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In utero and peripubertal exposure to phthalates and BPA in relation to female sexual maturation

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ABSTRACT

The age of pubertal onset for girls has declined over past decades. Research suggests that endocrine disrupting chemicals (EDCs) may play a role but exposure at multiple stages of development has not been considered. We examined *in utero* and peripubertal exposure to bisphenol-A (BPA) and phthalates in relation to serum hormones and sexual maturation among females in a Mexico City birth cohort. We measured phthalate metabolite and BPA concentrations in urine collected from mothers during their third trimester ($n=116$) and from their female children at ages 8–13 years ($n=129$). Among girls, we measured concurrent serum hormone concentrations, Tanner stages for breast and pubic hair development, and collected information on menarche onset. We used linear and logistic regression to model associations between *in utero* and peripubertal measures of exposure with hormones and sexual maturation, respectively, controlling for covariates. An interquartile range (IQR) increase in *in utero* urinary mono-2-ethylhexyl phthalate (MEHP) was positively associated with 29% (95% CI: 9.2–52.6%) higher dehydroepiandrosterone sulfate (DHEA-S), an early indicator of adrenarche, and 5.3 (95% CI: 1.13–24.9) times higher odds of a Tanner stage >1 for pubic hair development. Similar relationships were observed with other *in utero* but not peripubertal di-2-ethylhexyl phthalate (DEHP) metabolites. IQR increases in *in utero* monobenzyl phthalate (MBzP) and monoethyl phthalate (MEP) were associated with 29% and 25% higher serum testosterone concentrations (95% CI: 4.3–59.3; 2.1–54.1), respectively. In addition, we observed suggestive associations between *in utero* and peripubertal MEP concentrations and increased odds of having undergone menarche, and between peripubertal MnBP concentrations and increased odds of having a Tanner stage >1 for both breast and pubic hair development. BPA was not associated with *in utero* or peripubertal serum hormones or sexual maturation.

Our findings suggest *in utero* phthalate exposure may impact hormone concentrations during peripubescence and timing of sexual maturation. Efforts to control phthalate exposure during pregnancy should be of high priority.

Abbreviations: BBzP, butylbenzyl phthalate; BMI-Z, body mass index *Z* score for age and sex; BPA, bisphenol A; CDC, Centers for Disease Control and Prevention; CLASS, Clinical Ligand Assay Service Satellite; DEHP, di-2-ethylhexyl phthalate; DHEA-S, dehydroepiandrosterone sulfate; EDC, endocrine disrupting compound; ELEMENT, Early Life Exposure in Mexico to Environmental Toxicants; GM, geometric mean; ID–LC–MS/MS, isotope dilution–liquid chromatography–tandem mass spectrometry; IQR, interquartile range; LH, luteinizing hormone; LOD, limit of detection; MBzP, monobenzyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; MEP, monoethyl phthalate; MiBP, mono-isobutyl phthalate; MnBP, mono-n-butyl phthalate; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; SG, specific gravity; SHBG, sex hormone-binding globulin

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1. Introduction

Population trends since the mid-1900s show shifts in the age of onset and progression of puberty in both boys and girls (Aksglaede et al., 2009; Euling et al., 2008a). There is concern that *in utero* and early life exposure to endocrine disrupting compounds (EDCs), such as bisphenol A (BPA) and phthalates, could play a role in this shift (Euling et al., 2008b; Meeker, 2012). Early onset of puberty is an important public health issue since it is associated with increased risk of psychological and social issues during adolescence (Short and Rosenthal, 2008), as well as metabolic (Frontini et al., 2003), cardiovascular (Lakshman et al., 2009), and endocrine-related diseases or cancers (Apter et al., 1989; Gail et al., 1989; Lacey et al., 2009) in adulthood. Exposure to phthalates and BPA has been hypothesized to be associated with earlier puberty in both boys and girls (Buck Louis et al., 2008; Mouritsen et al., 2010; Schoeters et al., 2008).

Phthalates are used in a wide range of consumer products, resulting in ubiquitous exposure (Silva et al., 2004; Teitelbaum et al., 2008). In humans, studies of adults have linked phthalate exposure with altered reproductive steroid (Meeker et al., 2009; Pan et al., 2006; Sathyanarayana et al., 2014) and thyroid (Meeker et al., 2007; Meeker and Ferguson, 2011) hormone concentrations, although most studies to date have focused on men. Findings from previous studies evaluating exposure to various phthalates and sexual maturation in girls have not been consistent. In a cross-sectional evaluation of sexual development among 1151 U.S. girls at 6–8 years of age, exposure to low molecular weight phthalates (sum of monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), and mono-isobutyl phthalate (MiBP)) was weakly associated with a higher prevalence of breast and pubic hair development (thelarche and pubarche, respectively), while exposure to high molecular weight phthalates (sum of mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-2-ethylhexyl phthalate (MEHP), monobenzyl phthalate (MBzP), and mono-3-carboxypropyl phthalate (MCPP)) was associated with lower prevalence of pubarche (Wolff et al., 2010). In a recent 7-year follow-up of these participants, high molecular weight phthalate metabolites measured at enrollment were again associated with later pubic hair development (Wolff et al., 2014). In a cross-sectional study of 725 Danish girls aged 5–19 years, girls in the highest quartiles of urinary MBzP and MnBP plus MiBP concentrations entered pubarche at significantly later ages compared to girls in the lowest quartiles (Frederiksen et al., 2012). Conversely, in a study evaluating *in utero* exposure among 121 Australian girls, di-2-ethylhexyl phthalate (DEHP) metabolite concentrations in maternal serum during pregnancy were associated with earlier age of menarche in female offspring (Hart et al., 2014). Animal and *in vitro* studies have demonstrated that several phthalates and/or their metabolites are anti-androgenic (Autian, 1973; Borch et al., 2006), estrogenic (Chen et al., 2014; Harris et al., 1997), and associated with adverse reproductive and developmental effects in males (Foster, 2006), but studies examining exposure related effects in females are limited. Recently, a study of female rats found that both neonatal and prepubertal dibutyl phthalate (DBP) exposure were associated with earlier pubertal onset (Hu et al., 2013).

BPA is a high production chemical used in the manufacture of polycarbonate plastics, epoxy resins, and thermal paper, and can be measured in the urine of almost everyone tested (Calafat et al., 2008). Human studies evaluating adult exposure to BPA have reported associations between exposure, serum estradiol concentrations, and oocyte number among women undergoing *in-vitro* fertilization (Ehrlich et al., 2012; Mok-Lin et al., 2010). However, no human studies have evaluated associations between early life exposure to BPA and sexual maturation. In animals, *in utero* and perinatal BPA exposure have been linked to early puberty onset (Adewale et al., 2009; Maffini et al., 2006).

To date there have been limited human studies to assess associations between EDC exposure and sexual maturation. In addition, previous studies have been primarily cross-sectional, although *in utero* exposure may play an important role in later development. Among studies that have looked at *in utero* exposure to EDCs, few have examined the impacts of exposure on the tempo of sexual maturation in girls. We examine phthalate and BPA exposure during two potentially sensitive developmental time points – *in utero* and peripubertal – in relation to serum hormone concentrations and physical measures of puberty, defined by breast development, and pubarche, defined by pubic hair development, in girls.

2. Materials and methods

2.1. Study population

Our study population comprises a subset of participants from the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) project, a longitudinal cohort study of pregnant women recruited from maternity hospitals in Mexico City and their offspring. Our analysis includes women from the second and third of three sequentially enrolled cohorts who were recruited from 1997 to 2004 during their first trimester. Mothers were followed throughout pregnancy, and both mothers and offspring were followed through postnatal visits as previously described (Lewis et al., 2013). During their third trimester, mothers provided a urine sample and completed interview-based questionnaires. In 2010, a subset of male and female child participants, who were now 8–13 years of age, were re-contacted to participate in follow-up studies ($n=250$), and subsequently provided spot urine samples, serum samples, anthropometry, and completed an interview-based questionnaire. In the present analysis we included 132 female children who had urinary phthalate metabolite and BPA measurements from their urine sample collected at follow-up ($n=129$) and/or their mother's urine sample collected during her third trimester ($n=116$). Urine samples were available from both *in utero* and peripubertal time points for 113 mother/female child pairs. One participant who did not have serum hormone measurements was only included in the sexual maturation analyses. 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 spot urine sample during her third trimester of pregnancy study visit as a measure of *in utero* exposure to the child, and each female child provided a spot urine sample during a follow-up study visit at 8–13 years of age as a measure of peripubertal exposure. Samples were collected in commercially available urine containers, frozen at −80 °C, and analyzed at NSF International (Ann Arbor, MI). Total (free+glucuronidated) BPA and nine phthalate metabolites, comprising MEP, MnBP, MiBP, MBzP, MCPP, MEHP, MEHHP, MEOHP, and MECPP were measured in urine using isotope dilution–liquid chromatography–tandem mass spectrometry (ID–LC–MS/MS) as previously described (Lewis et al., 2013). 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/$\sqrt{2}$ (Hornung and Reed, 1990).

2.3. Hormones

We measured total estradiol, total testosterone, inhibin B, and sex hormone-binding globulin (SHBG) as biomarkers of puberty and breast development, and dehydroepiandrosterone sulfate (DHEA-S) as a biomarker of adrenarche and pubic hair development. We collected fasting serum samples from children during follow-up visits at 8–13 years of age. Samples were sent to the Clinical Ligand Assay Service Satellite (CLASS) Laboratory at the University of Michigan (Ann Arbor, MI) for hormone analysis. DHEA-S, estradiol, SHBG, and total testosterone (Total T) 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/$\sqrt{2}$.