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Associations of urinary phthalate and phenol biomarkers with menarche in a multiethnic cohort of young girls

Mary S. Wolff^{a,*}, Ashley Pajak^a, Susan M. Pinney^b, Gayle C. Windham^c, Maida Galvez^a, Michael Rybak^d, Manori J. Silva^d, Xiaoyun Ye^d, Antonia M. Calafat^d, Lawrence H. Kushi^e, Frank M. Biro^f, Susan L. Teitelbaum^a, and the Breast Cancer and Environment Research Program¹

^aDepartment of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place Box 1057, New York, NY 10029, USA

^bDepartment of Environmental Health, Kettering Building, Room 208, University of Cincinnati College of Medicine, 3223 Eden Avenue, Cincinnati, OH 45267-0056, USA

^cDivision of Environmental and Occupational Disease Control, California Department of Public Health, 850 Marina Bay Pkwy, Bldg P, Richmond, CA 94804, USA

^dCenters for Disease Control and Prevention, 4770 Buford Hwy, MS-F17, Atlanta, GA 30341, USA

^eDivision of Research, Kaiser Permanente, 2000 Broadway, Oakland, CA 94612, USA

^fDivision of Adolescent Medicine, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue (ML-4000), Cincinnati, OH 45229-3029, USA

Abstract

To study potential environmental influences on puberty in girls, we investigated urinary biomarkers in relation to age at menarche. Phenols and phthalates were measured at baseline (6–8 years of age). Menarche was ascertained over 11 years for 1051 girls with menarche and biomarkers. Hazards ratios were estimated from Cox models adjusted for race/ethnicity and caregiver education (aHR, 95% confidence intervals [CI] for 5th vs 1st quintile urinary biomarker concentrations). 2,5-Dichlorophenol was associated with earlier menarche (aHR 1.34 [1.06–1.71]); enterolactone was associated with later menarche (aHR 0.82 [0.66–1.03]), as was mono-3-carboxypropyl phthalate (MCP) (aHR 0.73 [0.59–0.91]); the three p-trends were <0.05. Menarche differed by 4–7 months across this range. Enterolactone and MCP associations were stronger in girls with below-median body mass index. These analytes were also associated with age at breast development in this cohort. Findings from this prospective study suggest that some childhood exposures are associated with pubertal timing.

Keywords

Menarche; Environmental; Phenols; Phthalates; Phytoestrogens

*Corresponding author. mary.wolff@mssm.edu (M.S. Wolff).

¹<http://www.bcerp.org/index.htm>.

1. Introduction

Menarche, or the first menstrual period, marks the beginning of a woman's reproductive life. Earlier or later onset has been linked with risk of chronic disease in adulthood. Its decline from about 18–12 years of age over 300 years is explained by lifestyle changes that accompanied industrialization. Primary factors are a girl's age, adiposity, and race. Individual factors such as race and body mass index (BMI) account for 6–12 months difference in menarche [1,2]. Racial differences may reflect both genetic and environmental factors. Much but not all of variation in menarche is explained by genetics, perhaps 70% [3]. These observations, in combination with strong experimental evidence that some chemical exposures alter pubertal timing, have triggered research regarding whether environment influences menarche [4]. Most research has come from cross-sectional investigations that assess environmental exposures among girls over a range of ages. Cross-sectional designs may not be able to address adequately the question of temporality needed to evaluate exposures in relation to menarche.

We are interested in highly prevalent hormonally active environmental exposures that have been identified in the past 20 years [5]. Common sources are diet and the physical environment, including household and personal care products. Among these, a number of phthalates and phenols, including phytoestrogens, have exhibited agonist and antagonistic hormone activity as well as possible obesogenic and anti-obesogenic effects. Estrogen, androgen, thyroid, and peroxisome proliferator-activated receptor (PPAR) activity has been observed [6].

To examine relationships between such exposures and pubertal onset, the NCI and NIEHS established the Breast Cancer and Environment Research Program (BCERP) cohort of girls to enable longitudinal study during childhood [7]. Here we report associations of 19 urinary biomarkers of environmental exposures with menarche in the BCERP cohort.

2. Materials and methods

2.1. Study design and data collection

The BCERP Puberty Study is a cohort that enrolled girls between 2004 and 2007, concluding in 2015 (up to eleven years' follow-up or 9 annual visits). Study sites included Icahn School of Medicine at Mount Sinai (NYC) that recruited black or Hispanic girls mainly from East Harlem in New York City; Cincinnati Children's Hospital (Cincinnati) that recruited from the greater Cincinnati metropolitan area; and Kaiser Permanente Northern California (California) that recruited members of the KPNC Health Plan in the San Francisco Bay Area. Eligible girls were 6–8 years of age without serious endocrine medical conditions. Informed consent was obtained from parent or guardian and assent from the girl, administered by the institutional IRBs. The IRB of the Centers for Disease Control and Prevention (CDC) also approved the analysis of urine specimens, but CDC had no access to identifiers. A study timeline is provided in Supplemental Table 1. We obtained demographics, anthropometry, pubertal assessment, and urine at enrolment (baseline) and annually or semiannually thereafter. Race/ethnicity of the girl was self-reported and coded as black, non-black Hispanic, non-Hispanic white, or Asian. Anthropometry and breast stages

were determined by trained examiners to ascertain age at the first appearance of breast development (stage B2 vs B1, no development) as well as age- and sex-specific BMI percentiles (BMI%) calculated using the CDC growth charts [8]. Complete exam protocols and laboratory methods have been described previously along with quality control measures [7,9]. At the second annual visit after enrolment (NYC and California) or the 5th year (Cincinnati), the guardian was asked for the age and date of the girl's menarche; the girl also reported menarche from 2012 to 2015. The first guardian-reported date was used to determine age at menarche (82%) unless it was not provided in which case the first girl-reported date was used (11%). Reported ages were used if dates were not reported. Sensitivity analyses comparing guardian vs daughter reports found that 78% of reported dates agreed within 6 months.

Urine specimens collected within a year of enrolment were analyzed at the CDC National Center for Environmental Health laboratory. Results and analytic procedures including quality control measures have been reported in previous papers investigating pubertal onset [9]. Analytes included phenols (benzophenone-3, enterolactone, bisphenol A, methyl-, ethyl-, propyl-parabens, 2,5-dichlorophenol, triclosan, genistein, and daidzein) and phthalate metabolites (monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), mono-isobutyl phthalate, monobenzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCP), mono(2-ethyl-5-carboxypentyl) phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethylhexyl) phthalate (MEHP)). Phenols were detected in >80% of samples except for butyl paraben (48%). As described in previous papers, we summed the paraben metabolites based on molecular weight (micromoles/L), expressing the paraben sum as propyl paraben (molecular weight 180.2 g/mol). We also combined the phthalate metabolites into groups based on similar sources and biologic activity, low- (<250 Da, or "low-MWP") and high-molecular weight (>250 Da, "high-MWP") [9]. Low-MWP include MEP, MBP and MIBP. High-MWP include the four di(2-ethylhexyl)phthalate (DEHP) metabolites (Σ DEHP: MECPP, MEHHP, MEOHP and MEHP), MBzP, and MCP. We expressed low-MWP molar sum as MEP (molecular weight 194) and high-MWP as MEHP (molecular weight 278) so that units were the same as the single analytes ($\mu\text{g/L}$). Phthalate metabolites were detected in >98% of samples, except for MEP (>80%). Concentrations below the limit of detection (LOD) were assigned the value $\text{LOD}/2$. Concentrations ($\ln\text{-}\mu\text{g/L}$) were normalized for urine dilution in linear models using $\ln\text{-creatinine}$ as a covariate or, in models with quantiles, using cut-points based on creatinine-corrected biomarkers ($\mu\text{g/g-creatinine}$ or $\mu\text{g/gCr}$). LOD imputation and creatinine adjustment have been described earlier in more detail [10]. There were 1051 girls with at least one exposure biomarker and menarche information including censored values for 139 girls (no menarche reported at last followup). To assess intraindividual reliability of biomarkers over time, assays were repeated in additional samples taken approximately one and three years after enrolment (ca. 100 per site, except for phytoestrogens which were done for two sites; total 927 serial samples except for phytoestrogens, $n = 465$).

2.2. Statistical analyses

Analyses were performed with SAS (version 9.4; SAS Institute, Inc). Using Cox Proportional Hazards models to compute hazard ratios (HR) and 95% confidence intervals

(CI), we modeled relative risk for menarche associated with urinary biomarker concentrations as both continuous and quantiles (quintiles or tertiles) using PROC PHREG. The Cox model allows for censored observations; therefore the 139 girls with no menarche report were included in all analyses. We computed median age at menarche for quantiles of urinary concentrations using the baseline survivor function of multivariable adjusted Cox models. From these models we graphed adjusted Kaplan-Meier survival curves for menarche by quantiles of biomarkers using output produced with the STRATA statement. We tested proportionality assumptions by including quintiles as a time-dependent variable as well as using the *Assess* function in PROC PHREG which analyzes martingale residuals. To resolve violations of proportionality, the covariate race/ethnicity was included in the STRATA statement of PROC PHREG. For quintile models with non-linear associations, such as U-shaped, we verified fit using splines to verify the effect patterns (PROC PHREG and PROC PLM). To obtain p-trend for quintiles, we substituted the natural log of the medians of quintile urinary biomarker concentrations as a continuous variable. Potential confounders were identified by examining the exposure-menarche pathway for biological plausibility and correlations among the variables, i.e. those in Table 1 as well as season at urine donation. We retained the two covariates that altered the estimates by more than 10% or materially improved precision of models (girl's race/ethnicity and her caregiver's education). Study site was not considered a confounder as it is not causally related to menarche nor biomarker concentrations, and as it was collinear with the main covariates (race/ethnicity and caregiver education). In addition, our multi-site study was designed to include geographic variation in order to provide a range of exposures [7].

In order to explore possible multiple exposure effects, we computed Cox models for mutually adjusted tertiles of 2,5-dichlorophenol, enterolactone, and MCP. The risk estimates were almost identical to models for single biomarkers, though slightly attenuated, and significance was unaffected, with the CIs shifting by less than 2%. Although these results suggested independent effects of the biomarkers, we also examined joint models of dichotomized 2,5-dichlorophenol and enterolactone, by creating 3 categories of girls, those having two high risk tertiles (n = 136; low enterolactone, high 2,5-dichlorophenol), low (reversed, n = 152) and the remainder (n = 763). Results were similar to the individual estimates. Therefore only the single biomarker models are presented.

Because BMI% may be in the biological pathway, we did not include it as a confounder that could overadjust the models. Instead, we investigated whether BMI mediated the exposure-menarche relationship. We compared models with and without including BMI%. For this purpose, we used the average of all available BMI% data for each girl (up to 16 visits from Cincinnati, 9 in NYC and California). We also tested effect modification by BMI% using an interaction term in the Cox models (biomarker concentration $\times$ average BMI% dichotomized at the median, or 70th percentile). To illustrate interaction effects, we computed adjusted HRs and menarche ages (survival until menarche after exposure measurement) from joint variables for BMI% $\times$ tertiles to optimize power, using a categorical six-level variable for dichotomous BMI% and tertiles of biomarkers. We conducted sensitivity analyses to rule out other explanations for associations, including race, site, and extremes of creatinine.

Within-person reliability of biomarkers was assessed by analyzing three samples taken successively (baseline, one and three years later; $n = 927$). We computed the intraclass correlation coefficient (ICC) and surrogate analysis as described in earlier biomarker studies [11,12]. The ICC assesses the within-subject agreement of biomarkers using continuous variables over 3 years, as a proportion of between- divided by total-subjects variance. Surrogate analysis evaluates whether a single exposure rank (surrogate tertile) in one year represents the average exposure over the timeframe. We calculated 3-year geometric mean concentrations for a girl's three samples, to represent her average exposure over the interval. We created surrogate tertiles for each single year's samples ("surrogates" at baseline, one and three years later). For each surrogate tertile, the averages of the individual grand-means of girls in that tertile were then computed. A monotonic increase indicates that a single sample's concentration is predictive of the 3-year average [13,14]. We include here summary results that have been reported elsewhere [15].

3. Results

The BCERP cohort is a culturally diverse group of girls who were enrolled at 6–8 years of age and followed through 2015, for eight-to-eleven years depending on year of enrolment. Of the 1051 girls in our analysis, menarche occurred at 12.2 years of age among 912 girls (median 146 months, IQR 135–156). Girls who reported menarche were 137 months old at the pre-menarche visit immediately before (median, IQR 126–148; $n = 912$). Girls who never reported having a period ($n = 139$) were somewhat younger at their last visit, chiefly because they dropped out before menarche (median 128 months, IQR 114–144; $n = 139$, Table 1). Compared with our earlier reports with 7 years of data, breast stage 2 or higher had been observed by 2015 among 89% compared to 88% of girls (not shown) [10,16]. Exposure biomarkers varied by the characteristics in Table 1, including race/ethnicity and caregiver education as reported previously (not shown) [9].

In bivariate comparisons, age of menarche was later with increasing levels of two phenols (benzophenone-3 and enterolactone) and was younger for two other phenols (2,5-dichlorophenol and combined parabens) as well as low-MWP (Table 2). In Cox models with continuous biomarkers (ln- $\mu\text{g/L}$ adjusted only for ln-creatinine), seven of the biomarkers were associated with menarche: four phenols including enterolactone, low-MWP and its main constituent MEP, and MCP (all $p < 0.01$, Table 3). Associations were attenuated after adjustment for race/ethnicity and education, such that the HRs shifted by 3–8% toward the null with only enterolactone and MCP remaining significant or borderline in relation to continuous biomarkers ($p = 0.06$ and $p = 0.018$ respectively). Associations of the individual phthalate metabolites with menarche were consistent with those of the molecular sums except for MCP which was associated with later menarche while the high-MWP association was null (Table 3). Effect modification by BMI% was seen for continuous biomarkers only for enterolactone (p -interaction 0.010, not shown).

Further analyses were conducted using quintiles of exposure biomarkers. Associations were seen for three analytes (2,5-dichlorophenol, enterolactone, MCP; Table 4, Fig. 1). Comparing girls in the 5th vs 1st quintile concentrations of biomarkers, adjusted median ages for menarche were 7 months earlier for 2,5-dichlorophenol, 4 months later for

enterolactone, and 5 months later for MCP. For all three compounds, tests for trend indicated a dose-response for HRs and median ages, with 34% increased risk of menarche for 5th vs 1st quintiles of 2,5-dichlorophenol, 18% reduced risk for enterolactone, and 27% lower risk for MCP. Low-MWP and MEP associations, suggestive as continuous variables, were null in adjusted quintile models.

BMI% modified the enterolactone association with menarche (Table 4, Fig. 2). For the joint variable [BMI $\times$ tertile] (Fig. 2), menarche occurred later (HR < 1) with a dose-response for enterolactone among the leaner girls (BMI% < 70) but not heavier girls. This may represent greater precision in models for leaner girls, as the difference in median menarche age (1st vs 3rd tertile) was similar in both BMI% groups, i.e. 6 months later in leaner and 4 months later in heavier girls, although the HRs did not differ by exposures in heavier girls (Fig. 2). Thus for the 3rd vs 1st tertile of enterolactone, risk of menarche was 25% lower among lean girls, while the HR was null among heavy girls. The model with joint BMI-enterolactone tertiles shown in Fig. 2 was not altered by further adjustment for BMI%. Similar BMI modification of the MCP-menarche association was observed (Fig. 2). Other biomarker models did not exhibit exposure modification by BMI%.

To compare relationships of these biomarkers with menarche and breast development, we reran models for breast development (age-at-B2) in relation to phenol, phytoestrogen and phthalate urinary biomarkers (continuous, ln- μ g/L) using the latest dataset with 2 more years of followup than our earlier reports [10,16]. Findings were unchanged for the continuous biomarkers. Enterolactone and 2,5-dichlorophenol were associated with both breast onset and menarche (Table 5, Fig. 3). Benzophenone-3 and triclosan were associated with breast development but not menarche. No phthalate biomarker was associated with breast development or menarche aside from MCP. MEP comparisons were similar to those for low-MWP with slightly narrower CIs for MEP (note CIs in Fig. 3).

Knowledge about biomarker reliability over time is essential in order to appreciate the window of action, or interval between exposure and outcome. To address this question, we examined intraindividual consistency of biomarkers over time among a subset of 309 girls with urine collected at baseline, one and three years later (Supplemental Fig. 1) [15]. We applied commonly used criteria for ICCs indicating 0.21–0.40 fair, 0.41–0.60 moderate, and 0.61–0.80 substantial agreement [17]. By this approach, as continuous variables, BPA, Σ DEHP and its constituents except MEHP were poor biomarkers; MEHP, MBzP, MCP were fair; low-MWP was fair except for its component MEP (moderate); triclosan and the paraben sum were fair while benzophenone-3 was moderate and 2,5-dichlorophenol was substantial (ICC 0.69). The isoflavones were fair and enterolactone (ICC 0.52) was moderate. However, surrogate analysis indicated identical ranking of the 3-year-averages (geometric mean, GM) of a girl's average biomarkers within tertiles for any single year's samples (Suppl Fig. 1). Hence, 3-year-average biomarkers increased monotonically within every surrogate year's tertiles. Furthermore, the 3-year-averages within a tertile (baseline, one and three years later) were similar at each timepoint, i.e. in the lowest tertile the 3-year GMs were similar in each surrogate year. For example, BPA had a poor ICC of 0.17, but within three first surrogate tertiles the 3-year-averages were similar (1.4, 1.5, 1.4 μ g/gCr for first, second, and fourth years), middle (2.4, 2.2, 2.5 μ g/gC) and third (3.8, 3.9, 3.6 μ g/gCr)

tertiles at each timepoint respectively. In this example the corresponding trend of the overall GM at the baseline year with increasing tertiles was 1.4, 2.4, 3.8 µg/gCr. Therefore, as indicated by surrogate analysis, exposure would likely not be misclassified for ranked exposure variables if a single spot urine sample in any of the four years was used to classify girls over this time period.

4. Discussion

Age of menarche in the BCERP cohort was associated with two urinary phenols and one phthalate metabolite which had been measured several years earlier. Menarche on average occurred 7 months younger with higher 2,5-dichlorophenol and 4–5 months older with enterolactone and MCPP. Exposure effects were similar for age at first breast development (adjusted; Table 5) but not pubarche, which is not estrogen dependent. Pubertal changes associated with these exposures were reflected in hazards ratios that changed by 20–30% between first and fifth quintiles representing 6–>100-fold ranges of concentrations. Compared with a representative sample of American children from the National Health and Nutrition Examination Survey (NHANES) of the same age, our cohort had similar levels of enterolactone and MCPP, but higher 2,5-dichlorophenol levels (median ~12 for BCERP vs 1 µg/gCr [18]; Table 2).

There have been at least twelve previous studies of menarche or age-at-B2 in relation to metabolites of phytoestrogens, phenols and phthalates or their dietary intake in both longitudinal and cross-sectional studies of reasonable size using acceptable biomarkers. For phytoestrogens, a few studies have observed associations of delayed puberty with higher biomarkers or dietary polyphenol intake in both longitudinal and cross-sectional papers, including ours [19–21]. Environmental phenol biomarkers in urine showed inconsistent associations in four papers, including two cross-sectional analyses using NHANES data and our reports with a longitudinal design to assess B2 and menarche; all found earlier menarche with higher 2,5-dichlorophenol [10,16,22,23]. In particular, our findings are consistent with analyses of NHANES data, showing earlier onset of both B2 and menarche with 2,5-dichlorophenol [22,23]. Phthalate metabolites have been investigated in cross-sectional and longitudinal studies of varying follow-up. Two cohorts reported earlier breast development with prenatal or child urinary phthalate metabolites, although one studied only non-oxidative monoesters (e.g. MEHP) [24,25]. Our cohort and the Danish cross-sectional study observed later pubarche with increased high-MWP [10,26]. In the current analysis we find MCPP, an oxidative metabolite of several parent phthalate diesters, associated with delay of both breast development and menarche.

To put the magnitude of these effects in perspective, age-at-B2 differs by BMI and race by 6–12 months. For example, normal-weight white girls started breast development 16 months later than overweight black girls in this cohort [27]. Also, in the late 20th century, menarche was earlier for blacks than whites by about 6 months [1,28–30]. Thus, the magnitude of environmental effects on girls' maturation suggested by our findings are smaller than some major risk factors, but are still ample. It has been suggested that breast development occurred at younger ages over the past several decades, more so than menarche, and thus may be more sensitive to risk factors such as obesity and environmental exposures [28,31].

However, a recent compilation shows similar ages of breast development (10–11 years) and menarche (12.5–13 years) over the past 50–60 years [30]. Small changes are likely not that significant considering how much methods differed for various studies over this time period, many being cross-sectional and having limited followup [1]. Besides BMI and race, other risk factors show small effects on pubertal timing. For example among twin girls reared apart, menarche differed by <0.4 years (<5 months [32]) suggesting a similar limit for environmental or lifestyle effects. Mouritsen et al. also suggest that menarche differs by a few months for the main risk factors, but as much as a year for age-at-B2 [30]. Therefore for environmental exposures to have an observed effect, there may need to be a minimal, biologically effective level of exposure as well as a wide exposure gradient to detect changes smaller than 4 months in menarche.

Several biologic mechanisms are consistent with effects of these exposures to advance or delay menarche, including hormones and other growth factors. For example, experimental evidence shows that enterolactone has anti-aromatase activity that can reduce estrogen production and thus delay puberty. Other phenols and phthalates may affect hormone action through thyroid or PPAR pathways [33]. In addition to aromatase antagonism, several steps in the steroid hormone synthetic pathway may be altered by endocrine-disrupting chemicals [34]. In the BCERP cohort, high-MWP including DEHP and MBzP biomarkers were associated with later pubarche but not B2-age or menarche, which may be related to anti-androgenic capacity of phthalates. MCPP poses a puzzle, as the only phthalate biomarker to be associated with age of menarche or B2. Reported associations of MCPP with other outcomes are also a bit unusual. For example, maternal prenatal urinary concentrations of MCPP, but no other phthalate metabolites, were associated with later childhood obesity [35]. MCPP is a non-specific metabolite of several phthalate diesters (e.g., dibutyl-, dioctyl-, other high-MWP) [36]. It also has a longer half-life than other phthalate metabolites. Lorber et al. suggest that MCPP behaves more like high- than low-MWP, and less like MBP [37]. We did not measure phthalate metabolites with side chains longer than eight carbons. An idea to explore is that there are occult sources of MCPP precursors, such as unrecognized household materials that contain long-chain phthalate diesters. MCPP may have dietary sources [38], while enterolactone comes exclusively through diet. Based on pharmaceutical analogs, MCPP is a possible SRD5A antagonist, supporting antiandrogenic activity [34]. The oddity of MCPP findings plus relatively low MCPP urinary concentrations raises the possibility of chance associations or residual confounding, which cannot be excluded in ours or other studies. Although the ICC for the within-person variability of urinary MCPP was low (0.26), the surrogate analysis showed identical rankings in the three-year analysis (Suppl Fig. 1).

We saw more biomarkers to be associated with breast development than menarche. 2,5-Dichlorophenol, enterolactone, and MCPP exhibited associations with both age-at-B2 and menarche, but triclosan and benzophenone-3 were associated only with B2-age (Table 5). Associations with breast development may have been more readily detectable because B2 occurred closer to the exposure biomarker measurement than did menarche. Urinary metabolites were measured approximately two years before breast development and five years before menarche (median age 7.4 years or 89 months at urine collection). Our ability to see small exposure effects upon pubertal ages may be challenged by measurement precision of both outcome and exposure. Age-at-B2 in our reports could not be estimated

more accurately than ± 6 months for girls at two sites, who were seen annually at most. Menarche was reported retrospectively as the month it occurred. As a result, detectable exposure effects may be slightly smaller for menarche than B2-age, as suggested by Table 5. For eight additional exposure biomarkers where associations were null, exposure effects for B2-age were 4–5 months for seven biomarkers, while for age at menarche they were 1–2 months (5th vs 1st quintile; not shown). The fact that exposures are associated with both B2 and menarche is natural as the two outcomes are correlated with each other, and they are on the same developmental trajectory [39]. However, recent data suggest that the correlation of B2 and menarche is less over the past several decades [28,30]. If so, environmental factors might affect the two pubertal milestones differently.

There was also likely a small loss in power for age at menarche, as there are about 10% fewer girls in the menarche than B2-age analyses. Indeed, effect estimates for B2-age ($n = 1171$) were unchanged when computed for the smaller subset with menarche ($n = 1051$), but in the smaller dataset CIs included the null for two of the four biomarkers in Table 4 (triclosan and enterolactone; not shown). Unlike our earlier report, this reanalysis with slightly more girls at stage B2 showed a delay in age-at-B2 with MCPP. Statistical power may also be affected by the disparate ranges of exposure biomarkers, especially existence of sufficiently high biomarker levels. Thus, on a molar basis the median concentrations in our study ranged from 0.1 to 1720 nmol/gCr for the four biomarkers in Table 5. Imprecision exists for exposure measurements in laboratory methods and for within-person exposure variation over time. Of interest, the two biomarkers with the best ICC in urine measurements were 2,5-dichlorophenol and enterolactone, for which we saw associations with both age-at-B2 and menarche.

Both enterolactone and MCPP associations were modified by BMI, but rather than having disparate effects in BMI strata, the actual estimates suggest that models are more stable in below-70th BMI% girls, as the HRs and the age shifts with exposure were quite similar for all girls and lower-BMI girls. With enterolactone, menarche was 6 months later for all girls, 3rd vs 1st tertile; 6 months later for low-BMI girls; and 4 months later for high BMI girls (Fig. 2). No similar BMI interaction was seen for pubarche, which is not estrogen-regulated. Notably, the two biomarkers that had BMI-interactions were associated with later menarche, whereas 2,5-dichlorophenol, which was not modified by BMI, was associated with earlier menarche. The BMI influence in early puberty may be stronger with respect to some environmental factors than in later ages of development, as we found in an earlier report that BMI changes more for leaner than heavier girls over the pubertal trajectory [16]. There is abundant literature showing relationships among BMI, enterolactone or lignans (dietary precursors of enterolactone), and other reproductive outcomes. For example, relationships of both lignans and soy with breast cancer risk were stronger in high-BMI women [40,41]. These and other studies show 20–60% reduced risk of breast cancer for women with highest exposures to phytoestrogens. Later menarche, by 2–4 years, has been associated with 40–80% reduced risk of young age or estrogen receptor-negative breast cancer by [42,43]. Others suggest that a year younger age at menarche may lead to 5% raised risk of breast cancer overall [44]. Exposures in our study were associated with about ½-year differences in pubertal onset, including menarche. Based on experimental data, pubertal timing can be affected differently by environmental exposures at various timepoints, including perinatally

through childhood [4,6]. Therefore, the critical window may not involve exposure during the years of childhood before menarche, but rather during an effective time period that could precede the first signs of pubertal onset, or even earlier, during gestational development. Even if the childhood window in our study is influential, there may still be exposure misclassification of exposure biomarkers if there is poor within-person reliability around the critical window of exposure. Only one biomarker in this study, 2,5-dichlorophenol, had “substantial” intraindividual consistency over four years, based on the ICC, although several biomarkers had “moderate” reliability, but the analyses using surrogate rankings had excellent agreement for tertiles for all biomarkers. A number of other studies have found similar reliability over a range of years [45–47]. Furthermore, if measures within 4 years offer fair within-person reliability, it is likely that the window extends at least one year before or after the first measurement.

5. Conclusion

In this prospective study of environmental biomarkers measured as long as ten years before puberty, we find three to be associated with timing of both breast development and menarche; two others were associated with breast development but not menarche. Six other biomarkers or composite indices were associated with neither. Little is known about the relative biological effects and exposure thresholds that govern the pubertal trajectory. The timing of exposure measurement in this study may represent but one of the possible causal windows.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.reprotox.2016.11.009>.

Abbreviations

aHR	adjusted hazards ratio
B2	breast stage 2
BMI%	body mass index percentile age and sex specific

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Fig. 1. Survival curves for menarche by 5th (black) and 1st (grey) quintiles of three exposure biomarkers, race and caregiver-education adjusted. Vertical lines are drawn at the median survival age; median ages and biomarker concentrations of 1st and 5th quintiles are given. Median menarche was 144 months (12 yr). See Table 5 for details.

Fig. 2.

Age at menarche associated with urinary 2,5-dichlorophenol (2,5-DCP), enterolactone or MCPP, and by BMI%. The median menarche of all girls was 144 months (survival analysis), similar to the median age for All girls in the 2nd tertiles. Cox proportional hazards models were run with a 6-level joint variable for tertile X BMI% (dichotomized at the median 70%). The 2,5-DCP effect was the same regardless of BMI; the difference between the high- and low- BMI plots for 2,5-DCP is the effect of higher BMI toward younger menarche. For enterolactone and MCPP, menarche was later among lower BMI girls with highest exposures. Further adjustment for BMI did not change these results. Note that the median concentrations of 2,5-DCP and MCPP 3rd tertiles are lower than the first tertile of enterolactone. Adjusted HRs, CIs and median ages for each group are provided in Supplemental Table 3.

Fig. 3.

HR (CI) for selected exposure biomarker associations with B2 age and menarche, BCERP, 2004–2015. HRs are from Table 2 for menarche, not shown for B2. Estimates for continuous biomarkers ln ug/L are adjusted for ln-creatinine, race/ethnicity, and caregiver education. Note that 2,5-dichlorophenol has an association with menarche in models with quantiles of exposure, though it is borderline for this continuous exposure measure. The y-axis is log10-scale.

Table 1

Characteristics of 1051 girls with a biomarker measured and with menarche data, BCERP 2004–2015.

	Girls with ascertained menarche Menarche median age (IQR), months	Right-censored (no menarche)
N	912	139
Menarche, months*	146 (135–156)	–
Age at enrolment, months	88 (82–94)	85 (79–92)
Age at urine collection	90 (84–95)	87 (81–93)
Age at premenarcheal visit*	137 (126–148)	128 (114–144) ^a
Months between urine donation and menarche*	56 (44–68)	47 (33–58) ^a
BMI% at:		
baseline*	71 (41–91)	64 (34–90)
last B1 visit*	71 (38–91)	68 (33–91)
pre-menarche*	73 (41–92)	63 (26–91)
average over study*	72 (45–91)	61 (33–90)
Race/ethnicity*	N (%)	N (%)
White	298 (33%)	44 (32%)
Asian	52 (6%)	2 (1%)
Hispanic	279 (31%)	49 (35%)
Black	283 (31%)	44 (32%)
Age at Breast Stage 2 ^b		
<9.3 Years Old	435 (49%)	60 (50%)
≥9.3 Years Old	453 (51%)	59 (50%)
Site*		
NYC	296 (32%)	67 (48%)
Cincinnati	239 (26%)	28 (20%)
California	377 (41%)	44 (32%)
Caregiver education ^c		
<High school	262 (30%)	50 (36%)
High School	625 (70%)	87 (64%)

Asterisks indicate characteristics where the K-W test of medians or chi-square of frequencies was significant ($p < 0.05$ by 3 menarche groups).

^a AGE at terminal visit, no menarche.

^b The number excludes girls lost to followup before the median age (9.3 years).

^c Caregiver education was missing on 27 participants.

Table 2

Urinary biomarker concentrations by age at menarche for 1051 girls with at least one biomarker, BCERP 2004–2015.

	Girls with ascertained menarche, median (IQR)			Right-censored (no menarche)
	<11 yr (<132 mo)	11–13 yr (132–156 mo)	13+ yr (156 + mo)	
N	162	501	249	139
Biomarker, ug/gr Cr				
Benzophenone–3 *	18 (6–62)	32 (8–150)	38 (11–142)	17 (6–97)
bis-Phenol A	2.3 (1.4–4.3)	2.4 (1.5–4.3)	2.4 (1.5–4.1)	2.4 (1.5–3.9)
Paraben Sum ^{a,*}	99 (29–339)	96 (35–320)	75 (28–197)	81 (31–287)
2,5-Dichlorophenol *	31 (7–100)	11 (3–54)	7 (2–40)	21 (3–73)
Triclosan	13 (5–39)	15 (5–42)	13 (6–49)	16 (7–56)
Enterolactone *	436 (198–733)	494 (223–1008)	574 (300–1179)	512 (196–1122)
Genistein	40 (16–141)	42 (17–176)	59 (17–217)	44 (14–136)
Daidzein	90 (43–331)	107 (36–416)	100 (36–380)	99 (36–271)
Low-MWP ^{a,*}	155 (91–288)	139(83–250)	131 (79–235)	155 (90–289)
MEP *	124 (54–300)	103 (54–236)	89 (49–178)	128 (55–240)
MBP	47 (32–72)	47 (27–72)	48 (29–77)	50 (34–90)
MIBP *	16 (9–26)	14 (8–23)	12 (7–21)	17 (10–30)
High-MWP ^a	192 (121–348)	186 (115–336)	189 (123–319)	228 (121–390)
MEHP	4.1 (2.4–8.2)	4.3 (2.3–8.3)	4.3 (2.6,8.7)	5 (2.4–10.4)
MEOHP	27 (15–55)	28 (16–54)	28 (18,50)	37 (18–62)
MEHHP	44 (26–95)	47 (26–89)	43 (28,82)	56 (29–98)
MECPP	74 (46–140)	73 (44–141)	79 (47,135)	87 (51–160)
MBzP	28 (17–46)	29 (14–54)	31 (15,53)	29 (17–61)
MCPP	5.4 (3.6–8.6)	5.9 (3.8–10.3)	7 (4.7,10.8)	6.2 (4.3–10.5)
Creatinine, mg/dL *	101 (69–141)	91 (55–124)	86 (51–116)	92 (60–125)

^aLow-MWP is the molar sum of MEP, MBP, MIBP expressed as MEP; high-MWP is the sum of DEHP metabolites, MEHP, MEOHP, MEHHP, MECPP plus MBzP, MCPP expressed as MEHP. Paraben sum is the molar sum of methyl-, ethyl-, propyl-parabens expressed as propyl paraben.

* Kruskal-Wallis test of biomarker medians differed across the 3 menarche age-groups, $p < 0.05$.

Table 3

Association of continuous urinary biomarkers (ln-ug/L) with age of menarche, BCERP cohort 2004–2015.

Phenol Metabolites, ln-ug/L	median, ug/g creatinine	Adjusted for ln-urine creatinine		Adjusted for ln-urine creatinine, race/ethnicity and caregiver education	
		HR (95% CI)	P-value	HR (95% CI)	P-value
Benzophenone-3	28	0.95 (0.93–0.98)	0.002	0.99 (0.95–1.02)	0.415
bisPhenol A	2.4	1.05 (0.97–1.13)	0.223	1.04 (0.96–1.12)	0.327
Paraben sum	89	1.08 (1.03–1.13)	0.001	1.03 (0.98–1.08)	0.261
2,5-Dichlorophenol	12	1.08 (1.04–1.11)	<0.001	1.03 (0.99–1.06)	0.140
Triclosan	14	1.01 (0.97–1.05)	0.695	1.01 (0.97–1.05)	0.767
Enterolactone	512	0.92 (0.87–0.97)	0.002	0.95 (0.90–1.00)	0.058
Genistein	45	0.97 (0.93–1.01)	0.108	1.00 (0.96–1.04)	0.948
Daidzein	100	0.98 (0.94–1.02)	0.231	1.01 (0.97–1.05)	0.582
Phthalate Metabolites, ln-ug/L					
Low-MWP sum	141	1.15 (1.07–1.24)	<0.001	1.07 (0.98–1.16)	0.140
MEP	68	1.13 (1.07–1.20)	<0.001	1.06 (0.99–1.14)	0.100
MIBP	48	1.06 (0.99–1.14)	0.117	1.01 (0.94–1.09)	0.718
MBP	14	1.00 (0.92–1.09)	0.984	0.98 (0.90–1.06)	0.567
High-MWP sum	192	1.03 (0.95–1.12)	0.460	0.99 (0.91–1.08)	0.886
High MWP Metabolites, DEHP sum	142	1.00 (0.94–1.07)	0.885	1.00 (0.94–1.06)	0.896
MEHP	4.0	1.00 (0.94–1.07)	0.963	0.96 (0.89–1.02)	0.204
MEHHP	46	1.03 (0.96–1.11)	0.379	0.99 (0.93–1.07)	0.884
MEOHP	28	1.02 (0.95–1.09)	0.641	0.98 (0.91–1.06)	0.666
MecPP	76	1.03 (0.96–1.11)	0.405	1.01 (0.93–1.08)	0.893
High MWP Metabolites, not DEHP					
MBzP	21	0.98 (0.92–1.05)	0.610	0.98 (0.92–1.05)	0.630
MCPP	6.0	0.85 (0.78–0.93)	<0.001	0.89 (0.81–0.98)	0.018

Estimates were obtained from Cox proportional hazards models with menarche as outcome and continuous urinary biomarkers as the exposure (ln-ug/L). Results for the creatinine-only adjusted models, in the subset of adjusted models that had fewer observations, were almost identical to those in the complete set shown. Adjusted benzophenone-3, paraben sum, bisphenol A, 2,5-dichlorophenol, triclosan, high-MWP sum, DEHP sum, low-MWP sum, MEP, MIBP, MBP, MECPP, MEHHP, MEOHP, and MCPP models excluded 27 observation due to missing caregiver education. Adjusted enterolactone, genistein, and mBzP models excluded 23 observations with missing caregiver education.

Paraben sum is the molar sum of methyl-, ethyl-, and propyl-parabens, expressed as propylparaben (mol wt 180).

Low-MWP sum is the molar sum of MEP, MBP, and MIBP expressed as MEP (mol wt 194).

High-MWP sum is the molar sum of MECPP, MEHHP, MEOHP, MCPP and MBzP expressed as MEHP (mol wt 278).

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Table 4

Association of quintile urinary biomarkers (ug/gr Cr) with menarche for three biomarkers. BCERP cohort 2004–2015.

2,5-Dichlorophenol									
Quintile	Median µg/gr Cr	Unadjusted		Adjusted		N	Median Survival Age, mo	N	Median Survival Age, mo
		nm/gCr	HR (95% CI)	HR (95% CI)	HR (95% CI)				
1	1.6	9.8	Ref.	Ref.	Ref.	210	208	149	
2	4.5	28	1.12 (0.91–1.37)	1.04 (0.84–1.28)	1.04 (0.84–1.28)	210	205	148	
3	12.2	75	1.56 (1.27–1.91)	1.34 (1.07–1.66)	1.34 (1.07–1.66)	210	200	143	
4	43.1	260	1.64 (1.33–2.02)	1.28 (1.01–1.63)	1.28 (1.01–1.63)	210	207	144	
5	199	1220	1.72 (1.40–2.11)	1.34 (1.06–1.71)	1.34 (1.06–1.71)	210	203	142	
p-class									
<0.001									
p-trend for ln medians									
<0.001									
p-interaction quintile × BMI									
0.082									
0.214									
Enterolactone									
Quintile	µg/gr Cr	Unadjusted		Adjusted		N	Median Survival Age, mo	N	Median Survival Age, mo
		nm/gCr	HR (95% CI)	HR (95% CI)	HR (95% CI)				
1	98.2	330	Ref.	Ref.	Ref.	202	196	143	
2	280	940	1.06 (0.86–1.31)	1.15 (0.92–1.43)	1.15 (0.92–1.43)	202	197	142	
3	512	1720	0.86 (0.69–1.06)	0.89 (0.71–1.10)	0.89 (0.71–1.10)	202	197	144	
4	892	2990	0.88 (0.71–1.09)	0.95 (0.76–1.18)	0.95 (0.76–1.18)	202	199	146	
5	1868	6260	0.74 (0.60–0.91)	0.82 (0.66–1.03)	0.82 (0.66–1.03)	202	198	147	
p-class									
0.008									
p-trend for ln medians									
0.002									
p-interaction quintile × BMI									
0.007									
<0.001									
MCPP									
Quintile	µg/gr Cr	Unadjusted		Adjusted		N	Median Survival Age, mo	N	Median Survival Age, mo
		nm/gCr	HR (95% CI)	HR (95% CI)	HR (95% CI)				
1	2.5	10	Ref.	Ref.	Ref.	210	206	142	
2	4.4	18	0.92 (0.75–1.14)	0.92 (0.74–1.14)	0.92 (0.74–1.14)	210	203	146	
3	6.2	25	0.78 (0.64–0.96)	0.87 (0.70–1.07)	0.87 (0.70–1.07)	210	207	146	
4	9.2	36	0.74 (0.60–0.91)	0.83 (0.67–1.03)	0.83 (0.67–1.03)	211	207	146	
5	16.2	64	0.67 (0.54–0.82)	0.73 (0.59–0.91)	0.73 (0.59–0.91)	210	201	147	

2,5-Dichlorophenol							
Quintile	Median	Unadjusted		Adjusted		N	Adjusted Median Survival Age, mo
	µg/gr Cr	nm/gCr	HR (95% CI)	HR (95% CI)			
	p-class						
	p-trend for ln medians						
	p-interaction quintile × BMI						

Estimates were produced from Cox proportional hazards models with menarche as the outcome and quintile urinary biomarkers as the exposure. Adjusted models included caregiver education and race/ethnicity. Adjusted models excluded 27 observation due to missing caregiver education (23 for enterolactone).

P-class was derived from models that include quintiles as a class variable.

P-trends for ln medians were derived from models that include ln-quintile medians as a continuous variable.

P-interaction for quintile × BMI% was derived from models that include a quintile × average median BMI% (< and 70%) interaction term.

Table 5

Estimated median ages at attainment of breast stage 2 vs stage 1 and menarche, adjusted for race/ethnicity and caregiver education, for 1st and 5th quintiles of five urinary metabolites determined at baseline, with eight to eleven year follow-up (2004–2007 through 2015).

	Median, all (1st–5th quintile) nm/grC	Age at B2, mo; median by quintile		Menarche, mo; median by quintile	
		1st–5th ages	Q1–Q5 effect	1st–5th ages	Q1–Q5 effect
Benzophenone-3	124 (16–3560)	106–112.5	+6.5 mo	143–147	(+4)
Enterolactone	1720 (329–6270)	104.5–110	+5.5 mo	143–147	+4 mo
MCPP	25 (10–64)	104–109.5	+5.5 mo	142–147	+5 mo
2,5-Dichlorophenol	75 (10–1220)	112–103	–9 mo	149–142	–7 mo
Triclosan	50 (9–560)	109.5–104.5	–5 mo	145–145	(0)

“Effect” is not computed, but is the simple difference between the adjusted medians for the 1st and 5th quintiles, that are shown. “Effect” shown in parentheses for menarche age median indicates the HR included the null (benzophenone-3 and triclosan). HRs for B2-age all exclude the null. Median biomarkers are the same as those in Table 3, as nanomolar concentrations (ng/gCr divided by molecular weight). Age at B2 was rerun with the most recent dataset, so ages may differ slightly from previous reports. [10] HRs are in Table 3 except for benzophenone-3 and menarche, HR 1.02 [0.81–1.28] for the 5th vs 1st quintile of the model; for triclosan, the HR was 1.01 [0.82–1.24]. For menarche, Ns are the same as Table 2. For B2, N = 1169 (BP3, DCP), 1147 enterolactone, 1157 triclosan, 1170 MCPP. Adjusted median ages for the subset of B2 that overlaps the menarche dataset were almost identical, so we report the larger dataset.