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Phthalate and bisphenol A exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls

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Abstract

Background—Over the past several decades, the age of pubertal onset in girls has shifted downward worldwide. As early pubertal onset is associated with increased risky behavior and psychological issues during adolescence and cardiometabolic disease and cancer in adulthood, this is an important public health concern. Exposure to endocrine disrupting chemicals during critical windows of in utero development may play a role in this trend. Our objective was to investigate trimester-specific phthalate and BPA exposure in relation to pubertal development among girls in the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) birth cohort.

Methods—We measured maternal urinary phthalate metabolites and BPA in samples collected during the first, second, and third trimesters of pregnancy. To assess reproductive development among their female children, we measured serum testosterone, estradiol, dehydroepiandrosterone sulfate (DHEA-S), inhibin B, and sex hormone-binding globulin (SHBG), and assessed sexual

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maturation, including Tanner staging for breast and pubic hair development and menarche status, at age 8–13 years (n=120). We used linear and logistic regression to examine measures of trimester-specific in utero exposure as predictors of peripubertal hormone levels and pubertal onset, respectively. In secondary analyses, we evaluated estimated exposure at the midpoint of the first trimester and rates of change in exposure across pregnancy in relation to outcomes.

Results—Several phthalate metabolites measured throughout in utero development were associated with higher serum testosterone concentrations, while a number of metabolites measured in the third trimester were associated with higher DHEA-S. For example, an interquartile range (IQR) increase in mean monoethyl phthalate (MEP) levels across pregnancy was associated with 44% higher peripubertal testosterone (95% CI: 13–83%), while an IQR increase in di-2-ethylhexyl phthalate metabolites (ΣDEHP) specifically in the third trimester was associated with 25% higher DHEA-S (95%CI: 4.7–47%). In IQR increase in mean mono-2-ethylhexyl phthalate (MEHP) levels across pregnancy was associated with lower odds of having a Tanner Stage >1 for breast development (OR=0.32, 95%CI: 0.11–0.95), while MEHP in the third trimester was associated with higher odds of having a Tanner Stage >1 for pubic hair development (OR=3.76, 95%CI: 1.1–12.8). Results from secondary analyses were consistent with findings from our main analysis.

Conclusion—These findings suggest that female reproductive development may be more vulnerable to the effects of phthalate or BPA exposure during specific critical periods of in utero development. This highlights the need for comprehensive characterizations of in utero exposure and consideration of windows of susceptibility in developmental epidemiological studies. Future research should consider repeated measures of in utero phthalate and BPA exposure within each trimester and across pregnancy.

Keywords

adrenarche; bisphenol A; in utero; phthalates; pregnancy; puberty; windows of susceptibility

1. Introduction

In recent decades, the average age of girls entering puberty has shifted downward worldwide (Aksglaede et al. 2008; Aksglaede et al. 2009; Biro et al. 2013; Euling et al. 2008a; Ma et al. 2009). Early onset of puberty is associated with increased risk of alcohol and substance use, health risk behaviors (Collado-Rodriguez et al. 2014; Kaltiala-Heino et al. 2011; Patton et al. 2004), and psychological and social issues during adolescence (Hamilton et al. 2014; Klump 2013; Mendle et al. 2007; Mendle et al. 2012; Short and Rosenthal 2008; Whittle et al. 2015), as well as increased risk of metabolic syndrome and type 2 diabetes (Frontini et al. 2003; He et al. 2010; Janghorbani et al. 2014; Widen et al. 2012), cardiovascular disease (Jacobsen et al. 2009; Lakshman et al. 2009; Prentice and Viner 2013), and endocrine-related cancers (Ali 2014; Beral et al. 2012; Jordan et al. 2005; Walvoord 2010) in adulthood. Improved nutritional status (Cheng et al. 2012; Villamor and Jansen 2016; Wyshak and Frisch 1982; Zacharias and Wurtman 1969) and increased prevalence of childhood obesity (Anderson et al. 2003; Kaplowitz 2008; Lee et al. 2007; Rosenfield et al. 2009; Shalitin and Kiess 2017) may contribute to the downward trend in age of pubertal onset. Early life and prepubertal exposure to endocrine disrupting chemicals, such as bisphenol A (BPA) and phthalates, are thought to also play a role (Buck Louis et al. 2008;

Euling et al. 2008b; Mouritsen et al. 2010; Schoeters et al. 2008), possibly via disruption of the hypothalamus-pituitary-gonadal (HPG) axis, disruption of metabolic homeostasis, or a combination of these mechanisms. Because in utero development is a crucial period of organogenesis and increased hormonal activity, exposure during this time may result in effects not observed with exposure at other life stages.

Phthalates and BPA are used in a range of consumer products, including personal care products, plastics, food packaging, and thermal receipt paper, resulting in widespread human exposure (Calafat et al. 2008; Silva et al. 2004; Teitelbaum et al. 2008). Previous studies have shown associations between markers of phthalate and BPA exposure and altered steroid hormone levels in adults (Ehrlich et al. 2012; Meeker et al. 2009; Mok-Lin et al. 2010; Pan et al. 2006; Sathyanarayana et al. 2014; Sathyanarayana et al. 2017), as well as associations between in utero or early life exposure and hormone levels in infants (Araki et al. 2014; Lin et al. 2011; Main et al. 2006). However, few studies have investigated relationships between phthalate or BPA exposure during in utero development and subsequent hormone levels during puberty, a time at which steroid hormones play an essential role in reproductive development. One study of in utero exposure, measured by pooling maternal serum samples from 18 and 34–36 weeks gestation, reported associations between DEHP and earlier age of menarche (Hart et al. 2014), although prior studies examining cross-sectional relationships between urinary markers of phthalate and BPA exposure and pubertal outcomes in adolescents have had conflicting findings (Frederiksen et al. 2012; Wolff et al. 2010; Wolff et al. 2014).

Recently, we reported that urinary mono-2-ethylhexyl phthalate (MEHP) levels during the third trimester of in utero development were associated with increased odds of adrenarche, and third trimester levels of monobenzyl phthalate (MBzP) and monoethyl phthalate (MEP) were associated with increased serum testosterone concentrations, in girls at 8 to 13 years of age (Watkins et al. 2014b). However, we hypothesized that there may be critical windows of susceptibility earlier in pregnancy, during which exposure may have a distinct impact on pubertal reproductive development given that the HPG axis is first established early in gestation (Bordini and Rosenfield 2011). To test this hypothesis, we measured urinary phthalate metabolite and BPA concentrations in maternal samples collected during the first and second trimesters of pregnancy and assessed relationships with peripubertal steroid hormone levels and measures of pubertal onset among female children within this same Mexico City birth cohort. We then compared our present findings to the previously reported associations between third trimester exposure, peripubertal reproductive hormone levels, and pubertal onset. In secondary analyses, we assessed modeled exposure levels at seven weeks gestation, the midpoint of the first trimester, and rates of change in exposure across pregnancy in relation to outcomes using advanced statistical methods.

2. Materials and Methods

2.1 Study Population

Participants are part of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) project, a longitudinal cohort study of pregnant women in Mexico City and their children. Our analysis includes women who were recruited from maternity hospitals

during their first trimester between 1997 and 2004 and their children as previously described (Lewis et al. 2013). Mothers provided a urine sample and completed interview-based questionnaires at up to three different prenatal visits (mean gestational age at visit 1: 13.5 (range: 9–24) weeks, visit 2: 25.1 (range: 19–37) weeks, visit 3: 34.4 (range: 28–43) weeks). In 2011, a subset of their children, who were then 8 to 13 years of age, were selected based on the availability of maternal prenatal urine samples and re-contacted to participate in follow-up studies (n=250). Children provided fasting serum samples, anthropometry, and reported demographic information via an interview-administered questionnaire. Age-specific BMI z-scores were calculated based on the World Health Organization child reference curves for age and sex (WHO 2007). In the current analyses, we included female children for whom we had maternal urinary phthalate metabolite and BPA measurements from at least one prenatal study visit (n=120). Distributions of child age and BMI z-score at follow-up are shown in Supplementary Table 1S. Research protocols were approved by the ethics and research committees of the Mexico National Institute of Public Health and the University of Michigan, and all participants provided informed consent prior to enrollment.

2.2 Urinary Phthalate Metabolites and BPA

Each mother provided a second morning void urine sample during at least one of the three prenatal study visits (visit 1 n=107, visit 2 n=109, visit 3 n=117), with most women providing a sample at all three time points (n=97). Children also provided a urine sample at the peripubertal visit as a measure of concurrent exposure. Samples were frozen and stored at −80°C and then transported to the University of Michigan until analysis at NSF International (Ann Arbor, MI, USA). BPA and nine phthalate metabolites, including monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP) were measured using isotope dilution liquid chromatography tandem mass spectrometry (ID LC MS/MS) as previously described (Lewis et al. 2013). Briefly, this method was developed based on the Centers for Disease Control and Prevention methods for measuring BPA and phthalates in urine (Calafat et al. 2008; Silva et al. 2007) and was evaluated against the acceptance criteria established within CDC methods. Samples first underwent enzymatic deconjugation of glucuronidated species, then ID-LC-MS/MS was performed using a Thermo Scientific (Waltham, MA, USA) Transcend TXII Turbulent Flow System interfaced with Thermo Scientific Vantage triple quadrupole mass spectrometer using multiple reaction monitoring in negative ionization mode. We calculated a DEHP metabolite summary measure (ΣDEHP) for each sample by dividing individual MEHP, MEHHP, MEOHP, and MECPP concentrations by their molar mass and summing them. Specific gravity (SG) was measured using a handheld digital refractometer (Atago Co., Ltd., Tokyo, Japan) at the time of sample analysis. Values below the limit of detection (LOD) were replaced with the LOD/√2.

2.3 Hormones

Children provided fasting blood samples during the follow-up visit at age 8–13 years. Serum aliquots were separated and frozen at -80°C, and then sent to the Clinical Ligand Assay

Service Satellite (CLASS) Laboratory at the University of Michigan (Ann Arbor, MI) for hormone analysis. We measured estradiol, testosterone, inhibin B, and sex hormone-binding globulin (SHBG) as biomarkers of puberty, and dehydroepiandrosterone sulfate (DHEA-S) as a biomarker of adrenarche. Estradiol, total testosterone, SHBG, and DHEA-S were measured using an automated chemiluminescent immunoassay (Bayer Diagnostics ACS: 180). Active inhibin B was assayed using Gen II ELISA (Beckman Coulter, Webster, TX). Values below the limit of detection (LOD) were replaced with the LOD/√2.

2.4 Pubertal Onset

Two pediatricians (CB-G and AM-G), trained according to standard methods (Chavarro et al. 2017), evaluated Tanner staging in female offspring using standardized protocols. We used breast developmental stage (BD) as an indicator of puberty and pubic hair stage (PH) as an indicator of adrenarche, with stage 1 corresponding to no development and stage 5 corresponding to full development (Marshall and Tanner 1969). Girls were also asked if they had had their first period (menarche) as an additional marker of pubertal onset.

2.5 Statistical Methods

Serum hormone and urinary phthalate metabolite and BPA concentrations were natural log-transformed prior to analysis. We used mixed models to calculate intraclass correlation coefficients (ICCs) to examine the variability of phthalate and BPA measurements across pregnancy within individuals with more than one exposure measurement (Hertzmark et al. 2016). ICC 95% confidence intervals were calculated as previously described (Hankinson et al. 1995; Hertzmark et al. 2016). Linear regression was used to separately assess associations between visit-specific urinary phthalate metabolite and BPA concentrations and peripubertal serum hormone concentrations. To assess overall exposure during pregnancy, we calculated geometric mean (GM) phthalate metabolite and BPA concentrations for each individual using all available measurements from prenatal visits 1, 2, and 3. Individual GM values were then entered into regression models to assess relationships between overall in utero exposure and peripubertal hormone levels. Results are presented as the percent difference in hormone (95% confidence interval) per interquartile range (IQR) increase in phthalate metabolite or BPA. All results are calculated based on the IQR of GM concentrations across pregnancy in order to more easily compare visit-specific effect estimates.

We used logistic regression to examine associations between visit-specific urinary phthalate metabolite or BPA concentrations and the odds of pubertal onset or adrenarche. Pubertal onset was defined by having a BD Tanner stage >1 or having undergone menarche, and adrenarche was defined by having a PH Tanner stage >1. Individual GM phthalate metabolite and BPA values were also entered into logistic regression models to assess relationships of overall in utero exposure with odds of pubertal onset and adrenarche. Results are presented as odds ratios (OR, 95% confidence interval), which express the change in odds per IQR increase in exposure, with the IQR based on GM concentrations across pregnancy.

In both linear and logistic regression models, we adjusted for child age and BMI z-score at follow-up as they have been associated with both exposure (Yang et al. 2017; Zota et al. 2014) and pubertal onset (Anderson et al. 2003; Kaplowitz 2008; Lee et al. 2007; Rosenfield et al. 2009; Shalitin and Kiess 2017). SG was included in all models as a measure of urinary dilution. In sensitivity analyses, we ran three separate sets of models that additionally adjusted for gestational age at the time of each prenatal visit based on mother's reported last menstrual period, children's concurrent urinary phthalate metabolite or BPA levels, or year of conception.

In secondary analyses, we utilized data from all participants and all visits in a single model to examine whether changes in exposure during pregnancy were associated with hormone concentrations or pubertal onset (Sanchez et al. 2011). This analysis complements our main analyses because it (a) explicitly accounts for variation in the weeks of gestation at which exposures were measured, (b) sheds light on how correlation among exposure measurements across pregnancy could influence what we see in the main analyses by helping to answer the question "after accounting for first-trimester exposure, does changing the exposure in subsequent trimesters matter?" (Sanchez et al. 2011), and (c) incorporates all available exposure data into a single model for each health outcome. To carry out these analyses, we first used mixed effects models with gestational age as a predictor of SG-corrected phthalate or BPA concentrations to estimate, for each woman and each compound, a random intercept and slope that described the compound's trajectory during the course of pregnancy. Prior to entering gestational age into the model, it was centered at the midpoint of the first trimester of pregnancy (seven weeks), such that an individual's intercept represents their estimated average urinary phthalate metabolite or BPA concentrations during the first trimester. An individual's slope indicates the rate of change in urinary phthalate metabolite or BPA across pregnancy: a positive slope indicates increased exposure over time, and a negative slope indicates decreased exposure over time. For each exposure, we then entered the estimated individual-specific slope and intercept into regression models as predictors of either hormone concentrations or measures of pubertal onset. However, if the slope and intercept for a specific phthalate metabolite or BPA were highly correlated (Pearson $r>0.8$), only the intercept was used. Findings were considered statistically significant at $p<0.05$, and all analyses were performed using SAS version 9.4 (Cary, NC).

3. Results

3.1 Phthalate Metabolite and BPA Variation across Pregnancy

With the exception of MiBP and BPA, the majority of phthalate metabolites were detected in >90% of urine samples (Table 1). Population geometric mean concentrations of BPA significantly decreased across pregnancy, while MBzP and MiBP significantly increased specifically in the third trimester. The reliability of one urinary measurement to reflect exposure across pregnancy was highest for MEP (ICC=0.42) and lowest for DEHP metabolites (range ICC: 0.24–0.28) (Table 1). Distributions of phthalate metabolite and BPA levels in each trimester are presented in Supplementary Figure 1S, and associations with the covariates child age and child BMI-z-score are presented in Table 2.

3.2 In utero phthalate and BPA exposure and peripubertal hormone concentrations

Several phthalate metabolites measured during in utero development were associated with higher peripubertal serum testosterone concentrations after adjustment for child age and BMI z-score (Table 3). IQR increases in the geometric means of MEP and MiBP across pregnancy were associated with 42% (95%CI: 6.6, 88.9) and 44% (95%CI: 12.8, 83.5) higher testosterone levels, respectively. This association was consistent across trimesters for MEP, but only significant for MiBP in the second trimester. BPA in the second trimester and MBzP in the third trimester were also associated with higher testosterone. In addition, several phthalate metabolites measured in the third trimester were associated with higher peripubertal DHEA-S concentrations, including the ΣDEHP summary measure, MBP, MiBP, and MCPP. In utero phthalate metabolites and BPA were not associated with peripubertal serum estradiol, SHBG, or inhibin B concentrations. When we included estimated gestational age at each prenatal visit into models, associations of third trimester phthalates with DHEA-S and inhibin B were slightly attenuated, but all other associations were either unchanged or slightly strengthened (not shown). In addition, when we included children's concurrent urinary phthalate or BPA levels or year of conception in models, associations between prenatal exposure measures and peripubertal hormones did not change (not shown).

3.3 In utero phthalate and BPA exposure and pubertal onset

Among the 120 study participants, 29% had a Tanner stage >1 for breast development, 21% had a Tanner stage >1 for pubic hair development, and 18% had undergone menarche (Supplementary Table 2S). DEHP metabolites measured across pregnancy, particularly MEHP, were associated with lower odds of having a Tanner Stage >1 for breast development at age 8–13 years, adjusting for child age and BMI z-score (Table 4). Interestingly, an IQR increase in MEHP in the third trimester was also associated with 3.8 times higher odds of having a Tanner Stage >1 for pubic hair development (95%CI: 1.1, 12.8), indicating adrenarche. MEP concentrations, primarily in the first trimester, were associated with higher odds of menarche (OR/IQR: 3.9; 95%CI: 1.1, 14.2), while BPA concentrations in the second trimester were associated with higher odds of having a Tanner Stage >1 for breast development (OR/IQR: 2.2; 95%CI: 1.0, 4.5). When we additionally included either gestational age at each prenatal visit, peripubertal urinary phthalate and BPA levels, or year of conception in models, the associations between prenatal exposure and pubertal onset did not change (not shown).

3.4 Secondary analysis

Distributions of individual exposure intercepts and slopes (random effects) and population means (fixed effects) from mixed models evaluating the effects of gestational age on prenatal phthalate and BPA levels are presented in Supplementary Table 3S. Although several phthalate metabolites were associated with increased testosterone in our main analyses, only the MEP and MiBP intercepts, indicating estimated average concentrations during the first trimester, were significantly associated with higher peripubertal testosterone in secondary analyses (Table 5). Consistent with findings from the third trimester in the main analyses, the slopes for ΣDEHP, MBP, and MCPP, which represent change in exposure across pregnancy, were each associated with higher peripubertal DHEA-S. In models

including both exposure intercept and slope as predictors of pubertal onset, effect estimates for slopes were unstable (infinite 95% confidence intervals), possibly due to the small number of girls who had undergone menarche or had Tanner stages >1, which limited the number of covariates we could reliably enter into models (Peduzzi et al. 1996; Vittinghoff and McCulloch 2007). Thus, we included only the intercept variable as a measure of first trimester exposure in final models (Table 6). Findings were consistent with first trimester associations observed in our main analyses. For example, an IQR increase in estimated MEP during the first trimester (intercept), as well as an IQR increase in measured MEP at the first prenatal visit, were both associated with 3.9 times higher odds of menarche at 8–13 years. In addition, urinary MEHP in the first trimester was associated with lower odds of Tanner stage >1 for breast development, regardless of whether it was modeled as the intercept at seven weeks gestation or measured in samples collected at the first prenatal visit.

4. Discussion

The goal of this analysis was to investigate potential windows of susceptibility during in utero development for the effect of phthalate and BPA exposure on female reproductive development during puberty. Building on our previous work, we assessed maternal urinary phthalate metabolite and BPA concentrations during the first, second, and third trimesters of pregnancy with subsequent peripubertal steroid hormone levels and measures of pubertal onset in female offspring. We found that testosterone concentrations and breast development were associated with specific phthalates across pregnancy, while markers of adrenarche, such as DHEA-S concentrations and pubic hair development, were associated with specific phthalates measured only during the third trimester. These findings suggest that certain aspects of reproductive development may have windows of susceptibility to phthalate or BPA exposure, while exposure throughout fetal development may be more important for others.

We previously reported associations between third trimester in utero phthalate exposure and altered reproductive hormone levels and pubertal onset in girls within this cohort (Watkins et al. 2014b). However, by looking only at phthalate exposure late in pregnancy, we missed important relationships between early in utero phthalate exposure and female reproductive development. For example, associations between first trimester MEP and increased odds of menarche and second trimester BPA and increased odds of breast development would have been missed if only measuring exposure in the third trimester. In addition, BPA and MiBP in the second trimester and MEP in the first and second trimesters were all associated with increased testosterone, while associations with third trimester exposure levels were either not observed or much weaker. These findings highlight the need for comprehensive characterizations of in utero exposure when investigating effects on development and health later in life.

One previous study has evaluated in utero phthalate exposure and age at menarche, reporting a marginally significant association between average in utero DEHP metabolite concentrations and earlier age of onset (Hart et al. 2014). We did not see a similar relationship in the current study, and the relationship between in utero MEP and earlier menarche reported here was not seen by Hart et al. These discrepancies could be due to

several important differences in study design. First, the study population in the previous study was older and more developed than our cohort. Second, Hart et al. measured phthalate metabolites in serum, which typically has relatively low phthalate concentrations and reflects very short-term exposure, while we measured urinary phthalate metabolites, which are present in relatively higher concentrations and reflect slightly longer-term exposure (Calafat et al. 2015). Although Hart et al. did pool two maternal serum samples collected at 18 and 34–36 weeks gestation for phthalate analysis, this measure is still unlikely to represent average exposure across pregnancy.

Two previous studies of urinary markers of childhood phthalate exposure reported associations between high molecular weight phthalates and delayed adrenarche (Frederiksen et al. 2012; Mouritsen et al. 2013; Wolff et al. 2010; Wolff et al. 2014), while in the present study, in utero DEHP exposure was associated with earlier adrenarche. However, Wolff et al. also reported that childhood exposure to low molecular weight phthalates (MEP, MnBP, MiBP) was associated with earlier breast development and adrenarche, (Wolff et al. 2010; Wolff et al. 2014). While we did observe similar, slightly higher odds of breast and pubic hair development onset with in utero MEP levels, these findings were not statistically significant. A recent study in China reported significant cross-sectional relationships between urinary DEHP metabolites and earlier onset and faster progression of breast development (Shi et al. 2015; Zhang et al. 2015), which is in contrast to our findings of later onset of breast development with higher in utero MEHP levels. The inconsistencies in findings between studies evaluating childhood vs. in utero phthalate exposure again emphasize the importance of timing of exposure, suggesting that exposure during distinct periods of development can have differential impacts on reproductive development. For example, we previously reported no significant associations between concurrent phthalate and BPA levels and sexual maturation in this same population (Watkins et al. 2014b), and additional adjustment for concurrent phthalate and BPA levels in models evaluating in utero exposure and peripubertal hormone concentrations did not alter our results (not shown).

Third trimester in utero phthalate exposure could influence the timing of adrenarche via changes in fetal development of the adrenal gland. During the third trimester, the definitive zone of the fetal adrenal gland, which later becomes the adult adrenal cortex, develops and become functionally and physically distinct from the fetal adrenal cortex, which disappears soon after birth (Xing et al. 2015). Phthalate exposure during this time may alter development of the adrenal cortex, which could become evident in periadolescence when growth of the adrenal zona reticularis (ZR; inner region of the adrenal cortex) and production of DHEA-S accelerates, stimulating pubic hair growth and other secondary sex characteristics (Xing et al. 2015). Females may be more sensitive than males to the effects of phthalate exposure on adrenal gland development as the ZR is their primary source of DHEA and DHEA-S, which is generally converted to testosterone in peripheral tissues. In males, adrenal androgens are far surpassed by androgens produced by the testes, so effects on the adrenal gland may not be evident. Animal studies have demonstrated that the adrenal gland is indeed a target for in utero DEHP exposure, with females being more sensitive to exposure than males and effects that last into adulthood (Martinez-Arguelles and Papadopoulos 2016). One hypothesis for how this may occur is through changes in fetal epigenetic programming (Martinez-Arguelles and Papadopoulos 2016).

Associations of in utero phthalate and BPA exposure with pubertal timing could be mediated through disruption of metabolic homeostasis, as previous studies have demonstrated associations between exposure and altered peripubertal leptin levels (Volberg et al. 2013; Watkins et al. 2016). Higher levels of leptin are necessary for the onset of puberty, as this signals that energy stores are adequate for reproductive development and maturity (Bordini and Rosenfield 2011). Previous findings also suggest that in utero phthalate and BPA exposure are associated with increased risk of childhood obesity and adiposity (Buckley et al. 2015; Deierlein et al. 2016; Hoepner et al. 2016; Valvi et al. 2013; Yang et al. 2017), key predictors of earlier pubertal development (Anderson et al. 2003; Kaplowitz 2008; Lee et al. 2007; Rosenfield et al. 2009; Shalitin and Kiess 2017). In addition, in utero phthalate exposure could affect pubertal timing via altered peripubertal levels of kisspeptin, a neurotransmitter that plays a crucial role in initiating GnRH release from the hypothalamus at the beginning of the pubertal transition (Hu et al. 2013), possibly by influencing neural development of the hypothalamus.

This analysis had a number of limitations, including a somewhat small sample size with only 20–30% of participants having begun the pubertal transition, resulting in imprecise effect estimates. However, an additional follow-up study of a larger subset of this cohort is currently underway, which will allow us to compare these early results to findings in the larger and older cohort. In addition, because we measured several phthalate metabolites, we made a large number of comparisons which likely increased the likelihood of chance findings. In the US, decreases in exposure to some phthalates has been observed since the early 2000s (Watkins et al. 2014a; Zota et al. 2014), so timing of the mother's pregnancy may have also influenced our findings. Specifically, older girls who are more likely to have begun puberty may have also been more likely to have higher *in utero* exposure to certain phthalates. However, it is not known if these same exposure trends occurred in Mexico during the time of mother's enrollment in this study (1997–2004), and when we additionally adjusted our models for year of conception, our results did not materially change. As urinary phthalate and BPA levels have been shown to vary over relatively short periods of time (Braun et al. 2012; Cantonwine et al. 2014; Meeker et al. 2013), one urine sample collected during each trimester may not fully characterize exposure during each specific window of development or trends in exposure across pregnancy. However, by calculating geometric mean phthalate levels across all three trimesters, we are able to more reliably characterize overall exposure during in utero development compared to utilizing one measure late in pregnancy. In addition, similar findings from our main and sensitivity analyses demonstrate that our results are robust to the method used. The possibility of contamination of urine samples with BPA and phthalates from outside sources cannot be ruled out, although samples were collected in BPA and phthalate-free containers using a standardized protocol and levels were well within NHANES reported ranges. In addition, some phthalate metabolites (MEHHP, MEOHP, and MECPP) that were significantly associated with outcomes in the present study are only present in biological samples and are not contaminants from the outside environment. Finally, serum steroid hormone concentrations were measured using standardized immunoassays, rather than the gold standard method, LC/MS-MS (Auchus 2014; Rosner et al. 2007), which may have resulted in a slight increase in the number of hormone levels below their respective LODs.

5. Conclusion

These findings suggest that aspects of reproductive development may be more vulnerable to the effects of phthalate or BPA exposure during specific critical periods of in utero development, but additional research is needed. This work highlights the need for comprehensive characterizations of in utero exposure and consideration of windows of susceptibility in epidemiological studies of early development and health later in life. Future research should consider repeated measures of in utero phthalate and BPA exposure within each trimester and across pregnancy, specifically in larger, more reproductively mature study populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BD	Tanner breast developmental stage
BPA	bisphenol A
DBP	dibutyl phthalate
DEHP	di-2-ethylhexyl phthalate
DHEA-S	dehydroepiandrosterone sulfate
ELEMENT	Early Life Exposure in Mexico to Environmental Toxicants
GM	geometric mean
HPG	hypothalamus-pituitary-gonadal
ICC	intraclass correlation coefficient
ID-LC-MS/MS	isotope dilution liquid chromatography tandem mass spectrometry
IQR	interquartile range
LOD	limit of detection

MBzP	monobenzyl phthalate
MCPP	mono-3-carboxypropyl phthalate
MECPP	mono-2-ethyl-5-carboxypentyl phthalate
MEP	monoethyl phthalate
MEHHP	mono-2-ethyl-5-hydroxyhexyl phthalate
MEHP	mono-2-ethylhexyl phthalate
MEOHP	mono-2-ethyl-5-oxohexyl phthalate
MiBP	monoisobutyl phthalate
MnBP	mono-*n*-butyl phthalate
PH	Tanner pubic hair stage
SHBG	sex hormone-binding globulin
SG	specific gravity

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Highlights

- Investigated exposure during in utero windows of development and female puberty
- In utero phthalate exposure was associated with higher peripubertal testosterone
- 3rd trimester phthalate exposure was associated with higher peripubertal DHEA-S
- In utero MEHP was associated with lower odds of breast development

Table 1

Distribution of unadjusted urinary phthalate metabolites and BPA across pregnancy (μg/L) among ELEMENT mothers who gave birth to female infants.

		Visit 1 (n=107)		Visit 2 (n=109)		Visit 3 (n=117)		Prenatal GM[a] (n=120)		Variability	
	%<LOD[b]	GM (GSD)	Max	GM (GSD)	Max	GM (GSD)	Max	GM (GSD)	Max	ICC	(95%CI)
BPA[c]	20.7	1.12 (2.52)	9.34	1.06 (2.55)	13.2	0.79 (2.38)	18.7	0.97 (2.03)	6.92	0.36	(0.25, 0.48)
MEHP	8.1	4.95 (3.00)	125	4.86 (2.93)	152	5.06 (2.76)	54.7	5.04 (2.13)	23.6	0.26	(0.16, 0.39)
MEHHP	0	17.5 (2.86)	158	18.8 (3.31)	1290	19.2 (2.97)	161	19.2 (2.23)	94.3	0.25	(0.15, 0.39)
MEOHP[c]	0	9.43 (2.89)	85	11.3 (3.28)	730	11.7 (2.95)	133	11.2 (2.22)	59.2	0.24	(0.14, 0.38)
MECPP	0	31.4 (2.56)	252	34.1 (2.96)	2650	31.2 (2.84)	251	33.1 (2.11)	172	0.28	(0.18, 0.41)
MBzP[c]	3.0	2.65 (3.72)	69.6	2.36 (3.43)	38.5	4.11 (2.71)	109	3.09 (2.38)	20.9	0.25	(0.15, 0.39)
MBP	0	63.6 (4.01)	1340	55.4 (3.80)	1390	55.0 (3.58)	1190	58.9 (2.65)	468	0.31	(0.20, 0.44)
MiBP[c]	19.2	1.09 (4.58)	18.5	0.82 (4.72)	26.5	2.03 (2.95)	33.9	1.25 (2.95)	20.9	0.32	(0.21, 0.45)
MCPP	8.7	1.12 (3.00)	17.8	1.07 (2.97)	15	1.06 (2.84)	11.1	1.10 (2.25)	6.55	0.34	(0.23, 0.46)
MEP	0.3	147 (4.19)	4480	121 (4.14)	4980	115 (4.72)	9810	129 (3.25)	2223	0.42	(0.31, 0.54)
ΣDEHP[d]		0.22 (2.65)	1.67	0.24 (3.03)	16.0	0.23 (2.74)	1.84	0.24 (2.10)	1.12	0.26	(0.16, 0.40)

LOD=limit of detection, GM=geometric mean, GSD=geometric standard deviation, ICC=intraclass correlation coefficient, CI=confidence interval.

[a]Geometric mean of phthalate or BPA measurements for each individual across pregnancy using all available measurements from prenatal visits 1, 2, and 3.

[b]LODs: BPA=0.4, MEHP, MEP=1.0, MEHHP, MEOHP=0.1, MECPP, MBzP, MiBP, MCPP=0.2, MBP=0.5;

[c]Concentrations were significantly different across prenatal visits after standardization for urinary specific gravity and consideration of intra-individual correlation across visits ($p<0.05$);

[d]ΣDEHP=molecular sum of MEHP, MEHHP, MEOHP, and MECPP (μmol/L).

Table 2

Associations between prenatal urinary phthalate metabolite and BPA concentrations (μg/L) and child age and BMI z-score at follow-up.

	Child Age (years)			Child BMI z-score		
	β[a]	(95% CI)	p-value	β[a]	(95% CI)	p-value
BPA	−0.061	(−0.130, 0.007)	0.08	−0.010	(−0.094, 0.074)	0.82
MEHP	0.019	(−0.057, 0.095)	0.62	−0.060	(−0.151, 0.031)	0.20
MBzP	0.048	(−0.046, 0.142)	0.31	−0.091	(−0.204, 0.021)	0.11
MBP	0.022	(−0.074, 0.118)	0.65	−0.017	(−0.133, 0.099)	0.77
MiBP	−0.145	(−0.253, −0.037)	0.01	0.044	(−0.089, 0.178)	0.51
MCPP	0.013	(−0.066, 0.092)	0.74	−0.018	(−0.114, 0.077)	0.70
MEP	−0.001	(−0.127, 0.125)	0.99	−0.015	(−0.167, 0.137)	0.85
ΣDEHP[b]	0.039	(−0.032, 0.109)	0.28	−0.004	(−0.089, 0.081)	0.92

[a] Difference in ln-transformed geometric mean prenatal phthalate metabolite or BPA concentration across pregnancy associated with a 1-unit increase in child age or BMI z-score, adjusted for urinary specific gravity.

[b] ΣDEHP=molecular sum of MEHP, MEHHP, MEOHP, and MECPP (μmol/L).

Table 3

Among female ELEMENT children, percent difference in peripubertal hormone levels associated with an interquartile range (IQR) increase in phthalate metabolite or BPA concentration during trimester specific in utero development, adjusted for child age, BMI z-score, and urinary specific gravity.

	Visit 1 (n=106) %Δ/IQR[b] (95%CI)	Visit 2 (n=108) %Δ/IQR[b] (95%CI)	Visit 3 (n=116) %Δ/IQR[b] (95%CI)	Prenatal GM[a] (n=119) %Δ/IQR[b] (95%CI)
Estradiol[c]				
BPA	0 (−14.6, 17.0)	14.9 (−1.9, 34.6)	−3.5 (−20.3, 17.0)	6.0 (−8.3, 22.4)
MBzP	0 (−12.9, 14.8)	−3.7 (−19.3, 14.9)	−2.7 (−13.1, 9.0)	−2.3 (−15.0, 12.4)
MBP	−1.3 (−13.7, 12.9)	0.3 (−15.5, 18.9)	0.5 (−11.7, 14.3)	−1.6 (−14.4, 13.2)
MiBP	7.5 (−12.2, 31.8)	−4.7 (−25.0, 21.0)	7.0 (−7.1, 23.2)	4.8 (−11.1, 23.4)
MCPP	−1.3 (−12.4, 11.1)	0.7 (−14.9, 19.1)	3.4 (−11.1, 20.3)	1.3 (−12.2, 16.8)
MEP	−10.8 (−22.1, 2.1)	−5.4 (−20.6, 12.7)	0.9 (−9.5, 12.6)	−4.1 (−16.7, 10.4)
ΣDEHP[d]	8.1 (−5.5, 23.7)	−5.7 (−21.3, 12.9)	9.5 (−5.0, 26.4)	7.9 (−6.4, 24.5)
Testosterone[c]				
BPA	−0.7 (−28.1, 37.3)	**33.2 (0.3, 77.0)** *	3.2 (−27.0, 46.0)	11.5 (−13.8, 44.4)
MBzP	0.8 (−24.2, 34.0)	7.1 (−22.0, 47.2)	**26.7 (3.8, 54.7)** *	12.8 (−12.1, 44.6)
MBP	15.8 (−12.0, 52.4)	14.1 (−16.0, 54.9)	13.3 (−10.1, 42.9)	19.1 (−7.0, 52.5)
MiBP	40.9 (−6.9, 113)	**87.7 (24.2, 184)** *	18.8 (−7.9, 53.2)	**41.9 (6.6, 88.9)** *
MCPP	1.8 (−20.3, 30.1)	12.3 (−16.9, 51.9)	8.6 (−17.3, 42.6)	6.4 (−17.5, 37.1)
MEP	**35.1 (2.6, 78.0)** *	**63.0 (20.7, 120)** *	**24.2 (2.4, 50.7)** *	**43.9 (12.8, 83.5)** *
ΣDEHP[d]	8.3 (−18.0, 43.1)	9.4 (−21.0, 51.4)	4.0 (−19.8, 34.7)	7.7 (−16.6, 39.1)
SHBG[c]				
BPA	3.6 (−10.5, 19.9)	6.0 (−7.2, 21.1)	−2.7 (−17.1, 14.4)	2.2 (−9.5, 15.5)
MBzP	−5.1 (−16.5, 7.9)	−4.6 (−17.6, 10.5)	−1.9 (−10.8, 7.8)	−5.2 (−15.7, 6.6)
MBP	3.3 (−8.8, 17.1)	−1.8 (−14.8, 13.2)	0.9 (−9.4, 12.5)	1.6 (−9.7, 14.2)
MiBP	10.9 (−8.2, 33.8)	11.9 (−8.2, 36.4)	1.1 (−10.3, 13.9)	6.1 (−7.5, 21.8)
MCPP	1.2 (−9.4, 13.1)	2.1 (−11.2, 17.4)	−0.7 (−12.5, 12.7)	−0.1 (−11.3, 12.6)
MEP	−10.1 (−20.8, 1.9)	1.9 (−11.9, 17.9)	−3.0 (−11.5, 6.3)	−6.2 (−16.7, 5.5)
ΣDEHP[d]	5.0 (−7.4, 19.1)	−1.9 (−15.6, 14.1)	−2.9 (−13.9, 9.5)	−0.2 (−11.5, 12.6)
DHEA-S[c]				
BPA	4.7 (−15.3, 29.4)	−6.2 (−21.9, 12.8)	14.6 (−9.3, 44.6)	5.0 (−11.8, 25.1)
MBzP	−6.0 (−22, 13.2)	−9.0 (−25.6, 11.4)	0.2 (−12.7, 15.1)	−7.3 (−21.7, 9.8)
MBP	−9.2 (−24.2, 8.7)	−4.0 (−21.1, 16.7)	**17.4 (0.5, 37)** *	2.7 (−13.3, 21.6)
MiBP	4 (−21, 36.8)	−4.7 (−27.6, 25.4)	**19.4 (0.7, 41.6)** *	3.6 (−15, 26.4)
MCPP	−8.9 (−22.4, 6.8)	−13.1 (−28.2, 5.2)	**20.5 (0.5, 44.5)** *	−0.9 (−16.6, 17.8)
MEP	17.3 (−2.2, 40.7)	13.7 (−6.8, 38.9)	9.9 (−3.8, 25.4)	18.1 (−0.2, 39.8)
ΣDEHP[d]	−1.8 (−18.2, 17.9)	−0.5 (−19.1, 22.4)	**24.2 (4.7, 47.3)** *	10.5 (−7.1, 31.3)
Inhibin B[c]				

	Visit 1 (n=106) %Δ/IQR[b] (95%CI)	Visit 2 (n=108) %Δ/IQR[b] (95%CI)	Visit 3 (n=116) %Δ/IQR[b] (95%CI)	Prenatal GM[a] (n=119) %Δ/IQR[b] (95%CI)
BPA	−4.1 (−20.8, 16.2)	−0.8 (−19.7, 22.5)	4.0 (−20.3, 35.7)	3.1 (−15.5, 25.7)
MBzP	1.0 (−14.7, 19.5)	−9.8 (−28.5, 13.6)	0.4 (−14.2, 17.5)	−3.5 (−20.3, 16.8)
MBP	8.9 (−7.5, 28.1)	−4.0 (−23.3, 20.1)	−2.2 (−18.3, 16.9)	1.0 (−16.6, 22.3)
MiBP	1.2 (−21.0, 29.7)	−11.8 (−35.6, 20.7)	4.1 (−14.5, 26.7)	−3.0 (−22.6, 21.4)
MCPP	7.8 (−6.7, 24.6)	5.7 (−15.3, 31.8)	11.2 (−9.8, 37.0)	8.8 (−10.4, 32.1)
MEP	−4.8 (−19.4, 12.4)	−11.7 (−29.8, 11.1)	7.3 (−7.7, 24.8)	0.5 (−17.2, 22.0)
ΣDEHP[d]	−1.9 (−16.9, 15.7)	−6.6 (−26.4, 18.4)	20.5 (−1.0, 46.6)	6.0 (−12.9, 28.9)

[*] p<0.05; CI=confidence interval;

[a] Geometric mean of phthalate or BPA measurements for each individual across pregnancy using all available measurements from prenatal visits 1, 2, and 3.

[b] Percent difference in hormone concentration per IQR increase in phthalate metabolite or BPA concentration.

[c] Testosterone=7.6% of samples <LOD (2.0ng/dL); DHEA-S=11%<LOD (15.0 μg/dL); inhibin B=21%<LOD (10.0 or 11.0, depending on batch), SHBG=0%<LOD (1.6 nmol/L); estradiol=0%<LOD (3.8 pg/mL).

[d] ΣDEHP=molecular sum of MEHP, MEHHP, MEOHP, and MECPP (μmol/L).

Table 4

Among female ELEMENT children, odds of Tanner stage >1 or menarche associated with an interquartile range (IQR) increase in in utero urinary phthalate metabolite or BPA concentration, adjusted for child age, BMI z-score, and urinary specific gravity.

	Visit 1 (n=107) OR/IQR[b] (95%CI)	Visit 2 (n=109) OR/IQR[b] (95%CI)	Visit 3 (n=117) OR/IQR[b] (95%CI)	Prenatal GM[a] (n=120) OR/IQR[b] (95%CI)
Menarche				
BPA	0.62 (0.20, 1.86)	1.23 (0.43, 3.51)	0.62 (0.26, 1.46)	0.7 (0.22, 2.19)
MEHP	0.70 (0.23, 2.15)	0.82 (0.27, 2.51)	0.93 (0.39, 2.21)	0.91 (0.24, 3.46)
MBzP	0.78 (0.33, 1.85)	0.96 (0.29, 3.23)	0.88 (0.32, 2.46)	0.92 (0.26, 3.25)
MBP	0.57 (0.21, 1.58)	0.70 (0.21, 2.27)	0.92 (0.39, 2.21)	0.73 (0.22, 2.41)
MiBP	0.56 (0.19, 1.69)	0.54 (0.17, 1.77)	0.35 (0.07, 1.66)	0.39 (0.09, 1.70)
MCPP	0.71 (0.24, 2.09)	0.63 (0.16, 2.52)	1.05 (0.40, 2.75)	1.00 (0.27, 3.68)
MEP	**3.92 (1.08, 14.2)** *	1.13 (0.34, 3.70)	2.44 (0.92, 6.52)	**4.33 (1.25, 15.0)** *
ΣDEHP[c]	1.24 (0.42, 3.66)	0.76 (0.22, 2.60)	1.33 (0.61, 2.86)	1.89 (0.57, 6.22)
Breast Development				
BPA	0.92 (0.47, 1.82)	**2.15 (1.04, 4.46)** *	1.40 (0.64, 3.06)	1.95 (0.82, 4.60)
MEHP	**0.26 (0.10, 0.68)** *	**0.38 (0.15, 0.93)** *	0.89 (0.43, 1.86)	**0.32 (0.11, 0.95)** *
MBzP	0.93 (0.50, 1.74)	1.25 (0.55, 2.85)	1.93 (0.82, 4.56)	1.58 (0.63, 3.96)
MBP	1.27 (0.61, 2.68)	0.60 (0.26, 1.41)	0.97 (0.48, 1.95)	1.06 (0.41, 2.71)
MiBP	0.89 (0.37, 2.15)	1.20 (0.51, 2.80)	0.65 (0.18, 2.26)	1.21 (0.38, 3.90)
MCPP	1.18 (0.54, 2.54)	0.87 (0.34, 2.24)	1.19 (0.54, 2.61)	1.37 (0.52, 3.62)
MEP	1.32 (0.59, 2.94)	1.58 (0.55, 4.59)	1.05 (0.51, 2.17)	1.49 (0.57, 3.88)
ΣDEHP[c]	0.67 (0.31, 1.47)	0.59 (0.24, 1.42)	0.81 (0.43, 1.51)	0.66 (0.26, 1.69)
Pubic Hair Development				
BPA	n/a	1.88 (0.61, 5.85)	1.08 (0.38, 3.02)	1.05 (0.30, 3.68)
MEHP	0.14 (0.01, 3.44)	1.65 (0.48, 5.68)	**3.76 (1.1, 12.8)** *	5.21 (0.83, 32.6)
MBzP	1.42 (0.35, 5.73)	2.63 (0.65, 10.6)	1.62 (0.49, 5.37)	3.89 (0.95, 15.8)
MBP	2.04 (0.31, 13.6)	0.96 (0.27, 3.46)	1.72 (0.67, 4.44)	2.6 (0.65, 10.46)
MiBP	0.48 (0.05, 4.36)	0.97 (0.30, 3.07)	1.68 (0.26, 10.7)	1.65 (0.30, 9.03)
MCPP	1.38 (0.19, 9.87)	1.31 (0.30, 5.61)	1.75 (0.61, 4.98)	2.32 (0.56, 9.58)
MEP	1.50 (0.24, 9.40)	1.56 (0.39, 6.21)	1.91 (0.74, 4.89)	2.31 (0.67, 7.94)
ΣDEHP[c]	1.80 (0.31, 10.4)	0.74 (0.19, 2.84)	1.94 (0.83, 4.53)	2.15 (0.62, 7.48)

* p<0.05; OR=odds ratio; CI=confidence interval;

[a] Geometric mean of phthalate or BPA measurements for each individual across pregnancy using all available measurements from prenatal visits 1, 2, and 3.

[b] Odds ratio associated with an IQR increase in phthalate metabolite or BPA concentration.

[c] ΣDEHP=molecular sum of MEHP, MEHHP, MEOHP, and MECPP (μmol/L).

Table 5

Among female ELEMENT children, the percent difference in hormone concentrations per interquartile range (IQR) increase in estimated exposure intercept and slope across in utero development at 8–13 years of age, adjusted for child age and BMI z-score (n=118).

		Estradiol %Δ/IQR[a] (95%CI)	Testosterone %Δ/IQR[a] (95%CI)	SHBG %Δ/IQR[a] (95%CI)	DHEA-S %Δ/IQR[a] (95%CI)	Inhibin B %Δ/IQR[a] (95%CI)
BPA[b]	Intercept	6.9 (−4.8, 19.9)	6.1 (−13.6, 30.3)	3.0 (−6.5, 13.4)	2.0 (−11.2, 17.3)	−1.9 (−16.2, 14.9)
	Slope	n/a	n/a	n/a	n/a	n/a
MBzP[b]	Intercept	−0.6 (−11.6, 11.9)	5.3 (−14.6, 29.8)	−4.1 (−13.0, 5.8)	−5.6 (−18.1, 8.7)	−2.8 (−17.2, 14.1)
	Slope	n/a	n/a	n/a	n/a	n/a
MBP	Intercept	−3.9 (−18.1, 12.8)	18.0 (−11.1, 56.7)	−0.3 (−12.8, 13.9)	10.0 (−8.7, 32.6)	−3.8 (−22.7, 19.7)
	Slope	−2.8 (−16.0, 12.4)	5.0 (−18.8, 35.9)	−5.7 (−16.4, 6.5)	**25.7 (6.1, 49.0)** *	1.5 (−24.7, 12.0)
MiBP[b]	Intercept	0.3 (−12.1, 14.5)	**34.3 (6.9, 68.6)** *	5.7 (−5.3, 18.0)	5.6 (−9.9, 23.7)	−0.6 (−16.9, 19.0)
	Slope	n/a	n/a	n/a	n/a	n/a
MCPP	Intercept	−1.2 (−13.6, 12.9)	6.9 (−15.7, 35.6)	−0.7 (−11.2, 11.0)	4.1 (−10.9, 21.6)	8.3 (−9.8, 29.9)
	Slope	−1 (−15.4, 15.8)	9.1 (−17.5, 44.2)	−4.5 (−16.2, 8.9)	**24.5 (3.7, 49.5)** *	5.6 (−14.8, 30.8)
MEP	Intercept	−6.4 (−18.0, 6.9)	**40.6 (11.9, 76.7)** *	−5.9 (−15.8, 5.1)	**17.3 (0.1, 37.3)** *	−1.4 (−17.8, 18.2)
	Slope	1.6 (−8.3, 12.5)	11.8 (−6.3, 33.2)	0.9 (−7.3, 9.9)	3.6 (−8.3, 16.9)	7.5 (−6.5, 23.6)
ΣDEHP[c]	Intercept	4.9 (−6.7, 18.0)	8.9 (−11.6, 34.2)	0.9 (−8.5, 11.2)	7.1 (−6.8, 23.0)	1.3 (−13.6, 18.8)
	Slope	−1.2 (−12.5, 11.7)	−1.0 (−20.4, 23.0)	−6.7 (−15.7, 3.3)	**17.6 (1.8, 35.9)** *	13.1 (−4.2, 33.5)

*p<0.05, CI=confidence interval;

[a]Percent difference in hormone concentration per IQR increase in estimated exposure at 7 weeks gestation (intercept) and change in exposure across in utero development (slope).

[b]Intercept and slope highly correlated (BPA r=−0.82, MBzP r=−0.99, MiBP r=−0.96), only intercept included in model.

[c]ΣDEHP=molecular sum of MEHP, MEHHP, MEOHP, and MECPP (μmol/L).

Table 6

Among ELEMENT girls, odds ratios of having a Tanner stage >1 or having undergone menarche at 8–13 years of age associated with an interquartile range (IQR) increase in estimated in utero exposure at the midpoint of first trimester (intercept), adjusted for child age and BMI z-score (n=119).

	Menarche OR/IQR[a] (95%CI)	Breast Development OR/IQR[a] (95%CI)	Pubic Hair Development OR/IQR[a] (95%CI)
Intercept			
BPA	0.78 (0.29, 2.12)	1.31 (0.66, 2.59)	0.58 (0.17, 1.98)
MEHP	0.83 (0.30, 2.34)	**0.22 (0.09, 0.55)***	1.03 (0.34, 3.16)
MBzP	0.79 (0.25, 2.55)	1.05 (0.47, 2.34)	2.19 (0.63, 7.58)
MBP	0.75 (0.25, 2.21)	0.96 (0.41, 2.26)	2.11 (0.55, 8.14)
MiBP	0.43 (0.13, 1.42)	1.03 (0.40, 2.67)	1.16 (0.27, 4.87)
MCPP	0.84 (0.32, 2.20)	1.01 (0.50, 2.05)	1.4 (0.47, 4.18)
MEP	**3.88 (1.24, 12.1)***	1.34 (0.58, 3.12)	1.97 (0.63, 6.20)
ΣDEHP	1.43 (0.48, 4.30)	0.49 (0.22, 1.11)	1.32 (0.41, 4.29)

* p<0.05; OR=odds ratio; CI=confidence interval;

[a] Odds ratio associated with an IQR increase in estimated exposure at 7 weeks gestation, the 1st trimester midpoint (intercept).