HHS Public Access

Author manuscript

Placenta. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

Placenta. 2015 June ; 36(6): 699–703. doi:10.1016/j.placenta.2015.04.002.

Phthalate metabolites and bisphenol-A in association with circulating angiogenic biomarkers across pregnancy

Kelly K. Ferguson[a], **Thomas F. McElrath**[b], **David E. Cantonwine**[b], **Bhramar Mukherjee**[c], and **John D. Meeker**[a]

[a]Department of Environmental Health Sciences, University of Michigan School of Public Health, 1415 Washington Heights, Ann Arbor, MI, 48109-2029, USA

[b]Division of Maternal-Fetal Medicine, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA, 02115, USA

[c]Department of Biostatistics, University of Michigan School of Public Health, 1415 Washington Heights, Ann Arbor, MI, 48109-2029, USA

Abstract

Introduction—Phthalates and bisphenol-a (BPA) are endocrine disrupting compounds with widespread exposure that have been linked in a number of epidemiologic studies to adverse birth outcomes and developmental effects. We hypothesized that these associations may be mediated in part through altered placental development and function consequent to exposure. To investigate this question, we examined associations between plasma biomarkers of angiogenesis and urinary biomarkers of exposure to phthalates and bisphenol-a (BPA) measured at repeated time points across pregnancy.

Methods—We utilized a nested case-control population consisting of 130 mothers who delivered preterm and 352 who delivered term from a prospective birth cohort. Placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured in plasma samples collected from up to four visits during pregnancy (median 10, 18, 26, and 35 weeks). Phthalate metabolites and BPA were measured in urine samples collected at the same visits as indices of exposure.

Results—In linear mixed effects models adjusted for urine dilution and gestational age at sample collection, oxidized di-2-ethylhexyl phthalate (DEHP) metabolites were associated with decreases in PlGF as well as increases in the sFlt-1 to PlGF ratio. These results were slightly attenuated in fully adjusted models. Other phthalate metabolites did not show consistent relationships with

Correspondence: John D. Meeker, ScD, University of Michigan School of Public Health, Department of Environmental Health Sciences, 1835 SPH I, 1415 Washington Heights, Ann Arbor, MI 48109-2029, Phone: 1-734-764-7184, Fax: 1-734-936-7283, meekerj@umich.edu.

Conflict of interest statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

either sFlt-1 or PlGF. BPA, however, was associated with increased sFlt-1 as well as the sFlt-1 to PlGF ratio in both crude and adjusted models.

Discussion—We observed associations between urinary DEHP metabolites and BPA and biomarkers of angiogenesis during pregnancy that may be indicative of disrupted placental development and/or function during gestation.

Keywords

Endocrine disrupting compounds; environment; epidemiology; placenta; preeclampsia

1. Introduction

Phthalate diesters and bisphenol-A (BPA) are chemicals used in a wide variety of consumer products that humans worldwide come into contact with daily, making exposure a common occurrence. Both are classified as endocrine disrupting compounds because of their anti-androgenic and estrogenic properties, respectively. Much research on the human health effects of exposure to these compounds has focused on birth outcomes and fetal development, as they have been shown to cross the placental barrier [1]. Particularly, maternal exposures to phthalates and BPA during pregnancy have been linked to fetal growth parameters [2], preterm birth [3], and altered neurodevelopment in infants [4] and children [5].

Growth of the fetus during pregnancy is strongly tied to the successful implantation, development and functioning of the placenta. Poor implantation in the first trimester can lead to inadequate villous perfusion and has been associated with conditions such as preeclampsia, intrauterine growth restriction, miscarriage, and stillbirth. On a more subtle level, function of the placenta and adequate substrate supply to the fetus may be important for optimal development, particularly of the brain [6]. While a number of these outcome measures have been studied in association with environmental exposures, few have examined biomarkers of the more subtle but nevertheless consequential changes.

Angiogenic markers have been explored in obstetric research as a means to identify and predict preeclampsia [7, 8]. Placental growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, is a protein that plays a role in vascularization of the placenta early in pregnancy and is secreted in increasing quantities as pregnancy progresses [9]. Lower than average levels may indicate poor placental development and/or function. Soluble fms-like tyrosine kinase-1 (sFlt-1, also known as sVEGFR-1), binds to VEGF with consequent anti-angiogenic activity [10]. Thus sFlt-1 levels higher than average signal problematic placentation. In addition to observed associations with preeclampsia, these biomarkers may provide further evidence for more subtle complications in placental development or function. In the present study we examine the relationships between urinary phthalate metabolites or BPA and each of these plasma angiogenic biomarkers utilizing repeated measures across pregnancy.

2. Methods

2.1. Study population

The pregnant women in this nested case-control study were selected from an ongoing prospective cohort of women recruited early in gestation at Brigham and Women's Hospital in Boston, MA, between 2006 and 2008. As part of the parent study mothers provided demographic and anthropometric information and informed consent along with blood and urine samples at up to four study visits (median 10, 18, 26, and 35 weeks gestation) [11]. Gestational dating was based on last menstrual period with verification by first trimester ultrasound. Retrospectively, 130 women who delivered live, singleton, preterm births were selected from this population along with 352 random controls for a study designed to assess the relationship between phthalate exposure during pregnancy and preterm birth.

2.2. Urinary phthalate metabolite and BPA measurement

In 2011 maternal urine samples were extracted from −80 degrees Celsius storage for analysis of 9 phthalate metabolites, including mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-benzyl phthalate (MBzP), mono-*n*-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), mono-ethyl phthalate (MEP), and mono-(3-carboxypentyl) phthalate (MCPP), as well as total (free plus conjugated) BPA. Analysis was performed by NSF International (Ann Arbor, MI) using high performance liquid chromatography and tandem mass spectrometry methods described in detail elsewhere [12]. Values below the limit of detection (LOD) were replaced with the LOD divided by the square root of 2 [13]. To adjust for urine dilution, specific gravity was measured in all samples at the time of analysis. For di-2-ethylhexyl phthalate (DEHP) metabolites (including MEHP, MEHHP, MEOHP, and MECPP), we additionally created a summed measured based on nanomolar concentrations for modeling purposes (∑DEHP) [11].

2.3. Plasma angiogenic marker measurement

Biomarkers of angiogenic function were measured in plasma samples from all pregnant women in the parent cohort study [8]. Measurement was performed using ARCHITECT immunoassays by Abbott Laboratories (Abbott Park, IL). Total (free and bound) soluble fms-like tyrosine kinase-1 was detected from 0.10 to 150 ng/mL and free PlGF was detected from 1 to 1500 pg/mL. As with exposure measures, levels below the LOD were replaced with the LOD divided by the square root of 2 [13]. In addition to examining these biomarkers individually, we also examined a ratio of sFlt-1 to PlGF as an increase in this measure is thought to be a stronger predictor than either measure alone for placental disorders like preeclampsia [14].

2.4. Statistical analysis

All statistical analyses were performed using R version 3.0.2. The goal of the present analysis was to examine the relationship between urinary phthalate metabolites and BPA and circulating biomarkers of placental function in a generalizable population of pregnant

women. This was a secondary goal to the primary aim of the study, to examine the relationship between urinary phthalate metabolites and preterm birth in a nested case-control study. Thus, for all statistical analyses, we utilized inverse probability weightings created from the probability of selection from the parent study population for cases (90.1 percent) and controls (33.9 percent) [15]. This adjustment negates the effect of oversampling preterm births and makes results generalizable to pregnant women in the base cohort population, regardless of birth outcome [16]. Angiogenic biomarker levels by demographic covariates and by study visit were examined using selected percentiles, and differences in levels between groups were tested using linear mixed models (LMM) with random intercepts and slopes. To examine the relationships between exposures and angiogenic biomarkers, we created LMM with one angiogenic biomarker predicted by one phthalate metabolite (or BPA) per model with adjustment for subject-specific random intercepts and slopes. Both exposure and outcome biomarkers were right skewed and hence natural log transformed for analysis. Crude models were created with adjustment for gestational age at sample collection and urinary specific gravity only. Full models additionally included covariates that were significantly associated with exposures and outcomes in bivariate analyses and that altered effect estimates by greater than 10 percent. Because of the log transformation of the exposure and outcome variables, beta estimates and standard errors obtained from regression models were converted to percent change in angiogenic biomarker in association with an interquartile range (IQR) increase in exposure for interpretability. Because this was an exploratory analysis, we did not correct for multiple comparisons.

As a sensitivity analysis, we also created models with an interaction term between the exposure and the visit of sample collection to investigate whether relationships were stronger at any particular time point during pregnancy.

3. Results

In the nested case-control population, there were 1611 time points where subjects (N=457) had both exposure and angiogenic biomarker measurements. The present analysis was restricted to these measures. sFlt-1 was above the detection limit in all but 6 samples; PlGF was detected in all samples measured. Phthalate metabolites were highly detectable (most metabolites >99%) [3] and BPA was slightly less so (83%). Other characteristics of urinary phthalate metabolites and BPA, including distributions overall, by study visit, and by demographic characteristics are presented in detail elsewhere [11, 15, 17].

Selected percentiles of plasma angiogenic biomarkers measured in all samples by demographic characteristics are presented in Table 1. Based on LMM, PlGF levels were significantly higher in mothers who were African American or Other race/ethnicity compared to White, who had public compared to private health insurance providers, who had previously had a child, and who had a term pregnancy. Levels were significantly lower in mothers who were obese (BMI >30 kg/m^2) compared to mothers with lower BMI. Few differences by demographic characteristics were observed for sFlt-1, but mothers who were obese or non-nulliparous had significantly lower plasma concentrations.

Distributions of sFlt-1 and PlGF in all samples and by study visit are presented in Table 2. Levels were similar to those observed in the parent population study [8]. For sFlt-1, concentrations were slightly but significantly higher at visits 2-4 compared to visit 1, and for PlGF levels were markedly increased later in pregnancy. Results from LMM adjusted for urinary specific gravity and gestational age only are presented in Table 3. An IQR increase in BPA was associated with a 7.08% increase in sFLT-1 (95% CI=2.04, 12.4, p=0.006) but no associations with phthalate metabolites were detected. For PlGF, inverse associations were observed for the oxidized DEHP metabolites MEHHP, MEOHP, and MECPP as well as ∑DEHP, and the associations for MECPP and ∑DEHP were statistically significant. However, a positive association was observed between PlGF and MEP. Associations between exposures and the ratio of sFlt-1/PlGF were stronger in magnitude than for either individual measure alone. A positive association was observed with DEHP metabolites and also with BPA.

Fully adjusted models additionally included maternal age, health insurance provider, and BMI (time-varying) as covariates. Addition of prematurity as a covariate did not alter associations by more than 10%. Effect estimates were generally in the same direction as those observed in crude models, but were somewhat attenuated (Table 4). The relationship between sFlt-1 and BPA remained statistically significant, as did the inverse association between PlGF and MECPP. As with the crude models, associations with the sFlt-1/PlGF ratio were strongest, and significantly elevated ratios were observed in subjects with higher urinary concentrations of MEHHP, MECPP, ∑DEHP, and BPA. It should be noted that due to a large number of comparisons, some of these associations may have been due to chance. Thus, the significant associations observed should be interpreted with caution.

In a sensitivity analysis, we also examined an interaction term between study visit and exposures in the prediction of each angiogenic biomarker in an attempt to identify potential windows of susceptibility. None of the interactions were statistically significant (data not shown).

4. Discussion

In a study of repeated measures of exposure and outcomes across pregnancy, we observed associations between urinary metabolites of DEHP and decreased PlGF, and an increase in the ratio of sFlt-1 to PlGF. We also observed that BPA was associated with increased sFlt-1 and an increase in the ratio of sFlt-1 to PlGF. These findings indicate that exposure to DEHP and BPA may result in changes in angiogenic factors indicative of altered placentation and trophoblast function, with potential adverse consequences for pregnancy.

PlGF is a pro-angiogenic protein produced by the syncytiotrophoblast of the placenta that plays a crucial role in vascularization [20]. Decreased expression is associated with impaired trophoblast invasion with consequent deficient placental perfusion. sFlt-1, on the other hand, binds to PlGF and other VEGF proteins making it anti-angiogenic in nature [10]. Lower circulating levels of PlGF and higher levels of sFlt-1 during pregnancy are associated with an increased risk of developing gestational diseases related to inadequate trophoblast function, such as preeclampsia [21] or intrauterine growth restriction [22]. Additionally,

some studies suggest that the ratio of sFlt-1 to PlGF may be an even stronger predictor of these outcomes [14].

To our knowledge, exposure to phthalates and BPA during pregnancy has not been previously examined in association with angiogenic biomarkers. However, some studies have investigated the relationships with pregnancy outcomes that may result from altered placentation. Associations between prenatal phthalate exposure and fetal growth endpoints (e.g., birthweight, size for gestational age, head circumference, etc.) have generally been null [1, 23-25]. In the present study population, we observed generally null associations between urinary phthalate metabolites and preterm births that originated from placental dysfunction [11]. On the other hand, a number of studies observed significant associations between prenatal BPA exposure and reduced birth weight [2, 25, 26], as well as preeclampsia [27].

Consistent with the studies of fetal growth, we observed few associations between phthalate exposure and changes in angiogenic biomarkers, although we did see slight decreases in PlGF and the sFlt-1 to PlGF ratio in association with the DEHP metabolites. This may suggest that measuring biomarkers of these changes may more sensitively detect relationships with exposures than measuring gross clinical endpoints. While the associations observed here are subtle, they may still be important for fetal development. Suboptimal placental function and perfusion during pregnancy may affect fetal neurodevelopment, for example [6]. For BPA we observed stronger associations with angiogenic biomarkers, which is consistent with the literature that suggests poorer placental development and/or function in mothers exposed during pregnancy. One potential explanation for the stronger effect of BPA compared to phthalates is that estrogens are particularly important in angiogenesis [28]. BPA could interfere with estrogen signaling by binding to the estrogen receptor, although *in vitro* studies show that this affinity may be low [29].

A weakness of this study is the inability to draw conclusions about temporality of effect. If exposures are causing changes in these biomarkers, there are two explanations: A) the exposures may be impacting the angiogenic biomarkers directly, which could consequently lead to alterations in placental function; or B) through other mechanisms, the exposures could be causing changes in placental function that subsequently altering concentrations of circulating maternal angiogenic biomarkers. The only *in vitro* evidence for A) opposes what we observe here; MEHP, DEHP, butyl benzyl phthalate (BBP, which can be metabolized into MBzP and MBP) have been shown to increase VEGF secretion in several cell lines [30, 31]. Thus B) seems more likely, that through other mechanisms exposures to DEHP metabolites and BPA are causing impaired placentation and consequently alterations in circulating cytokines. This is also supported by the fact that the strongest associations observed were for the ratio of sFlt-1 to PlGF, rather than either marker individually.

This study was also limited by the use of a case-control population designed to examine relationships with preterm birth; weighting the analysis makes our results generalizable to a more general pregnant population but also effectively decreases the sample size. Nevertheless, our analysis had substantial power to detect effects because of the availability of repeated measures of both exposure and outcome biomarkers across gestation. Finally,

this analysis involved multiple comparisons and some associations observed may have been due to chance.

In conclusion, we observed associations between repeated measures of some maternal urinary phthalate metabolites and BPA and plasma angiogenic biomarkers in a population of pregnant women. Changes in these angiogenic factors may be indicative of disrupted placental development and/or function during gestation. These findings will be useful for future studies investigating mechanisms by which phthalate and/or BPA exposure may lead to adverse pregnancy outcomes.

Acknowledgements

We thank Kurtis Kneen, Scott Clipper, Gerry Pace, David Weller, and Jennifer Bell of NSF International in Ann Arbor, Michigan, USA, for urine phthalate analysis. Funding for this project was provided by the National Institute of Environmental Health Sciences, National Institutes of Health (R01ES018872, P42ES017198, P01ES022844, and P30ES017885), and by Abbott Diagnostics Division (9MZ-04-06N03) for angiogenic biomarker analysis.

Abbreviations

BPA	bisphenol-A
PlGF	placental growth factor
VEGF	vascular endothelial growth factor
sFlt-1	soluble fms-like tyrosine kinase-1
MEHP	mono-2-ethylhexyl phthalate
MEHHP	mono-(2-ethyl-5-hydroxyhexyl) phthalate
MEOHP	mono-(2-ethyl-5-oxohexyl) phthalate
MECPP	mono-(2-ethyl-5-carboxypentyl) phthalate
MBzP	mono-benzyl phthalate
MBP	mono-*n*-butyl phthalate
MiBP	mono-isobutyl phthalate
MEP	mono-ethyl phthalate
MCPP	mono-(3-carboxypentyl) phthalate
DEHP	di-2-ethylhexyl phthalate
LOD	limit of detection
LMM	linear mixed models
IQR	interquartile range
BMI	body mass index
CI	confidence interval

References

1. Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. In utero exposure to di-(2-ethylhexyl) phthalate and duration of human pregnancy. Environmental health perspectives. 2003; 111(14):1783. [PubMed: 14594632]
2. Snijder CA, Heederik D, Pierik FH, Hofman A, Jaddoe VW, Koch HM, et al. Fetal growth and prenatal exposure to bisphenol A: the generation R study. Environmental health perspectives. 2013; 121(3):393–8. [PubMed: 23459363]
3. Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. JAMA pediatrics. 2014; 168(1):61–7. [PubMed: 24247736]
4. Engel SM, Zhu C, Berkowitz GS, Calafat AM, Silva MJ, Miodovnik A, et al. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. Neurotoxicology. 2009; 30(4):522–8. [PubMed: 19375452]
5. Téllez-Rojo MM, Cantoral A, Cantonwine DE, Schnaas L, Peterson K, Hu H, et al. Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. Science of The Total Environment. 2013; 461:386–90. [PubMed: 23747553]
6. Hsiao EY, Patterson PH. Placental regulation of maternal-fetal interactions and brain development. Developmental neurobiology. 2012; 72(10):1317–26. [PubMed: 22753006]
7. Levine RJ, Maynard SE, Qian C, Lim K-H, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. New England Journal of Medicine. 2004; 350(7):672–83. [PubMed: 14764923]
8. McElrath TF, Lim K-H, Pare E, Rich-Edwards J, Pucci D, Troisi R, et al. Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy. American journal of obstetrics and gynecology. 2012; 207(5):407. e1-. e7. [PubMed: 22981320]
9. Regnault T, Galan H, Parker T, Anthony R. Placental development in normal and compromised pregnancies—a review. Placenta. 2002; 23:S119–S29. [PubMed: 11978069]
10. Coolman M, Timmermans S, de Groot CJ, Russcher H, Lindemans J, Hofman A, et al. Angiogenic and fibrinolytic factors in blood during the first half of pregnancy and adverse pregnancy outcomes. Obstetrics & Gynecology. 2012; 119(6):1190–200. [PubMed: 22617584]
11. Ferguson KK, McElrath TF, Ko Y-A, Mukherjee B, Meeker JD. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. Environment International. 2014; 70:118–24. [PubMed: 24934852]
12. Lewis RC, Meeker JD, Peterson KE, Lee JM, Pace GG, Cantoral A, et al. Predictors of urinary bisphenol A and phthalate metabolite concentrations in Mexican children. Chemosphere. 2013; 93(10):2390–8. [PubMed: 24041567]
13. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Applied occupational and environmental hygiene. 1990; 5(1):46–51.
14. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, et al. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. American journal of obstetrics and gynecology. 2012; 206(1):58. e1-. e8. [PubMed: 22000672]
15. Ferguson KK, McElrath TF, Chen YH, Mukherjee B, Meeker JD. Urinary Phthalate Metabolites and Biomarkers of Oxidative Stress in Pregnant Women: A Repeated Measures Analysis. Environ Health Perspect. 2014
16. Jiang Y, Scott AJ, Wild CJ. Secondary analysis of case-control data. Statistics in medicine. 2006; 25(8):1323–39. [PubMed: 16220494]
17. Cantonwine DE, Ferguson KK, Mukherjee B, McElrath TF, Meeker JD. Variability of urinary bisphenol-A levels during pregnancy and risk of preterm birth. submitted
18. Remy S, Govarts E, Bruckers L, Paulussen M, Wens B, Den Hond E, et al. Expression of the sFLT1 gene in cord blood cells is associated to maternal arsenic exposure and decreased birth weight. PloS one. 2014; 9(3):e92677. [PubMed: 24664213]
19. van den Hooven EH, Pierik FH, de Kluizenaar Y, Hofman A, van Ratingen SW, Zandveld PY, et al. Air pollution exposure and markers of placental growth and function: the generation R study. Environmental health perspectives. 2012; 120(2):1753–9. [PubMed: 22922820]

20. Welch PC, Amankwah KS, Miller P, McAsey ME, Torry DS. Correlations of placental perfusion and PlGF protein expression in early human pregnancy. American journal of obstetrics and gynecology. 2006; 194(6):1625–9. [PubMed: 16635470]
21. Widmer M, Villar J, Benigni A, Conde-Agudelo A, Karumanchi SA, Lindheimer M. Mapping the theories of preeclampsia and the role of angiogenic factors: a systematic review. Obstetrics & Gynecology. 2007; 109(1):168–80. [PubMed: 17197602]
22. Regnault T, Orbus R, De Vrijer B, Davidsen M, Galan H, Wilkening R, et al. Placental expression of VEGF, PlGF and their receptors in a model of placental insufficiency—intrauterine growth restriction (PI-IUGR). Placenta. 2002; 23(2):132–44. [PubMed: 11945079]
23. Huang Y, Li J, Garcia JM, Lin H, Wang Y, Yan P, et al. Phthalate levels in cord blood are associated with preterm delivery and fetal growth parameters in Chinese women. PloS one. 2014; 9(2):e87430. [PubMed: 24503621]
24. Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, et al. Exposure to phthalates and phenols during pregnancy and offspring size at birth. Environmental health perspectives. 2012; 120(3):464–70. [PubMed: 21900077]
25. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, et al. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect. 2008; 116(8):1092–7. [PubMed: 18709157]
26. Snijder CA, Roeleveld N, te Velde E, Steegers EA, Raat H, Hofman A, et al. Occupational exposure to chemicals and fetal growth: the Generation R Study. Human reproduction. 2012:der437.
27. Lee BE, Park H, Hong YC, Ha M, Kim Y, Chang N, et al. Prenatal bisphenol A and birth outcomes: MOCEH (Mothers and Children's Environmental Health) study. International journal of hygiene and environmental health. 2014; 217(2-3):328–34. [PubMed: 23911140]
28. Losordo DW, Isner JM. Estrogen and angiogenesis a review. Arteriosclerosis, thrombosis, and vascular biology. 2001; 21(1):6–12.
29. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocrine reviews. 2009; 30(1):75–95. [PubMed: 19074586]
30. Buteau-Lozano H, Velasco G, Cristofari M, Balaguer P, Perrot-Applanat M. Xenoestrogens modulate vascular endothelial growth factor secretion in breast cancer cells through an estrogen receptor-dependent mechanism. Journal of Endocrinology. 2008; 196(2):399–412. [PubMed: 18252963]
31. Vetrano AM, Laskin DL, Archer F, Syed K, Gray JP, Laskin JD, et al. Inflammatory effects of phthalates in neonatal neutrophils. Pediatric research. 2010; 68(2):134–9. [PubMed: 20453712]

Research Highlights

- We examined repeated biomarkers of exposure and angiogenesis during pregnancy.
- Some phthalates and BPA were associated with adverse changes in sFlt-1 and PlGF.
- Exposure to these compounds may disrupt placental development and/or function.

Table 1

Weighted study population demographic characteristics and plasma angiogenic biomarker distributions (median, [25th, 75th percentiles]) within demographic groups (N=457 subjects; 1611 observations).

Demographic characteristic (weighted percent of total population)		sFlt-1 (ng/mL)	PlGF (pg/mL)
Race/ethnicity	White (58)	6.4 (3.9, 9.6)	142 (34.5, 410)
	African-American (16)	7.0 (4.4, 11.0)	**198 (57.1, 456)**[a]
	Other (26)	6.7 (4.3, 10.4)	**169 (49.7, 430)**
Education	High school (14)	6.7 (4.3, 9.7)	181 (52.1, 478)
	Technical school (16)	7.0 (4.1, 11.4)	171 (44.3, 473)
	Junior college or some college (30)	5.5 (3.8, 9.7)	136 (36.7, 354)
	College graduate (40)	6.9 (4.5, 9.8)	153 (41.3, 420)
Health insurance	Private insurance/HMO/Self-pay (81)	6.5 (4.1, 9.8)	148 (37.3, 404)
	Medicaid/SSI/MassHealth (19)	6.8 (4.3, 11.3)	**209 (52.9, 486)**
BMI at initial visit	Less than 25 kg/m^2 (54)	6.7 (4.3, 10.3)	159 (41.2, 452)
	25 to less than 30 kg/m^2 (26)	7.1 (4.4, 10.4)	163 (46.5, 415)
	Greater than 30 kg/m^2 (20)	**5.1 (3.3, 8.1)**	**124 (34.3, 325)**
Tobacco use	Smoked during pregnancy (6)	5.7 (3.5, 8.2)	173 (40.0, 371)
	No smoking during pregnancy (94)	6.6 (4.2, 10.0)	153 (40.9, 420)
Alcohol use	Alcohol use during pregnancy (5)	8.1 (4.2, 10.6)	158 (39.3, 427)
	No alcohol use during pregnancy (95)	6.5 (4.1, 9.9)	153 (40.9, 419)
Parity	Nulliparous (45)	7.3 (4.4, 12.1)	141 (39.2, 380)
	Non-nulliparous (55)	**6.0 (3.9, 8.7)**	**171 (41.2, 455)**
Fetal gender	Male (44)	6.8 (4.3, 11.1)	151 (41.2, 396)
	Female (56)	6.2 (3.9, 9.6)	162 (40.1, 438)
Case status	Preterm (11)	5.5 (3.8, 9.2)	120 (30.8, 296)
	Term (89)	6.6 (4.1, 10.0)	**159 (41.2, 429)**

[a] Bolding indicates significant difference ($p<0.05$) in angiogenic biomarker concentrations in demographic group compared to reference (first category listed). Results drawn from linear mixed models adjusting for subject-specific random intercepts and slopes. Abbreviations: sFlt-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; HMO, health maintenance organization; SSI, supplemental security income.

Table 2

Weighted study population distributions of plasma sFlt-1 (ng/mL) and PlGF (pg/mL) in all samples measured and by study visit.

		N	Geometric mean (geometric SD)	25th	50th	75th	90th	95th	Maximum
sFlt-1	All samples	1611	6.35 (2.19)	4.1	6.5	9.9	15.2	20.6	274
	Visit 1	450	4.42 (2.35)	3.2	5.0	7.3	10.3	12.5	37.4
	Visit 2	396	**6.18 (1.85)**[a]	3.9	6.1	8.9	13.8	18.2	62.3
	Visit 3	397	**5.93 (2.00)**	3.9	5.9	9.4	14.3	17.7	48.3
	Visit 4	368	**10.6 (1.94)**	6.9	9.9	14	23.3	32.6	274
PlGF	All samples	1611	134 (4.06)	40.7	153	420	751	1037	4267
	Visit 1	450	**21.6 (1.70)**	14.2	19.7	30.8	46.1	59.8	159
	Visit 2	396	**133 (1.71)**	93.9	135	191	261	304	582
	Visit 3	397	**422 (1.98)**	280	448	640	936	1204	2737
	Visit 4	368	**345 (2.80)**	155	389	749	1233	1751	4267

[a] Bolding indicates significant difference ($p<0.05$) in angiogenic biomarker concentrations compared to Visit 1. Abbreviations: sFlt-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; SD, standard deviation. Results drawn from linear mixed models adjusting for subject-specific random intercepts and slopes.

Table 3

Results from crude[a] linear mixed models in weighted study population (N=457 subjects; N=1611 observations) representing percent change in plasma angiogenic biomarker concentration in association with an interquartile range increase in urinary phthalate metabolite concentration.

	sFlt-1		PlGF		sFlt-1/PlGF ratio	
	percent change (95% CI)	**p**	**percent change (95% CI)**	**p**	**percent change (95% CI)**	**p**
MEHP	−1.83 (−5.98, 2.50)	0.402	3.74 (−1.94, 9.74)	0.202	−3.80 (−10.9, 3.89)	0.323
MEHHP	1.71 (−2.57, 6.19)	0.439	−5.55 (−10.8, 0.05)	0.052	9.01 (0.76, 17.9)	0.032
MEOHP	1.19 (−2.93, 5.49)	0.576	−3.47 (−8.73, 2.09)	0.217	6.62 (−1.24, 15.1)	0.101
MECPP	2.08 (−2.56, 6.93)	0.386	−8.42 (−13.8, −2.67)	0.005	12.3 (3.32, 22.0)	0.006
ΣDEHP	1.88 (−2.68, 6.64)	0.425	−6.58 (−12.1, −0.69)	0.029	10.4 (1.56, 20.0)	0.020
MBzP	1.33 (−4.39, 7.39)	0.656	1.79 (−5.06, 9.14)	0.618	0.87 (−8.17, 10.8)	0.857
MBP	−0.62 (−5.80, 4.86)	0.821	0.98 (−5.20, 7.56)	0.762	0.83 (−7.37, 9.77)	0.848
MiBP	−2.08 (−8.15, 4.39)	0.519	2.61 (−5.32, 11.2)	0.530	−2.98 (−13.0, 8.19)	0.586
MEP	−0.28 (−5.17, 4.85)	0.912	6.79 (0.13, 13.9)	0.046	−4.42 (−12.4, 4.29)	0.310
MCPP	−2.94 (−6.92, 1.20)	0.162	−2.19 (−7.41, 3.33)	0.430	1.30 (−5.98, 9.15)	0.734
BPA	7.08 (2.04, 12.4)	0.006	−3.68 (−9.82, 2.88)	0.265	12.3 (2.62, 22.8)	0.012

[a]Models were adjusted for urinary specific gravity and gestational age at sample collection and included random intercepts and slopes for each study subject. Abbreviations: sFlt-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor. Note: P-values were not corrected for multiple comparisons.

Table 4

Results from adjusted[a] linear mixed models in weighted study population (N=444 subjects; N=1565 observations) representing percent change in plasma angiogenic biomarker concentration in association with an interquartile range increase in urinary phthalate metabolite concentration.

	sFlt-1		PlGF		sFlt-1/PlGF ratio	
	percent change (95% CI)	**p**	**percent change (95% CI)**	**p**	**percent change (95% CI)**	**p**
MEHP	−2.51 (−6.68, 1.84)	0.254	3.79 (−1.92, 9.82)	0.198	−5.13 (−12.3, 2.59)	0.187
MEHHP	1.87 (−2.45, 6.39)	0.402	−4.59 (−9.91, 1.04)	0.109	8.37 (0.09, 17.3)	0.048
MEOHP	1.20 (−2.95, 5.53)	0.577	−2.69 (−7.98, 2.90)	0.339	5.67 (−2.20, 14.2)	0.163
MECPP	1.98 (−2.68, 6.87)	0.412	−7.30 (−12.8, −1.51)	0.014	10.9 (1.96, 20.6)	0.016
ΣDEHP	1.74 (−2.85, 6.55)	0.465	−5.48 (−11.1, 0.45)	0.070	8.99 (0.19, 18.6)	0.045
MBzP	0.84 (−5.06, 7.10)	0.786	−0.22 (−7.27, 7.36)	0.952	4.25 (−5.72, 15.3)	0.418
MBP	−0.94 (−6.16, 4.57)	0.733	−0.62 (−6.73, 5.89)	0.847	2.12 (−6.41, 11.4)	0.638
MiBP	−3.35 (−9.46, 3.17)	0.307	−1.68 (−9.47, 6.79)	0.688	−1.20 (−11.8, 10.7)	0.834
MEP	−1.25 (−6.19, 3.95)	0.630	3.25 (−3.27, 10.2)	0.337	−1.80 (−10.3, 7.46)	0.693
MCPP	−2.82 (−6.86, 1.40)	0.188	−2.27 (−7.56, 3.31)	0.418	1.08 (−6.37, 9.12)	0.784
BPA	6.43 (1.31, 11.8)	0.013	−4.98 (−11.1, 1.61)	0.136	14.5 (4.33, 25.6)	0.004

[a]Models were adjusted for urinary specific gravity, gestational age at sample collection, maternal age, health insurance provider, and body mass index (time-varying), and included random intercepts and slopes for each study subject. Abbreviations: sFlt-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor. Note: P-values were not corrected for multiple comparisons.