## Original Contribution

# Maternal Urinary Metabolites of Di-(2-Ethylhexyl) Phthalate in Relation to the Timing of Labor in a US Multicenter Pregnancy Cohort Study

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Di-(2-ethylhexyl) phthalate (DEHP) is a plasticizer used in consumer and medical products that can cross the placenta, disrupt steroid hormone synthesis, and activate peroxisome proliferator-activated receptor $\gamma$. The authors examined DEHP exposure in relation to the timing of labor in a pregnancy cohort study of 283 women recruited in 4 US states (California, Iowa, Minnesota, and Missouri) between 2000 and 2004. The authors estimated associations between concentrations of DEHP metabolites and gestational age at delivery using linear regression models and associations between DEHP metabolites and clinical outcomes using logistic regression models. After covariate adjustment, women at the 75th percentile of DEHP metabolite concentrations had a 2-day-longer mean length of gestation than women at the 25th percentile (95% confidence interval: 1.4, 3.3). Log-unit increases in mono-2-ethylhexyl phthalate and mono-2-ethyl-5-oxohexyl phthalate concentrations were associated with increased odds of cesarean section delivery (30% and 50% increased odds, respectively), increased odds of delivering at 41 weeks or later (100% and 120% increased odds), and reduced odds of preterm delivery (50% and 60% decreased odds). These data suggest that DEHP may interfere with signaling related to the timing of parturition.

creatinine; diethylhexyl phthalate; endocrine disruptors; gestational age; parturition; placenta; PPAR $\gamma$; pregnancy

Di-(2-ethylhexyl) phthalate (DEHP) is a plasticizer that is widely used in consumer products, such as polyvinyl chloride flooring, carpeting, roofing, vinyl, upholstery, clothing, and packaging (1). DEHP exposure occurs through ingestion of food and water (2, 3) and inhalation of household dust (4), as well as parenterally from medical devices (1). DEHP metabolites have been detected in 95% of the US population aged $\geq$6 years (5).

The hydrolytic metabolite of DEHP, mono-(2-ethylhexyl) phthalate (MEHP), can cross the placenta and enter fetal circulation (6, 7). Metabolites of DEHP have been measured in umbilical cord blood (8), amniotic fluid (9), maternal urine (10), placental tissue (7, 11), and neonatal urine and meconium (12, 13). MEHP is further metabolized into oxidative metabolites, including mono-2-ethyl-5-hydroxyhexyl

phthalate (MEHHP) and mono-2-ethyl-5-oxohexyl phthalate (MEOHP). MEHP and its oxidative metabolites can be glucuronidated and excreted in urine and feces. These 3 metabolites account for approximately 50% of the received dose of DEHP (14).

Among rodents, DEHP suppresses fetal testosterone synthesis in males (15, 16) and inhibits ovarian aromatase transcription in adult females (17, 18). Aromatase, which is responsible for the conversion of androgens to estradiol, is expressed in several tissues, including the human placenta (19). Inhibition of steroid hormone synthesis in the ovary of the adult rodent, fetal testis, and fetal brain has been linked to binding and activation of the transcription factor peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) (20–22). MEHP can activate PPAR$\gamma$ in primate fibroblasts, in rodent

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granulosa and trophoblast cells, and in human cervical cancer cells (21–24).

PPARγ activation increases early in pregnancy to promote zygote implantation and placental development (25–27). During pregnancy, high levels of placental PPARγ contribute to uterine quiescence by down-regulating cyclooxygenase 2 and inhibiting prostaglandin synthesis. By contrast, at parturition, PPARγ activation decreases in order to allow for increased prostaglandin production, which is essential in the stimulation of uterine contractions (26, 28). It has been hypothesized that exposure to PPARγ ligands during pregnancy reduces the risk of preterm labor by suppressing the inflammatory response in fetal tissues (27).

We tested the hypothesis that exposure to DEHP alters the timing of labor by measuring the associations between maternal urinary concentrations of DEHP metabolites during pregnancy and gestational age at delivery, as well as between these metabolites and risk of cesarean delivery, preterm birth, and delivering at greater than 41 weeks' gestation.

## MATERIALS AND METHODS

Women included in this analysis were recruited into the Study for Future Families (SFF) between March 2000 and August 2004 at 4 US sites. Details on the SFF are available elsewhere (29). Briefly, women were recruited at prenatal clinics associated with university hospitals in Los Angeles, California (Harbor-UCLA and Cedars-Sinai); Minneapolis, Minnesota (University of Minnesota Health Center); Columbia, Missouri (University of Missouri School of Medicine); and Iowa City, Iowa (University of Iowa School of Medicine). Women were eligible if they were at least 18 years of age, conception had been natural, and the pregnancy was not medically threatened.

Of the 783 pregnant women enrolled in the SFF between 2000 and 2004, only those who were fully enrolled in a follow-up study for postnatal evaluation of their babies were eligible for inclusion in the phthalate study ($n = 441$). For a woman to be eligible for follow-up, the pregnancy had to end in a livebirth, the baby had to be 2–36 months of age, the mother and baby had to live within 50 miles (80 km) of the clinic, and the mother had to attend at least 1 study visit. Reasons for exclusion were unlikely to be related to either phthalate exposure or gestational age at delivery (except possibly a nonviable birth outcome, which was extremely rare). The current analysis ($n = 283$) was restricted to mothers for whom we had urinary phthalate concentrations ($n = 304$) and complete medical record data ($n = 298$) after excluding twin births (12 babies), 2 babies with missing data, and 1 baby born at 30 weeks with eclampsia. Women provided 1 urine sample at the time of recruitment, which was on average 12.2 weeks (standard deviation, 7.6 weeks) before delivery or the beginning of the third trimester.

Human subject committees at each of the participating centers approved study procedures, and all participants gave signed informed consent. Data were stripped of identifying information before analysis.

Gestational age at delivery was calculated in 2 ways. First, the date of the last menstrual period as reported by the woman at study entry was subtracted from the date of delivery to obtain an estimate in days. Second, the clinical estimate recorded by the obstetrician was abstracted directly from the birth record. The clinical estimate was based on ultrasound data, examination of the newborn, and dates reported by the mother and was rounded up to the next-highest week. There was a high correlation between the 2 estimates ($r = 0.92$, $n = 283$) after we substituted the clinical estimate for cases for which there was a discrepancy of 14 or more days (30). We used the clinical estimate in the current analysis.

Urinary phthalate metabolite concentrations were measured using analytical methods described in detail elsewhere (31). Briefly, the phthalate metabolites were first enzymatically deconjugated and then extracted from the urine using automated on-line solid phase extraction, separated by high-performance liquid chromatography, and detected by isotope-dilution tandem mass spectrometry. Each analytical run included calibration standards, reagent blanks, and quality control materials of high and low concentration to monitor for accuracy and precision. The limits of detection were 0.98 ng/mL (MEHP), 0.95 ng/mL (MEHHP), and 1.07 ng/mL (MEOHP).

Because of the high correlation and pharmacokinetic similarities between MEOHP and MEHHP ($r = 0.99$), only estimates for MEOHP are described below.

### Statistical analysis

Geometric mean values for urinary phthalates and their 95% confidence intervals, unadjusted and adjusted for creatinine concentration, were calculated and compared with US population estimates for women of reproductive age (i.e., 18–40 years) derived from 1999–2000 and 2001–2002 National Health and Nutrition Examination Survey (NHANES) data (32, 33). We also calculated geometric mean values and 95% confidence intervals for US pregnant women using the NHANES data ($n = 209$ for MEHP and $n = 104$ for MEOHP, MEHHP, and %MEHP (defined below)). Because the NHANES sample was nonrandom, we used the recommended weights to correctly estimate variances (34). Phthalate metabolite concentrations, which were all right-skewed, were transformed using the natural logarithm. For concentrations below the limit of detection, we assigned a value equal to the limit of detection divided by the square root of 2 (35). A potential phenotypic marker of DEHP metabolism, %MEHP, was calculated as the ratio of MEHP concentration to the sum of the 3 DEHP metabolite concentrations (in nanomoles) and transformed using the natural logarithm (36).

We adjusted for urinary dilution in 2 ways. For comparisons with NHANES data, we used metabolite concentrations divided by creatinine concentrations (μg/g creatinine). However, because creatinine is associated with demographic and physiologic parameters that may be on the causal pathway from exposure to outcome (37, 38), we included a square root transformation of creatinine (mg/dL) as a covariate in all regression models rather than dividing the concentration by creatinine.

Spearman correlation coefficients were used to estimate pairwise associations among phthalate concentrations. Multivariate linear regression models were used to evaluate associations between urinary phthalate concentrations and gestational age at delivery. Although the distribution of gestational ages was slightly skewed, a sensitivity analysis using generalized estimating equations, which is valid even under departure from normality, produced similar results. Logistic regression was used to calculate associations of phthalate concentrations with binary outcomes, such as cesarean section delivery.

Covariates, including demographic characteristics (race, geographic site, mother's education, mother's age, mother's employment status during pregnancy), sample characteristics (timing of urine sample, creatinine), previous pregnancy history (parity, history of miscarriage), sex of the baby, maternal and paternal smoking, job-related stress, and mother's prepregnancy health (nongestational diabetes, thyroid disorders, fibroids, high blood pressure, and respiratory conditions (asthma and chronic obstructive pulmonary disease)) were considered as potential confounders (Table 1) and were retained in the model if they were significant at $P \leq$ 0.15. Potential effect modifiers evaluated included sex of the baby, %MEHP status (low/high), geographic location, timing of the urine sample, and parity. We had no information on mother's weight prior to pregnancy, body mass index, or weight gain during pregnancy.

Statistical significance was defined by a (2-sided) $P$ value of 0.05 or lower and was expressed as a 95% confidence interval. SAS 9.1 software (SAS Institute Inc., Cary, North Carolina) was used to conduct all analyses.

## RESULTS

Demographic and other sample characteristics varied by geographic site (Table 1), with significant differences in parity, race, education, history of fibroids, and gestational age. After creatinine adjustment, DEHP metabolite concentrations varied across geographic locations, with significantly higher concentrations of MEHP among women from Missouri and Minnesota than among women from California and Iowa. White non-Hispanic mothers, who made up 84% of the study population, had marginally higher concentrations of MEHP and MEOHP than Hispanics. Women with some college education had somewhat lower concentrations of MEOHP than women with a high school education or less. Higher levels of job-related stress were slightly associated with higher MEHP concentrations. Women with a previous miscarriage had significantly lower urinary concentrations of MEHP. Metabolite and creatinine concentrations were not related to the timing of the urine sample.

The unadjusted mean concentrations of all 3 DEHP metabolites in the SFF women were somewhat lower than the NHANES US population estimates for pregnant women and women of reproductive age (Table 2), but only the mean MEHHP concentration in SFF women was significantly lower than that for nonpregnant women (11.9 ng/mL vs. 19.8 ng/mL). Neither the unadjusted nor the creatinine-adjusted mean values for pregnant women differed significantly between the SFF and the NHANES.

Associations of the DEHP metabolites with gestational age, unadjusted and adjusted for covariates, are presented in Table 3 and Figures 1 and 2. Overall, a log-unit increase in the urinary concentrations of metabolites was associated with a 1.3- to 1.5-day increase in gestational age. The effect size was slightly greater for MEOHP than for MEHP, and we saw no association between %MEHP and gestational age. Effect estimates were somewhat attenuated after adjustment for potential confounders. Each tertile increase in MEHP concentration was associated with a 1.8-day (95% confidence interval (CI): 0.2, 3.4) increase in gestational age, and each tertile increase in MEOHP concentration was associated with a 1.9-day increase (95% CI: 0.1, 3.8). We compared estimates after removing 1 outlier for urinary MEHP concentration (1,600 ng/mL) and 1 outlier for gestational age (32 weeks) (Figure 1). The effect estimates did not change appreciably. Since we did not see any reasons, medical or otherwise, for excluding these subjects from the analysis, they were included.

The odds of giving birth at greater than 41 weeks increased by 100% and 120% with each log-unit increase in MEHP and MEOHP concentrations, respectively, after adjustment for covariates (Table 4). Conversely, we observed 50% and 60% reductions in the odds of preterm delivery with a log-unit increase in MEHP and MEOHP concentrations, respectively. The frequency of premature delivery (6%) was slightly lower than the NHANES US population estimate of 11.5% for whites, because we excluded twins and an eclampsia case, which would bring the overall prevalence to 9.5%. The higher socioeconomic status of this cohort also contributed to the slightly lower prevalence. The odds of a cesarean section delivery increased by 50% with each log-unit increase in MEOHP concentration and by 30% with each log-unit increase in MEHP concentration. We explored potential explanations for the association of cesarean section with DEHP metabolite concentrations. Women who had ''failure of labor to progress'' listed as a reason for cesarean section delivery had a mean MEOHP concentration that was 0.33 log units higher (95% CI: 0.08, 0.57) than that of all other women, after controlling for other reasons for cesarean section. We found no associations of DEHP metabolite concentrations with breech presentation ($n = 8$), repeat cesarean section ($n = 15$), or medically induced ($n = 85$) or augmented ($n = 55$) labor or with duration of labor measured in minutes.

We conducted stratified analyses by geographic site and %MEHP status (low/high). In Missouri, Iowa, and California, gestational age at delivery tended to increase with higher MEHP and MEOHP concentrations, while in Minnesota gestational age tended to decrease (though not significantly). After adjustment for creatinine, mother's education, parity, and job-related stress, the difference in slopes for the 2 groups (California, Iowa, and Missouri vs. Minnesota) was significant for MEOHP ($P = 0.02$) and marginally significant for MEHP ($P = 0.06$).

The association of MEOHP concentrations with gestational age among women in the low-%MEHP category was approximately 3 times stronger in magnitude than in

**Table 1.** Characteristics of Participants ($n = 283$) in the Study for Future Families, a US Multicenter Pregnancy Cohort Study, 2000–2004

| Characteristic | Overall | | California | | Iowa | | Minnesota | | Missouri | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. or Mean | % | No. or Mean | % | No. or Mean | % | No. or Mean | % | No. or Mean | % |
| No. of subjects | 283 | 100 | 48 | 17 | 80 | 28 | 87 | 31 | 68 | 24 |
| Time between urine collection and delivery, weeks[a] | | | | | | | | | | |
| Mean | 12 (8)[b] | | 14 (8) | | 11 (8) | | 12 (8) | | 12 (6) | |
| First trimester | 5 | 2 | 1 | 2 | 0 | 0 | 4 | 5 | 0 | 0 |
| Second trimester | 119 | 42 | 24 | 51 | 34 | 43 | 34 | 39 | 27 | 40 |
| Third trimester | 157 | 56 | 22 | 47 | 45 | 57 | 49 | 56 | 41 | 60 |
| Sex of baby | | | | | | | | | | |
| Male | 143 | 51 | 24 | 50 | 38 | 47 | 45 | 52 | 36 | 53 |
| Female | 140 | 49 | 24 | 50 | 42 | 53 | 42 | 48 | 32 | 47 |
| Parity (no. of prior livebirths)*** | | | | | | | | | | |
| 0 | 145 | 51 | 30 | 63 | 32 | 40 | 52 | 60 | 31 | 46 |
| 1 | 87 | 31 | 11 | 23 | 25 | 31 | 27 | 31 | 24 | 35 |
| ≥2 | 51 | 18 | 7 | 15 | 23 | 29 | 8 | 9 | 13 | 19 |
| Race[c],*** | | | | | | | | | | |
| White | 238 | 84 | 22 | 46 | 72 | 91 | 84 | 97 | 60 | 88 |
| Hispanic | 26 | 9 | 20 | 42 | 2 | 3 | 0 | 0 | 4 | 6 |
| Other | 18 | 6 | 6 | 13 | 5 | 6 | 3 | 3 | 4 | 6 |
| Education* | | | | | | | | | | |
| High school or less | 25 | 9 | 9 | 19 | 6 | 8 | 3 | 3 | 7 | 10 |
| Some college | 63 | 22 | 19 | 40 | 14 | 18 | 20 | 23 | 10 | 15 |
| College graduation | 106 | 38 | 10 | 21 | 34 | 43 | 33 | 38 | 29 | 43 |
| Graduate school | 89 | 31 | 10 | 21 | 26 | 33 | 31 | 36 | 22 | 32 |
| History of miscarriage (yes/no) | 60 | 21 | 9 | 19 | 20 | 25 | 14 | 16 | 17 | 25 |
| Employed during pregnancy (yes/no) | 231 | 82 | 33 | 69 | 63 | 79 | 79 | 91 | 56 | 82 |
| Job-related stress | | | | | | | | | | |
| No work-related stress | 52 | 18 | 15 | 31 | 17 | 21 | 8 | 9 | 12 | 18 |
| Not at all stressful | 17 | 6 | 2 | 4 | 4 | 5 | 6 | 7 | 5 | 7 |
| Not too stressful | 80 | 28 | 14 | 29 | 21 | 26 | 29 | 33 | 16 | 24 |
| Somewhat stressful | 105 | 37 | 12 | 25 | 32 | 40 | 35 | 40 | 26 | 38 |
| Very stressful | 29 | 10 | 5 | 10 | 6 | 8 | 9 | 10 | 9 | 13 |
| Maternal health | | | | | | | | | | |
| Diabetes | 14 | 5 | 4 | 8 | 6 | 8 | 1 | 1 | 3 | 4 |
| Thyroid disorders | 17 | 6 | 5 | 10 | 5 | 6 | 2 | 2 | 5 | 7 |
| Fibroids* | 16 | 6 | 1 | 2 | 8 | 10 | 2 | 2 | 5 | 7 |
| High blood pressure | 19 | 7 | 4 | 8 | 5 | 6 | 5 | 6 | 5 | 7 |
| Respiratory conditions | 26 | 9 | 4 | 8 | 10 | 13 | 5 | 6 | 7 | 10 |
| Mean creatinine level, mg/dL | 92 (61) | | 99 (63) | | 93 (64) | | 82 (51) | | 97 (66) | |
| Mean maternal age, years* | 30.2 (6.0) | | 28.3 (6.0) | | 30.6 (4.6) | | 30.8 (5.3) | | 30.3 (4.6) | |
| Gestational age at delivery (clinical estimate), weeks[d] | | | | | | | | | | |
| Mean** | 39.2 (1.5) | | 38.9 (1.3) | | 38.9 (1.3) | | 39.7 (1.3) | | 39.1 (1.8) | |
| <37 | 14 | 5 | 3 | 6 | 4 | 5 | 2 | 2 | 5 | 7 |
| 37–41 | 262 | 93 | 45 | 94 | 76 | 95 | 81 | 93 | 60 | 88 |
| >41 | 7 | 2 | 0 | 0 | 0 | 0 | 4 | 5 | 3 | 4 |

\* $P < 0.05$; \*\*$P < 0.01$; \*\*\*$P < 0.001$ ($P$ for difference between at least 2 of the 4 study centers).
[a] Information on timing of urine sample collection was missing for 2 participants.
[b] Numbers in parentheses, standard deviation.
[c] Information on race was missing for 1 participant.
[d] Clinical estimate of gestational age abstracted from the birth medical record; values were reported rounded to the next-highest week. Information was missing for 1 participant.

**Table 2.** Urinary Concentrations (ng/mL) of Di-(2-Ethylhexyl) Phthalate Metabolites Among Participants (*n* = 283) in the Study for Future Families (2000–2004) as Compared With Participants in the National Health and Nutrition Examination Survey (1999–2002)

| Di-(2-Ethylhexyl) Phthalate Metabolite | % of Samples With Values Greater Than Limit of Detection | Percentile | | | | | Study for Future Families | | NHANES Pregnant Women[a] | | NHANES Reproductive-Age Women[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5th | 25th | 50th | 75th | 95th | GM | 95% CI | GM | 95% CI | GM | 95% CI |
| MEHP | 76 | 0.6 | 1.1 | 3.5 | 8.2 | 40.2 | 3.6 | 3.1, 4.3 | 4.8 | 3.8, 6.0 | 4.5 | 4.0, 5.0 |
| MEHHP | 97 | 1.1 | 5.6 | 11.2 | 25.5 | 99.4 | 11.9 | 10.1, 13.9 | 19.0 | 13.5, 26.7 | 19.8 | 14.9, 26.2 |
| MEOHP | 96 | 1.2 | 5.1 | 9.9 | 24.6 | 68.4 | 10.9 | 9.3, 12.6 | 15.4 | 11.3, 21.1 | 13.8 | 10.4, 18.3 |
| %MEHP[c] | NA | 4 | 8 | 14 | 23 | 48 | 14 | 12, 15 | 17 | 13, 20 | 13 | 11, 14 |

Abbreviations: CI, confidence interval; GM, geometric mean; MEHHP, mono-2-ethyl-5-hydrohexyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; NA, not applicable; NHANES, National Health and Nutrition Examination Survey.

[a] NHANES data on pregnant women, 1999–2000 (MEHP) and 2001–2002 (MEHP, MEOHP, and MEHHP) (*n* = 209 for MEHP and *n* = 104 for MEHHP, MEOHP, and %MEHP).

[b] NHANES data on women aged 18–40 years, 1999–2000 (MEHP) and 2001–2002 (MEHP, MEOHP, and MEHHP) (*n* = 853 for MEHP and *n* = 437 for MEHHP, MEOHP, and %MEHP).

[c] MEHP/$\Sigma$(MEHP, MEOHP, MEHHP) $\times$ 100.

the high-%MEHP group after adjustment for creatinine, geographic site, mother's education, and job-related stress. When modeled as a multiplicative interaction term, the *P* value was 0.10 for differences in slope between the 2 groups (Figure 3). There was no difference in MEHP slope between the low- and high-%MEHP categories.

## DISCUSSION

In approximately 300 pregnant women from 4 US locations, we found that gestation was 1.1 days and 1.3 days longer with each log-unit increase in urinary concentrations of the DEHP metabolites MEHP and MEOHP, respectively. Women at the 75th percentile of urinary MEHP concentration had a duration of gestation that was 2.3 days (95% CI: 1.4, 3.3) longer than that of women at the 25th percentile of exposure, after we controlled for urinary

creatinine concentration, demographic factors, maternal health, stress, and parity. DEHP exposure in this cohort was similar to the NHANES US population estimate for pregnant females.

The clinical or population significance of an exposure-related shift of 2–3 days in gestational length is difficult to evaluate. For this reason, we also estimated associations with clinical outcomes and saw increased odds of delivering after 41 weeks, decreased odds of preterm delivery, and increased odds of delivering by cesarean section. Delivery after 41 weeks is associated with an increase in perinatal mortality due to meconium aspiration, fetal distress, asphyxia, pneumonia, malformations, shoulder dystocia, and traumatic injuries (39, 40). Women who undergo cesarean section are at increased risk in subsequent pregnancies of malpresentation, abnormal placentation, antepartum hemorrhage, placenta accreta, prolonged labor, uterine rupture, preterm birth, low birth weight, and stillbirth (41). The

**Table 3.** Relations of Urinary Concentrations of Di-(2-Ethylhexyl) Phthalate Metabolites in Pregnant Women to Gestational Age at Delivery in Linear Regression Models (*n* = 283), Study for Future Families, 2000–2004[a],*

| Model | MEHP | | MEOHP | | MEHHP | |
|---|---|---|---|---|---|---|
| | Estimate | 95% CI | Estimate | 95% CI | Estimate | 95% CI |
| Adjusted for creatinine | 0.18 | 0.04, 0.32 | 0.21 | 0.05, 0.38 | 0.19 | 0.05, 0.34 |
| Adjusted for creatinine + demographic factors[b] | 0.17 | 0.03, 0.32 | 0.20 | 0.03, 0.36 | 0.18 | 0.02, 0.33 |
| Adjusted for above factors + maternal health[c] | 0.17 | 0.03, 0.31 | 0.19 | 0.03, 0.35 | 0.17 | 0.02, 0.32 |
| Adjusted for above factors + parity | 0.16 | 0.02, 0.30 | 0.19 | 0.03, 0.35 | 0.16 | 0.01, 0.31 |

Abbreviations: CI, confidence interval; MEHHP, mono-2-ethyl-5-hydrohexyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate.

* $P < 0.05$ for all results shown in table.

[a] Change in gestational age at delivery (weeks) per log-unit increase in urinary di-(2-ethylhexyl) phthalate metabolite concentration.

[b] Geographic center, mother's educational level, and job-related stress.

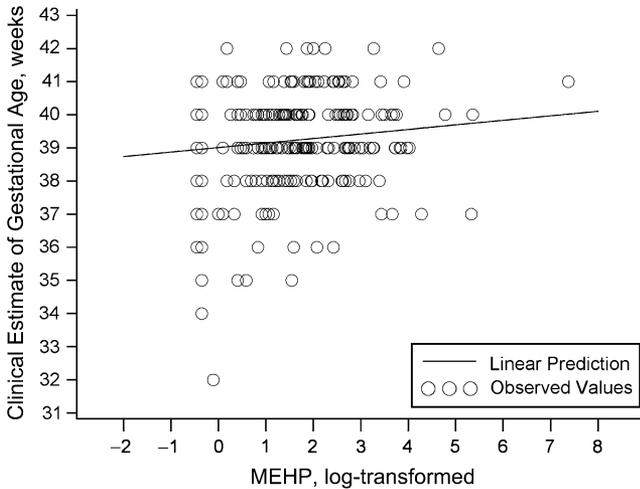[c] Nongestational diabetes, thyroid disorders, and fibroids.

**Figure 1.** Clinically estimated gestational age as a function of log urinary mono-2-ethylhexyl phthalate (MEHP) concentration (ng/mL) ($n = 283$) after adjustment for creatinine, Study for Future Families, 2000–2004.
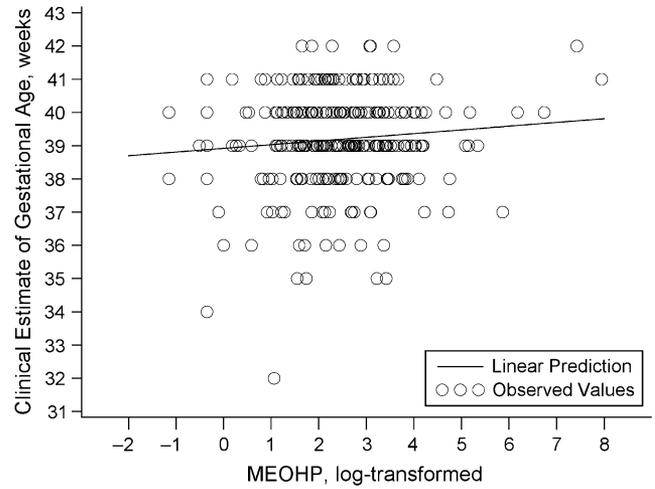


**Figure 2.** Clinically estimated gestational age as a function of log urinary mono-2-ethyl-5-oxohexyl phthalate (MEOHP) concentration (ng/mL) ($n = 283$) after adjustment for creatinine, Study for Future Families, 2000–2004.

observed decrease in the risk of preterm delivery may be protective or may be indicative of abnormal function of the placenta (26); in this study, we could not distinguish between these possibilities.

A prior study using MEHP concentration in umbilical cord blood from 84 Italian mother/newborn pairs found an association that pointed in the opposite direction than was observed here (8). Latini et al. (8) reported that gestational

**Table 4.** Relations of Urinary Concentrations of Di-(2-Ethylhexyl) Phthalate Metabolites in Pregnant Women to Clinical Outcomes Related to Parturition and Labor in Logistic Regression Models ($n = 283$), Study for Future Families, 2000–2004[a]

| Outcome | No. of Subjects | % | MEHP | | MEOHP | | MEHHP | |
|---|---|---|---|---|---|---|---|---|
| | | | OR | 95% CI | OR | 95% CI | OR | 95% CI |
| Gestational age >41 weeks | 7 | 2 | | | | | | |
| Adjusted for creatinine | | | 1.8* | 1.1, 3.0 | 2.1** | 1.2, 3.4 | 1.9* | 1.1, 3.2 |
| Adjusted for creatinine + covariates[b] | | | 2.0* | 1.1, 3.5 | 2.2** | 1.3, 4.0 | 2.1* | 1.3, 3.7 |
| Preterm delivery (<37 weeks) | 14 | 5 | | | | | | |
| Adjusted for creatinine | | | 0.5* | 0.3, 0.9 | 0.5* | 0.2, 0.9 | 0.5* | 0.3, 0.9 |
| Adjusted for creatinine + covariates[c] | | | 0.5* | 0.3, 0.9 | 0.4* | 0.2, 0.9 | 0.5* | 0.3, 0.9 |
| Cesarean section delivery | 62 | 22 | | | | | | |
| Adjusted for creatinine | | | 1.3 | 1.0, 1.6 | 1.4* | 1.1, 1.9 | 1.4* | 1.1, 1.8 |
| Adjusted for creatinine + covariates[d] | | | 1.3* | 1.0, 1.6 | 1.5** | 1.1, 1.9 | 1.4* | 1.1, 1.8 |
| Failure to progress as reason for cesarean section delivery | 18 | 6 | | | | | | |
| Adjusted for creatinine | | | 1.3 | 0.9, 1.8 | 1.6* | 1.1, 2.4 | 1.5* | 1.1, 2.2 |
| Adjusted for creatinine + covariates[e] | | | 1.2 | 0.9, 1.7 | 1.6* | 1.1, 2.3 | 1.5* | 1.0, 2.1 |

Abbreviations: CI, confidence interval; MEHHP, mono-2-ethylhexyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; OR, odds ratio.

* $P < 0.05$; ** $P < 0.01$.

[a] Change in log odds per log-unit increase in urinary di-(2-ethylhexyl) phthalate metabolite concentration.

[b] Adjusted for geographic site (Minnesota vs. California, Iowa, and Missouri) and respiratory conditions.

[c] Adjusted for high blood pressure and nongestational diabetes.

[d] Adjusted for mother's age (≥35 years vs. <35 years), geographic site (Minnesota vs. California, Iowa, and Missouri), nongestational diabetes, and fibroids.

[e] Adjusted for parity, thyroid conditions, and high blood pressure. Information on the reason for cesarean section was missing for 2 subjects.
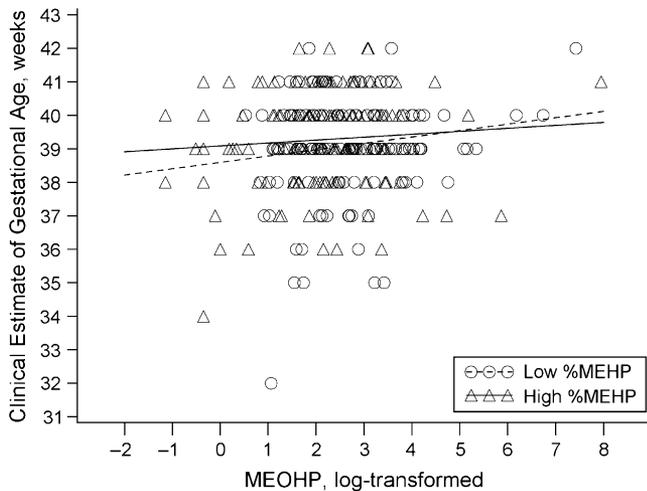
**Figure 3.** Clinically estimated gestational age as a function of log urinary mono-2-ethyl-5-oxohexyl phthalate (MEOHP) concentration (ng/mL), by percent mono-2-ethylhexyl phthalate (%MEHP) status (low/high) ($n = 283$), after adjustment for creatinine and covariates, Study for Future Families, 2000–2004. %MEHP was calculated as the ratio of MEHP concentration to the sum of the concentrations of 3 di-(2-ethylhexyl) phthalate metabolites (MEHP, MEOHP, and mono-2-ethylhexyl phthalate) (in nanomoles) and transformed using the natural logarithm.

age was shorter by 1.2 weeks in the MEHP-positive pairs than in the MEHP-negative subjects. In that study, they measured MEHP and DEHP in umbilical cord blood, which may have been subject to contamination by DEHP in the sampling and analytic equipment (42). Measuring MEHP in blood is not recommended because of its short half-life (14). In that analysis, exposure status was dichotomized as exposed and nonexposed. Given that more than 95% of the general US population has detectable urinary metabolites of DEHP (5), this approach could have resulted in misclassification among the nonexposed subjects. The discrepant results between these 2 studies could be due to differences in study design, exposure assessment, and/or the underlying characteristics of the populations.

When we stratified SFF subjects by geographic location, gestational age tended to increase with increasing phthalate metabolite concentrations for all sites except Minnesota, where it tended to remain flat or decrease slightly. The women from Minnesota tended to have higher MEOHP concentrations and %MEHP, higher gestational age, higher maternal age, and more education than women from other study centers, most markedly relative to California, and they were predominantly white (97%). We can speculate on 2 possible explanations. It is possible that the dose-response curve was nonlinear and essentially reversed at the higher doses among the Minnesota subjects. It is also possible that the site-specific populations differed in ways that modified the relation between DEHP exposure and placental function. The significant differences in race, education, maternal age, and parity between study centers could be proxies for other unmeasured effect

modifiers, such as nutritional factors, coexposures, and/or lifestyle factors.

Other known causes of prolonged gestation include fish oil consumption during pregnancy (43, 44), deficiency in placental sulfatase, which is another cause of decreased estrogen synthesis (45), and living in a highly polluted area (46). Fish oil contains n-3 long-chain polyunsaturated fatty acids, which are also ligands of PPARγ and may contribute to a suppressed inflammatory response late in pregnancy (47, 48). If this were the case, it is possible that a competitive interaction between DEHP and fatty acids in the diet exists. We could not test this hypothesis, since fish consumption among our subjects was generally low (88% of those who consumed fish had 2 or fewer servings per week). We did not have information on the type of fish consumed or on how it was prepared.

We hypothesized that the association between DEHP exposure and timing of labor could also differ depending on a woman's ability to metabolize and excrete DEHP. To test this, we dichotomized %MEHP values at the median and compared metabolite associations within the low and high strata. The association of MEOHP concentrations with longer gestation was 3-fold stronger in the low-%MEHP group than in the high-%MEHP group. The association of MEOHP concentration with the timing of labor might be due to disruption in parturition and signaling by DEHP metabolites, but it might also be due to differences in a woman's ability to metabolize and excrete DEHP. In a previous report, we found %MEHP to be approximately twice as reproducible within a woman over the last 6 weeks of pregnancy as DEHP metabolites (10), suggesting that %MEHP may reflect stable interindividual differences that could be relevant to the metabolism and excretion of these compounds in pregnancy.

Concern exists about the potential for systematic error in estimating gestational age using the last-menstrual-period approach (49, 50). We addressed this by also using the clinical estimate, which takes into account ultrasound data and examination of the newborn in cases where there are inconsistencies in last-menstrual-period dating and clinical presentation, but the clinical estimate may have been less precise, given that it was rounded up. We found results to be consistent using both measures when including all gestational ages and less consistent when modeling associations with preterm and postterm delivery. Misclassification of gestational age by last menstrual period is most problematic among preterm and postterm births, with the degree of misclassification being associated with maternal race, age, education, parity, month that prenatal care began (51), and regularity of the menstrual period (52). Given that some of these factors are also related to phthalate exposure and pregnancy outcomes, we relied on the clinical estimate to model associations with DEHP metabolite concentrations.

Some misclassification of DEHP exposure was present in our data, given that we had a single spot urine sample for characterizing exposure. In a previous analysis, we showed that DEHP metabolite concentrations are not highly reproducible in pregnant women over the last 6 weeks of pregnancy, probably because of physiologic changes occurring

in the third trimester (10). Of the SFF subjects, 55% were sampled in the third trimester. In addition to misclassification, there may have been other exposures and risk factors associated with urinary DEHP metabolite concentrations and birth outcomes that we were not able to adequately control for, resulting in residual confounding.

In conclusion, we observed an association between increased concentrations of DEHP metabolites in maternal urine measured during pregnancy and gestational age in a US multicenter pregnancy cohort study. The direction and size of the effect appeared to differ depending in part on geographic location and a woman's ability to metabolize and eliminate DEHP. Our results support the hypothesis that DEHP exposure may alter the dialogue between the maternal and fetal compartments that is essential for normal labor. Consistent with this hypothesis, urinary DEHP metabolite concentrations were associated with an increased risk of cesarean section delivery and of delivering at more than 41 weeks. The binding affinity of the metabolite MEHP for PPARγ and the central role PPARγ plays in regulating placental function may provide an explanation for this association, but this was not directly explored in the present study.

These results need to be replicated in other populations. There is likewise a need for more in vitro and in vivo research to better understand molecular mechanisms by which DEHP may alter placental development and/or function.

The findings and conclusions presented in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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