Prenatal Exposure to Polycyclic Aromatic Hydrocarbons and Gestational Age at Birth

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Public Health

University of Washington

2021

Committee:

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Program Authorized to Offer Degree:

Epidemiology

University of Washington

Abstract

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Background. Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds that are ubiquitous, probable human carcinogens. Maternal PAH exposure has potential effects on fetal development. We examined associations of prenatal PAH exposure with spontaneous preterm birth (PTB) and gestational age at birth among term births. Given the sex-specific endocrine disrupting properties of PAHs, we additionally examined the extent to which infant sex modifies the association of PAH exposure with spontaneous PTB and gestational age at birth.

Methods. Study participants included 1,677 non-smoking women with singleton pregnancies from three cohorts (CANDLE, TIDES, and GAPPS) in the ECHO PATHWAYS Consortium. Seven PAH metabolites (1-hydroxynaphthalene [1-nap], 2-hydroxynaphthalene [2-nap], 2-hydroxyphenanthrene [2-phen], 3-hydroxyphenanthrene [3-phen], combined 1- and 9-hydroxyphenanthrene [1/9-phen], combined 2-, 3-, and 9-hydroxyfluorene [2/3/9-fluo], and 1-hydroxypyrene [1-pyr]) were measured in second trimester maternal urine. PAH values below the limit of detection were imputed using censored likelihood multiple imputation. Logistic and linear regression models were fit for spontaneous PTB and gestational age (days, among terms births), respectively, with individual $\log_{10}$-transformed PAH metabolites as the exposure, adjusted for specific gravity, maternal age, site, race, urinary cotinine, education, income, season of birth, infant sex, alcohol use during pregnancy, pre-pregnancy BMI, parity, and prior PTB. Effect modification by infant sex was assessed using interaction terms and comparison of marginal sex-specific estimates.

Results. Most participants were between the ages of 18-29 (N=827, 49.3%) or 30-39 (N=762, 45.4%) and were high school (N=540, 32.2%) or college graduates (N=570, 34.0%). PAH levels varied by cohort and metabolite, with CANDLE having the highest mean levels of all metabolites overall. Overall percent detection was highest for 2-nap (99.8%) and the lowest for 1-pyr (65.2%). The overall prevalence of spontaneous PTB was 5.5% (overall PTB: 9.9%) and cohort-specific prevalence of spontaneous PTB were 6.0%, 3.5% and 8.9% for CANDLE, TIDES, and GAPPS, respectively (overall PTB: 9.7%, 10.4%, 9.4%). Among term births, the mean gestational age was 39.5 weeks (SD= 1.09), with similar means observed across cohorts. We did not find statistically significant associations between PAH exposure and spontaneous PTB, although there was suggestive evidence of a an inverse association with 2-nap. A ten-fold higher exposure to 2-nap was associated with a 1.13-day (95% CI: -2.19, -0.13) lower gestational age at birth. Associations of 1-pyr with PTB and 2-nap and 2/3/9-fluo with gestational age were significant among females (1-pyr: OR=1.96 [95% CI: 1.11, 3.46]; 2-nap: -2.13 days [95% CI: -3.41, -0.85], 2/3/9-fluo: -1.55 days [95% CI: -2.96, -0.14]) but not males (1-pyr: OR=0.99 [95% CI: 0.60, 1.62]; 2-nap: -0.28 [95% CI: -1.50, 0.93]; 2/3/9-fluo: -1.55 [95% CI: -2.96, -0.14]) (interaction term p-values <0.05). Other interactions (interaction term p-values <0.05) were observed for 2-phen on PTB and 2-phen, 3-phen, and 1/9-phen on gestational age, although marginal estimates were not significant among males or females.

Discussion. In this large cohort study, we observed inverse associations of exposure to 2-nap with gestational age as well as suggestive evidence that female fetuses may be more susceptible to PTB or shorter gestational age following exposure to PAH metabolites. This study is the first to assess PAH toxicity in relation to spontaneous PTB and gestational age among term births in a sex-specific manner. This study suggests maternal PAH exposure may be a modifiable environmental determinant of gestational age at birth, particularly for female infants.

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INTRODUCTION

Gestational age at birth is an important predictor of life course health (1,2). Preterm birth (PTB), defined as birth at less than 37 completed weeks of gestation, accounts for approximately 10 percent of births in the United States (3). PTB complications are the leading causes of death among children under five years of age (4). PTB complications persist into adulthood and include neurodevelopmental impairments (5), behavioral problems (6), and hearing and visual deficits (5,7). Recent evidence also indicates that risk of neonatal complications is heterogeneous within the term period (8), with a doubling of infant morbidity for each decreasing gestational week prior to 38 weeks of gestational age (9). Early term births (37 to 38 6/7 weeks of completed gestation) are associated with a higher risk of infant mortality (8), childhood cognitive impairment (10), and respiratory morbidities (11) compared to full term births (39 to 40 6/7 weeks). While known demographic and lifestyle factors (e.g. maternal age, psychosocial stress, and tobacco use) and medical indications contribute to PTBs and early term births, accumulating evidence suggests potential roles for environmental exposures (12). Identifying these modifiable environmental risk factors may contribute to public health strategies aimed to promote full-term pregnancies and resulting improved newborn health.

Polycyclic aromatic hydrocarbons (PAH) are a group of environmental contaminants and several PAH compounds have been classified as probable human carcinogens (13). Major sources of exposure include consumption of charred foods, occupational hazards, ambient air pollution, and tobacco smoke. PAH exposure is ubiquitous and well-documented in most populations, including pregnant individuals (14). Prenatal exposure to PAH may impact risk of PTB through changes in uterine physiology associated with labor induction (15,16). One study found that pregnant rats exposed to Benzo-a-pyrene (BaP) versus no PAH experienced higher rates of PTB and had higher levels of inflammatory biomarkers (inflammatory cytokines IL-1β, IL-8, and TNFα) and expression of genes related to uterine contractions (connexin 43, cox-2, and prostaglantin receptor 2α) (15). Another study found exposure of uterine cells to BaP was associated with alterations in oxytocin-induced calcium oscillations, which can contribute to PTB (16). PAH exposure is also linked to higher levels oxidative stress biomarkers in the placenta (17), which have been implicated in spontaneous PTB (18,19). Finally, PAH exposure has been associated with epigenetic changes (e.g. DNA methylation) in the placenta (20,21) that have been related to both spontaneous (22) and overall PTB (23).

A small number of epidemiological studies have demonstrated associations between maternal exposure to PAH and PTB (12,17,21,24–27). Findings from these studies were mixed and studies had several limitations. A study that examined National Health and Nutrition Examination Surveys (NHANES) data found that a one standard deviation increase in urinary 1-hydroxypyrene was associated with 80% higher odds (95% CI: 1.1, 2.8) of PTB. However, the study relied on cross-sectional data and did not account for potential confounding by smoking. Studies examining placental tissue collected at birth found statistically significant higher levels of benzo(b)fluoranthene (21,24), benzo(a)pyrene (17,21), fluoranthene (24) and dibenz(a,h)anthracene (21) among preterm versus term births. However, these studies lack information on timing of PAH exposure and its potential developmental-specific effect on the fetus. Ambient air monitoring-based studies have demonstrated associations between proxy measures of trimester-specific PAH exposure and PTB (25–27), but exposure misclassification is a major limitation of these studies, given the proxy measures, lack of spatial resolution from air monitors, and exclusion of important PAH exposure pathways, such as diet. This study addresses challenges in PAH exposure ascertainment by measuring PAH from maternal urine, thus providing a time-specific measure that capture all sources of PAH. We also address previous limitations in sample size, generalizability, and potential confounding by using data from a large, geographically diverse, and well-characterized cohort.

Male and female fetuses respond differently to adverse environmental exposures and sex-specific differences in gestational age at birth related to environmental exposures has been previously reported (28,29). While sex-specific associations have not been explored related to PAH, there is well-documented evidence of sex-specific differences in DNA methylation of the placenta in response to endocrine disrupting chemical exposures (30), which may play a role in fetal programming and gestational age at birth (31). Sex-specific differences in the association of PAH, an endocrine disruptor, with gestational age may also be driven by differences in circulating hormone levels (32), particularly hormones important for labor induction (17β estradiol, estriol, estrone) (32)

Using a large, prospective multi-site study that combines several cohorts, we examined associations of seven maternal urinary PAH metabolites, collected during the second trimester in pregnancy, with spontaneous PTB and gestational age at birth among term births. We also examined potential effect modification role of offspring sex in the associations of PAH with spontaneous PTB and gestational age at birth.

METHODS

Study setting and study population

The current study was based on information from mother-child dyads enrolled in three prenatal cohorts that constitute the ECHO PATHWAYS Consortium: Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) (34), The Infant Development and Environment Study (TIDES) (35,36), and Global Alliance to Prevent Prematurity and Stillbirth (GAPPS).

Participants in the CANDLE cohort were recruited from the University of Tennessee Health Science Center in Memphis, TN between 2006 and 2011. Participants were eligible if they were 16 years of age or older, enrolled between 16 and 27 weeks of gestation, had uncomplicated pregnancies at enrollment (e.g. no diagnosis of gestational diabetes, high blood pressure, or other condition), were able to speak and write in English, and planned to deliver at a study site.

Participants in the TIDES cohort were recruited from the following institutions between 2010 and 2012: University of California, San Francisco (UCSF); University of Rochester Medical Center (URMC); University of Minnesota (UMN); and University of Washington/Seattle Children's Hospital (UW/SCH). Participants were eligible for the TIDES cohort if they enrolled before 13 weeks of gestation, were 18 years of age or older, did not have a medically threatened pregnancy, were able to speak and write in English, and planned to deliver at a study site.

Participants in the GAPPS cohort were recruited from the following institutions between 2011 and 2014: University of Washington Medical Center (UWMC) and Swedish Medical Center in Seattle, WA and Yakima Valley Memorial Hospital in Yakima, WA. Participants were eligible to enroll in the GAPPS cohort if they were 18 years of age or older, English-speaking, and planned to deliver at a study sites. All study participants provided informed consent and study protocols were approved by respective institutions where the studies were based.

For the current analysis, we included mother-child dyads from CANDLE, TIDES, and GAPPS cohorts with data available for both gestational age at birth and PAH metabolites. We excluded pregnant individuals with multiple pregnancies, stillbirths, or a history of smoking during pregnancy—defined as either self-reporting smoking behavior or having urinary cotinine levels of >200 ng/mL (37,38). Active smokers were excluded due to evidence of strong associations of smoking with PAH exposure (39) and gestational age (40). After exclusions, 1,677 participants were eligible for analysis, including 867 (51.7%)

participants from CANDLE, 597, (35.6%) participants from TIDES, and 213 (12.7%) participants from GAPPS. The ECHO-PATHWAYS study protocol was approved by the University of Washington IRB.

Data Collection

Data collection procedures for CANDLE (34) and TIDES (35,36) have been previously described. Data collection for all three cohorts occurred during routine study visits, occurring approximately once per trimester. During these visits, participants completed health behavior and medical history questionnaires and provided spot urine samples. Birth and medical data were abstracted from medical records obtained from the participating clinics.

Gestational Age and Spontaneous Preterm Birth

Gestational age was calculated for CANDLE participants based on reported last menstrual period (LMP) and confirmed with ultrasound. Gestational age at delivery was calculated for TIDES and GAPPS participants based on ultrasound, or if unavailable, self-reported LMP. PTB was defined as <37 completed weeks of gestation. Birth indications were abstracted from medical records. The PTB outcome was restricted to spontaneous PTB, defined as either idiopathic preterm births or births resulting from preterm premature rupture of membranes (PPROM). PTBs with medical indications were excluded from this analysis. For the analysis examining gestational age as an outcome, the dataset was restricted to term births (occurring on or between 38 and 42 weeks of gestation).

Maternal Urinary PAH Metabolite Analysis

The primary exposure of interest was maternal PAH exposure, characterized using single PAH metabolites obtained through spot urine samples. Samples were stored at -80 degrees C in study biorepositories until the time of analysis at the Wadsworth Laboratory located at the New York State Department of Health. Concentrations of the following 12 PAH metabolites were assessed: Two metabolites of naphthalene (1-hydroxynaphthalene [1-nap], 2-hydroxynaphthalene [2-nap]), four metabolites of phenanthrene (2-hydroxyphenanthrene [2-phen], 3-hydroxyphenanthrene [3-phen], 4-hydroxyphenanthrene [4-phen], combined 1/9-hydroxyphenanthrene [1/9-phen]), combined 2/3/9-hydroxyfluorene (2/3/9-fluo), 1-hydroxypyrene (1-pyr), 3-hydroxybenzo[c]phenanthrene (3-bcp), two metabolites of hydroxychrysene (1-hydroxychrysene [1-chry], 6-hydroxychrysene [6-chry]), and 1-hydroxybenz[a]anthracene (1-baa).

Specific laboratory methods for analyzing PAH metabolites have been previously described (41). Briefly, urine samples (500 µL) were fortified with 10 ng each of an isotopically labeled internal standard mixture and mixed with 1 mL of 0.5 M ammonium acetate buffer containing 200 units/mL of β-glucuronidase/sulfatase enzyme (MP Biomedicals, LLC, Solon, OH, USA). The samples were mixed and incubated overnight at 37°C, and then diluted with 2 mL of HPLC-grade water and extracted with 7 mL of 80% pentane: 20% toluene, by shaking on a reciprocating shaker for one hour, centrifuged at 3600 x g for 20 minutes, the supernatant was transferred into a new glass tube for instrumental analysis. The chromatographic separation of PAH metabolites was conducted using a Waters Acquity I-Class UPLC system (Waters; Milford, MA, USA) connected with an Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 µm, Waters; Milford, MA, USA). Identification and quantification of PAH metabolites was performed on an ABSCIEX 5500 triple quadrupole mass spectrometer (Applied Biosystems; Foster City, CA, USA). Quality assurance protocols included analysis of two Standard Reference Materials (SRM 3672, SRM 3673) containing certified values for several PAH metabolites. Recoveries of analytes in SRMs ranged from 79 to 109%. A 13-point standard calibration curve (0.02 – 200 ng/mL) was prepared prior to the injection of samples. Calibration standards were injected periodically throughout the sample run to ensure instrument stability. The limits of detection (LOD) were metabolite- and cohort-specific and ranged from 0.017 to 0.48 ng/mL. PAH metabolites for which at least 50 percent of the pooled study population was above the limit of detection (LOD) were included in final analyses. The following seven PAH metabolites had at least 50 percent detection in the pooled study population and were included in final analyses: 1-nap, 2-nap, 2-phen, 3-phen, 1/9-phen, 2/3/9-fluo, and 1-pyr.

Covariates

Covariates included specific gravity (sg, continuous), maternal age (years, continuous), site (Memphis, San Francisco, Minnesota, Rochester, Seattle-TIDES, Seattle-GAPPS, and Yakima), race (White, Black/African American, Asian, Other), ethnicity (Non-Hispanic White, Hispanic/Latinx), urinary cotinine (ng/mL, continuous), education (<high school, high school graduate, graduated college or technical school, some graduate work), annual household income (<$20,000, $20,000-45,000, $45,000-75,000, >$75,000), season of birth (Autumn, Spring, Summer, Winter), infant sex (male, female), alcohol use during pregnancy (any, none), pre-pregnancy body mass index (BMI, kg/m^2, continuous), parity (nulliparous, parous), and prior preterm birth (yes, no). Specific gravity and maternal cotinine were measured from urine samples obtained during the second trimester visit. Immediately after urine collection, sg was recorded using a hand-held refractometer to measure urine dilution. Continuous

maternal cotinine was included as a measure of passive smoking exposure (active smokers were excluded from analysis). Season of birth and infant sex were obtained from medical record abstraction. All other covariates were obtained from questionnaires completed by mothers during prenatal visits. Race was included as a proxy for the impact of racist practices and structural inequities on adverse environmental exposures and birth outcomes. The following groups were included in the "other" category for race to improve model fit: Native American/Other Pacific Islander (<1% of study population), American Indian/Alaskan Native (<1% of study population), multiple race (2.4% of study population), and self-reported other race (3.9% of study population).

Statistical Analysis

Demographic and behavioral characteristics of mother-offspring dyads were summarized overall and by cohort. The distribution of PAH metabolites was characterized for each cohort by examining limit of detection (LOD), percent detection, median, interquartile range, geometric means and confidence intervals, overall and by cohort.

All regression models included $\log_{10}$-transformed, unadjusted PAH metabolites as the exposure and specific gravity as a covariate to account for urinary dilution. Separate multivariable logistic regression models for each PAH metabolite were fit for spontaneous PTB to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) associated with a 10-fold higher maternal PAH exposure. Covariates were selected based on a Directed Acyclic Graph constructed from prior literature. The minimal model (Model 1) included the following covariates: sg, maternal age, site, race, urinary cotinine (smoke exposure), education, income, season of birth, and infant sex. The main model (Model 2) included all Model 1 covariates plus alcohol use during pregnancy, pre-pregnancy BMI, parity, and prior PTB. To assess the extent to which restriction to spontaneous PTB may have influenced results, we conducted a sensitivity analysis examining all PTBs as the outcome. To examine potential interaction by offspring sex, we added an interaction term including sex and the PAH exposure variable of interest in each metabolite model. The p-value for the interaction term was used to determine whether results significantly differed by sex at the alpha=0.05 level. We present marginal estimates from the interaction models if any of the interaction terms were statistically significant.

Variables with high levels of missingness and considered theoretically appropriate for imputation (satisfies missing at random assumption, related to other variables in imputation model) were imputed using Multiple Imputation by Chained Equations (MICE) (42). Ten imputations with 50 iterations were

performed. Cotinine (7.6% missing), Income (5.0% missing), and pre-pregnancy BMI (1.4% missing) were imputed. The following variables were included as predictors in all of the imputation models: gestational age, maternal education, study site, race, ethnicity, maternal age, prior preterm birth, parity, pre-pregnancy BMI, income, alcohol use during pregnancy, urinary cotinine, season of birth, and seven PAH metabolites. Model checking included comparison of imputed covariates to observed covariates and examination of trace plots to observe trends in the mean and standard deviation of the imputed values.

Using the datasets obtained from MICE imputations, PAH values below the LOD were imputed using censored likelihood multiple imputation (CLMI) techniques available in the R Lodi package (43)**.** The CLMI method addresses differences in cohort-specific LODs of PAHs that may result in variable misclassification of exposure. For a given PAH metabolite, ten imputations were performed using the same variables included in the MICE imputation models. Regression models were computed for each of the ten imputed datasets and pooled into a single output using Rubin's Rules. This method was repeated for each of the seven PAH metabolites. CLMI requires complete datasets. Therefore, the analytic dataset was restricted to participants who had no missing data in datasets obtained from the MICE imputations. Because CLMI is a relatively new approach, CLMI results were compared to analyses for which PAH values below the LOD were substituted with the LOD/sqrt(2).

The gestational age among term births analysis was analogous to the PTB analysis with respect to exposure characterization and covariate inclusion. We used multivariable linear regression as the model and continuous gestational age (days) as the outcome to obtain the mean difference of gestational age at birth associated with 10-fold higher maternal PAH exposure. An interaction term with $\log_{10}$PAH and infant sex were added to the main gestational age models to assess effect modification by infant sex. Similar to the PTB analysis, we assessed statistical significance of the interaction terms at the α =0.05 level and presented marginal estimates if any of the interaction terms were statistically significant. All analyses were conducted in RStudio Version 1.4.1106 and significance was assessed at an α level of 0.05.

RESULTS

Descriptive Statistics

Most participants were between the ages of 18-29 (N=827, 49.3%) or 30-39 (N=762, 45.4%) and were high school (N=540, 32.2%) or college graduates (N=570, 34.0%). Participants in the CANDLE cohort were more likely to be younger, Black/African American, high school graduates or less, or have lower

income compared to participants in the TIDES and GAPPS cohorts (Table 1). The prevalence of overall PTB was highest in the TIDES cohort (N=62, 10.4%), followed by CANDLE (N=84, 9.7%) and GAPPS (N=20, 9.4%). After excluding individuals with medically-indicated PTB (N= 74) and missing covariate data (N=64), the analytic dataset for the PTB analysis included 1,529 mother-child dyads and 87 spontaneous PTBs.

The mean weeks of gestational age at birth among term births in the CANDLE, TIDES, and GAPPS cohort was 39.3 (SD=1.0), 39.7 (SD=1.2), and 39.5 (SD=1.1), respectively. Of the 1,677 mothers eligible for analysis, 1,511 (90.1%) gave birth during the term period. After restricting to participants with no missing covariates, the analytic dataset for the gestational age analysis included 1,435 mother-child dyads.

Percent detection and PAH levels varied considerably across metabolites and cohorts (Table 2). Overall percent detection was highest for 2-nap (99.8%) and the lowest for 1-pyr (65.2%). The TIDES cohort had only 39.8% and 36.0% detection for 2/3/9-fluo and 1-pyr, while CANDLE had 73.0% and 88.5% detection for these metabolites, respectively. CANDLE participants had the highest geometric median and mean levels for all PAH metabolites compared to participants in TIDES and GAPPS.

Spontaneous PTB

In the main analysis, we observed no significant association between a 10-fold higher maternal PAH exposure and spontaneous PTB (Table 3). There was suggestive evidence of a positive association between 1-pyr and spontaneous PTB (OR: 1.32, 95% CI: 0.88, 1.99) and negative association between 2-nap and spontaneous PTB (OR: 0.68, 95% CI: 0.38, 1.23). Comparison of CLMI results to single imputation methods suggests that single imputation did not substantially change results (Supplementary Figure 1). While some directions of association changed when examining overall PTB as the outcome, no estimates were statistically significant (Supplementary Figure 3).

In models examining potential interaction between $\log_{10}$PAH and infant sex on spontaneous PTB, we observed significant multiplicative interaction for 2-phen (p=0.03) and 1-pyr (p=0.04) (Figure 1). Marginal estimates suggest positive associations of odds of spontaneous PTB with maternal PAH metabolites (2-phen, 1-pyr) among females and inverse (2-phen) or no (1-pyr) association among males (Figure 1), although statistically significant association was observed only for 1-pyr among females. Among females, 10-fold higher maternal exposure to 1-pyr was associated with 1.94-fold higher odds

(95% CI: 1.11, 3.46) of spontaneous PTB. The same exposure among males was null (OR: 0.99, 95% CI: 0.60, 1.62).

Gestational Age at Birth

In our main, fully adjusted model, we observed that ten-fold higher maternal concentration of 2-nap was associated with 1.13 days earlier gestational age at birth (95% CI: -2.15, -0.11). Associations for all other PAH metabolites were null at the 95% confidence level (Table 4). Replacement of CLMI with single imputation produced similar results (Supplementary Figure 2).

In models examining potential interaction between $\log_{10}$PAH and infant sex on gestational age at birth, we observed significant multiplicative interaction for the following metabolites: 2-nap (p=0.01), 2-phen (p=0.04), 3-phen (p=0.048), 1/9-phen (p=0.03), and 2/3/9-fluo (p=0.01) (Figure 2). Similar to the spontaneous PTB results, associations of these PAH metabolites with gestational age at birth trended inverse (all five) among females while associations among males trended in general positive (1/9-phen, 2-phen, and 3-phen) or null (2-nap and 2/3/9-fluo). Marginal estimates were statistically significant for 1-nap and 2/3/9-fluo among mothers carrying female infants. Among this group, 10-fold higher maternal levels of urinary 2-nap and 2/3/9-fluo were associated with a mean lowering of gestational age at birth by 2.13 days (95% CI: -3.41, -0.85) and 1.55 days (95% CI: -2.96, -0.14), respectively (Figure 2).

DISCUSSION

Using prospective, pooled data from three geographically diverse cohorts, we examined associations of second trimester maternal urinary PAH biomarkers with spontaneous PTB risk and gestational age at birth among term infants. Among all participants, we observed inverse association between 2-nap and gestational at birth. We also found suggestive sex-specific associations of PAH metabolites with spontaneous PTB and gestational age at birth. Females, not males, appeared to have higher odds of PTB (for 1-pyr) and shorter gestational age among term births (for 2-nap, and 2/3/9-fluo) with higher exposure of PAH metabolites.

Comparison of our primary PTB results to prior studies is somewhat limited because we restricted outcomes to spontaneous PTB. We chose to exclude medically-indicated PTBs because they include a heterogenous set of conditions that are typically not informative, in aggregate, of potential mechanisms underlying PAH and PTB. Spontaneous PTB is associated with placental inflammation (44), changes in factors that promote uterine contraction (expression of connexin 43, cox-2, and prostaglantin receptor 2α genes) (45), and higher rates of placental DNA methylation (22)—all hypothesized biological effects that have been related to *in utero* PAH exposure (15,16,20,21). To assess the extent to which restriction to spontaneous PTB may have influenced results and to produce estimates comparable to prior literature, we conducted a sensitivity analysis including all PTBs as an outcome. Findings from these sensitivity analyses had confidence intervals that were too wide and no results were statistically significant. Interestingly, our observations of positive association of 1-pyr with PTB risk among females replicated reports from an NHANES study that one standard deviation increase in urinary biomarkers of 1-pyr was associated with 80% higher odds (95% CI: 1.1, 2.8) of PTB. Compared to our findings (10-fold change related to 96% increase in odds of spontaneous PTB), the observed association in the NHANES study is likely an overestimate because they did not control for maternal smoking history, a major confounder in PAH and PTB. Three studies that controlled for maternal smoking found that infants born preterm (versus term) had statistically significant higher mean levels of benzo(b)fluoranthene (21,24), benzo(a)pyrene (17,21), fluoranthene (24) and dibenz(a,h)anthracene (21) in placenta measurements collected at birth. Associations were null for other PAHs observed, which may be due to small sample sizes ranging from 42 to 84 total subjects and 22 to 29 preterm deliveries. Two of these studies examined naphthalene, phenanthrene, fluorene, and pyrene, but comparability with our study is limited because they assessed parent compounds instead of specific PAH metabolites. In an air traffic study,

researchers found that an interquartile range increase of summed naphthalene was associated with 29% higher odds of PTB (95% CI: 1.14, 1.45) (25). We did not see similar associations in our study, although 2-nap was associated with shorter gestational age among term born infants. While there is some suggestion of higher risk of PTB associated with several PAH metabolites in these studies, comparability to the present study is limited because prior studies used different PAH exposure ascertainment (primarily placenta measurements or air monitoring), these studies assessed overall PTB rather than spontaneous PTB, and these studies used different (mostly limited) sets of adjustment variables than we used in the current study.

This is the first study to examine PAH exposure and gestational age at birth among term births. Prior studies suggest PTB and early term birth may share same mechanisms such as placental inflammation and ischaemia (44,46,47), but risk factors for early term birth are relatively understudied. Our findings suggest exposure to some PAH metabolites, particularly 2-nap, may be a risk factor for early term birth. While our observation that 10-fold higher 2-nap is associated with one day earlier gestational age at birth may appear small, such a difference may have important impacts at the population level. The potential significance of this shortening of gestational age on growth, development, and programming and eventually the life course health of the offspring needs further research.

We observed significant interactions between sex and several PAH metabolites on PTB and gestational age among term births. The direction of marginal estimates suggested that females experience higher odds of spontaneous PTB and lower mean gestational age among term births as a result of higher PAH exposure. The consistent trend raises the possibility of sex-specific differences in biological effects of PAH metabolites. Prior research suggests the placenta mediates fetal programming in a sex-specific manner (48) and females experience more inflammation and DNA methylation in the placenta in response to maternal environmental stressors (31). While this relationship has not been explored for PAH, sex-specific differences in epigenetic changes in the placenta in response to endocrine disrupting chemicals are well-documented (30). In addition, sex-specific effects of PAH on gestational age may be mediated by differences in hormonal changes associated with exposure to endocrine disruptors (32). Accumulating evidence suggests changes in circulating hormone levels in response to endocrine disruptor exposure may be time- and sex-dependent (32,49). Hormones with estrogenic effects are important for regulating labor and birth through effects on uterine physiology and expression of oxytocin receptors (33). One study found females fetuses are more sensitive to estriol from BPA exposure during the second trimester compared to male fetuses (32). A population-based cohort study

examining birth outcomes found that exposure to bisphenol A (BPA) and two BPA analogs were associated with shorter gestational age and reduced growth parameters among females (50). Animal studies have demonstrated similar relationships in which exposure to BPA was associated with higher risk of PTB in females compared to males (51).

There are limitations to this study. First, extrapolating second trimester PAH exposure based on a single measurement may lead to misclassification. The half-life of PAH metabolites ranges from 2.5 to 6.1 hours, and one study found that PAH concentrations return to baseline levels within 24-48 hours after exposure (52). A measurement from a single point in the second trimester may not capture the entire exposure window of interest. Second, examination of seven PAH metabolites for multiple aims raises the possibility that some associations may arise due to chance. We did not conduct any statistical corrections for multiple comparisons, so findings should be interpreted cautiously. Third, exclusion of participants with missing covariates may have led to bias, especially if missingness was associated with PAH exposure and gestational age. To mitigate this problem and preserve the sample size, we imputed income, BMI, and cotinine variables. Multiple imputation relies on the assumption that data is missing at random (MAR). If data were missing not at random, multiple imputation may not have effectively reduced bias. Finally, heterogeneity in laboratory methods (e.g. varying LODs by cohort) and sociodemographic characteristics across cohorts raises the concern that results may be driven by cohort- or site-specific effects. We attempted to address this concern by 1) adjusting for study site in all analyses and 2) comparing main analyses to models that iteratively excluded a single site or cohort from the study population. Results changed slightly in sensitivity analyses, but the direction of association was consistent throughout different combinations of sites and cohorts. The challenges of pooling cohort data are outweighed by several advantages, including having urinary measures of PAHs vs. proxy estimates and a large, diverse sample population with participants from seven study sites spanning urban and rural US regions.

Approximately 10% and 26.5% of all U.S. infants are born preterm and early term, respectively (3) and both conditions have been associated with several adverse neonatal and life course outcomes (4,9,53). Identifying modifiable environmental determinants of PTB and early term births may have substantial public health impact. Our findings suggest that PAH exposure is not associated with spontaneous PTB in the general population, though some adverse effects were observed in females. Maternal exposure to 2-napthalene during the second trimester was associated with earlier gestational age at birth among term births in the general population. Sex-specific effects were observed for a number of metabolites across

both analyses, suggesting that females may be more vulnerable to adverse effects of maternal PAH exposure. Additional research is needed to understand the relationship and mechanisms associated with sex-specific impacts of maternal PAH exposure during pregnancy on birth outcomes.

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TABLES AND FIGURES

Table 1. Selected characteristics of study participants overall and by cohort

	All (N=1677)	**CANDLE (N=867)**	**TIDES (N=597)**	**GAPPS (N=213)**
	N (%)	N (%)	N (%)	N (%)
Site, ***Missing = 0***				
Memphis	867 (51.7)	867 (100)		
San Francisco	157 (9.4)	-	157 (26.3)	
Minnesota	162 (9.7)	-	162 (27.1)	-
Rochester	139 (8.3)	-	139 (23.3)	-
Seattle - TIDES	139 (8.3)	-	139 (23.3)	-
Seattle - GAPPS	96 (5.7)	-	-	96 (45.1)
Yakima	117 (7.0)	-	-	117 (54.9)
Age, ***Missing = 20 (1.2%)***				
<18	11 (<1)	11 (1.3)	0 (0)	0 (0)
18-29	827 (49.3)	547 (63.1)	200 (33.5)	80 (37.6)
30-39	762 (45.4)	283 (32.6)	360 (60.3)	119 (55.9)
≥40	57 (3.4)	8 (1.0)	37 (6.2)	12 (3.8)
Race ***Missing = 17 (1.0%)***				
White	871 (51.9)	266 (30.7)	432 (72.4)	173 (81.2)
Black/African American	618 (36.9)	550 (63.4)	64 (10.7)	4 (1.9)
Asian	51 (3.0)	9 (1.0)	35 (5.9)	7 (3.3)
Other	120 (7.2)	42 (4.8)	55 (9.2)	23 (10.8)
Ethnicity, ***Missing=9 (<1%)***				
Hispanic or Latinx	94 (5.6%)	14 (1.6)	51 (8.5)	29 (13.6)
Not Hispanic or Latinx	1574 (93.9%)	853 (98.4)	538 (90.1)	183 (85.9)
Education, ***Missing = 8 (<1%)***				
<High School	112 (6.7)	73 (8.4)	32 (5.4)	7 (3.3)
High school graduate	540 (32.2)	397 (45.8)	89 (14.9)	54 (25.4)
Graduated college or technical school	570 (34.0)	278 (32.1)	200 (33.5)	92 (43.2)
Some graduate work, graduate/professional degree	447 (26.7)	118 (13.6)	270 (45.2)	59 (27.7)
Income, ***Missing = 84 (5.0%)***				
<$20k	383 (22.8)	254 (29.3)	109 (18.3)	20 (9.4)
$20k-45k	308 (18.4)	231 (26.6)	50 (8.3)	27 (12.7)
$45k-75k	328 (19.6)	175 (20.2)	107 (17.9)	46 (21.6)
>$75k	574 (34.2)	153 (17.6)	311 (52.1)	110 (51.6)
Pre-pregnancy BMI, ***Missing = 23 (1.4%)***				
Underweight (<18.5)	50 (3.0)	37 (4.3)	7 (1.2)	6 (2.8)
Normal (18.5-24.9)	728 (43.4)	328 (37.8)	310 (51.9)	90 (42.3)
Overweight (25.0-29.9)	405 (24.2)	203 (23.4)	142 (23.8)	60 (28.2)

Obese (>=30.0)	471 (28.1)	295 (34.0)	128 (21.4)	48 (22.5)
Infant Sex, ***Missing = 0***				
Male	809 (48.2)	422 (48.7)	282 (47.2)	105 (49.3)
Season of birth, ***Missing = 18 (1.1%)***				
Autumn	480 (28.6)	246 (28.4)	182 (30.5)	52 (24.4)
Spring	359 (21.4)	179 (20.6)	122 (20.4)	58 (27.2)
Summer	452 (27.0)	236 (27.2)	164 (27.5)	52 (24.4)
Winter	368 (21.9)	188 (21.7)	129 (21.6)	51 (23.9)
Alcohol use during pregnancy ***Missing = 4 (<1%)***				
Yes	159 (9.5)	62 (7.2)	75 (12.6)	22 (10.3)
Parity ***Missing = 16 (<1%)***				
Nulliparous	741 (44.2)	349 (40.3)	321 (53.8)	71 (33.3)
Prior PTB ***Missing = 0***				
Yes	134 (8.0)	65 (7.5)	59 (9.9)	10 (4.7)
PTB				
Yes	166 (9.9)	84 (9.7)	62 (10.4)	20 (9.4)
Spontaneous PTB	*92 (5.5%)*	*52 (6.0%)*	*21 (3.5%)*	*19 (8.9%)*
Medically indicated PTB	*74 (4.4%)*	*32 (3.7%)*	*41 (6.9%)*	*1 (<1%%)*
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Gestational Age at Birth (weeks)				
Mean (SD) *Term births*	39.5 (1.09)	39.3 (1.0)	39.7 (1.2)	39.5 (1.1)
Raw cotinine value (ng/mL) ***Missing = 127***				
Mean (SD)	2.21 (9.6)	2.87 (10.1)	1.59 (9.6)	0.06 (0.19)
Specific gravity ***Missing = 26 (1.6%)***				
Mean (SD)	1.017 (0.01)	1.017 (0.01)	1.014 (0.01)	1.028 (0.01)

Table 2. Characterization of specific gravity-adjusted PAH Exposure[a], Overall and by Cohort

PAH Metabolite	N	LOD	Percent Detection	50th (25th, 75th)	Geometric mean (95% CI)
1-OH-Naphthalene					
Overall	1,677		87.2	0.61 (0.27, 1.38)	0.55 (0.51, 0.60)
CANDLE	867	0.02	100	1.02 (0.54, 2.08)	1.20 (1.11, 1.29)
TIDES	597	0.04	64.7	0.25 (0.04, 0.61)	0.20 (0.17, 0.22)
GAPPS	213	0.017	98.1	0.39 (0.23, 0.67)	0.42 (0.37, 0.48)
2-OH-Naphthalene					
Overall	1,677		99.8	3.66 (2.02, 6.64)	3.61 (3.45, 3.78)
CANDLE	867	0.025	99.8	4.90 (2.86, 7.90)	4.89 (4.62, 5.18)
TIDES	597	0.017	99.7	2.46 (1.29, 4.72)	2.48 (2.29, 2.68)
GAPPS	213	0.018	100.0	3.01 (1.70, 5.17)	2.96 (2.62, 3.34)
2-OH-Phenanthrene					
Overall	1,677		88.1	0.07 (0.04, 0.12)	0.07 (0.07, 0.07)
CANDLE	867	0.03	85.7	0.09 (0.06, 0.13)	0.09 (0.09, 0.10)
TIDES	597	0.003	98.2	0.06 (0.03, 0.09)	0.06 (0.05, 0.06)
GAPPS	213	0.017	70.0	0.04 (0.03, 0.07)	0.05 (0.04, 0.05)
3-OH-Phenanthrene					
Overall	1,677		88.0	0.07 (0.04, 0.11)	0.07 (0.07, 0.07)
CANDLE	867	0.03	85.6	0.09 (0.06, 0.14)	0.10 (0.09, 0.10)
TIDES	597	0.003	97.2	0.05 (0.03, 0.08)	0.05 (0.04, 0.05)
GAPPS	213	0.018	72.3	0.04 (0.03, 0.06)	0.05 (0.05, 0.05)
1/9-OH-Phenanthrene					
Overall	1,677		86.6	0.18 (0.07, 0.39)	0.16 (0.15, 0.17)
CANDLE	867	0.08	83.4	0.34 (0.18, 0.57)	0.28 (0.26, 0.30)
TIDES	597	0.007	89.1	0.10 (0.05, 0.19)	0.09 (0.08, 0.10)
GAPPS	213	0.017	92.5	0.10 (0.06, 0.15)	0.10 (0.09, 0.10)
2/3/9-OH-Fluorene					
Overall	1,677		73.0	0.66 (0.36, 1.16)	0.63 (0.61, 0.66)
CANDLE	867	0.12	96.3	0.89 (0.59, 1.43)	0.93 (0.89, 0.98)
TIDES	597	0.48	30.8	0.53 (0.34, 0.93)	0.59 (0.55, 0.62)
GAPPS	213	0.017	96.2	0.14 (0.10, 0.20)	0.15 (0.13, 0.16)
1-OH-Pyrene					
Overall	1,677		65.2	0.09 (0.02, 0.20)	0.07 (0.07, 0.08)
CANDLE	867	0.03	88.5	0.14 (0.08, 0.23)	0.14 (0.13, 0.15)
TIDES	597	0.009	36.0	0.02 (0.01, 0.14)	0.03 (0.03, 0.03)
GAPPS	213	0.02	52.6	0.05 (0.02, 0.07)	0.04 (0.04, 0.05)

Abbreviations: CI, confidence interval

a. All PAH values are expressed in ng/mL

Table 3. Odds ratios (95% confidence intervals) for spontaneous preterm birth associated with 10-fold higher urinary PAH metabolites[a]

PAH Metaboliteb	Crude[c]	Model 1[d]	Model 2[e]
1-OH-Naphthalene	1.18 (0.87, 1.59)	1.02 (0.70, 1.49)	1.05 (0.71, 1.56)
2-OH-Naphthalene	0.93 (0.54, 1.59)	0.69 (0.39, 1.21)	0.68 (0.38, 1.23)
2-OH-Phenanthrene	1.01 (0.52, 1.96)	0.86 (0.41, 1.81)	0.88 (0.42, 1.88)
3-OH-Phenanthrene	1.32 (0.68, 2.55)	1.12 (0.51, 2.46)	1.04 (0.48, 2.29)
19-OH-Phenanthrene	0.92 (0.58, 1.46)	0.77 (0.44, 1.33)	0.78 (0.45, 1.37)
2/3/9-OH-Fluorene	0.86 (0.51, 1.45)	0.72 (0.35, 1.51)	0.67 (0.31, 1.43)
1-OH-Pyrene	**1.45 (1.06, 1.99)**	1.33 (0.88, 2.01)	1.32 (0.88, 1.99)

a. Analytic dataset included 1,529 mother-child pairs, including 87 spontaneous PTBs
b. PAH compounds were measured in ng/mL and $\log_{10}$-transformed.
c. Adjusted for specific gravity.
d. Adjusted for maternal age, site, race, ethnicity, cotinine measurements, education, income, urinary specific gravity, infant sex, and season of birth
e. Adjusted for model 1 covariates plus alcohol use during pregnancy, pre-pregnancy BMI, parity, and prior preterm birth.

Table 4. Mean change in gestational age at birth (days) associated with 10-fold higher urinary PAH metabolites[a]

PAH Metabolite[b]	Crude[c]	Model 1[d]	Model 2[e]
1-OH-Naphthalene	**-0.44 (-0.98, 0.09)**	0.32 (-0.32, 0.97)	0.22 (-0.41, 0.85)
2-OH-Naphthalene	**-1.51 (-2.48, -0.54)**	-0.97 (-1.99, 0.06)	**-1.13 (-2.15, -0.11)**
2-OH-Phenanthrene	-0.16 (-1.33, 1.01)	0.33 (-0.89, 1.55)	0.17 (-1.04, 1.38)
3-OH-Phenanthrene	-0.44 (-1.63, 0.75)	0.21 (-1.07, 1.48)	0.10 (-1.16, 1.35)
19-OH-Phenanthrene	**-0.86 (-1.67, -0.05)**	0.22 (-0.71, 1.14)	0.15 (-0.76, 1.07)
2/3/9-OH-Fluorene	**-1.51 (-2.47, -0.55)**	-0.67 (-1.89, 0.54)	-0.67 (-1.88, 0.55)
1-OH-Pyrene	**-0.71 (-1.26, -0.16)**	0.05 (-0.65, 0.76)	0.06 (-0.62, 0.74)

a. Analytic dataset included 1,435 mother-child pairs
b. PAH compounds were measured in ng/mL and $\log_{10}$-transformed.
c. Adjusted for specific gravity.
d. Adjusted for maternal age, site, race, ethnicity, cotinine measurements, education, income, urinary specific gravity, infant sex, and season of birth
e. Adjusted for model 1 covariates plus alcohol use during pregnancy, pre-pregnancy BMI, parity, and prior preterm birth.

Figure 1. Marginal male and female odds ratios and 95% confidence intervals of spontaneous preterm birth associated with 10-fold higher maternal PAH (ng/mL)

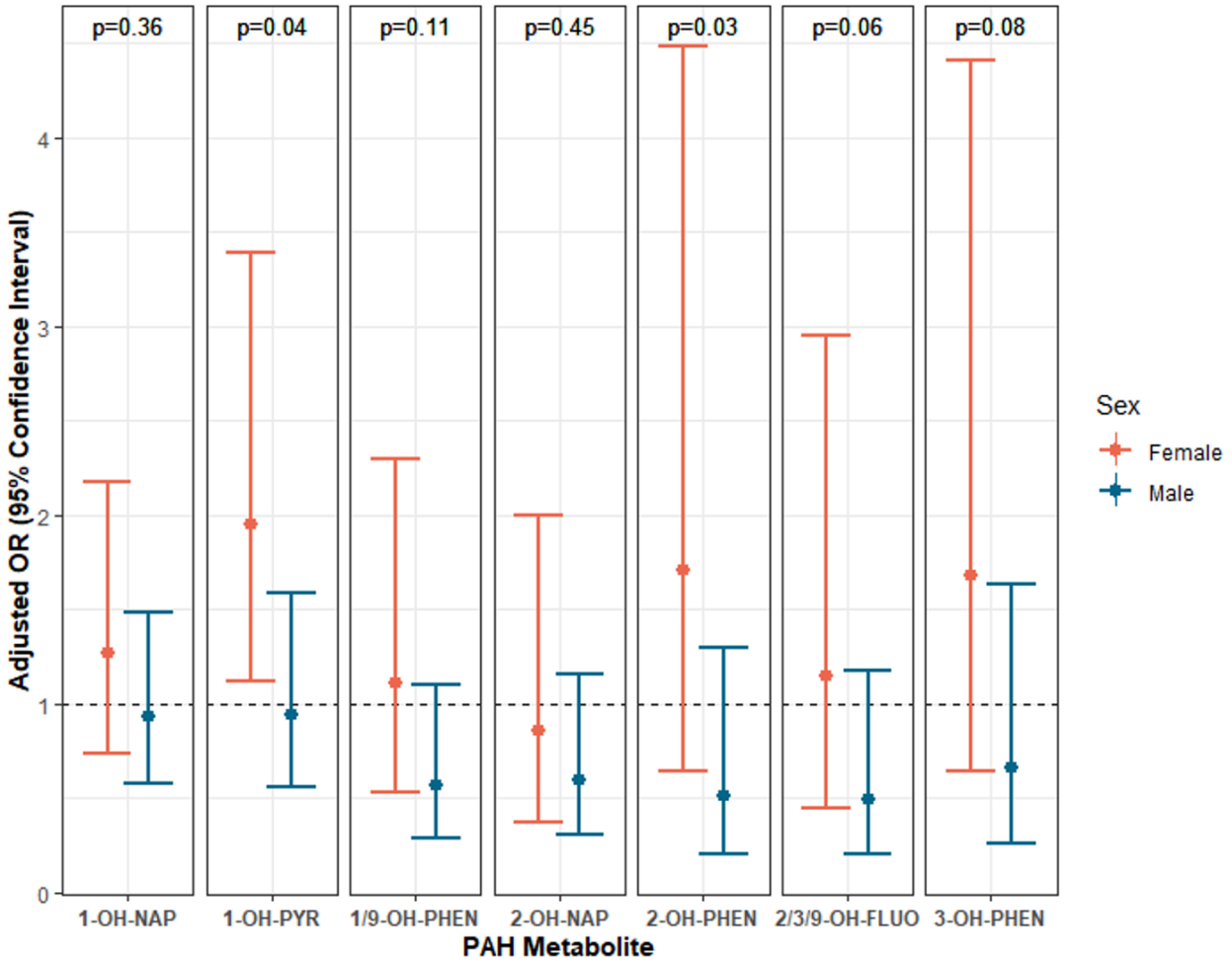
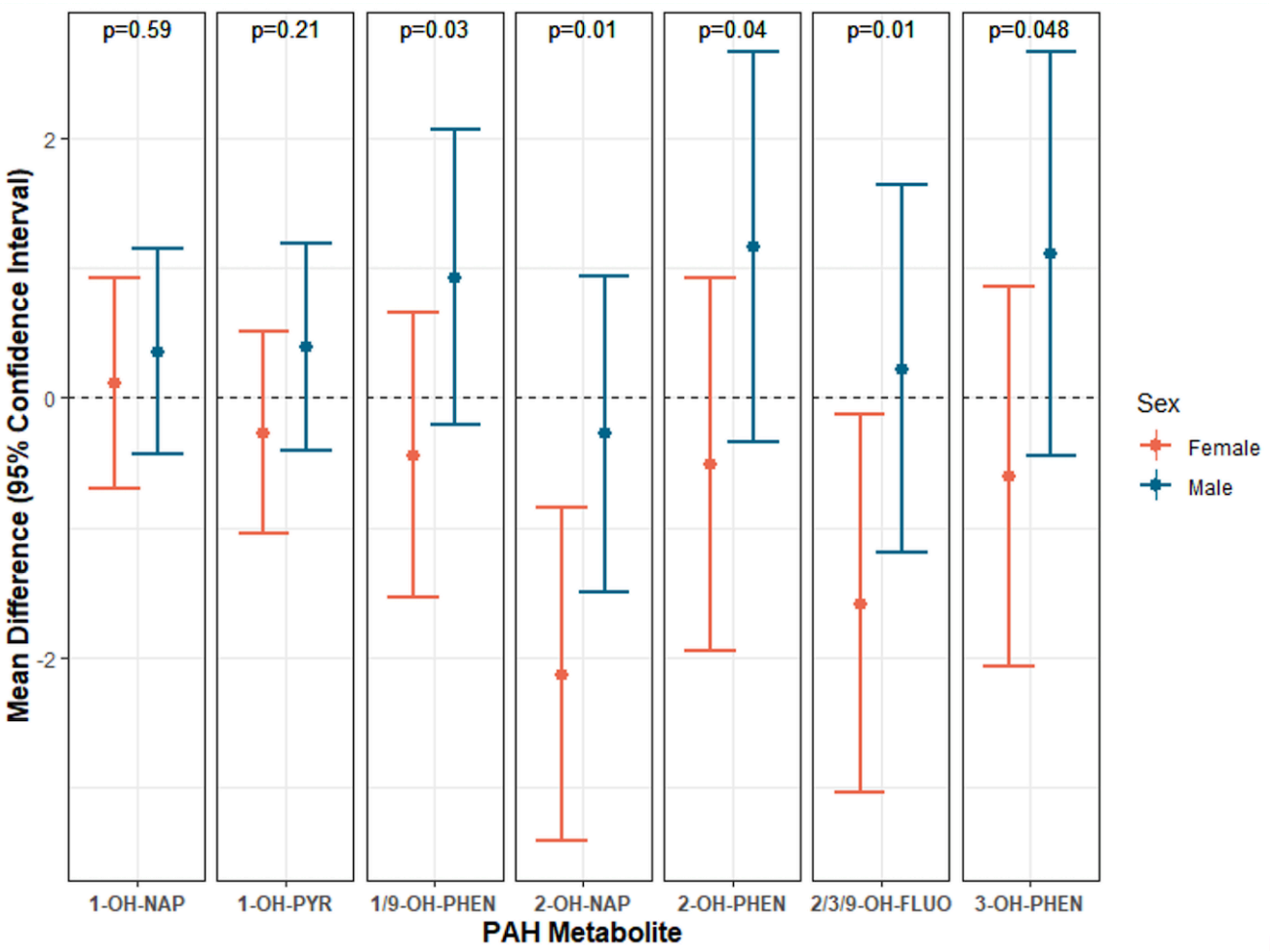
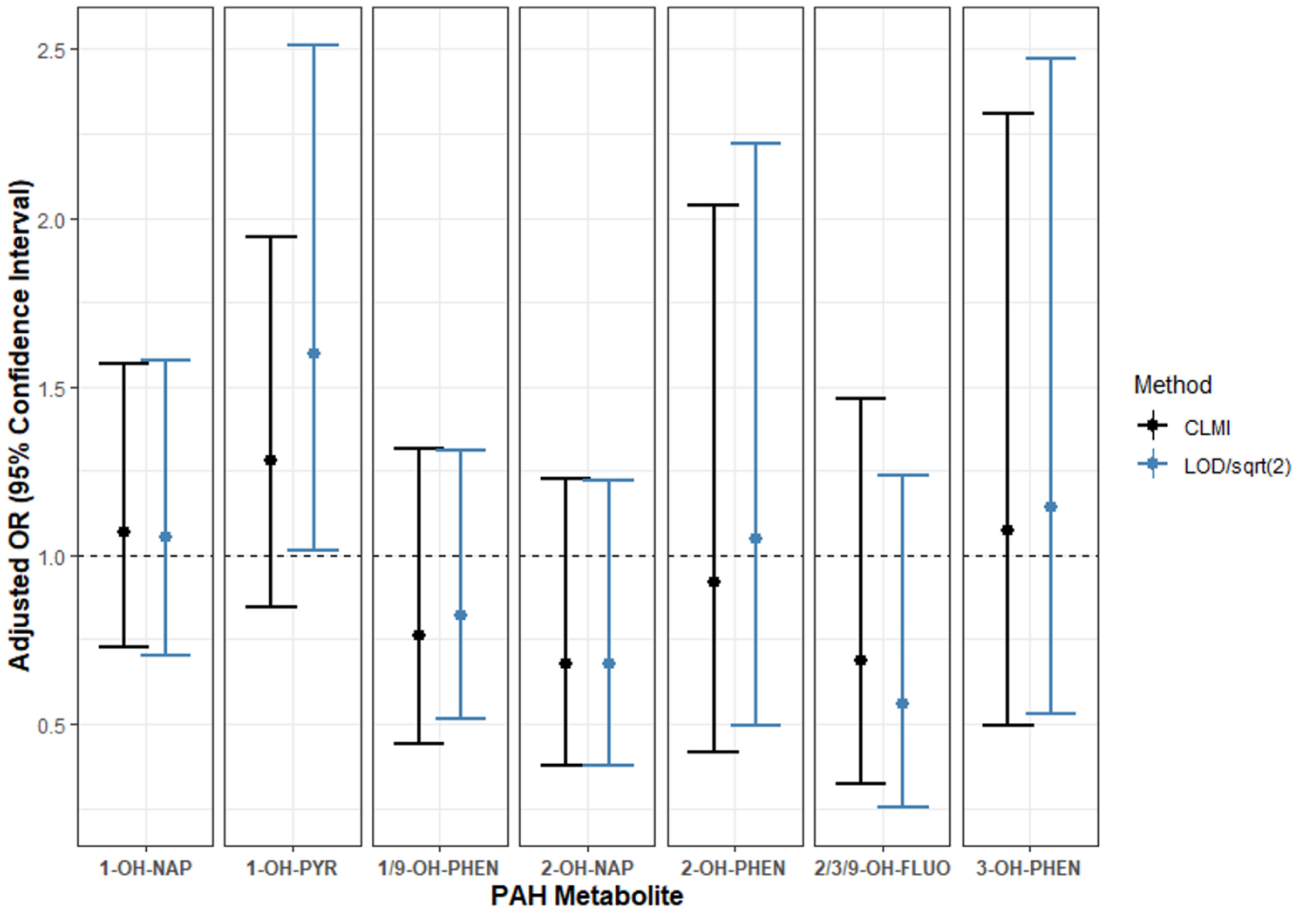


Figure 2. Marginal male and female mean difference in gestational age at birth (days) and 95% confidence intervals associated with 10-fold higher maternal PAH (ng/mL)

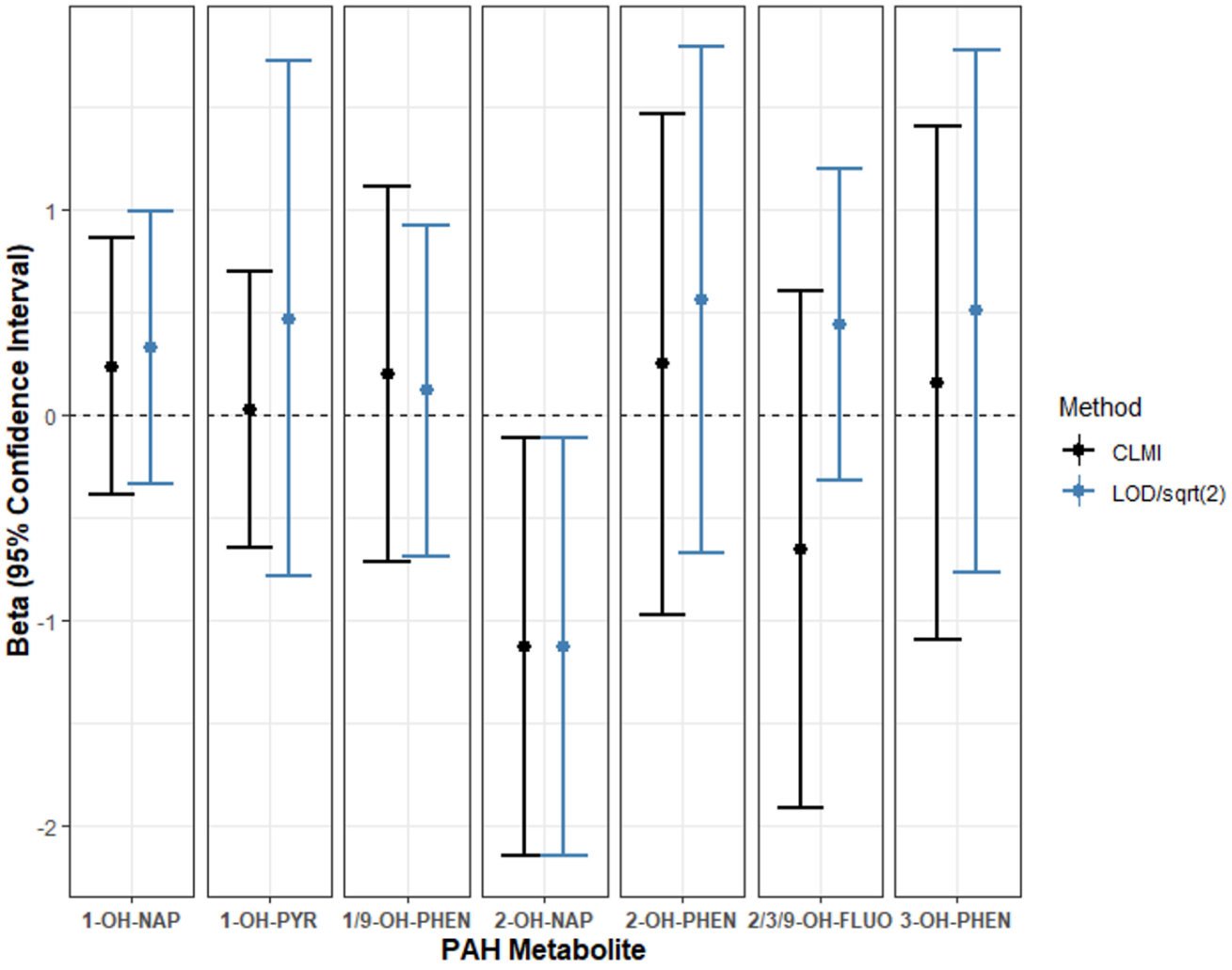


Supplementary Tables and Figures

Supplementary Figure 1. Comparison of CLMI and Single Imputation Results for Spontaneous PTB Analysis



Supplementary Figure 2. Comparison of CLMI and Single Imputation Results For Gestational Age Among Term Births Analysis



Supplementary Figure 3. Comparison of Spontaneous PTB to All PTB results