2	
Missing	21	9.6	25	10.9	
Mother's age at delivery					0.9201
<20 years	1	0.5	7	3.0	
20-24 years	43	19.7	41	17.8	
25-29 years	83	38.1	81	35.2	
30-34 years	65	29.8	75	32.6	
≥35 years	24	11.0	25	10.9	
Missing	2	0.9	1	0.4	
Child birth order					0.048
First born	110	50.46	97	42.17	
Second born or later	94	43.12	122	53.04	
Missing	14	6.42	11	4.78	

[a] Achieving menarche before 11.5 years (cases)

[b] Achieving menarche at, or after, 11.5 years (controls)

Table 1. (Continued)
Characteristics of study population.

Characteristic	Early Menarche[a] (N = 218) N	%	Not early menarche[b] (N = 230) N	%	p-value for difference
Child ethnic background					0.0156
White	199	91.3	214	93.0	
Non-White	12	5.5	3	1.3	
Missing	7	3.2	13	5.7	
Child's BMI at 8 years					<0.0001
<13.6	0.0	-	1.0	0.4	
13.6-18.4	72.0	33.0	140.0	60.9	
18.4-20.6	45.0	20.6	24.0	10.4	
≥20.6	41.0	18.8	16.0	7.0	
Missing	60.0	27.5	49.0	21.3	
Child's birth weight					
<2,500 g	7	3.2	10	4.4	0.2077
2,500 - 3,999 g	184	84.4	197	85.7	
>4,000 g	24	11	18	7.8	
Missing	3	1.4	5	2.2	
Child's gestational age					
<37 weeks (preterm)	4	1.8	10	4.4	0.4678
37-40 weeks (term)	136	62.4	141	61.3	
>40 weeks (over term)	76	34.9	78	33.9	
Missing	2	0.9	1	0.4	
<u>Child menarche status and age at menarche</u>					
Number achieving menarche by age 13	218	100	113	49.1	-
	Median	IQR	Median	IQR	
Age at Menarche	11.08	10.75 - 11.33	12.42	12.08 - N/A	-

[a] Achieving menarche before 11.5 years (cases)

[b] Achieving menarche at, or after, 11.5 years (controls)

Table 2.

Groupings of PCB congeners used in data analysis

2a. Grouped by proposed endocrine action

Proposed Endocrine Action	**PCB Congeners**
Estrogenic	28, 52, 66, 74, 99, 110, 128
Anti-Estrogenic	138-158, 153, 170, 196-203, 199
Dioxin-like[a]	105, 118, 156, 157, 167

[a] Dioxin-like PCBs were converted to their toxic equivalency to 2,3,7,8-TCDD based on a TEF of 0.0003 (46).

2b. Grouped by structural homologs (same chlorine number)

Number of Chlorines	**PCB Congeners**
3	28
4	44, 49, 52, 66, 74
5	87, 99, 101, 105, 110, 118
6	128, 138-158, 146, 149, 151, 153, 156, 157, 167
7	170, 172, 177, 178, 180, 183, 187, 189
8	194, 195, 196-203, 199, 206
9	209

Table 3.

Gestational serum PCB concentrations (lipid adjusted, ng/g lipid) among mothers of girls with and without earlier age at menarche.

	Total (N=448)[a]	Early Menarche (N=218)[a]	Not Early Menarche (N=230)[a]
Congener Group	Median (IQR)	Median (IQR)	Median (IQR)
Total PCBs	309.58 (241.30 - 394.20)	300.12 (238.47 - 388.49)	319.78 (242.58-397.14)
Common PCBs[b]	165.00 (125.10 - 219.55)	160.85 (123.10 - 220.00)	173.35 (126.30 - 218.30)
Most Prevalent in Sample[c]	184.10 (139.60 - 244.10)	178.10 (139.00 - 244.70)	194.25 (144.10-241.20)
Estrogenic PCBs	34.62 (26.13 - 49.20)	34.82 (26.54 - 47.30)	34.36 (25.97-49.5)
Anti-Estrogenic PCBs	182.60 (138.20 - 244.80)	175.10 (135.40 - 248.00)	191.25 (142.00-242.20)
Dioxin-like PCBs	0.0085 (0.0063 - 0.011)	0.0083 (0.0063 - 0.011)	0.0085 (0.0064 - 0.012)
Homologs			
4 Chlorines	20.67 (14.71 - 29.95)	20.49 (14.69 - 29.45)	20.85 (14.80 - 31.00)
5 Chlorines	33.80 (25.10 - 45.68)	33.75 (25.10 - 44.40)	33.80 (25.10 - 47.80)
6 Chlorines	125.09 (93.22 - 162.15)	121.49 (92.73 - 162.40)	129.56 (93.92 - 161.80)
7 Chlorines	89.15 (68.13 - 120.30)	87.35 (64.00 - 120.70)	92.90 (71.44 - 117.40)
8 Chlorines	24.90 (19.05 - 34.75)	24.10 (17.91 - 33.20)	26.25 (20.03 - 35.20)

[a] Of those individuals selected for the study, 20 total samples could not be analyzed, 8 among the cases and 12 among the controls.

[b] PCBs most commonly detected in serum in other studies: PCB118, PCB138_158, PCB153, PCB180

[c] PCBs that were most common in our sample: PCB118, PCB138_158, PCB153, PCB170, PCB180

Table 4.

Median and interquartile range of total maternal serum PCB[a] concentration during gestation by maternal and child characteristics.

Group	Median (IQR)
Mother's highest education	
CSE/none	258.0 (191.9 - 347.6)
Vocational	298.3 (248.1-359.7)
O-Level	266.0 (206.7-352.6)
A-Level	328.9 (273.9-423.3)
Degree	386.7 (306.8-483.5)
Mother's social class	
Lower	315.9 (245.6-429.3)
Middle	307.8 (241.6-388.8)
Upper	321.0 (267.4-364.4)
Mother's age at menarche	
9-11 years	315.9 (245.6-429.3)
12-14 years	307.8 (241.6-364.4)
≥15 years	321.0 (267.4-364.4)
Mother's pre-pregnancy BMI	
<18.5	348.3 (307.4-483.5)
18.5 - 24.99	319.8 (248.5-396.3)
25-29.99	264.3 (215.2-373.7)
≥30	269.3 (211.9-354.4)
Mother's age at delivery	
<20 years	173.8 (163.2-207.3)
20-24 years	219.8 (181.0-283.4)
25-29 years	282.5 (238.5-344.3)
30-34 years	367.7 (301.2-479.4)
≥35 years	441.6 (322.7-534.3)
Child birth order	
First born	312.8 (228.1-397.1)
Second born or later	313.0 (247.1-395.7)
Child ethnic background	
White	311.4 (241.8-394.8)
Non-White	342.0 (242.6-445.3)
Child's BMI at 8 years	
<13.6	488.1 (488.1-488.1)
13.6-18.4	322.7 (246.1-420.3)
18.4-20.6	306.8 (243.1-385.8)
≥20.6	311.9 (228.7-414.5)
Child's birth weight	
<2,500 g	350.8 (312.8-445.3)
2,500 - 3,999 g	310.0 (243.0-395.7)
>4,000 g	246.6 (190.1-322.7)
Child's gestational age	
<37 weeks (preterm)	344.3 (307.7-534.3)
37-40 weeks (term)	309.2 (228.7-390.1)
>40 weeks (over term)	306.8 (251.0-393.6)

[a] Summation of concentration of total lipid adjusted PCBs (ng/g lipid)

Table 5a.

Logistic regression analysis for the association between maternal serum PCB concentrations (ng/g lipid) during gestation and earlier age at menarche in daughters.

Congener Group	Continuous[a]	
	Unadjusted[b] OR (95% CI)	Adjusted[c] OR (95% CI)
Total PCBs	0.70 (0.40, 1.21)	0.68 (0.34, 1.34)
Estrogenic PCBs	0.96 (0.61, 1.51)	0.87 (0.53, 1.41)
Anti-Estrogenic PCBs	0.66 (0.39, 1.11)	0.65 (0.34, 1.23)
Dioxin-like PCBs	0.85 (0.52, 1.40)	0.75 (0.42, 1.34)
Common PCBs[d]	0.69 (0.42, 1.16)	0.67 (0.36, 1.25)
Most Prevalent in Sample[e]	0.68 (0.41, 1.15)	0.67 (0.36, 1.25)
Homologs		
4 Chlorines	0.89 (0.59, 1.33)	0.82 (0.53, 1.26)
5 Chlorines	0.99 (0.65, 1.52)	0.90 (0.56, 1.43)
6 Chlorines	0.72 (0.43, 1.21)	0.71 (0.38, 1.29)
7 Chlorines	0.65 (0.39, 1.09)	0.67 (0.34, 1.29)
8 Chlorines	0.63 (0.38, 1.02)	0.63 (0.33, 1.22)

[a]Continuous models use log transformations of lipid adjusted PCB levels as the exposure.

[b]Unadjusted models included 335 observations (164 cases and 171 controls). Same exclusion criteria as for adjusted model.

[c]Adjusted for maternal age at menarche, pre-pregnancy BMI, maternal age at delivery and parity. Adjusted models had 335 observations (164 cases and 171 controls).

[d] PCBs most commonly detected in serum in other studies: PCB118, PCB138_158, PCB153, PCB180.

[e] PCBs that were most common in our sample: PCB118, PCB138_158, PCB153, PCB170, PCB180.

Table 5b.

Logistic regression analysis for the association between maternal serum PCB concentrations (ng/g lipid) during gestation and earlier age at menarche in daughters.

Congener Group	Tertiles		
	Range of Concentrations	Unadjusted[b] OR (95% CI)	Adjusted[c] OR (95% CI)
Total PCBs	361.01-1,054.85	0.79 (0.47, 1.33)	0.77 (0.30, 1.49)
	259.01-361.00	0.96 (0.57, 1.64)	1.10 (0.61, 1.98)
	63.83-259.00	1.00	1.00
Estrogenic PCBs	42.01-203.90	1.00 (0.59, 1.70)	0.90 (0.50, 1.60)
	28.81-42.00	1.35 (0.80, 2.29)	1.31 (0.75, 2.27)
	7.00-28.80	1.00	1.00
Anti-Estrogenic PCBs	215.01-568.20	0.69 (0.41, 1.16)	0.69 (0.35, 1.34)
	154.01-215.00	0.74 (0.43, 1.27)	0.86 (0.47, 1.56)
	15.01-154.00	1.00	1.00
Dioxin-like PCBs	0.011-0.043	0.92 (0.55, 1.55)	0.79 (0.42, 1.49)
	0.0071-0.010	0.91 (0.53, 1.57)	0.87 (0.48, 1.56)
	0.0015-0.007	1.00	1.00
Common PCBs[d]	196.01-568.10	0.77 (0.46, 1.30)	0.74 (0.38, 1.43)
	140.01-196.00	0.93 (0.54, 1.59)	1.05 (0.58, 1.89)
	12.30-140.00	1.00	1.00
Most Prevalent in Sample[e]	218.01-617.00	0.74 (0.44, 1.24)	0.71 (0.37, 1.37)
	158.01-218.00	0.98 (0.58, 1.68)	1.12 (0.62, 2.01)
	14.60-158.00	1.00	1.00
Homologs			
4 Chlorines	25.81-166.30	0.90 (0.53, 1.52)	0.79 (0.45, 1.40)
	16.51-25.80	0.93 (0.55, 1.58)	0.87 (0.50, 1.51)
	5.02-16.50	1.00	1.00
5 Chlorines	41.01-165.70	0.93 (0.55, 1.59)	0.86 (0.48, 1.53)
	27.61-41.00	1.18 (0.69, 1.99)	1.20 (0.69, 2.10)
	3.96-27.60	1.00	1.00
6 Chlorines	144.81-412.57	0.86 (0.51, 1.45)	0.87 (0.46, 1.65)
	102.81-144.80	1.16 (0.68, 1.97)	1.39 (0.78, 2.47)
	11.31-102.80	1.00	1.00
7 Chlorines	105.41-256.17	0.64 (0.38, 1.09)	0.69 (0.35, 1.37)
	74.91-105.40	0.56 (0.33, 0.96)	0.66 (0.35, 1.23)
	13.71-74.90	1.00	1.00
8 Chlorines	30.81-109.50	0.69 (0.41, 1.17)	0.77 (0.38, 1.56)
	20.71-30.80	0.79 (0.46, 1.34)	0.89 (0.48, 1.63)
	5.53-20.70	1.00	1.00

[b]Unadjusted models included 335 observations (164 cases and 171 controls).

[c]Adjusted for maternal age at menarche, pre-pregnancy BMI, maternal age at delivery and parity. Adjusted models had 335 observations (164 cases and 171 controls).

[d] PCBs most commonly detected in serum in other studies: PCB118, PCB138_158, PCB153, PCB180.

[e] PCBs that were most common in our sample: PCB118, PCB138_158, PCB153, PCB170, PCB180.

Table 6.

Logistic regression analysis for the association between maternal serum PCB concentrations (ng/g lipid) during gestation and earlier age at menarche in daughters when adjusting for covariates possibly on the causal pathway.

Congener Group	Continuous[a]	
	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
Total PCBs	0.62 (0.29, 1.34)	0.60 (0.26, 1.36)
Estrogenic PCBs	0.87 (0.50, 1.52)	0.90 (0.51, 1.61)
Anti-Estrogenic PCBs	0.58 (0.28, 1.21)	0.54 (0.24, 1.19)
Dioxin-like PCBs	0.75 (0.39, 1.45)	0.68 (0.34, 1.36)
Common PCBs	0.61 (0.30, 1.24)	0.57 (0.27, 1.23)
Most Prevalent in Sample	0.60 (0.29, 1.23)	0.56 (0.26, 1.22)
Homologs		
4 Chlorines	0.86 (0.52, 1.42)	0.89 (0.53, 1.48)
5 Chlorines	0.94 (0.56, 1.59)	0.94 (0.54, 1.64)
6 Chlorines	0.65 (0.33, 1.30)	0.62 (0.30, 1.29)
7 Chlorines	0.60 (0.27, 1.22)	0.53 (0.23, 1.20)
8 Chlorines	0.57 (0.27, 1.21)	0.53 (0.24, 1.17)

[a]Continuous models use log transformations of lipid adjusted PCB levels as the exposure

[b] Model 1 is adjusted for maternal age at menarche, pre-pregnancy BMI, maternal age at delivery, parity, and maternal education. It includes 253 observations (120 cases and 133 controls) and has the same exclusion criteria as Model 2.

[c] Model 2 is adjusted for maternal age at menarche, pre-pregnancy BMI, maternal age at delivery, parity, maternal education, child BMI at 8 years, child birth weight, and child gestational age at delivery. It includes 253 observations (120 cases and 133 controls).

Figure 1.

Flowchart of eligibility and exclusions

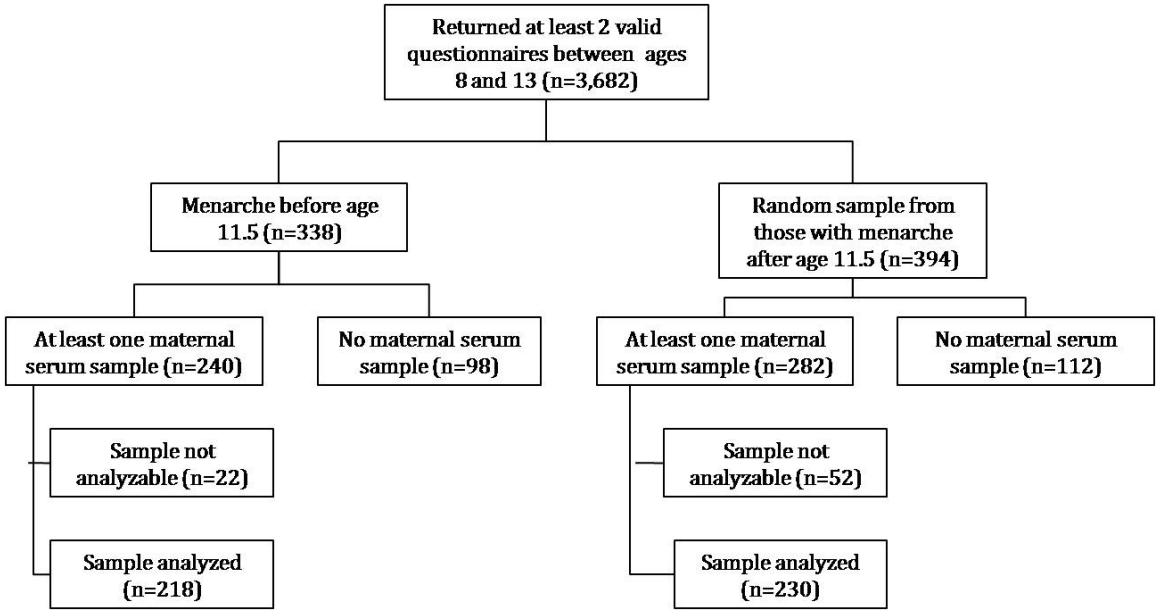
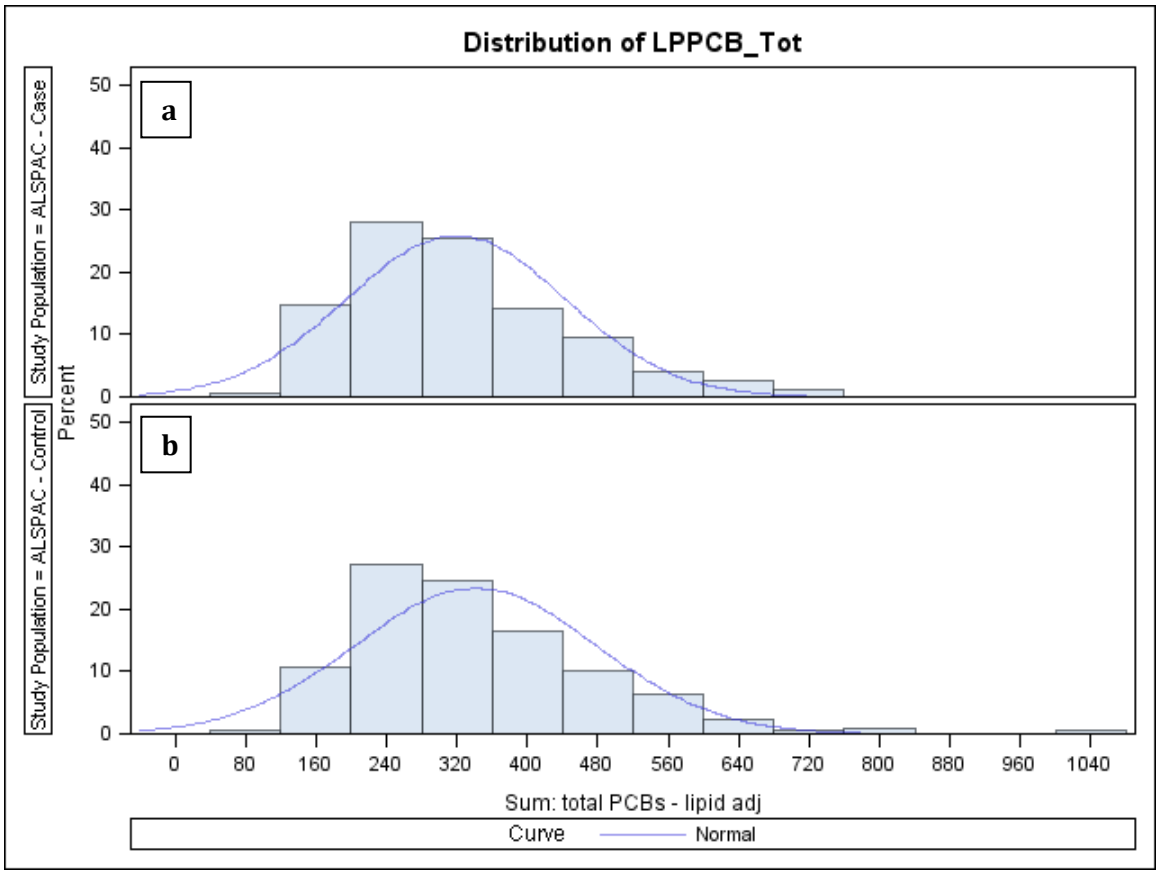
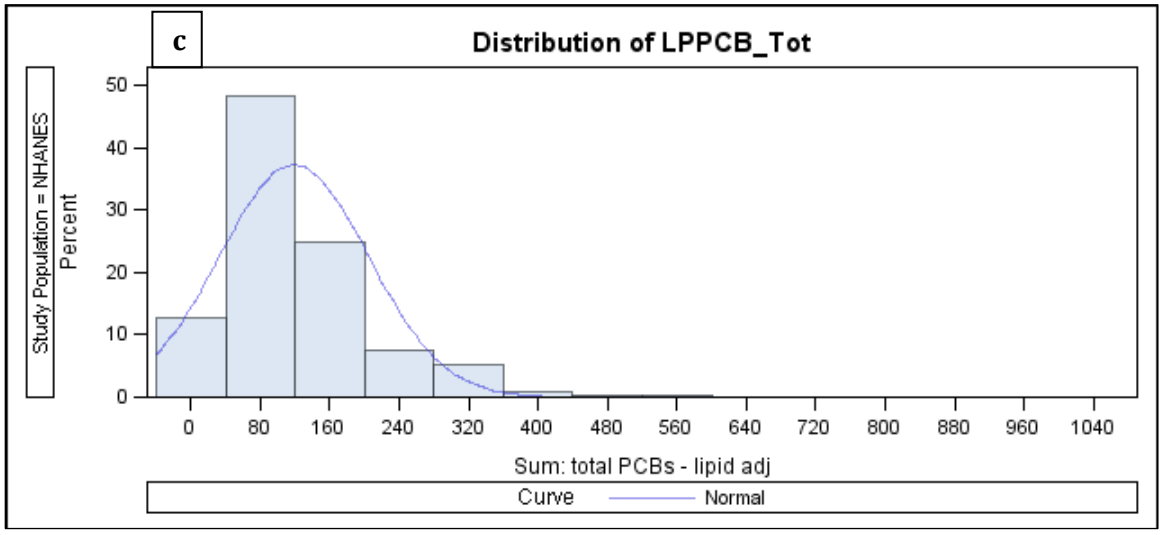


Figure 2.

Histogram comparing total lipid adjusted PCB levels among ALSPAC cases (a) and controls (b) to white women ages 18-55 years in NHANES 2003-2004 (c) (43-45).





Appendix

Table 1.

PCB congeners measured in maternal serum

IUPAC Number	Chemical Structure	Proposed Endocrine Action
PCB28	2,4,4'-triCB	Estrogenic
PCB44	2,2' ,3,5'-tetraCB	
PCB49	2,2',4,5'-tetraCB	
PCB52	2,2' ,5,5' -tetraCB	Estrogenic
PCB66	2,3' ,4,4' -tetraCB	Estrogenic
PCB74	2,4,4',5-tetraCB	Estrogenic
PCB87	2,2' ,3,4,5'-pentaCB	
PCB99	2,2',4,4',5-pentaCB	Estrogenic
PCB101	2,2',4,5,5'-pentaCB	
PCB105	2,3,3',4,4'-pentaCB	Dioxin-like
PCB110	2,3,3',4' ,6-pentaCB	Estrogenic
PCB118	2,3' ,4,4',5-pentaCB	Dioxin-like
PCB128	2,2',3,3',4,4'-hexaCB	Estrogenic
PCB138-158	2,2',3,4,4',5'-hexaCB and 2,3,3'.4,4',6-hexaCB	Anti-estrogenic
PCB146	2,2',3,4',5,5'-hexaCB	
PCB149	2,2' ,3,4',5',6-hexaCB	
PCB151	2,2',3,5,5',6-hexaCB	
PCB153	2,2' ,4,4' ,5,5'-hexaCB	Anti-estrogenic
PCB156	2,3,3' ,4,4' ,5-hexaCB	Dioxin-like
PCB157	2,3,3',4,4',5'-hexaCB	Dioxin-like
PCB167	2,3',4,4',5,5'-hexaCB	Dioxin-like
PCB170	2,2' ,3,3',4,4' ,5-heptaCB	Anti-estrogenic
PCB172	2,2',3,3',4,5,5'-heptaCB	
PCB177	2,2',3,3',4',5,6-heptaCB	
PCB178	2,2',3,3',5,5',6-heptaCB	
PCB180	2,2',3,4,4',5,5'-heptaCB	Anti-estrogenic
PCB183	2,2',3,4,4',5',6-heptaCB	
PCB187	2,2',3,4',5,5',6-heptaCB	
PCB189	2,3,3',4,4' ,5,5' -heptaCB	Dioxin-like
PCB194	2,2',3,3',4,4',5,5'-octaCB	
PCB195	2,2',3,3',4,4' ,5,6-octaCB	
PCB196-203	2,2',3,3',4,4',5',6-octaCB and 2,2',3,4,4',5,5',6-octaCB	Anti-estrogenic
PCB199	2,2',3,3' ,4,5,6,6'-octaCB	Anti-estrogenic
PCB206	2,2',3,3',4,4',5,5',6-nonaCB	
PCB209	decaCB	

Table 2.

Additional logistic regression analysis for the association between maternal serum PCB concentrations (ng/g lipid) during gestation and earlier age at menarche in daughters.

log-PCB group (lipid adjusted)	Unadjusted Model OR (95 % CI)	Model 1 OR (95 % CI)	Model 2 OR (95 % CI)	Model 3 OR (95 % CI)	Model 4 OR (95 % CI)
Total	0.70 (0.40, 1.21)	0.68 (0.34, 1.34)	0.66 (0.29, 1.52)	0.62 (0.29, 1.34)	0.71 (0.35, 1.43)
Estrogenic	0.96 (0.61, 1.51)	0.87 (0.53, 1.41)	0.88 (0.50, 1.54)	0.87 (0.50, 1.52)	0.89 (0.54, 1.46)
Anti-Estrogenic	0.66 (0.39, 1.11)	0.65 (0.34, 1.23)	0.69 (0.56, 1.48)	0.58 (0.28, 1.21)	0.68 (0.35, 1.30)
Dioxin-like	0.85 (0.52, 1.40)	0.75 (0.42, 1.34)	0.75 (0.38, 1.49)	0.75 (0.39, 1.45)	0.77 (0.42, 1.40)
Most Prevalent	0.69 (0.42, 1.16)	0.67 (0.36, 1.25)	0.68 (0.31, 1.52)	0.61 (0.30, 1.24)	0.70 (0.37, 1.32)
Most Prevalent in Sample	0.68 (0.41, 1.15)	0.67 (0.36, 1.25)	0.68 (0.30, 1.52)	0.60 (0.29, 1.23)	0.69 (0.36, 1.32)
Homologs					
4 Chlorines	0.89 (0.59, 1.33)	0.82 (0.53, 1.26)	0.81 (0.50, 1.32)	0.86 (0.52, 1.42)	0.84 (0.54, 1.31)
5 Chlorines	0.99 (0.65, 1.52)	0.90 (0.56, 1.43)	0.86 (0.50, 1.47)	0.94 (0.56, 1.59)	0.94 (0.58, 1.51)
6 Chlorines	0.72 (0.43, 1.21)	0.71 (0.38, 1.29)	0.72 (0.33, 1.55)	0.65 (0.33, 1.30)	0.73 (0.39, 1.36)
7 Chlorines	0.65 (0.39, 1.09)	0.67 (0.34, 1.29)	0.67 (0.20, 1.54)	0.60 (0.27, 1.22)	0.69 (0.35, 1.37)
8 Chlorines	0.63 (0.38, 1.02)	0.63 (0.33, 1.22)	0.59 (0.29, 1.23)	0.57 (0.27, 1.21)	0.65 (0.33, 1.26)

Unadjusted model includes no covariates (335 observations: 164 cases and 171 controls).

Model 1 is adjusted for maternal age at menrche, maternal age at delivery, pre-pregnancy BMI and parity (335 observations: 164 cases and 171 controls).

Model 2 is adjusted for maternal age at menarche, maternal age at delivery, pre-preg BMI, parity, social class (middle class=ref), maternal education (O-level = ref) (290 observations: 138 cases and 152 controls)

Model 3 is adjusted for maternal age at menarche, maternal age at delivery, pre-preg BMI, parity, maternal education (O-level = ref) (253 observations:120 cases and 133 controls).

Model 4 is adjusted for maternal age at menarche, maternal age at delivery, pre-preg BMI, parity, daughter race (332 observations: 163 cases and 169 controls)

Table 3a. Association between covariates and outcome (odds of earlier menarche compared to not earlier menarche) using binary logistic regression

Covariate	OR	95% Confidence Interval Lower Limit	Upper Limit	Width	Association
Maternal age at menarche (continuous)	0.68	0.57	0.79	1.38	Moderate
Mother's pre-pregnancy BMI (continuous)	1.08	1.03	1.14	1.11	Weak
Mother's age at delivery (continuous)	0.99	0.95	1.03	1.08	Weak
Mother's highest education (categorical)					Moderate
O-Level	1.00				
CSE/none	1.30	0.70	2.41	3.44	
Vocational	1.24	0.57	2.67	4.66	
A-Level	1.01	0.62	1.63	2.61	
Degree	0.95	0.54	1.68	3.11	
Mother's social class (categorical)					Weak
Middle	1.00				
Lower	1.06	0.52	2.17	4.18	
Upper	1.01	0.65	1.55	2.39	
Birth order (dichotomous): First born vs. not	1.47	1.00	2.16	2.153	Moderate
Child's BMI at 8 years (continuous)	1.30	1.18	1.42	1.20	Moderate
Child's birth weight (continuous)	1.00	1.00	1.00	1.00	Weak
Child's gestational age (continuous)	1.01	0.90	1.14	1.26	Weak

Table 3b. Association between covariates and exposure (lipid adjusted PCBs, log-transformed for normality) using linear regression

Covariate	Exposure	Correlation[a]	p-value	
Maternal Age at Menarche (continuous)	Total	0.0234	0.7497	
Weak association	Estrogenic	-0.0111	0.8799	
	Anti-estrogenic	0.0347	0.6365	
	Dioxin-like (TEQ)	0.0124	0.8656	
	Most Prevalent	0.0375	0.6095	
Maternal Social Class (categorical)	Total	-0.0145	0.1104	Low: Mid
Middle class as referent		0.1881	0.0009	Upper: Mid
	Estrogenic	-0.1619	0.1633	Low: Mid
Strong association when upper social class is compared		0.1258	0.0791	Upper: Mid
to middle (higher PCBs)	Anti-estrogenic	-0.1672	0.0754	Low: Mid
Moderate association when lower social class is		0.2029	0.0005	Upper: Mid
compared to middle (lower PCBs)	Dioxin-like (TEQ)	-0.1397	0.1895	Low: Mid
		0.1542	0.0194	Upper: Mid
	Most Prevalent	-0.1652	0.0812	Low: Mid
		0.1944	0.0010	Upper: Mid
Maternal Highest Education (categorical)	Total	0.0116	0.8922	1 to 3
O-level as referent		0.0234	0.8232	2 to 3
		0.1596	0.0135	4 to 3
Weak association when lower education is compared to		0.3312	<0.0001	5 to 3
middle (lower PCBs)	Estrogenic	-0.0950	0.3753	1 to 3
Strong association when higher education is compared		-0.0458	0.7258	2 to 3
to middle (higher PCBs)		0.1068	0.1831	4 to 3
		0.2862	0.0025	5 to 3
	Anti-estrogenic	0.0185	0.8372	1 to 3
		0.0450	0.6818	2 to 3
		0.1798	0.0081	4 to 3
		0.3406	<0.0001	5 to 3
	Dioxin-like (TEQ)	0.0148	0.8817	1 to 3
		0.0527	0.6646	2 to 3
		0.1064	0.1541	4 to 3
		0.0345	0.0001	5 to 3
	Most Prevalent	0.0093	0.9185	1 to 3
		0.0451	0.6832	2 to 3
		0.1752	0.0102	4 to 3
		0.3304	<0.0001	5 to 3
Maternal Pre-pregnancy BMI (continuous)	Total	-0.1381	0.0542	
Moderate association, depending on the PCB	Estrogenic	0.0308	0.6692	
	Anti-estrogenic	-0.1834	0.0103	
	Dioxin-like (TEQ)	0.0009	0.9898	
	Most Prevalent	-0.1552	0.0303	
Maternal age at delivery (continuous)	Total	0.5982	<0.0001	
Strong association (positive correlation)	Estrogenic	0.2700	<0.0001	
	Anti-estrogenic	0.6251	<0.0001	
	Dioxin-like (TEQ)	0.5465	<0.0001	
	Most Prevalent	0.6117	<0.0001	
Birth order (dichotomous): First born vs. not	Total	0.0093	0.8944	
Moderate association, depending on the PCB	Estrogenic	0.1254	0.0711	
	Anti-estrogenic	-0.0197	0.7771	
	Dioxin-like (TEQ)	0.0389	0.5766	
	Most Prevalent	0.0022	0.9753	

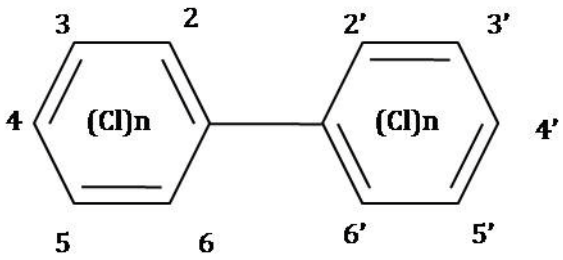
Table 3b Continued. Association between covariates and exposure (lipid adjusted PCBs, log-transformed for normality) using linear regression

Covariate	Exposure	Correlation[a]	p-value
Daugher Race (dichotomous): White vs. not	Total	0.0120	0.8641
Weak association	Estrogenic	0.0317	0.6505
	Anti-estrogenic	0.0143	0.8386
	Dioxin-like (TEQ)	0.0474	0.4991
	Most Prevalent	0.0210	0.7640
Mother Race (dichotomous): White vs. not	Total	0.0322	0.6438
Weak association	Estrogenic	0.0150	0.8295
	Anti-estrogenic	0.0402	0.5641
	Dioxin-like (TEQ)	-0.0093	0.8940
	Most Prevalent	0.0310	0.6563
Child's BMI at 8 years (continuous)	Total	-0.02109	0.0709
Weak association (negative correlation)	Estrogenic	-0.0303	0.6925
	Anti-estrogenic	-0.1658	0.0293
	Dioxin-like (TEQ)	-0.0465	0.5436
	Most Prevalent	-0.1592	0.0365
Child's birth weight (continuous)	Total	-0.1425	0.0377
Moderate association (negative correlation)	Estrogenic	-0.0302	0.6609
	Anti-estrogenic	-0.1753	0.0104
	Dioxin-like (TEQ)	-0.0863	0.2095
Child's gestational age (continuous)	Total	-0.0003	0.9618
Weak association, direction varies	Estrogenic	0.0607	0.3736
	Anti-estrogenic	-0.0276	0.6858
	Dioxin-like (TEQ)	0.012	0.8601
	Most Prevalent	-0.0211	0.7571

[a] Correlation coefficient for continous variables/beta for categorical

Figure 1.

Chemical structure of PCBs



Potential positions of the chlorines (Cl) on each benzene ring are represented by the numbers 1-6 at each carbon atom.

Ortho positions are 2, 2', 6 and 6'

Meta positions are 3, 3', 5 and 5'

Para positions are 4 and 4'

Figure 2.

Directed Acyclic Graphs (DAGs) of the relationship of potential covariates of interest with the exposure (*in utero* PCBs) and outcome (earlier age at menarche).

Figure 2a. All potential covariates.

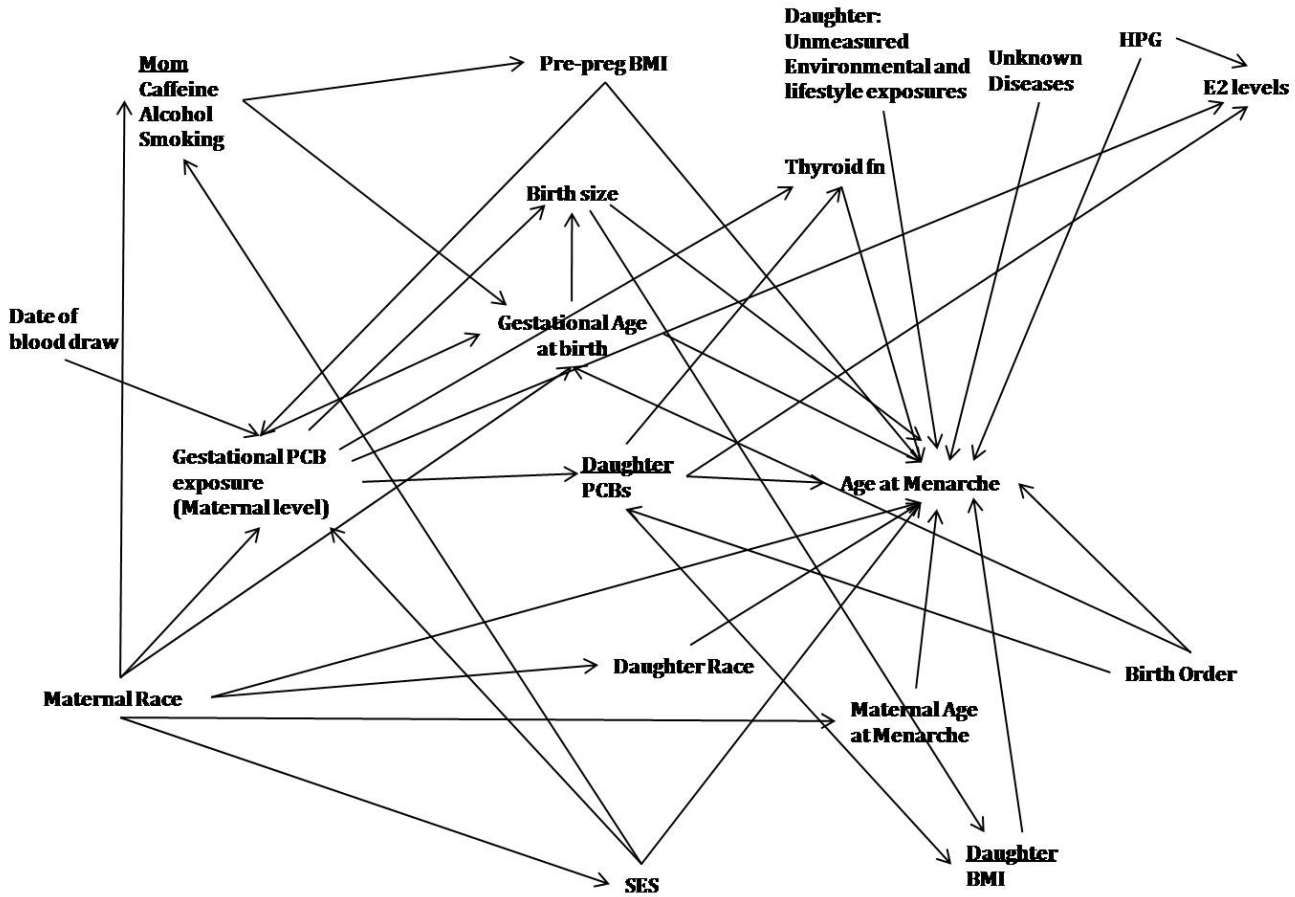


Figure 2b. Open back door paths between exposure and outcome – DAG identified confounders.

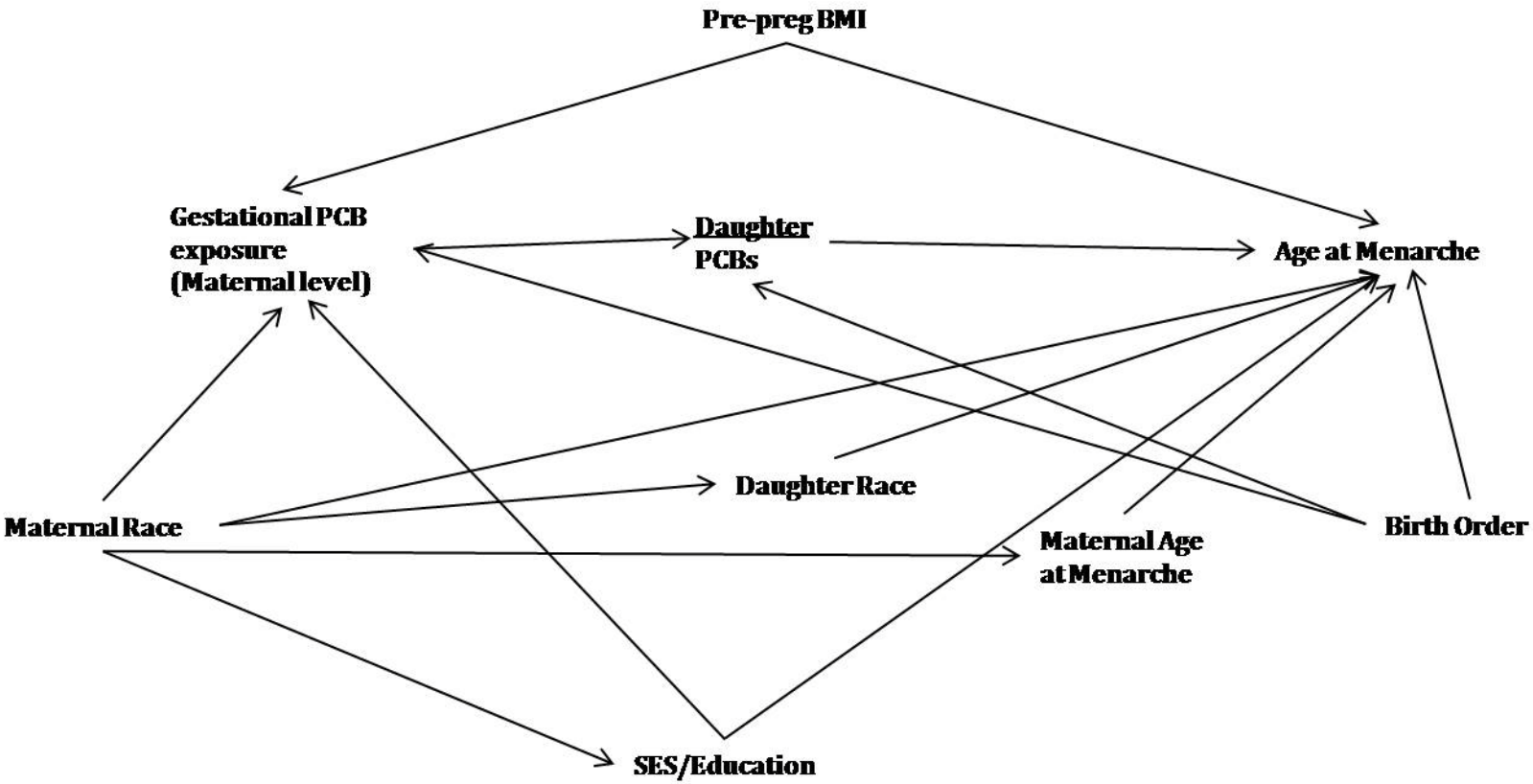


Figure 2c. Covariates potentially on the causal pathway between exposure (*in utero* PCB concentration) and outcome (age at menarche).