ENVIRONMENTAL PHTHALATE EXPOSURE, OXIDATIVE STRESS, AND PRETERM BIRTH

by

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ABSTRACT

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Preterm birth affects over 1 in 10 pregnancies in the US, is a leading contributor of neonatal mortality and morbidities, and has been linked to a number of adverse health outcomes later in life. Despite the severity of the problem, mechanisms of preterm birth are poorly understood, identified causes are few, and preventions are minimally effective. Environmental contributors to preterm birth are understudied but potentially important. Phthalates are a class of chemicals used commonly as plasticizers and solvents in various consumer products. Exposure in the US and elsewhere is ubiquitous. This dissertation provides evidence that maternal exposure to phthalates during pregnancy is associated with preterm birth and that phthalate-induced oxidative stress may play a partial role in mediating this relationship. The nested case-control population examined herein is drawn from a large prospective birth cohort of women recruited early in pregnancy and followed until delivery. Subjects provided urine samples at up to four visits across gestation, which were used for measurement of phthalate metabolites and oxidative stress biomarkers. At delivery, detailed birth outcome data was recorded, including information on preterm etiology. Based on these designations, cases were divided into two groups for the

majority of the analysis, including spontaneous preterm births following spontaneous preterm labor and/or preterm premature rupture of the membranes and placental preterm births resulting from preeclampsia and/or intrauterine growth restriction. The results indicated strong associations between maternal phthalate exposure during pregnancy and increased risk of preterm birth, and relationships were strongest in the spontaneous preterm subset. When patterns of urinary phthalate metabolite levels were examined longitudinally across gestation, it became clear that levels measured toward the end of pregnancy were most predictive of prematurity. Oxidative stress biomarkers measured in this study were strongly associated with phthalate metabolites as well as preterm birth. Mediation analysis demonstrated that oxidative stress accounted for 25-50 percent of the association between phthalate exposure and spontaneous preterm birth. These results provide evidence for not only association but causality in the relationship between urinary phthalate metabolites and prematurity. They suggest that women should take steps to decrease exposure levels during pregnancy.

CHAPTER I

INTRODUCTION

Preterm birth is a significant public health concern, as it is associated with high risk of infant mortality, various morbidities in both the neonatal period and later in life, and a significant societal economic burden (Behrman and Butler 2007; Cole et al. 2011; Guellec et al. 2011; Kochanek et al. 2012). Furthermore, despite a recent plateau, rates of preterm birth increased over 30% between 1981 and 2006 (Martin et al. 2011). Several causes of spontaneous preterm birth have been identified, including maternal stress, infection and inflammation, uterine distension (in pregnancies with multiple-births or abnormal amounts of amniotic fluid), cervical insufficiency, and placental dysfunction; however a large proportion with unknown etiology remain. In addition, although a variety of known risk factors predict preterm birth, including African American race, history of preterm delivery, low socio-economic status, and alcohol and cigarette use (Behrman and Butler 2007), these associations are largely unexplained. For these reasons the Institute of Medicine and the Surgeon General have called on the scientific community to investigate additional contributors to preterm birth risk, particularly those that may be related to environmental factors (Behrman and Butler 2007; Ashton et al. 2009).

Many environmental chemicals deserve investigation in this context because of (1) prevalent exposures, (2) demonstrated reproductive toxicities in animal studies, (3) ability to cross the placenta, and (4) association with other adverse birth outcomes that may result from related mechanisms. Despite this likelihood, the number of epidemiologic studies assessing relationships between environmental exposures and preterm birth are few, and the inconsistencies and limitations numerous. While some of these factors are overarching, many are specific to classes of chemicals. Below the studies to date that have examined associations between environmental contaminant

exposures and preterm birth are described in detail, along with major limitations and recommendations for future studies. The categories of chemicals examined include: persistent organic pollutants (POPs), drinking water contaminants, atmospheric pollutants, metals and metalloids, and other environmental contaminants.

PERSISTENT ORGANIC POLLUTANTS

Persistent organic pollutants (POP) including organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and hexachlorobenzene (HCB), polychlorinated biphenyls (PCB), dioxin, perfluorinated compounds such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), and, of more recent interest, polybrominated diphenyl ethers (PBDE), have been explored in a large number of studies for potential relationships with preterm birth (Table I.1, Figure I.1).

Organochlorine pesticides

Dichlorodiphenyltrichloroethane (DDT) is an organochlorine pesticide that was used commonly in agricultural settings in the US until 1972 when it was banned because of adverse effects on wildlife (ATSDR 2002a). However exposure still occurs in the US to a lesser degree through consumption of contaminated food and water. Additionally DDT is still used as a pesticide and for preventing the spread of malaria in many other places in the world (ATSDR 2002a). The primary component of DDT pesticides is the isomer is *p,p*-DDT but there are also smaller amounts of *o,p*-DDT. Furthermore, DDT is metabolized into dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) in humans. These metabolites as well as the parent compounds are measured in serum as markers of exposure.

Several small case-control studies published before 2000 found significantly higher levels of these organochlorines in cases of preterm delivery compared to controls. Notably, exposure levels in most of these studies were almost an order of magnitude higher than those measured recently in the US (median DDE for females from 2003-2004=1.25 ppb of serum; see Table I.1 for comparisons of study levels) (CDC 2009). The first study to examine this relationship by Saxena and colleagues (1981) (n=40) observed that placental tissue and maternal blood taken at delivery from mothers who delivered

preterm had higher levels of *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD compared to controls. These results were confirmed by another group (*n*=54) when exposure was measured in umbilical cord blood, but not when it was measured in maternal blood also taken at birth (Procianoy and Schvartsman 1981). However in another study by Wasserman et al. (1982) (*n*=27), where levels were examined in maternal serum during the third trimester, most isoforms of DDT, DDE and DDD were higher in cases compared to controls. Finally Berkowitz et al. (1996) performed a case-control study in New York City (*n*=40) where mothers had much lower exposure levels, and observed no significant differences in *p,p'*-DDE levels in maternal serum measured during the first trimester of pregnancy in cases of spontaneous preterm birth compared to controls.

The strongest evidence for a relationship between DDT exposure and preterm birth comes from a cohort study (*n*=2,380) conducted as part of the Collaborative Perinatal Project (CPP) in 11 US cities (Longnecker et al. 2001). Longnecker and colleagues (2001) found that increased maternal exposure to DDT, measured by DDE levels in third trimester serum, was significantly associated with increased OR of preterm delivery, and that there was a dose-dependent effect. Notably, women from the cohort were recruited from 1959-1965, a time when DDT use was at its peak in the US. The most pronounced statistical relationship between DDE and preterm birth was for women with greater than 60 μg/L in serum, about 40 fold higher than levels measured in US females in 2003-2004, compared to women with less than 15 μg/L in serum (OR=3.1, 95%CI=1.8 to 5.4) (CDC 2009; Longnecker et al. 2001). Another cohort study conducted during approximately the same time (1959-1967) failed to observe any significant associations in a population of women in San Francisco exposed to similar levels of DDT and DDE, but the sample size and number of preterm cases was much smaller (*n*=420, cases=33) (Farhang et al. 2005).

More recent studies that investigated relationships between DDT exposure and preterm birth at levels more consistent with those found currently in the US showed comparatively null results. Ribas-Fitó (2002) and colleagues observed higher levels of *p,p'*-DDE in cord serum of preterm newborns compared to those born full term in a small case-control study in Spain (*n*=70), but results were not adjusted for covariates and were observed in a population highly exposed to hexachlorobenzene (HCB). Another study

from Mexico City (*n*=233) measured *p,p'*-DDE levels in maternal serum at delivery and failed to find any significant differences in *p,p'*-DDE concentrations among preterm vs. term births in adjusted regression models (Torres-Arreola et al. 2003). Wood et al. (2007) examined differences in DDE levels in serum from primiparous women taken one day postpartum in a case-control study (*n*=78), and observed no significant association between exposure and odds of spontaneous preterm delivery, defined as <35 weeks gestation, in adjusted analyses. A case-control analysis by Pathak and colleagues (2009) in India measured *p,p*-DDT and *p,p*-DDE in both maternal and cord blood taken at delivery (*n*=46) and observed no significant differences in unadjusted comparisons of exposure levels from either matrix in cases vs. controls. Wojtynizk et al. (2010) examined three groups of women in Greenland (*n*=572), Ukraine (*n*=611), and Poland (*n*=258) to determine relationships between maternal serum levels of *p,p*-DDE at approximately 24 weeks gestation and odds of preterm birth. In adjusted models they failed to find any statistically significant associations, although in Poland, where the geometric mean of *p,p*-DDE exposure was 357 ng/g lipid (geometric mean for all US females measured from 2003-2004=241 ng/g lipid) a highly suggestive increase in the odds of preterm birth was reported (OR=2.44, 95%CI=0.99 to 6.06) (Wojtyniak et al. 2010).

The latest study, however, with median exposure levels below those currently observed in the US, found significantly elevated DDE in preterm vs. term biological samples. Bergonzi and colleagues (2011) examined *p,p*-DDE and *p,p*-DDD levels in maternal and cord serum, placenta, and subcutaneous adipose tissue all taken at delivery in a small cohort study in Italy (*n*=70). In adjusted models they observed higher levels of *p,p*-DDE in serum and higher *p,p*-DDT in adipose tissue of mothers who delivered preterm.

Several of the above studies also examined the association between preterm birth and hexachlorobenzene (HCB), an organochlorine pesticide used widely in the US until 1965 primarily for protection of wheat crops (ATSDR 2002b). Similar to DDT, exposure to HCB persists through consumption of contaminated foods, particularly fish, despite its discontinued use. Of the groups that examined HCB in association with preterm birth, only Ribas-Fitó and colleagues (2002) observed a significant difference in exposure levels between cases and controls. However, as mentioned previously, this study was

performed in a group of individuals in Spain who were subject to high levels of HCB through air pollution due to residence near an electrochemical factory. An additional study in an agricultural population, where exposures were again elevated compared to what is generally observed in the US, also found an inverse association between HCB exposure and gestational age (Fenster et al. 2006). Other studies, however, have not reported similar results (Torres-Arreola et al. 2003; Bergonzi et al. 2011).

Another organochlorine pesticide, hexachlorocyclohexane (HCH), is also consistently found in the environment though its use in agriculture has been discontinued in the US for over 20 years (ATSDR 2005). It takes on several isomeric forms, and when the γ-HCH isomer is present in >99% of the pesticide used it is more familiarly called lindane. HCH exposure was found at higher levels in cases of preterm birth compared to term births in the early study by Saxena and colleagues (1981), as well as in the Pathak et al. (2009) study where measurements were made in both maternal and cord serum. There was a similar suggestively increased odds of preterm birth in association with HCH exposure in the study in Mexico City, although the effect estimate did not reach statistical significance ($p=0.08$) (Torres-Arreola et al. 2003). Other organochlorine pesticides, including heptachlor/heptachlor epoxide and aldrin/dieldrin, discontinued in the late 1980s but persistent in soil and fatty food products (ATSDR 2002c, 2007a), were linked to preterm birth in the small case-control studies performed by Saxena et al. (1981) and Wasserman et al. (1982) but have not been explored in more recent studies of preterm birth. In the aforementioned study by Fenster and colleagues (2006), the only significant association observed in relation to gestational age was for HCB, and no relationships were observed for HCH or other organochlorine pesticides.

Polychlorinated biphenyls (PCBs)

In addition to pesticides, other chlorinated industrial chemicals no longer in use continue to be human health threats and may be factors in preterm birth. Polychlorinated biphenyls (PCB) are a group of 209 chemicals that were used in the US until 1977 as lubricants or coolants, electrical insulators, and in various building materials (ATSDR 2000). They do not degrade in the environment and accumulate in fish and marine animals. Exposure to humans occurs primarily through consumption of contaminated

foods, and is commonly measured in blood serum samples. The PCB congener 153 is the most highly detected in humans and is used frequently as a marker of overall PCB exposure.

Several of the small case-control studies introduced above have also observed significant differences in summed serum PCB levels in cases compared to controls. It is important to note that while many studies report findings in relation to change in summed PCB levels, this is indicative of a sum of the congeners *measured* which vary widely between studies. Wassermann and colleagues (1982) observed higher levels in maternal 3rd trimester serum in preterm cases compared to controls in unadjusted assessments. The study by Ribas-Fitó et al. (2002) in Spain also noted significantly higher levels in preterm cases compared to controls in measurements made in cord serum. However, Berkowitz and colleagues (1996) did not find marked differences in PCB levels measured in 1st trimester maternal serum by case/control status in a matched analysis.

In another analysis from the Collaborative Perinatal Project, a large US-based cohort study (*n*=1,034), Longnecker and colleagues (2005) also did not observe significant associations between summed PCB exposure and preterm birth, although a positive trend in OR was noted with increasing exposure categories in crude models (OR for 2-<3 μg/L vs. <2 μg/L=1.07, 95%CI=0.65 to 1.77; OR for 3-<4 μg/L vs. <2 μg/L=1.09, 95%CI=0.63 to 1.88; OR for 4+ μg/L vs. <2 μg/L=1.51, 95%CI = 0.91 to 2.52). Effect estimates were diminished and confidence intervals widened in adjusted models, particularly with the addition of *p,p*-DDE levels (Longnecker et al. 2005).

More recent studies have been null as well. Wojtynizk et al. (2010) did not find significantly altered odds of preterm birth in association with maternal serum levels of PCB-153 in Greenland, Ukraine, or Poland. The study by Bergonzi et al. (2011) in Italy did not observe significantly different levels of summed PCB in maternal or cord serum, placenta, or adipose tissue in samples from preterm vs. term pregnancies. However, as mentioned previously, this was a small cohort study with only 4 preterm deliveries.

Finally, in a large meta-analysis of studies from ENRIECO and OBELIX cohorts examining the relationships between POP exposure and several birth outcomes, no association was observed between PCB-153 and gestational age in any individual study or overall (Govarts et al. 2012).

Dioxin

Dioxins are compounds unintentionally released into the environment through various industrial processes, such as paper bleaching, drinking water disinfection, and incineration of waste (ATSDR 1998). Of the 75 dioxin congeners, the most toxic and commonly studied is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). This compound has been associated with many adverse reproductive outcomes in rodent studies, including fetal loss and birth defects (ATSDR 1998). Exposure to humans occurs primarily through consumption of contaminated foods, particularly meat, dairy, and fish (ATSDR 1998).

Despite the well-demonstrated toxicity in animals, no studies meeting our selection criteria have examined the relationship of dioxin exposure and preterm birth. Two studies did investigate this relationship, although they did not examine exposure specifically during pregnancy. However, because (1) half-life of TCDD in humans is long, (2) it is reasonable to argue that measures taken outside the time period of gestation may still be reflective of exposure during pregnancy, and (3) as dioxin has been associated with a range of adverse birth outcomes in animal studies, it is important to mention the exposure in this context. First, Eskenazi and colleagues (2003) examined the relationship between dioxin exposure and preterm birth in women from Seveso, Italy, where an extremely large amount of 2,3,7,8-TCDD was accidentally released in 1976. Most women were approximately 20 years old when the accidental exposure occurred and when serum TCDD levels were measured, and approximately 40 years old when the study was conducted to assess birth outcomes from the interim (*n*=510 women, 888 pregnancies). Despite high levels of exposure (median=46.6, range=2.5 to 9.14 ppt of lipid), the elevated odds of preterm birth observed in association with TCDD serum measures was not considered significant (adjusted OR for preterm birth in association with 10-fold increase in TCDD=1.5, 95%CI=0.7 to 3.2 for all pregnancies from 1976-1984) (Eskenazi et al. 2003).

A later study by Lin et al. (2006) examined effects of exposure to polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) released from a waste incinerator in Taipei, Taiwan. Annual exposure estimates for districts within Taipei were created from models of the incinerator plume, and rates of preterm birth from the exposed districts

were compared to rates in a nearby but unexposed region. In 1991, before the incinerator was built, there were no differences in rates of preterm birth between the exposed and unexposed districts; however in 1997, five years after the construction, OR were slightly elevated (OR for districts with 0.03-0.05 pg Toxic Equivalent [TEQ]/m^3 vs. <0.03 pg TEQ/m^3=1.12, 95%CI=0.94-1.32; OR for districts with >0.05 pg TEQ/m^3 vs. <0.03 pg TEQ/m^3=1.22, 95%CI=0.97-1.52) (Lin et al. 2006).

Two additional studies of accidental exposure to dioxin, with limited exposure and outcome measurements, have further suggested an association between this class of contaminants and preterm birth (Le and Johansson 2001; Revich et al. 2001). However, analyses with more precise exposure and outcome definitions are necessary to substantiate these results.

Perfluorinated compounds (PFC)

Perfluorinated chemicals repel oil, grease, and water and are used to treat carpets and clothing to prevent stains, and are also components of some food containers and wrappers (ATSDR 2009). Through product use and manufacturing they can be released into the environment, and they do not break down in soil or water. Exposure occurs through consumption of contaminated drinking water and food, inadvertent ingestion of indoor dust, and potentially through inhalation (ATSDR 2009). The two compounds produced in largest quantities include perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). Despite efforts to phase out use of these chemicals and/or decrease release into the environment, exposure is still widespread.

A number of recent studies examined the relationship between PFOA and PFOS exposure and preterm birth. Four studies examined associations in populations where exposure levels were relatively low and on the same order of magnitude as those observed in the general US population. Apelberg et al. (2007) measured PFOA and PFOS levels in cord serum from newborns in the Baltimore THREE study, where a relatively high proportion of births were preterm (*n*=293, preterm=28). No significant differences in median concentrations were detected, however, in term compared to preterm samples. Fei and colleagues (2007) examined levels of PFOS and PFOA in plasma taken during the first trimester from mothers participating in the Danish National Birth Cohort. Exposure

levels were divided into quartiles for analysis. The authors found that mothers with PFOS levels in the third quartile had significantly elevated OR of preterm birth compared to mothers from the first quartile of exposure (*n*=1,400, preterm=53). For mothers with levels of PFOA in the second quartile compared to the first OR were significantly higher as well (Fei et al. 2007). Other quartiles, however, were not significantly associated with change in OR, nor was there a significant trend in increasing quartiles for PFOS or PFOA.

Conversely, in the Norwegian Mother and Child Cohort Study, Whitworth and colleagues (2012) found a decreased odds of preterm birth for participants in the highest quartiles of PFOA and PFOS compared to the lowest, with exposure measured in maternal plasma at approximately 17 weeks gestation (*n*=901, preterm=35). A smaller study in Alberta, Canada with exposure levels measured in second trimester maternal serum showed no significant associations between PFOA or PFOS and preterm birth (*n*=252; preterm=21) (Hamm et al. 2010). Exposure levels were comparable in the studies by Hamm et al. (2010) (geometric mean for PFOA=1.3 ng/mL; geometric mean for PFOS=7.4 ng/mL) and Whitworth et al. (2012) (median for PFOA=2.2 ng/mL; median for PFOS=13 ng/mL), but were slightly higher in the study by Fei and colleagues (2007) (mean for PFOA=5.6 ng/mL; mean for PFOS=35.3 ng/mL).

Several studies of populations with high exposure to PFOA as a result of industry-related drinking water contamination in the Mid-Ohio valley, running between Ohio and West Virginia, also reported no significant relationships with preterm birth. In a study by Nolan and colleagues (2009) exposure levels were assigned by whether pregnant mothers resided in areas where drinking water was sourced from a contaminated facility only (*n*=150), partially sourced from that facility (*n*=190), or not at all sourced from that facility (*n*=1,017). No significant differences in frequency of preterm deliveries in mothers who consumed water from the contaminated source or partially from the contaminated source were noted compared to those who consumed no water from that source (Nolan et al. 2009). The C8 Health Project (C8 is a synonym for PFOA) examined this association in several studies in the same region. The original study to examine the odds of preterm birth in association with exposure in this population did not fit our search criteria, as exposure during pregnancy was estimated by serum PFOA and PFOS levels

measured at study enrollment some years later (Stein et al. 2009). It is also unlikely that current serum measures accurately reflect historical levels because of high variability in drinking water levels (Bartell et al. 2010; Olsen et al. 2007).

Follow-up papers by the same group, however, improved exposure assessment by using a fate-transport model to incorporate data on serum and historical drinking water PFOA levels into an estimate of maternal exposure during pregnancy. The first study examined these estimates in association with self-reported preterm birth in the C8 Health Project (*n*=11,737, preterm=1,843) with no significant results (Savitz et al. 2012a). The second utilized birth records from the same regions of Ohio and West Virginia in order to increase the study size and improve accuracy of outcome measures (cases=3,613, controls=3,695) (Savitz et al. 2012b). In this analysis maternal PFOA levels during early pregnancy were estimated using the same modeling techniques, with maternal residence based on addresses geo-coded from birth records. This, however, may have been associated with some exposure misclassification. Again, Savitz et al. (2012b) reported no significant change in odds of preterm birth associated with PFOA exposure. Finally, in the third analysis, cases of preterm birth from birth certificates were matched to C8 questionnaire data to maximize the quality of both exposure and outcome data (*n*=4,547, preterm=405) (Savitz et al. 2012b). In this analysis as well no significant change in odds of preterm birth in association with PFOA exposure was reported.

Another paper examining the association between exposure to perfluorinated compounds and preterm birth measured PFOS, PFOA, perfluorononanoic acid (PFNA), and perfluoroundecanoic acid (PFUA) in cord blood from mothers in a Taiwan cohort (*n*=429, preterm~40) (Chen et al. 2012). While no significant associations were observed for PFOA, PFNA, or PFUA, increased PFOS levels were associated with an increased odds of preterm delivery (OR for ln-unit increase in PFOS=2.45, 95%CI=1.47 to 4.08). Further, when PFOS exposure was divided into quartiles and modeled in relation to preterm birth, a significant trend was observed (p=0.001) (Chen et al. 2012). In a study from Ottawa, Canada, measuring levels of PFC in cord serum from women with scheduled C-sections, there were no differences observed in levels of PFC in cord serum from preterm compared to term births, however gestational age was inversely associated with PFOS levels (*n*=100, preterm=3) (Arbuckle et al. 2012).

Finally, a study in China examined the relationship between exposure to PFOA measured in maternal serum level and preterm birth (Wu et al. 2012). The population included one group residing in Guiyu, China (n=108) where there are many facilities and in-home workshops for recycling electronic waste, a process that results in a large amount of PFOA and other toxin release into the environment, and a control group in Chaonan, China (n=59). Wu and colleagues (2012) observed significantly higher levels of PFOA in maternal serum from preterm compared to term deliveries.

Polybrominated diphenyl ethers (PBDEs)

Mixtures of PBDE congeners are used as flame-retardants in many consumer goods, namely electronics and furniture. While use of the various commercial mixtures has been or is in the process of being phased out in the US and a number of other countries, many products containing these compounds remain. The continued use and disposal of products containing PBDEs results in their release into the environment and human exposure. One study examined the relationship between exposure to PBDE measured by umbilical cord serum levels and adverse birth outcomes, including but not exclusively preterm birth, in the same cohort mentioned previously in Guiyu and Chaonan, China (Wu et al. 2010). PBDE contamination, like PFC, is prevalent in the Guiyu area as a result of e-waste recycling. In this analysis Wu and colleagues (2010) observed significantly higher levels of various individual and summed PBDE congeners in cord blood from adverse birth outcome pregnancies (stillbirths or babies born preterm or low birth-weight) compared to normal pregnancies. Although the group did not examine preterm birth alone, this study provides suggestive evidence for future investigation of the association between PBDE exposure and preterm birth.

Limitations and recommendations for POPs

In summary, the literature suggests: (1) An association between high levels of DDT exposure and preterm birth; (2) The absence of an association when DDT exposure is at or below levels observed currently in the US; and (3) There is insufficient data to make conclusions about other POP at this time. DDT and its isomers, most commonly studied, were positively associated with OR of preterm birth in all but one of studies

where exposure was an order of magnitude above levels currently observed in the US. Studies of lower exposure levels primarily showed null results; although two exceptions provide grounds for further exploration of whether there is a threshold for effect. Studies of other organochlorine pesticides are too few for conclusions to be drawn, but results are similarly suggestive of an effect at higher exposure levels. With PCB, there is no strong evidence for an association with preterm birth, as the studies that did demonstrate effects were small and did not adjust for critical covariates. Literature on PFC and preterm birth was inconclusive when maternal exposures were estimated from levels measured in drinking water sources alone or in combination with data on exposure pathways. However, in studies using biomarkers, data is suggestive of an association between PFOS in particular and preterm birth. Finally, for PBDE exposure, although the single study is suggestive of an association with preterm birth among other adverse pregnancy outcomes, additional studies, particularly with a more specific definition for cases, are necessary.

Several study design aspects will be important to address in the future in order to better assess the relationship between POP exposure and preterm birth. First, exposures need to be measured at more time points during pregnancy, as most have focused on levels in the third trimester or at birth and some longitudinal studies demonstrated varying levels of persistent compounds during pregnancy (Bloom et al. 2007; Glynn et al. 2011). Second, more attention needs to be paid to body mass index (BMI) and other maternal anthropometric measures in these analyses. POP accumulate readily in the environment but also in human fatty tissues. Changes in maternal metabolism and adipose deposits during pregnancy could significantly alter blood and tissue lipid concentrations and consequently measures of POP (Hamel et al. 2003). The potential impact of these processes on the assessment of relationships between POP measures and birth outcomes need to be more carefully addressed. Finally, in the same vein, many of the more recent studies use exposure concentrations expressed on a lipid-basis in statistical analysis. However, using this method may produce bias and cloud real associations, and alternatively adjusting for serum lipids as a separate covariate in regression models may be preferable (Schisterman et al. 2005). These issues need particular attention in future study of POP exposure and preterm birth.

DRINKING WATER CONTAMINANTS

Many chemicals used for various purposes can be found in drinking water supplies. In addition, treatment of drinking water with chlorine results in the release of byproducts, such as trihalomethanes (THM), which can also be hazardous. A number of studies investigated the relationship between these contaminants and preterm birth (Table I.2, Figure I.2).

Chlorination disinfection byproducts

Drinking water is commonly treated with chlorine to kill bacteria. However the reaction of chlorine with various compounds found in the water may result in the formation of potentially hazardous byproducts (disinfection byproducts; DBP), including trihalomethanes (THM: chloroform, bromoform, bromodichloromethane, and dichlorobromomethane) and haloacetic acids (HAA: chloroacetic acid, dichloroacetic acid, trichloroacetic acid, bromoacetic acid, and dibromoacetic acid). Studies of relationships between exposure to THM and preterm birth were systematically summarized and meta-analyzed by Grellier and colleagues (2010). Of 9 studies chosen for inclusion in that study, none found significant associations with preterm birth (Dodds et al. 1999; Gallagher et al. 1998; Hoffman et al. 2008; Kramer et al. 1992; Lewis et al. 2007; Savitz et al. 1995; Wright et al. 2003, 2004; Yang et al. 2007). Likewise, the results of the meta-analysis were insignificant, although a decrease in odds of preterm delivery with increasing THM exposure was suggested (Grellier et al. 2010). Two of these studies also examined the relationship between preterm birth and HAA exposure but did not detect significant results (Hoffman et al. 2008; Wright et al. 2004).

Six other studies that examined odds of preterm birth in association with DBP exposure were excluded because exposures were either inadequately characterized or grouped into too few categories (Grellier et al. 2010). (These studies were similarly omitted from Table I.2 and Figure I.2 because of the limited value they add to the data on this topic.) Two studies that assigned maternal exposure levels by municipality use of chlorine for drinking water disinfection found a positive association between residence in these areas and odds of preterm birth (Yang et al. 2000; Yang 2004). One study that also used municipality of residence for exposure classification purposes found higher rates of

premature delivery in mothers who resided in municipalities where water was treated with chlorine dioxide compared to those who resided where water was treated with chlorine (Tuthill and Schwalm 1992). On the other hand, another analysis that used similar exposure assessment methods found that mothers living in areas where water was chlorinated had significantly decreased odds of preterm birth (OR=0.91, 95%CI=0.84 to 0.99) (Jaakkola et al. 2001). The last two of these 6 studies, again with exposure assigned by maternal water-source treatment with chlorine, did not find any statistically significant associations between chlorine exposure and preterm birth (Kanitz et al. 1996; Kallen and Robert 2000). One additional study that examined preterm birth in association with DBP exposure was excluded from the meta-analysis because of insufficient exposure categorization (Aggazzotti et al. 2004). This study examined levels of THM and other chlorination by-products in tap water samples from mothers' homes, and also incorporated information from a questionnaire. They found no association with chlorination by-products and preterm birth. Finally, two other studies mentioned in association with other birth outcomes in the review also examined preterm delivery and found no association with THM (Hinckley et al. 2005; Bove et al. 1995). One of these studies also examined levels of HAA in drinking water, but reported no significant relationships with odds of preterm birth (Hinckley et al. 2005). Overall, Grellier and colleagues (2010) concluded that based on the existing literature there was insufficient evidence to indicate an association between THM exposure and any birth outcome, including preterm birth. Similarly, no apparent relationships for HAA emerged, although examining effects of this exposure was not a primary aim in the study.

Since the publication of the meta-analysis by Grellier et al. (2010), 4 new papers have been published examining the relationship between DBP and preterm birth, all with more refined exposure assessment methods. Patelarou and colleagues (2011) examined births from the RHEA cohort in Crete, Greece (n=1,359). They measured THM in drinking water sources and administered in-depth questionnaires from which they calculated each subject's personal exposure levels by ingestion, dermal absorption, and inhalation. No significant differences were observed in odds of preterm birth when comparing mothers exposed to levels in the highest tertile compared to the lowest, whether exposure was measured in the 1st, 2nd, or 3rd trimester, or averaged across the

duration of pregnancy. In a mother-child cohort in Spain, exposure estimated in a similar fashion was also not associated with odds of preterm birth (*n*=2,074) (Villanueva et al. 2011).

A study of two southeastern US sites of either brominated or chlorinated DBP contamination, however, observed a positive association between DBP exposure and preterm birth. Horton and colleagues (2011) measured THM, HAA, and total organic halide (TOX) concentrations in drinking water weekly or biweekly in drinking water in both communities, and identified a significantly increased odds of preterm delivery for mothers in the 50^{th}-75^{th} percentile of TOX exposure compared to those $<50^{th}$ percentile. The group also observed a significantly increased odds of preterm birth in association with continuous TOX exposure (OR for 10 µg/L increase=1.09, 95%CI=1.03 to 1.16). This finding was within the population residing near a site of brominated-DBP contamination (preterm=401, term=3,109). No significant associations were observed for any other contaminant or any other exposure category (75^{th}-90^{th} vs. $<50^{th}$ percentile or $>90^{th}$ vs. $<50^{th}$ percentile) within that community or in the community with chlorinated-DBP contamination (Horton et al. 2011).

The most recently published study of DBP and preterm birth was the first to use a maternal biomarker of exposure. Costet and colleagues (2012) examined odds of preterm birth in a nested case-control study within the PELAGIE birth cohort in France (cases=114; controls=399). In addition to the previously used method of identifying THM levels in drinking water supplies and assigning individual exposure levels based on questionnaires designed to characterize ingestion, inhalation, and dermal absorption, they also measured maternal urinary levels of the HAA trichloroacetic acid (TCAA) during early pregnancy. While biomarkers of THM may only reflect very recent exposures, TCAA levels may be valid markers of long-term ingestion of chlorinated water (Kim et al. 1999). In this study urinary TCAA detection was low (6.7%), and concentrations correlated with THM ingestion estimates only in subjects with detectable levels (Costet et al. 2012). No significant results were reported in relation to preterm birth for either exposure assessment method. However, the use of a biomarker for DBP exposure may mark a promising new direction for this line of research.

Chlorinated solvents

In addition to DBP, many compounds not used for water-treatment purposes end up in groundwater and drinking water supplies. Trichloroethylene (TCE) and tetrachloroethylene (PCE) are solvents used commonly as metal degreasing agents, and the latter is also used for spot treatments in dry cleaning facilities (CDC 2009). TCE dissolves slightly in water and evaporates when it reaches the surface, but otherwise can remain in groundwater for long periods of time (ATSDR 1997a). PCE may be degraded more readily by microorganisms in water but can still be found in drinking water sources (ATSDR 1997b). Exposure to these compounds occurs through ingestion of contaminated water or inhalation of vapors.

Bove and colleagues (1995) first examined the relationship between TCE and PCE exposure and birth outcomes in four regions of northern New Jersey from 1985-1988 (*n*=80,938). They assigned exposure levels to pregnant mothers based on water samples analyzed twice annually for contaminant levels, and found no significant associations with preterm birth. Sonnenfeld and colleagues (2001) examined the same relationship in mothers residing on a US Marine Corps base at Camp Lejeune, North Carolina, where there was known contamination of drinking water with PCE (*n*=11,798). They observed slightly elevated odds of preterm delivery in mothers exposed to drinking water from the PCE-containing well for both 4-10 weeks (OR=1.3, 90%CI=1.0 to 1.7) and 11-20 weeks of pregnancy (OR=1.3, 90%CI=1.1 to 1.6) compared to no exposure during pregnancy. Aschengrau et al. (2008) utilized a leaching and transport model to estimate maternal PCE exposure near the time of conception in Cape Cod, Massachusetts, where drinking water pipe linings were found to be contaminated (*n*=2,125, preterm=96). No significant elevations in OR were detected. Finally, Forand and colleagues (2011) examined birth outcomes in mothers from Endicott, New York, where exposure to PCE and TCE was thought to occur largely through inhalation as a result of contaminant soil vapor intrusion (*n*=1,440). No elevated risks of preterm birth in women living in the PCE, TCE, or combined contaminated areas were noted compared to women living in non-contaminated areas.

Limitations and recommendations for drinking water contaminants

In summary, evidence on a relationship between exposure to any individual drinking water contaminant and preterm birth is currently inconclusive. In some early publications DBP were associated with a significant increase in odds of preterm delivery, but results from more recent studies with more precise exposure assessment methods have been generally null. Drinking water contaminated with chlorinated solvents has been examined in fewer studies, but there is some evidence that exposure to PCE via this route may be related to preterm birth.

These results suggest that studies of drinking water contaminants and preterm birth need more attention to exposure assessment methods. Many studies have been ecologic in nature, linking pregnancy outcomes to exposure indicated by maternal residence in proximity to contamination sources. This may result in a high degree of exposure misclassification, as water consumption varies significantly by individual. Also, ecologic studies are highly subject to confounding as a result of differences in socioeconomic status, additional environmental exposures, and other potentially important factors that differ by region. Data on drinking water contamination levels would best be combined with other known factors, such as water use habits, to provide more accurate exposure assessments. In addition, when possible, studies should consider the use of biomarkers of exposure. Another issue is that contamination of drinking water by one toxicant may be linked to contamination with another. Examination of exposure to mixtures of drinking water pollutants need to be addressed in the future.

ATMOSPHERIC POLLUTANTS

Studies of air pollutant associations with preterm birth have been reviewed in several recent publications. Criteria air pollutants, including ozone, particulate matter (of sizes ≤2.5 and ≤10 microns in aerodynamic diameter, $PM_{2.5}$ and PM_{10}, respectively), carbon monoxide (CO), oxides of nitrogen (NO_x), and sulfur dioxide (SO_2) received the most attention (Glinianaia et al. 2004; Shah and Balkhair 2011; Sram et al. 2005). While conclusions from individual reviews have been conflicting, the most recent assessment of the evidence asserted that a relationship exists between SO_2 and $PM_{2.5}$ exposures and

preterm birth (Shah and Balkhair 2011). Associations with other criteria air pollutants are less definitive (Stillerman et al. 2008).

Environmental tobacco smoke (ETS) exposure in relation to preterm birth has been examined extensively and reviewed recently as well. A meta-analysis that improved upon previous reviews by clearly excluding mothers who were active smokers observed an elevated odds of preterm delivery in relation to ETS exposure in models of crude data (OR=1.2, 95% CI=0.99 to 1.46) which was attenuated in an adjusted analysis (RR=1.07, 95%CI=0.93 to 1.22) (Salmasi et al. 2010). However, other assessments, including one by the US Department of Health and Human Services (2006), have concluded that ETS exposure decreases gestational duration (Stillerman et al. 2008; Wigle et al. 2008).

Other air contaminants, particularly polycyclic aromatic hydrocarbons (PAH) and volatile organic compounds (VOC), received less attention in previous reviews, and hence the literature on the relationships between these exposures and preterm birth was examined here (Table I.3, Figure I.3).

PAH are released in the combustion of coal, oil and gas, and other organic matter, and humans are exposed through inhalation of contaminated air (ATSDR 1995a). Major contributors are inhalation of ETS and PAH bound to particulate matter, which complicates estimation of PAH-specific effects. Additionally, exposure can occur via dietary sources of PAH, for example via consumption of charbroiled foods. Exposure assessment is most commonly performed via air monitoring, but more recently has moved toward biomonitoring with urine measures of hydroxylated PAH metabolites or blood measures of parent compounds or DNA-adducts.

Three studies have used air measurements to assess the relationship between PAH and preterm birth. Vassilev and colleagues (2001) used ambient air monitoring data in New Jersey between 1990 and 1991 to create estimates of average exposure to polycyclic organic matter (including PAHs, arenes, and polyhalo compounds) within each census tract (n=214,493). Significantly elevated odds of preterm birth in mothers residing in medium and high PAH-exposure areas compared to mothers residing in low exposure areas (OR for medium compared to low=1.09, 95%CI=1.04 to 1.14; OR for high compared to low=1.25, 95%CI=1.19 to 1.31) were noted in adjusted models. Since

maternal tobacco use appeared equally distributed across air pollution categories, no adjustments were made for this factor in the analysis.

In a more recent study in Los Angeles County, where exposures to PAH and other air pollutants are particularly high, Wilhelm et al. (2011) similarly found increased odds of preterm birth in association with an interquartile range increase in ambient total PAH levels averaged across the duration of pregnancy after adjustments for maternal age, race/ethnicity, education, and parity (OR=1.3, 95%CI=1.15 to 1.47; *n*=112,915). Significantly elevated OR were observed for individual PAH (benzo[a]pyrene, benzo[g,h,i]perylene, and naphthalene) as well. Due to the use of birth certificates in the study, they were unable to adjust for maternal smoking or exposure to ETS during pregnancy. A third study that employed the use of personal air monitoring data, collected among non-smoking women during the third trimester of pregnancy, found that African American mothers, but not Dominican mothers, had nearly a 5-fold rise in odds of preterm birth in association with an ln-unit increase in PAH exposure in New York City (OR=4.68, 95%CI=1.84 to 11.9; *n*=224) (Choi et al. 2008). These ORs were reported from models adjusted for maternal pre-pregnancy body mass index, infant sex and parity, season of delivery, and months of gestational ETS exposure.

Studies using various biomonitoring methods have similarly observed a positive association between PAH exposure and preterm birth. Singh and colleagues (2008) performed a small case-control study of non-smoking women in Lucknow, India, between 2005 and 2006, measuring PAH concentrations in placental tissue. Significantly higher levels of two individual PAH (fluoranthene and benzo(*b*)fluoranthene) in preterm cases (*n*=29) compared to controls (*n*=31) were found, although no adjustments were made for potential confounders. Also, in the aforementioned study of Guiyu, China, where e-waste recycling lead to high levels of environmental pollution, Guo and colleages (2012) measured 7 carcinogenic PAH in umbilical cord blood in deliveries from Guiyu and from Chaonan, an uncontaminated area, for comparison (*n*=183, adverse birth outcomes=18). They observed generally higher values of the PAH measured in cord blood from adverse compared to normal births (adverse birth outcomes included infants born preterm, low birth weight, and with congenital malformations, as well as stillbirths). Furthermore, 2 individual PAH (chrysene and benzo[a]anthracene) were inversely

associated with gestational age (Guo et al. 2012). Again, however, no adjustments were made for covariates, namely maternal smoking or ETS exposure.

Volatile organic compounds (VOC) are a large class of compounds that move readily from the liquid phase to air. These include some of the previously described drinking water contaminants, such as TCE and PCE, as well as many others such as acetone, benzene, and formaldehyde. Benzene is one VOC that has received significant attention because of its carcinogenic potential. It is released in many industrial processes into the air, and also from automobile emissions and tobacco smoke. Two studies examined the relationship between maternal benzene exposure and preterm birth. In the previously described study by Wilhelm et al. (2011), significantly increased odds of preterm birth were found in association with an interquartile range increase in benzene (adjusted OR=1.09, 95%CI=1.06 to 1.13). Further, a study in Valencia, Spain, examined benzene exposure measured via ambient air monitors and found elevated odds of preterm birth in individuals exposed to greater than 2.7 $\mu g/m^3$ across the duration of pregnancy (OR for 1 $\mu g/m^3$ increase in benzene exposure level = 6.46, 95%CI=1.58 to 26.4; *n*=785) (Llop et al. 2010). Formaldehyde exposure was examined in relation to preterm birth in one publication with no significant results (Maroziene and Grazuleviciene 2002), but otherwise no studies examined associations between other VOC exposures and preterm birth.

Limitations and recommendations for atmospheric pollutants

In summary, previous reviews strongly suggest associations between preterm birth and environmental exposures to (1) SO_2, (2) $PM_{2.5}$, and (3) ETS. Studies of PAH exposure and preterm birth indicate a relationship, although additional studies that address the importance of PAH compared to other components of the complex mixture, such as PM and ETS, would be useful. Studies on air pollutant exposures and preterm birth suffer from several limitations recently identified by Slama et al. (2008) at the International Workshop on Air Pollution and Human Reproduction. Moving forward, the workshop report called for (1) More prospective studies, (2) Attention to important confounders such as seasonality of exposure/delivery as well as maternal nutrition status (3) Advancement in exposure assessment methods, such as using biomarkers of exposure,

and identifying key exposure windows (e.g., first trimester of pregnancy), and (4) Exploration of potential toxicologic mechanisms to explain exposure-outcome relationships (Slama et al. 2008). Addressing these issues may help to better explore some of the associations observed to date between ambient air pollution exposures and preterm birth.

METALS AND METALLOIDS

Metal and metalloid exposures have long been studied in association with adverse reproductive outcomes, and some results indicate associations with preterm birth. Although many early studies focused on women with occupational exposures, some also examined the association in populations exposed through ambient levels in the environment (Table I.4, Figure I.4).

Lead

The largest number of studies examining the relationship between metal exposure and preterm birth has been for lead (Pb). Exposure to this metal has been well studied in association with adverse birth outcomes since its inclusion in gasoline resulted in high environmental exposures until it was phased out by the Environmental Protection Agency in 1975. Despite these efforts, exposure to Pb still occurs in the general population: It is released into air through various manufacturing processes and in combustion of fossil fuels, which results in inhalation exposures (ATSDR 2007b); use of Pb in pipes can lead to drinking water contamination; ceramics containing Pb glazes can be a source of exposure; and older Pb-based paints can flake leading to exposure via ingestion or inhalation of contaminated dusts. Pb has been associated with a range of adverse health outcomes, and, notably, there seems to be no threshold of exposure for most effects.

Lead exposure in relation to birth outcomes has been well-reviewed. Andrews and colleagues (1994) summarized early findings, including several occupational studies, reporting that Pb exposure was likely associated with increased risk of preterm birth, and that the effects were dose-dependent. The more recent publications are reviewed here.

In a case-cohort study in Mexico City from 1995, Torres-Sánchez et al. (1999) measured levels of Pb in umbilical blood of 161 preterm and 459 full term infants. They

found, like previous studies, that increasing Pb levels were significantly associated with preterm birth, but, interestingly, only in infants born to primiparous women. Similar to previous studies, exposure levels in this population were relatively high (mean levels in firstborn preterm infant cord blood=9.77 μg/dL, in firstborn term infants=8.24 μg/dL) (Torres-Sánchez et al. 1999). Another study examining deliveries between 1996-2002 in a population of primarily Hispanic Californian women found that elevated (≥10μg/dL) blood Pb levels during pregnancy were associated with significantly elevated odds of preterm delivery (OR=4.2, 95%CI=1.3 to 13.9; *n*=262) (Jelliffe-Pawlowski et al. 2006).

Since the elimination of Pb from gasoline, mean levels in human blood in the US have dropped almost 80% (Pirkle et al. 1994). Hence, there is particular interest in health effects associated with low exposures, or at blood levels less than 10 μg/dL. Studies of Pb exposure in lower ranges and preterm birth are less consistent. In 2002 Sowers and colleagues (2002) examined blood Pb levels at four time points throughout gestation in 705 pregnant women from Camden, New Jersey, where average blood Pb levels were approximately 1.2 μg/dL. No significant associations were noted with preterm birth, defined as <36 weeks gestation, in either cross-sectional or longitudinal analyses (Sowers et al. 2002). Similarly, in a large cohort study of births in upstate New York, Zhu et al. (2010) found that there was no significant increase in odds of preterm birth with maternal blood Pb measurements in the highest quartile of exposure compared to the lowest (3.1-9.9 μg/dL compared to ≤1 μg/dL; *n*=43,288). In this study Pb was measured in maternal blood at or before delivery date, and the average exposure level was 2.1 μg/dL (Zhu et al. 2010). Notably, exposure measurements were taken anywhere between last menstrual period and the date of delivery.

In 2010 Cantonwine et al. (2010a) pointed out that blood Pb levels during certain time points during pregnancy, particularly the first and second trimesters, may be especially predictive of preterm birth. In this study of mother-infant pairs in Mexico City conducted between 1997 and 1999, data showed that a one standard deviation increase in blood Pb levels measured during the second trimester was associated with significantly increased odds of preterm birth (OR=1.75, 95%CI=1.02 to 3.02; *n*=235) (Cantonwine et al. 2010a). However, associations with first or third trimesters, or with measures from umbilical cord blood, were not statistically significant (Cantonwine et al. 2010a).

Exposures in this population were somewhat elevated but still below the CDC threshold (mean in whole blood 6.3-7.2 μg/dL in first through third trimesters) (Cantonwine et al. 2010a).

Two other studies found significant associations with preterm birth and low Pb levels in first trimester maternal blood or in placenta. Vigeh and colleagues (2011) observed that elevated maternal first trimester blood Pb levels were significantly associated with increased odds of preterm birth in Tehran, Iran (OR=1.41 with an ln-unit increase in blood Pb level, 95%CI=1.08 to 1.84; *n*=348). Falcon et al. (2003) found significantly higher Pb levels in placenta of adverse pregnancies, including those with premature rupture of the membranes and preterm delivery, compared to normal pregnancies in Spain (*n*=89). Addressing critical windows of exposure is a particularly important aspect of understanding chemical exposure associations with preterm birth; however the evidence for a specific window of susceptibility in relation to Pb exposure is still inconclusive.

Cadmium, arsenic, and mercury

Cadmium (Cd) is found naturally in the earth, and is extracted for use in some products like batteries and metal coatings because of its anti-corrosive properties (ATSDR 2008). Low levels are also detected in nearly all foods, making ingestion the most common route of exposure in the general population. Results from studies examining the relationship between Cd exposure and preterm birth have been conflicting. An ecologic study conducted in southern Sweden between 1985 and 1990 found that there was no significant elevation in odds of shortened gestation in women who lived in areas with elevated exposure to Cd (*n*=38,718) (Landgren 1996). However, in a small case-control study from the early 1990s Fagher and colleagues (1993) found higher blood Cd levels in mothers who delivered preterm compared to term. The most recent study, conducted among a small cohort of women in China, found no significant associations between Cd measured in maternal whole blood, cord blood, or placenta and risk of preterm birth, defined as delivery ≤37 weeks gestation (*n*=44) (Zhang et al. 2004). Finally, one study examining Cd exposure in postnatal urine that did not fit within our inclusion criteria deserves mention since urinary Cd levels may be representative of long-

term exposure. Nishijo and colleages (2002) measured Cd levels 5-8 days following delivery in maternal urine in Toyama, Japan, an area of high Cd contamination (*n*=57, preterm=9). Mothers with high (≥2 µg/g creatinine) vs. low (<2 µg/g creatinine) urinary Cd concentrations had higher rates of preterm delivery.

Four studies investigated the relationship between preterm delivery and exposure to arsenic (As), a metalloid that is also found naturally in the environment. Exposure in small amounts may occur through ingestion of contaminated food and water, and higher exposures are possible in areas where As is found naturally at elevated levels in soil and groundwater, such as in certain regions of Bangladesh. All studies of As in relation to preterm birth have assigned exposure based on region of maternal residence, and thus had the aforementioned limitations of ecologic studies, although As species can be measured reliably in spot urine samples (Rivera-Nunez et al. 2010). The above study by Landgren (1996) found a slightly decreased odds of preterm birth in women who resided in a municipality with less than mean study exposure levels during pregnancy. In a study in Bangladesh, Ahmad and colleagues (2001) found that women residing in areas with high exposure to As (>0.05 mg/L in well water) had significantly higher preterm birth rates compared to women residing in low exposure areas (<0.02 mg/L in well water) (*n*=192). A study in Taiwan found an elevated, although non-significant, odds of preterm birth in association with residence in an area with a history of high well-water levels of As (OR=1.10, 95%CI=0.91 to 1.33; *n*=18,259) (Yang et al. 2003). In a small subset from a cross-sectional study in another region of Bangladesh, Mukherjee and colleages (2005) examined birth outcomes and observed no differences in rates of preterm birth in mothers exposed to drinking water with 284-400 µg/L (*n*=21, preterm=8) or 401-1474 µg/L (*n*=44, preterm=12) compared to those exposed to less than 3 µg/L As (*n*=18, preterm=2). A study in Inner Mongolia, China, where exposure was assigned to mothers by averaging well-water levels in her respective village, did not find a significant change in odds of preterm birth (OR=1.02, 95%CI=0.72 to 1.44) with residence in a village with high exposure (>50 µg/dL) (*n*=9,890) (Myers et al. 2010).

Finally, mercury (Hg) exposure, which occurs primarily through ingestion of contaminated fish, has been linked to preterm birth in two epidemiologic studies. The aforementioned study by Landgren (1996) mothers residing in municipalities with greater

than mean ground concentrations of Hg had slightly reduced OR for preterm birth. The Pregnancy Outcomes and Community Health (POUCH) Study, which recruited women in 5 communities throughout Michigan between their 15th and 27th weeks of pregnancy, assessed Hg exposure by measuring levels in hair samples (n=1,024) (Xue et al. 2007). After adjustment for covariates including fish consumption, a significantly elevated odds of very preterm birth (defined as <35 weeks gestation) was observed in association with Hg hair levels ≥90th percentile (OR=3.0, 95%CI=1.3 to 6.7), although the odds ratio for delivery <37 weeks gestation was not significant (OR= 1.55, 95%CI=0.7 to 2.9) (Xue et al. 2007).

Limitations and recommendations for metals and metalloids

Studies of metal and metalloid exposure and preterm birth provide evidence for an effect of Pb at higher levels, but studies at levels more consistent with current environmental exposure levels, or for Cd, As, or Hg, are inconclusive. Several limitations should be addressed in future studies examining these relationships. For one, investigation of other markers of exposure may be valuable. For example, blood Pb levels may be more representative of transient exposures, and not of exposures that are long-term or cumulative. Bone scans are superior for cumulative Pb exposure measurement; however, it may be dangerous to perform them during pregnancy. Exploration of ways to better characterize women's more complete Pb exposure profile before and during pregnancy are recommended. For As, utilizing urinary biomarkers of exposure in studies on preterm birth could strengthen the existing evidence. Future studies should also be designed to identify whether there are increased risks of preterm birth associated with exposure to metals at "background" levels common among the general population, as most studies to data have focused on areas with elevated exposures.

OTHER EVIRONMENTAL CONTAMINANTS

In addition to the toxicants described above, numerous others may be capable of causing adverse reproductive outcomes including preterm birth. Identifying any of these potential links is particularly important for those compounds with widespread exposure. Few studies have examined associations between these compounds and preterm birth in

particular, but in some instances there is preliminary data suggestive of an effect (Table I.5, Figure I.5).

Phthalates

Phthalate diesters are commonly used both as plasticizers in products such as polyvinyl chloride, and also as ingredients in personal care products such as lotions and perfumes. Exposure may occur through ingestion of contaminated food and water as well as product use and is ubiquitous in the general US population, with most metabolites detectable in over 99% of urine samples (Silva et al. 2004a). Research has identified phthalates as endocrine disruptors and reproductive toxicants, and two studies have examined the association between exposure and preterm birth. Adibi and colleagues (2009) examined the relationship between di(2-ethylhexyl) phthalate (DEHP) metabolites measured in 3rd trimester urine samples and odds of preterm birth in a cohort of women recruited between 2000 and 2004 in the Study for Future Families (SFF) (*n*=283). Significantly decreased odds of preterm birth in association with log-unit increases in the metabolites mono-(2-ethylhexyl) phthalate (MEHP; OR=0.5, 95%CI=0.3 to 0.9), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP; OR=0.5, 95%CI=0.3 to 0.9), and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP; OR=0.4, 95%CI=0.2 to 0.9) were reported. Notably, the proportion of preterm deliveries in this cohort was very low (preterm=14). On the other hand, in a small case-control study of women who gave birth in Mexico City, Meeker et al. (2009a) observed significantly increased odds of preterm birth in association with greater than median levels of the metabolites mono-butyl phthalate (MBP; OR=4.5, 95%CI=1.2 to 16.6), mono(2-ethyl-5-carboxypentyl) (MECPP; OR=3.4, 95%CI=1.0 to 12.0), and mono(3-carboxypropyl) phthalate (MCPP; OR=3.2, 95%CI=1.0 to 9.8) in adjusted models of levels from 3rd trimester urine samples (*n*=60). Results from studies examining exposures in relation to gestational age have been similarly conflicting (Suzuki et al. 2010a; Whyatt et al. 2009; Wolff et al. 2008a).

Bisphenol-A (BPA)

BPA is a component of polycarbonate plastics and epoxy resins which are used primarily in the production of hard plastic containers and to line food cans, respectively.

Exposure occurs primarily through the ingestion of contaminated food, and levels in urine were detected in over 92% of the US population (Calafat et al. 2008). BPA is weakly estrogenic, and may be associated with adverse reproductive health outcomes. However, only one study investigated the relationship between BPA and preterm birth. In the same case-control group of Mexican women in which urinary phthalate metabolites were measured, Cantonwine and colleagues (2010b) found that 3rd trimester total urinary BPA levels were suggestively associated with an increase in birth at ≤ 37 weeks gestation (*n*=30 cases). When case definition was defined as < 37 weeks (*n*=12 cases) the relationship became statistically significant (Cantonwine et al. 2010b). Furthermore, elevated OR remained in models additionally adjusted for urinary phthalate levels. Although more studies are needed, this evidence is suggestive of a link between BPA exposure and preterm birth.

Non-persistent pesticides

Persistent organochlorine pesticides have been replaced with a wide variety of non-persistent pesticides with potential effects on humans that are only beginning to be studied. The general population may be exposed to these compounds through ingestion of food and/or hand-to-mouth contact with contaminated surfaces. Inhalation and dermal exposures may also occur. Exposures to agricultural workers and individuals who reside in agricultural areas are also of concern. Further, for some compounds, contamination of drinking water supplies may pose a threat to the general population.

Organophosphate pesticides are one class of these compounds, including chlorpyrifos, diazinon, malathion, parathion, and others, that are currently in common use in agricultural settings. Eskenazi and colleagues (2004) investigated associations between exposure to these compounds and preterm birth within the Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS), in a region of high agricultural production and pesticide use. In the cohort of 488 women, parent compounds and metabolites of dialkyl phosphate, chlorpyrifos, malathion, and other organophosphate metabolites were measured in two urine samples collected during gestation. Levels of cholinesterase, which are thought to be depressed by organophosphate exposure, were measured in maternal and cord blood at birth (Eskenazi et al. 2004). Although neither

individual nor summed organophosphates or metabolites were associated with a change in odds of preterm birth, decreased cholinesterase (ChE) levels in umbilical and maternal blood were found to be associated with increased risk of preterm birth (adjusted OR=2.3, 95%CI=1.1 to 4.8, and OR=1.6, 95%CI=1.0 to 2.5 for μmol/min/mL increase in ChE, respectively) (Eskenazi et al. 2004). Although outside our inclusion criteria, the Agricultural Health Study examined exposure to organophosphates as well as other pesticides in relation to birth outcomes in a cohort of 2,246 births (Sathyanarayana et al. 2010). Exposure was based on mother's self-reported pesticide use, and no significant associations were identified.

Atrazine is one of the most used herbicides in agriculture. Although it is not bioaccumulative and breaks down readily in soil and air, it has a long half-life in water and hence poses a potential exposure threat to the general population (ATSDR 2003). Atrazine exposure can be assessed via biomarkers, but all three studies investigating its relationship with preterm birth thus far have estimated exposure by measuring levels in sources of drinking water and assigning subjects' exposure levels based on their proximity to those sources, potentially incurring ecologic bias. The first study examined rates of preterm birth in an agricultural district of France in association with atrazine levels in drinking water distribution units assessed regularly by the district health and social affairs bureau (*n*=3,510) (Villanueva et al. 2005). Slight increases in the odds of preterm birth in association with the mothers' assigned exposure level were noted, although the results did not reach statistical significance. Notably, a high proportion of atrazine measurements in water were below the detection limit in this study.

The second study, by Ochoa-Acuña and colleagues (2009), involved residents in Indiana whose atrazine exposure levels were designated by use of specific Community Water Systems (CWS) where measurements were previously made (*n*=24,154). In this analysis no significant change in prevalence of preterm delivery was identified in association with atrazine exposure category (Ochoa-Acuña et al. 2009). The third and most recent study, however, did report a significant rise in the odds of preterm birth in relation to increased atrazine levels in drinking water. This cross-sectional analysis of all births in Kentucky from 2004-2006 (*n*=71,768) assigned exposure to mothers with average levels measured in their county of residence from 2000-2008 (Rinsky et al.

2012). Despite a high % of measures below assay limits of detection, Rinsky et al. (2012) observed a significantly elevated OR for the group exposed to the highest levels of atrazine compared to the lowest (≥0.081 μg/L vs. 0 μg/L; OR=1.22, 95%CI=1.16 to 1.29).

Limitations and recommendations for other environmental contaminants

For non-persistent pesticides and potential endocrine disrupting compounds, very few studies exist examining their relationship with preterm birth. Evidence is conflicting for an effect of phthalates or atrazine; and although single studies identified relationships with preterm birth for BPA and organophosphate pesticides, additional studies to confirm or refute are necessary. Other issues that need to be addressed within this group of exposures include the following. First, more rigorous exposure assessment methods are necessary, particularly with respect to atrazine. Assigning subject-specific contamination levels by taking measurements in home water samples, adjusting for individual subject water-use habits (e.g., use of tap water vs. bottle water), or utilizing biomarkers of exposure would all improve on the current studies. For phthalates and BPA, exposure biomarkers have been utilized but studies have been small. In addition, exposure assessment using biomarkers is limited by the fact that these compounds are rapidly metabolized in the human body. Using a single urine sample as an exposure metric may hence lead to measurement error and bias in results. Utilization of multiple exposure measurements throughout pregnancy would improve these estimates, and also help to identify potentially sensitive time points in gestation. Second, as some of these compounds have the potential to act through similar mechanisms, and, as exposure to a single compound might frequently coincide with exposure to another, assessment of effects of mixed exposures needs to be addressed.

SYNOPSIS AND CONCLUSIONS

Overall, the current literature examining associations between environmental contaminant exposures and preterm birth indicates strongly suggestive evidence for effects of some exposures, while results for others are inconclusive: (1) For POP, evidence is strong for a relationship between DDE exposure at high levels and preterm

birth. Data is suggestive for DDE at lower levels and also for PFC, particularly in studies where biomarkers of exposure were used. Associations with other organochlorine pesticides, PCB, and PBDE are inconclusive; (2) For Drinking Water Contaminants examined in this review, including THM, HCA, PCE, and TCE, results from studies of preterm birth are inconclusive; (3) With regards to air pollution exposures, including criteria air pollutants and ETS (reviewed elsewhere), as well as PAH and VOC (reviewed here), support is strong for a relationship between SO_2, PM, ETS, and PAH exposure and preterm birth, whereas results for other contaminants, including ozone, CO, NO_x, and VOC are inconclusive; (4) Metal and metalloid studies suggest strong associations between high Pb exposure and preterm birth, but analyses with lower levels of Pb exposure, and those examining Cd, As, and HG, remain inconclusive; and finally (5) other environmental contaminants, including phthalates, BPA, organophosphate pesticides, and atrazine, have been insufficiently studied in this context and results are inconclusive.

Additional studies with robust study designs emphasizing larger numbers of preterm deliveries are necessary. A clear limitation for assessment of potential effects related to several exposures is the small number of studies, despite the call for research on potential environmental causes of preterm birth from the Surgeon General and the Institute of Medicine (Behrman and Butler 2007; Ashton et al. 2009). Additionally, many of the existing studies suffer from small sample sizes and/or small numbers of preterm deliveries. Interestingly, in many instances, the proportions of preterm births in cohort populations are lower than would be expected in their respective countries or communities. This could be a result of truly lower percentage in populations studied, lower participation from individuals with greater likelihood of delivering preterm, or a decrease in preterm deliveries due to selection criteria. Lower participation by individuals who eventually deliver preterm is a challenging issue to address, and may not be a significant problem if that probability is unrelated to exposure status. Selection criteria, on the other hand, need to be more carefully considered in study design. Some advantages to excluding subjects based on criteria that are unrelated to exposure exist. However in some instances there may be, despite being unidentified, relationships between risk factors for preterm birth and environmental exposures. For example,

hypertension during pregnancy, a common exclusion factor, may be related to environmental exposures as well, and in fact could be part of the mechanistic pathway underlying a relationship between that exposure and preterm birth. Investigating such variables during statistical analysis as opposed to initial exclusion could not only improve ability to detect associations but also provide insight into why those relationships exist. Information to be gained needs to be balanced with budget considerations, however, because in order to make a clear comparison between such subgroups a larger sample size is necessary.

Nested case-control populations may be particularly useful, but potential consequences of using this study design deserve more careful consideration. Case-control studies have similar issues with small sample size and potentially problematic exclusion criteria. The latter is compounded by the fact that most case-control studies measuring exposures during pregnancy, particularly those utilizing biomarkers, are nested within larger prospective cohorts. So, while case-control studies may be useful in offering more power in assessing associations between environmental exposures and preterm birth, a frequent consequence is the constraints of the design of the parent cohort study. The exclusion criteria and other study characteristics of the original study need to be carefully considered before undertaking a nested analysis. In general, however, when these limiting factors are minimized, nested case-control studies offer much power for assessing these associations. Further, power in these analyses can be amplified with the creation of collaborations between multiple studies, as has been done recently with European birth cohorts (Vrijheid et al. 2012).

Exposure assessment methods need to address effects of contaminants at a range of exposure levels and at additional time points during gestation. Exposure assessment approaches are limited across the literature on this topic as they are with others. These have been described in regard to individual exposures above. However, several overarching issues exist which should be addressed in future research. First, more precise exposure assessment metrics are necessary. In some instances this may require pairing measurements of contaminant levels in drinking water with questionnaire data to provide more complete information about maternal intake. It may also mean the use of biomarkers of exposure. These measures, however, should be interpreted with caution.

For example, maternal physiologic changes that occur during pregnancy can affect where a contaminant is compartmentalized and how diluted it is in various matrices which may greatly impact concentrations measured in biologic specimens. As some of these factors in turn may be related to preterm birth, there is potential for confounding or the possibility for reverse causation in observed associations. For non-persistent compounds, the nature of exposure sources and pathways as well as toxicokinetics for a particular agent, which can greatly impact within-person variability and likelihood for exposure measurement error, need to be carefully considered.

Second, timing of exposure needs to be considered more carefully (Makri et al. 2004). Whether it occurs during the first or third trimester, or uniformly across pregnancy, may be a particularly important component of these relationships. For instance, as suggested by Cantonwine et al. (2010a), Pb susceptibility may be particularly high during the second trimester of pregnancy, but few studies examined the exposure-preterm birth relationship with measures from that time-period. Therefore, real effects may be obscured by lack of studies using exposure measurements from the most sensitive time in gestation. Furthermore, better understanding sensitive time periods of exposure may also be crucial for identifying the mechanisms of effect; for example, exposures during the first trimester may be impairing placentation whereas those in the third trimester may be activating inflammation pathways directly feeding into preterm parturition pathways. While studies among highly exposed populations (i.e., those subjected to specific occupational or environmental sources of exposure) are vital for detecting associations and providing valuable dose-response information at the high end of the exposure distribution, close attention needs to also be paid to effects at lower levels of exposure. It will be especially important, as efforts are made to reduce particularly high exposures (as with Pb), to determine whether thresholds of effect exist for chemical associations with preterm birth, and to see what shape dose-response relationships follow.

Dichotomizing preterm birth by the 37 week cutoff may be insufficient. Studies have fairly consistently used the clinically recognized time point of 37 completed weeks gestation as a cutoff for indicating preterm birth. This is useful because it offers comparability in the literature both within the context of each exposure and also in relation to mortality and morbidities associated with being born preterm. However,

several different aspects of preterm birth may be important to measure in these studies as well. First, preterm births can be separated into multiple categories which few studies in this context have done previously. Preterm births can be both spontaneous, due to largely unknown factors, or they can be induced, which is generally the result of maternal or fetal complications. Combining these categories may be diluting effects. For example, if environmental exposures are playing a larger role in spontaneous preterm births, but not in those that are induced, pooling all cases may be causing, in effect, outcome misclassification and a null bias in results. Spontaneous preterm births can be further categorized as well. McElrath and colleagues (2008), using data from extremely preterm births (<28 weeks) suggested that these data should be divided into two groups based on etiology for epidemiologic studies. These groups include: (1) cases arising from intrauterine inflammation; and (2) those resulting from abnormal placentation. Other divisions, by biological mechanism or clinical presentation, have also been identified previously (Klebanoff and Shiono 1995; Savitz 2008; Savitz et al. 1991). Despite increases in cost and sample size, investigating these more specific outcomes might be helpful in identifying environmental chemical exposures that contribute to risk of preterm birth and also in explaining mechanisms of chemical toxicity.

A second question that arises in assessing preterm birth as an outcome is the 37 week cutoff. In addition to this division, many papers have also examined the relationship between environmental exposures and gestational age at delivery as a continuous outcome. The limitation to this approach is that a change in number of days gestation may be difficult to interpret in terms of clinical relevance, whereas preterm and very preterm have clear adverse associations. However, newer studies suggest that there may be no threshold for an effect of gestational age on neonatal mortality or adverse health outcomes later in life (Boyle et al. 2012; Clark et al. 2009; Zhang and Kramer 2009). A better understanding of the value in measuring associations with preterm birth compared to this continuous measure deserves attention.

A better understanding of pathways by which contaminants cause preterm birth is crucial. Another aspect that has been insufficiently recognized in this area is the understanding of mechanisms that may connect environmental exposures with preterm birth. Identifying the underlying pathways could help to significantly improve future

study in many ways: (1) As mentioned previously, understanding mechanism could improve study design and analysis, and the ability to detect true associations; (2) Identifying potential mechanisms in epidemiologic studies could fuel toxicological lab research on the same topic, and combining efforts in these fields could produce powerful results; and (3) Knowledge of a pathway connecting an environmental exposure to preterm birth could inform interventions for remediating effects, where reducing or eliminating exposures is not possible.

No studies using contaminant-specific exposure measurements have examined the relationship between preterm birth and mixed exposures. Assessing exposure to mixtures of chemicals is an important next step (de Rosa et al. 2004). Since pregnant women are exposed to many different toxicants throughout the duration of pregnancy, this is a more realistic approach to assessing the relationship between a mother's environment and preterm birth. In addition, if many chemicals are acting through similar or complementary mechanisms, there may be additive effects of these combinations that deserve to be explored.

In conclusion, many suggestive relationships have been demonstrated between environmental contaminants and preterm birth, but a larger number of well-designed studies are necessary to draw clear conclusions in order to positively impact public health and clinical practice.

HUMAN EXPOSURE TO PHTHALATES AND ASSOCIATED EFFECTS

Phthalate diesters differ by length of alkyl side chains, and can be divided into two groups based on their corresponding molecular weights. High molecular weight phthalates, such as di-2-ethylhexyl phthalate (DEHP) and di-n-octyl phthalate (DNOP), are used primarily to soften plastics for products like vinyl floor coverings, raincoats, food packaging materials, medical tubing, and blood storage bags (ATSDR 1997c, 2002d). Phthalates can be released into the environment easily during manufacture and use, or following disposal. Human exposure to these compounds occurs most commonly through ingestion of contaminated food or water. Low molecular weight phthalates, such as benzylbutyl phthalate (BzBP), dibutyl phthalate (DBP), and diethyl phthalate (DEP), can also be used as plasticizers but are frequently used as solvents in personal cosmetic

products, such as hairspray, nail polish, deodorants, and lotions, as well as paints, adhesives, and pesticides (ATSDR 1995b, 2001). Phthalates from these products can be volatilized into the air, and human exposure can occur orally as well as through inhalation and dermal absorption.

Once absorbed, diesters are hydrolyzed via phase I biotransformation (Hauser and Calafat 2005). For example, BzBP is broken down into mono-benzyl phthalate (MBzP). Some metabolites, such as mono-2-ethylhexyl phthalate (MEHP), a product of DEHP, are transformed further. MEHP is transformed into mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP). The monoester metabolites are thought to be the primary mediators of toxic effects.

Phthalate metabolites are excreted in urine, and levels are commonly measured as biomarkers of human exposure, both for assessment and epidemiologic purposes (Table I.6). While some studies have used other methods, such as personal air monitoring or proximity to phthalate-containing products (e.g., time spent in spaces with vinyl flooring), urinary analysis is preferred because it captures the individual's exposure via all possible routes. Also, urinary measures are preferred to those made in blood. Enzymes in serum or plasma that can hydrolyze parent diesters to their metabolites make monoester levels in this matrix too variable for an accurate exposure assessment.

In addition to measuring metabolites individually, some cumulative measures are used in assessing exposure. Summed high molecular weight or low molecular weight phthalates are used on occasion, although sums of all phthalate metabolites are not useful in epidemiologic studies because phthalate compounds may have dramatic differences in toxic effects. Summed metabolites of individual parent compounds, for example MEHP, MEHHP, MEOHP, and MECPP (metabolites of DEHP), or mono-n-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP) (metabolites of DBP) are used in some studies. Also, it has been hypothesized that MEHP%, a variable created from the proportion of MEHP compared to the oxidized metabolites of DEHP (MEHHP, MEOHP, and MECPP), is a valuable marker indicative of not only levels of exposure, because of its relatively low variability over time, but also, potentially, of the individual's ability to

metabolize DEHP and susceptibility to its toxic effects (Hauser 2008; Meeker et al. 2012).

Measuring phthalate metabolites in urine is not without complications. The most important issue is determining if the levels observed are representative of current exposure or, additionally, exposure over a longer period of time. Most analyses, including US biomonitoring in the National Health and Nutrition Examination Survey (NHANES), utilize a spot urine sample only. Two recent studies have suggested that levels of phthalates in spot urine samples may be predictive of daily averages, or at least that intra-individual variability is not improved by taking 24-hour or first-morning void measures (Christensen et al. 2012; Preau et al. 2010). However, some metabolites may be more variable than others and adjusting for time of day of sample collection is crucial (Aylward et al. 2011).

Because phthalates are rapidly metabolized and excreted in the urine within a few hours of exposure, levels measured in urine are not indicative of phthalate exposure more than a day before sample. However, because exposure corresponds highly to dietary and personal product use habits, a single measurement of exposure may be correlated with levels over a broader period of time. Hauser and colleagues examined correlations between multiple spot urine samples taken over a three-month period in men of reproductive age, and concluded that a single measurement may be moderately predictive of an individual's average exposure (Hauser et al. 2004). Also, a group that studied variability in the phthalate levels in women before and during pregnancy found that single spot urine measures of MnBP and MEP were predictive of average levels across gestation (Braun et al. 2012). The authors also observed, however, that metabolite measurements were more variable during pregnancy than before, which may complicate studies on *in utero* exposures and birth outcomes.

Another issue in utilizing phthalate metabolites levels in urine samples to represent individual exposure is how to adjust for urine dilution. Many studies use a creatinine-correction approach, dividing phthalate measurements by creatinine levels for a final value in nanograms per gram creatinine. These values are used for analyzing data for population distributions and some bivariate associations, and in regression models creatinine is included as a covariate (Barr et al. 2005). However, due to the high

variability of creatinine levels in urine, particularly in pregnant women, this method may be inappropriate (Boeniger et al. 1993; Lorber et al. 2011). Adjustment using urine specific gravity, which reflects changes in urine concentration but not metabolism, is preferable. Phthalate metabolite levels can be corrected for specific gravity using the following equation: $P_c = P[(SG_{median} -1)/SG – 1)]$, where P_c is the corrected phthalate level, P is the originally measured level in an individual urine sample, SG_{median} is the median specific gravity measurement in the population of interest, and SG is the specific gravity measurement for the individual urine sample (Meeker et al. 2009b). As with creatinine, it is appropriate to use these specific gravity-corrected phthalate levels for examining population distributions and for performing bivariate analysis, and in multivariate regression analysis including specific gravity as a separate covariate is preferable.

With that being said, a number of studies in various populations have utilized urinary biomarkers to examine exposure to phthalates. Overall, these have unequivocally demonstrated ubiquitous exposure in both the US and elsewhere. In pregnant women, exposure is similarly widespread. Furthermore, an analysis of maternal phthalate levels before and during pregnancy in the same women showed that some urinary metabolites levels were significantly higher during pregnancy than they were before (Braun et al. 2012). These differences could be due to increased phthalate exposure during pregnancy, for example, if pregnant women use particular phthalate-containing products more than non-pregnant women, or it could be due to a change in phthalate metabolism that occurs during gestation. Further exploration deserves closer attention in exposure research studies.

Some of the wide variability in urinary phthalate levels in human populations corresponds to several demographic factors. Differences exist by sex, with women having significantly higher levels of MEP and MBzP compared to males, although both groups are similar for measures of MEHP (Silva et al. 2004b). There is also variation by age group; adolescent MBP, MBzP and MEHP levels are higher than those observed in adults, and MEP levels are lower (Silva et al. 2004b). Furthermore, metabolite levels differ by an individual's race and ethnicity as well as their body mass index (BMI) (Silva

et al. 2004b; Hatch et al. 2008). Hence these variables are important to consider either as covariates or effect modifiers in epidemiologic studies.

Human studies have identified a range of adverse human health outcomes that may be associated with phthalate exposure. Some of the seminal literature on phthalate exposure and human health examined maternal levels of urinary metabolites during pregnancy and observed associations with decreased anogenital distance in male infants (Swan et al. 2005), which displayed in humans the anti-androgenic effects of phthalates that had been observed previously in rodent studies (Foster et al. 2000; Gray et al. 2000; Parks et al. 2000). Other studies have confirmed this association in humans (Bustamante-Montes et al. 2013; Huang et al. 2009), and some have additionally identified relationships between *in utero* exposure and other developmental abnormalities of the reproductive tract, such as cryptorchidism, but those findings have been conflicting (Chevrier et al. 2012; Gaspari et al. 2011). Similar studies in females have been sparse (Kay et al. 2013).

Cross-sectional relationships between phthalate exposure measurements and circulating endocrine hormones and/or related outcomes have been identified in a number of studies in adolescents and adults. In adult men, urinary phthalate metabolite levels have been linked to reduced testosterone levels as well as other sex hormones (Duty et al. 2005; Meeker et al. 2009c) and also to reduced semen quality parameters (Duty et al. 2003a; Duty et al. 2003b; Hauser et al. 2006; Hauser et al. 2007). Similar associations between phthalates and hormone levels have been identified in male children and adolescents (Durmaz et al. 2010; Mieritz et al. 2012; Mouritsen et al. 2013). Again, studies in women are few, but some have identified associations between phthalate levels and hormone related disease, such as endometriosis and uterine leiomyomata (Huang et al. 2010; Upson et al. 2013; Weuve et al. 2010). Studies in female children have identified associations between exposure and premature thelarche as well as changes in sex hormone levels (Mouritsen et al. 2013; Colon et al. 2000).

Although the mechanism is less clear, phthalates have also been linked to alterations in thyroid hormone levels in children and adults (Meeker et al. 2007; Meeker and Ferguson 2011) as well as in pregnant women (Huang et al. 2007), potentially through antagonistic action on the thyroid hormone receptor (Sugiyama et al. 2005).

These changes may be important for a number of downstream health effects, but of particular concern are the effects of *in utero* thyroid hormone levels on child neurodevelopment. This concern has sparked a number of studies examining prenatal phthalate exposure in relation to cognition, intelligence, and autism spectrum disorders among other neurological endpoints in infants and young children (Cho et al. 2010; Engel et al. 2010; Engel et al. 2009; Kim et al. 2009).

A final set of endpoints which have received significant attention in association with phthalate exposure are birth outcomes. As discussed above, the previous studies of phthalate exposure and preterm birth or gestational age at delivery showed conflicting results, potentially due to limitations in exposure assessment methods, samples sizes, and inability to examine different subtypes of preterm birth by clinical presentation. In addition, a number of papers have examined the relationship between phthalate exposure during pregnancy and other adverse birth outcomes, such as fetal growth. One study found significant relationships between summed low molecular-weight phthalates as well as MEP alone and increased head circumference at birth, and also between MBzP and increased birth length (Wolff et al. 2008b). A study of women with occupational exposures to phthalates corroborated these findings and additionally found an association between phthalates and reduced placental weight (Snijder et al. 2012). However, two other studies found no significant relationships between phthalate metabolites and birth weight (Suzuki et al. 2010b; Philippat et al. 2012). A major limitation of these studies is the use of measurements at delivery only to examine fetal growth.

OXIDATIVE STRESS AS A MECHANISM FOR PHTHALATE ACTION

The mechanisms underlying the observed relationships between phthalate exposure and adverse birth outcomes may be diverse, and are currently poorly understood. Disrupted endocrine activity may certainly be a contributing factor. Particularly, effects of phthalates on circulating maternal thyroid hormone levels may be important as thyroid hormones are crucial for normal fetal development and growth. However, an additional mechanism of interest which has been understudied is the action of phthalates through induction of oxidative stress.

The ability of phthalates to bind and activate peroxisome proliferator activated receptors (PPARs) has been well-characterized (Lapinskas et al. 2005; Maloney and Waxman 1999). Binding may cause increased intracellular oxidative stress by overly activating certain enzymes involved in reactive oxygen species (ROS) generation but only slightly activating those involved in their degradation (Citron 1995; Reddy and Rao 1989). This action may lead to systemic increases in oxidative stress which could have a range of downstream effects. For example, this mechanism could lead to altered metabolism, obesity, and development of type II diabetes. These outcomes have been linked to phthalate exposure in a handful of epidemiologic studies (Hatch et al. 2008; Stahlhut et al. 2007). Also, as oxidative stress is linked to increased inflammation, effects of phthalates on asthma and allergic symptoms are possible. Such associations have been identified in cross-sectional studies of adults (Hoppin et al. 2004; Jaakkola et al. 2006) and also in children with *in utero* exposures (Bornehag et al. 2004; Kolarik et al. 2008).

Systemic changes in oxidative stress levels could also play a role in the relationship between phthalate exposure and preterm birth. One hypothesis is that increased oxidative stress levels, particularly near the end of pregnancy, may cause an inflammatory response that prematurely initiates a cascade of events, including release of pro-inflammatory cytokines, prostaglandins, and physiological changes such as cervical ripening that lead to preterm parturition (Challis et al. 2009). Results from some epidemiologic studies support the concept that maternal oxidative stress could lead to preterm birth. Stein and colleagues showed increased 8-hydroxydeoxyguanosine (8-OHdG) levels, indicative of oxidative DNA damage, to be associated with lower infant birth weight as well as shorter gestational duration (Peter Stein et al. 2008). The group also found that increased isoprostane levels, associated with lipid oxidative damage, were associated with increased risk of preeclampsia, a strong predictor of preterm birth (Peter Stein et al. 2008). The latter finding was further supported by work done by Mehendale et al. who reported an increase in circulating serum antioxidants, such as α-tocopherol and ascorbic acid, and decrease in oxidative stress, indicated by serum malondialdehyde (MDA) levels, in preeclamptic mothers (Mehendale et al. 2008). Lastly, using MDA as a marker, one group observed higher levels of oxidative stress in women who delivered preterm compared to those who delivered full term (Joshi et al. 2008; Pathak et al. 2010).

On top of existing cellular and animal data, there is some human evidence suggesting an association between exposure to phthalates and increases in circulating oxidative stress levels. Three large cross-sectional studies observed positive associations between various metabolites and the oxidative stress markers MDA, 8-OHdG, gamma-glutamyl transferase (GGT) and bilirubin (Ferguson et al. 2011a; Hong et al. 2009; Ferguson et al. 2011b). In a "temple-stay" study in Korea researchers found a decrease in MDA levels in association with decreased urinary phthalate metabolite levels in participants following a strict diet regimen over a five-day period (Ji et al. 2010). Also a study in infants showed a significant increase in circulating DEHP levels, and associated increase in MDA levels, following parenteral nutrition treatment with phthalate-containing materials (Kambia et al. 2011). These relationships, though suggestive, are tenuous. Improved data could be obtained by examining repeated measures of both phthalate exposure and oxidative stress biomarkers.

Combined, these data provide strongly suggestive evidence that phthalate effect on reproductive outcomes could occur through the induction of oxidative stress. Unfortunately, exploration of this mechanism in animal studies is not possible because of the lack of suitable animal model for studying preterm birth. Therefore, investigation of these relationships in humans is necessary.

RESEARCH AIMS AND HYPOTHESES

It is the aim of this dissertation to address how environmental exposure to phthalates during pregnancy contributes to the critical issue of preterm birth, and to examine the role of oxidative stress in mediating that relationship. It improves on previous studies of phthalates and also on many other environmental contaminants by examining this question in a large sample size with multiple exposure measurements taken over pregnancy, and adds substantially to the literature by investigating a poorly understood mechanism that has not been previously tested in humans. The specific aims and hypotheses for this work are as follows. First, I examine the association between average exposure to phthalates across pregnancy in relation to preterm birth, with the hypothesis that maternal exposure is associated with increased risk of preterm delivery. Second, with closer attention to the changes in phthalate exposure across gestation, I

explore sensitive windows of exposure for the relationship between urinary phthalate metabolites and prematurity. For this aim I hypothesize that phthalate excretion patterns are somewhat variable across pregnancy, but levels later in pregnancy are most strongly associated with increased risk of preterm birth. Third and finally I investigate the role of oxidative stress in mediating the relationship between phthalate exposure and preterm birth with biomarkers measured in urine at multiple time points during pregnancy. Analyses include assessment of the relationship between those biomarkers of phthalate exposure and of oxidative stress as well as the relationship between oxidative stress biomarkers and preterm birth. Here I hypothesize that urinary phthalate metabolites are associated with increased oxidative stress biomarkers which are predictive of increased risk of preterm birth. These concerted findings provide evidence for a mechanistic link between phthalate exposure and prematurity.

Table I.1 Studies of persistent organic pollutants in relation to preterm birth

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Saxena et al. 1981	Case-control	Lucknow, India	*n*=40 preterm=15	Delivery	Maternal blood and placental tissue	*p,p'*-DDT	4.5±4.1 ppb
						p,p'-DDE	12.6±7.0 ppb
						p,p'-DDD	6.9±7.9 ppb
						HCB	52.2±18.2 ppb
						Lindane	18.9±8.80 ppb
						Aldrin	11.1±7.30 ppb
Procianoy and Schvartsman 1981	Case-control	Sao Paulo, Brazil	*n*=54 preterm=24	Delivery	Maternal and cord blood	*p,p'*-DDT	10.5±10.6 μg/L
						p,p'-DDE	20.0±13.6 μg/L
Wassermann et al. 1982	Case-control	Jerusalem, Israel	*n*=27 preterm=17	3rd trimester to delivery	Maternal serum	*p,p'*-DDT	2.9±3.0 ppb
						p,p'-DDE	10.7±6.1 ppb
						p,p'-DDD	3.3±2.2 ppb
						∑PCBs	19.3±10.3 ppb
						HCB	4.3±4.8 ppb
						Dieldrin	1.1±1.4 ppb
						Heptachlor	3.0±4.2 ppb
Berkowitz et al. 1996	Case-control	New York, NY	*n*=40 preterm=20	1st trimester	Maternal serum	*p,p'*-DDE	1.35 ng/mL†
						∑PCBs	1.70 ng/mL†
Longnecker et al. 2001	Cohort	11 US cities	*n*=2,380 preterm=361	3rd trimester	Maternal serum	*p,p'*-DDE	25.0 μg/L†
Ribas-Fitó et al. 2002	Cohort	Flix, Spain	*n*=72 preterm=4	Delivery	Maternal and cord serum	*p,p'*-DDE	0.85 ng/mL†
						∑PCBs	0.27 ng/mL†
						HCB	1.13 ng/mL†
						β-HCH	0.54 ng/mL†

Table I.1 *(Continued)*

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Torres-Arreola et al. 2003	Case-cohort	Mexico City, Mexico	*n*=233 preterm=100	Delivery	Maternal serum	*p,p'*-DDE	153 ng/g lipid[†]
						HCB	45.8 ng/g lipid[†]
						β-HCH	54.3 ng/g lipid[†]
Farhang et al. 2005	Cohort	San Francisco, CA	*n*=420 preterm=33	2nd trimester to delivery	Maternal serum	DDT	11 μg/L[†]
						DDE	43 μg/L[†]
Longnecker et al. 2005	Cohort	11 US cities	*n*=1,034 preterm=132	3rd trimester	Maternal serum	∑PCBs	2.80 μg/L[†]
Apelberg et al. 2007	Cross-sectional	Baltimore, MD	*n*=293 preterm=38	Delivery	Cord serum	PFOA	1.6 ng/mL[†]
						PFOS	5 ng/mL[†]
Fei et al. 2007	Cohort	Denmark	*n*=1,400 preterm=53	1st trimester, 2nd trimester, and delivery	Maternal and cord plasma	PFOA	5.6±2.5 ng/mL
						PFOS	35.3±13.0 ng/mL
Wood et al. 2007	Case-control	Alberta, Canada	*n*=78 preterm=26	1 day postpartum	Maternal serum	DDE	69.3 ng/g lipid[†]
Nolan et al. 2009	Cross-sectional	Washington County, Ohio	*n*=1,555 preterm=200	Entire pregnancy	Drinking water	PFOA	0.0-5.7 μg/L[†]
Pathak et al. 2009	Case-control	Delhi, India	*n*=46 preterm=23	Delivery	Maternal and cord blood	*p,p'*-DDT	1.66±1.18 ng/mL
						p,p'-DDE	3.70±2.63 ng/mL
						∑HCH	8.59±8.11 ng/mL
Hamm et al. 2010	Cohort	Alberta, Canada	*n*=252 preterm=21	2nd trimester	Maternal serum	PFOA	1.3±2.9 ng/mL[‡]
						PFOS	7.4±2.0 ng/mL[‡]
						PFHxS	1.1±3.0 ng/mL[‡]

Table I.1 *(Continued)*

Study				Study			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Wojtyniak et al. 2010	Cohort	Greenland	*n*=572 preterm=28	Entire pregnancy	Maternal serum	*p,p'*-DDE	274±2.9 ng/g lipid‡
						PCB-153	105±2.8 ng/g lipid‡
		Kharkiv, Ukraine	*n*=611 preterm=12			*p,p'*-DDE	653±1.8 ng/g lipid‡
						PCB-153	25.7±1.9 ng/g lipid‡
		Warsaw, Poland	*n*=258 preterm=12			*p,p'*-DDE	357±1.9 ng/g lipid‡
						PCB-153	9.0±2.1 ng/g lipid‡
Bergonzi et al. 2011	Cohort	Brescia, Italy	*n*=70 preterm=4	Delivery	Maternal serum and adipose tissue, cord serum, placental tissue	*p,p'*-DDE	124 ng/g lipid‡
						∑PCBs	229 ng/g lipid‡
						HCB	20 ng/g lipid‡
Chen et al. 2012	Cohort	Taipei and New Taipei, Taiwan	*n*=429 preterm~40	Delivery	Cord blood	PFOA	1.84±2.23 ng/mL‡
						PFOS	5.94±1.95 ng/mL‡
						PFNA	2.36±4.74 ng/mL‡
						PFUA	10.3±3.07 ng/mL‡
Arbuckle et al. 2012	Cohort	Ottawa, Canada	*n*=100 preterm=3	Delivery	Cord serum	PFOA	1.47 ng/mL‡
						PFOS	4.44 ng/mL‡
						PFNA	0.36 ng/mL‡
						PFHxS	0.58 ng/mL‡
Savitz et al. 2012a	Cohort	Ohio and West Virginia	*n*=11,737 preterm=1,843	Entire pregnancy	Maternal serum concentrations*	PFOA	6.0-15.9 ng/mL†
Savitz et al. 2012b Study I	Cohort	Ohio and West Virginia	*n*=7,308 preterm=3,613	Early pregnancy	Maternal serum concentrations*	PFOA	7.7 ng/mL†
Savitz et al. 2012b Study II	Cohort	Ohio and West Virginia	*n*=4,547 preterm=405	Early pregnancy	Maternal serum concentrations*	PFOA	13.4 ng/mL†

Table I.1 *(Continued)*

Study				Study			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Whitworth et al. 2012	Cohort	Norway	*n*=901 preterm=35	2nd trimester	Maternal plasma	PFOA	2.2 ng/mL†
						PFOS	13.0 ng/mL†
Wu et al. 2012	Cohort	Guiyu and Chaonan, China	*n*=167 cases=8	Delivery	Maternal serum	PFOA	9.76±5.05 ng/mL

Note. Levels represent means±standard deviations, medians (†), or geometric means±geometric standard deviations (‡) of chemicals measured in all subjects pooled or in controls alone where pooled information was not available. If exposure was assessed in multiple matrices, levels from the bolded matrix are presented. *Maternal serum concentrations in studies by Savitz et al. were calculated from statistical models. Ranges represent minimum to maximum median levels from study subgroups where overall medians not presented. For drinking water levels averages that included values below the limit of detection were preferentially presented. See Figure 1 for results. Abbreviations: Dichlorodiphenyltrichloroethane (DDT); dichlorodiphenyldichloroethylene (DDE); polychlorinated biphenyls (PCBs); hexachlorobenzene (HCB); hexachlorohexane (HCH); perfluorooctane sulfonic acid (PFOS); perfluorohexane sulfonate (PFHxS); perfluorononanoic acid (PFNA); perfluoroundecanoic acid (PFUA); perfluorohexanesulfonate (PFHxS); brominated diphenyl ether (BDE); parts per billion (ppb).

Table I.2 Studies of drinking water contaminants in relation to preterm birth

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Kramer et al. 1992	Case-control	Iowa	*n*=2,052 preterm=342	Entire pregnancy	Drinking water	Chloroform	1 μg/L[†]
Bove et al. 1995	Cross-sectional	New Jersey	*n*=80,938 preterm=7,167	Entire pregnancy	Drinking water	TCE	55 ppb
						PCE	14 ppb
						TTHMs	144 ppb
Savitz et al. 1995	Case-control	North Carolina	*n*=577 preterm=244	3rd trimester	Drinking water	TTHMs	Not reported
Gallagher et al. 1998	Cohort	Denver, CO	*n*=1,893 preterm=68	3rd trimester	Drinking water	TTHMs	Not reported
Dodds et al. 1999	Cohort	Nova Scotia, Canada	*n*=49,842 preterm=3,173	3rd trimester	Drinking water	TTHMs	Not reported
Sonnenfeld et al. 2001	Cross-sectional	Camp Lejeune, NC	*n*=11,798 preterm=832	Entire pregnancy	Drinking water	PCE	Not reported
Wright et al. 2003	Cross-sectional	Massachusetts	*n*=56,513 preterm=3,173	Entire pregnancy	Drinking water	TTHMs	25.5-34.8 μg/L[†]
Wright et al. 2004	Cross-sectional	Massachusetts	*n*=196,000 preterm=11,580	3rd trimester	Drinking water	TTHMs	38.2±27.0 μg/L
						HAAs	31.4±13.6 μg/L
Aschengrau et al. 2008	Cohort	Massachusetts	*n*=2,125 preterm=96	Conception	Drinking water	PCE	0.9 g/month per residence
Lewis et al. 2007	Case-control	Massachusetts	*n*=37,498 preterm=2,813	1st-3rd trimester and pregnancy average	Drinking water	TTHMs	Not reported
Yang et al. 2007	Cohort	Taiwan	*n*=90,848 preterm=2,818	Entire pregnancy	Drinking water	TTHMs	Not reported

Table I.2 *(Continued)*

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Hoffman et al. 2008	Cohort	3 US sites	*n*=2,039 preterm=185	2nd trimester	Drinking water	TTHMs	42.4±32.4 μg/L
						HAA5	20.0±17.3 μg/L
						TOX	119±79.8 μg/L
Forand et al. 2011	Cross-sectional	New York	*n*=1,090 preterm=93	Delivery	Indoor air	TCE	0.18-140 $\mu g/m^3$
			n=350 preterm=20			PCE	0.1-24 $\mu g/m^3$
Horton et al. 2011	Cohort	US site with brominated DBP contamination	*n*=3,946 preterm=438	2nd trimester	Drinking water	TTHMs	60.4±20.7 μg/L
						HAAs	21.5±5.9 μg/L
						TOX	186±35.1 μg/L
		US site with chlorinated DBP contamination	*n*=27,177 preterm=2,201			TTHMs	63.3±23.1 μg/L
						HAAs	33.2±12.1 μg/L
						TOX	171±37.3 μg/L
Patelarou et al. 2011	Cohort	Crete, Greece	*n*=1,359 preterm=156	Each trimester and pregnancy average	Drinking water	TTHMs	3.71±5.75 μg/L
Villanueva et al. 2011	Cohort	5 sites in Spain	*n*=2,074 preterm=77	Each trimester and pregnancy average	Drinking water	TTHMs	5.9-114.7 μg/L
Costet et al. 2012	Case-control	Brittany, France	*n*=513 preterm=114	1st trimester	Maternal urine	TCAA	0.03 mg/L†
				3rd trimester	Drinking water	TTHMs	41.6±16.1 μg/L

Note. Levels represent means±standard deviations, medians (†), or geometric means±geometric standard deviations (‡) of chemicals measured in all subjects pooled or in controls alone where pooled information was not available. Ranges represent medians across multiple seasons for Wright et al. (2003), range of indoor air concentrations observed for Forand et al. (2011), and medians across multiple sites for Villanueva et al. (2011). Table adapted in part from Grellier et al. (2010). Exposures estimated from drinking water levels were often combined with modeling of data on maternal water consumption and household use. In some studies levels for individual THM or HAA were presented, however they are not included here for the sake of brevity. See Figure 2 for results. Abbreviations: Trichloroethylene (TCE); tetrachloroethylene (PCE); total trihalomethanes (TTHMs); haloacetic acids (HAAs); total organic halides (TOX); trichloroacetic acid (TCAA); parts per billion (ppb).

Table I.3 Studies of atmospheric pollutants in relation to preterm birth

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Vassilev et al. 2001	Cross-sectional	New Jersey	*n*=214,493 preterm=13,989	Entire pregnancy	Ambient air monitoring	Polycyclic organic matter	0.49 μg/m^{3†}
Choi et al. 2008	Cohort	New York, NY	*n*=351 Dominicans preterm=8	3rd trimester	Personal air monitoring	∑PAHs	3.15±3.42 ng/m^3
			n=205 African Americans preterm=12			∑PAHs	3.32±3.38 ng/m^3
Singh et al. 2008	Case-control	Lucknow, India	*n*=60 preterm=29	Delivery	Placenta	Naphthalene	250±47.6 ppb
						Acenaphthylene	58.7±42.5 ppb
						Phenanthrene	378±79.5 ppb
						Anthracene	25.8±8.08 ppb
						Fluoranthene	209±21.9 ppb
						Pyrene	296±91.6 ppb
						Benzo(*f*) fluoranthene	29.9±22.3 ppb
						Benzo(*b*) fluoranthene	23.8±7.01 ppb
						Benzo(*a*)pyrene	8.83±5.84 ppb
						Dibenzo(*a*,*h*) anthracene	22.0±17.1 ppb
Maroziene and Grazuleviciene 2009	Cross-sectional	Kaunas, Lithuania	*n*=3,988 preterm=203	Entire pregnancy	Ambient air monitoring	Formaldehyde	3.14±2.36 μg/m^3
Llop et al. 2010	Cohort	Valencia, Spain	*n*=785 preterm=47	Each trimester	Ambient air monitoring	Benzene	2.2±0.6 μg/m^3

Table I.3 *(Continued)*

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Wilhelm et al. 2011	Cohort	Los Angeles, CA	*n*=112,915 preterm=10,265	Each trimester and pregnancy average	Ambient air monitoring	Napthalene	182±34.6 μg/m^3
						Benzo(*a*)pyrene	0.13±0.05 μg/m^3
						Benzo(*g*,*h*,*i*)perylene	0.32±0.08 μg/m^3
						∑PAHs	221±38.6 μg/m^3
						Benzene	0.66±0.16 μg/m^3

Note. Levels represent means±standard deviations or standard errors or medians (†) of chemicals measured for all subjects pooled or in controls alone where pooled information was not available. See Figure 3 for results. Abbreviations: Polycyclic aromatic hydrocarbons (PAHs).

Table I.4 Studies of metals and metalloids in relation to preterm birth

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Metal/metalloid	Level
Fagher et al. 1993	Case-control	Sweden	*n*=30 preterm=17	Delivery	Maternal blood, placenta, myometrium	Lead	11.2±2.9 ug/L
						Cadmium	1.1±1.7 ug/L
		Poland				Lead	37.9±17.2 ug/L
						Cadmium	1.8±1.3 ug/L
Landgren 1996	Cross-sectional	Sweden	*n*=38,718	Entire pregnancy	Ground concentration in municipality	Lead	151 μg/m^3
						Cadmium	1.6 μg/m^3
						Arsenic	93 μg/m^3
						Mercury	0.02 μg/m^3
Torres-Sanchez et al. 1999	Case-cohort	Mexico City, Mexico	*n*=459 preterm=161	Delivery	Cord blood	Lead	10.6±7.13 μg/dL
Ahmad et al. 2001	Cross-sectional	Katiarchar, Bangladesh	*n*=96 preterm=27.1 per 1,000 live births	Entire pregnancy	Drinking water	Arsenic	<0.02 mg/L
		Samta, Bangladesh	*n*=96 preterm=68.8 per 1,000 live births				0.24 mg/L
Sowers et al. 2002	Cohort	Camden, NJ	*n*=705 preterm=72	1st trimester	Maternal blood	Lead	1.22±0.04 μg/dL
				2nd trimester			1.08±0.05 μg/dL
				3rd trimester			1.10±0.03 μg/dL
				Delivery			1.32±0.03 μg/dL
Falcon et al. 2003	Cohort	Murcia, Spain	*n*=89, cases=18	Delivery	Placenta	Lead	103±49.5 ng/g
Yang et al. 2003	Cohort	Taiwan, Taiwan	*n*=14,387 unexposed preterm=494	Entire pregnancy	Drinking water	Arsenic	<0.9 ppb
			n=3,872 exposed preterm=145				>0.9 ppb
Zhang et al. 2004	Cohort	Da-Ye, China	*n*=44 preterm=7	Delivery	Maternal and cord blood, placenta	Cadmium	2.10±2.10 μg/L†

Table I.4 *(Continued)*

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Metal/metalloid	Level
Mukherjee et al. 2005	Cross-sectional	Murshidabad, Bangladesh	*n*=18 preterm=2	Entire pregnancy	Drinking water	Arsenic	<3 µ/L
			n=21 preterm=8				284-400 µ/L
			n=44 preterm=12				401-1474 µ/L
Jelliffe-Pawlowski et al. 2006	Cross-sectional	California	*n*=262 preterm=30	Entire pregnancy	Maternal blood	Lead	Not reported
Xue et al. 2007	Cohort	5 communities in Michigan	*n*=1,024 preterm=101	15^{th}-27^{th} week gestation	Maternal hair	Mercury	0.29 µg/g
Cantonwine et al. 2010a	Cohort	Mexico City, Mexico	*n*=235 preterm=22	1^{st} trimester	Maternal blood and plasma, cord blood	Lead	7.2±5.2 µg/dL
				2^{nd} trimester			6.3±4.3 µg/dL
				3^{rd} trimester			6.8±4.5 µg/dL
Myers et al. 2010	Cross-sectional	Inner Mongolia, China	*n*=9,890 preterm=289	Entire pregnancy	Drinking water	Arsenic	37.6±0.7 µg/L
Vigeh et al. 2011	Cohort	Tehran, Iran	*n*=348 preterm=44	1^{st} trimester	Maternal blood	Lead	3.8±2.0 µg/dL
Zhu et al. 2010	Cohort	New York	*n*=43,288 preterm=3,519	Entire pregnancy	Maternal blood	Lead	2.1 µg/dL

Note. Levels represent means±standard deviations or geometric means±geometric standard deviations (†) of chemicals measured in samples of all subjects pooled or in controls alone where pooled information was not available. If exposure was assessed in multiple matrices, levels from the bolded matrix are presented. For Torres-Sanchez et al. (1999) levels are for primiparous births. For Sowers et al. (2002) preterm birth is defined as <36 weeks gestation. For Zhang et al. (2004) preterm birth is defined as ≤37 weeks gestation. For Myers et al. (2010) levels are taken from Ning et al. (2007). See Figure 4 for results.

Table I.5 Studies of other environmental contaminants in relation to preterm birth

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Eskenazi et al. 2004	Cohort	Salinas Valley, CA	*n*=488 preterm=32	Entire pregnancy	Maternal urine	DAPs	136 nmol/L[†]
						MDA	0.2 µg/L[†]
						PNP	0.5 µg/L[†]
						TCPy	3.3 µg/L[†]
Villanueva et al. 2005	Cross-sectional	Finistére, France	*n*=3,510 preterm=137	Entire pregnancy	Drinking water	Atrazine	0.036 µg/L
Adibi et al. 2009	Cohort	4 US cities	*n*=283 preterm=14	3rd trimester	Maternal urine	MEHP	3.6 ng/mL
						MEHHP	11.9 ng/mL
						MEOHP	10.9 ng/mL
Meeker et al. 2009	Case-control	Mexico City, Mexico	*n*=60 preterm=30	3rd trimester	Maternal urine	MEHP	1.90 µg/L
						MEHHP	13.6 µg/L
						MEOHP	10.4 µg/L
						MECPP	29.7 µg/L
						MBzP	2.30 µg/L
						MBP	38.1 µg/L
						MiBP	1.9 µg/L
						MCPP	1.1 µg/L
						MCOP	Not calculated
						MCNP	Not calculated
						MEP	112 µg/L
Ochoa-Acuña et al. 2009	Cohort	Indiana	*n*=24,154 preterm=1,777	First and last months of pregnancy	Drinking water	Atrazine	0.011-0.996 µg/L[†]
Cantonwine et al. 2010b	Case-control	Mexico City, Mexico	*n*=60 preterm=30	3rd trimester	Maternal urine	BPA	1.52 µg/L

Table I.5 *(Continued)*

Study				Exposure			
Reference	Design	Location	Sample size	Timing	Assessment	Chemical	Level
Rinsky et al. 2012	Cross-sectional	Kentucky	*n*=71,768 preterm=8,915	Entire pregnancy	Drinking water	Atrazine	0.11±0.49 μg/L‡

Note. Levels represent geometric means±geometric standard deviations, medians or median ranges in multiple groups measured (†), or means±standard deviations (‡) of chemicals measured in maternal urine or drinking water of all subjects pooled or in controls alone where pooled information was not available. For Cantonwine et al. (2010b) preterm birth is defined as ≤37 weeks gestation. For Rinsky et al. (2012) mean includes values below the detection limit. For Eskenazi et al. (2004) levels of selected pesticide-specific metabolites are presented. See Figure 5 for results. Abbreviations: Dialkyl phosphates (DAPs); malathion dicarboxylic acid (MDA; malathion metabolite); 4-nitrophenol (PNP; parathion metabolite); 3,5,6-trichloro-2-pyridinol (TCPy; chlorpyrifos metabolite); mono(2-ethylhexyl) phthalate (MEHP); mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP); mono(2-ethyl-5-oxohoexyl) phthalate (MEOHP); mono(2-ethyl-5-carboxypentyl) phthalate (MECPP); monobenzyl phthalate (MBzP); mono-*n*-butyl phthalate (MBP); mono-isobutyl phthalate (MiBP); mono(3-carboxypropyl) phthalate (MCPP); monocarboxyisooctyl phthalate (MCOP); monocarboxyisononyl phthalate (MCNP); monoethyl phthalate (MEP); bisphenol-A (BPA).

Table I.6 Phthalate diesters and their primary metabolites

Di-2-ethylhexyl phthalate (DEHP)	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)
Di-n-octyl phthalate (DOP)	Mono-(3-carboxypropyl) phthalate (MCPP)
Benzylbutyl phthalate (BzBP)	Mono-benzyl phthalate (MBzP)
Di-*n*-butyl phthalate (DBP)	Mono-*n*-butyl phthalate (MnBP
Di-iso-butyl phthalate (DiBP)	Mono-iso-butyl phthalate (MiBP)
Diethyl phthalate (DEP)	Monoethyl phthalate (MEP)

Figure I.1 Odds ratios and 95% confidence intervals for studies of persistent organic pollutants in relation to preterm birth

Exposure	Reference	Exposure corresponding to OR
DDT	Farhang et al. 2005	IQR increase
DDE	Longnecker et al. 2001	15-29 vs. <15 µg/L
		30-44 vs. <15 µg/L
		45-59 vs. <15 µg/L
		≥60 vs. <15 µg/L
	Torres-Arreola et al. 2003	112-229 vs. <112 ng/g lipid
		>229 vs. <112 ng/g lipid
	Farhang et al. 2005	IQR increase
	Wood et al. 2007	Log-unit increase
	Wojtyniak et al. 2010	Two-fold increase (Greenland)
		Two-fold increase (Ukraine)
		Two-fold increase (Poland)
PCBs	Longnecker et al. 2005	2-<3 vs. <2 µg/L
		3-<4 vs. <2 µg/L
		4+ vs. <2 µg/L
	Wojtyniak et al. 2010	Two-fold increase (Greenland)
		Two-fold increase (Ukraine)
		Two-fold increase (Poland)
HCH	Torres-Arreola et al. 2003	24-77 vs. <24 ng/g lipid
		>77 vs. <24 ng/g lipid
HCB	Torres-Arreola et al. 2003	36-62 vs. <36 ng/g lipid
		>62 vs. <36 ng/g lipid
PFOA	Fei et al. 2007	3.91-5.20 vs. <3.91 ng/mL
		5.21-6.96 vs. <3.91 ng/mL
		≥6.97 vs. <3.91 ng/mL
	Nolan et al. 2009	Exposed vs. unexposed
		Partially exposed vs. unexposed
	Hamm et al. 2010	1.1-2.1 vs. <1.1 ng/mL
		>2.1 vs. <1.1 ng/mL

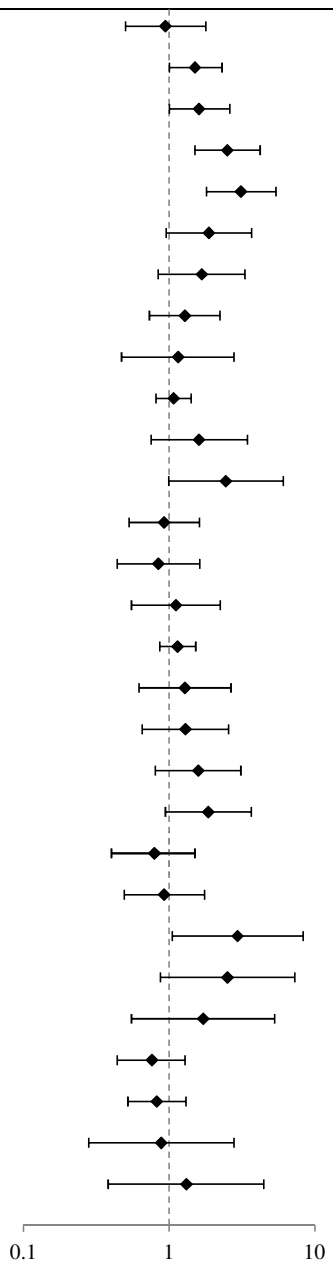
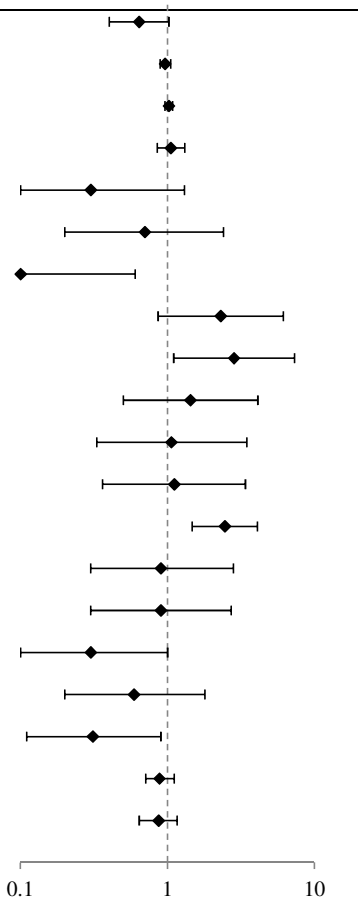


Figure I.1 *(Continued)*

Exposure	Reference	Exposure corresponding to OR
PFOA	Chen et al. 2012	Ln-unit increase
	Savitz et al. 2012a	IQR increase
	Savitz et al. 2012b Study I	IQR increase
	Savitz et al. 2012b Study II	IQR increase
	Whitworth et al. 2012	1.65-2.24 vs. <1.65 ng/mL
		2.25-3.03 vs. <1.65 ng/mL
		≥3.04 vs. <1.65 ng/mL
PFOS	Fei et al. 2007	26.1-33.3 vs. <26.0 ng/mL
		33.4-43.2 vs. <26.0 ng/mL
		≥43.3 vs. <26.0 ng/mL
	Hamm et al. 2010	6.1-10 vs. <6.1 ng/mL
		>10 vs. <6.1 ng/mL
	Chen et al. 2012	Ln-unit increase
	Whitworth et al. 2012	10.3-13.0 vs. <10.3 ng/mL
		13.0-16.6 vs. <10.3 ng/mL
		≥16.6 vs. <10.3 ng/mL
PFHxS	Hamm et al. 2010	0.69-1.4 vs. <0.69 ng/mL
		>1.4 vs. <0.69 ng/ml
PFNA	Chen et al. 2012	Ln-unit increase
PFUA	Chen et al. 2012	Ln-unit increase



Note. Adjusted odds ratios are presented where available. Results for Hamm et al. 2010 are risk ratios. For Longnecker et al. 2005 OR are for summed PCB. For Wojtyniak et al. 2010 OR are for PCB-153. Abbreviations: Dichlorodiphenyltrichloroethane (DDT); dichlorodiphenyldichloroethylene (DDE); polychlorinated biphenyls (PCBs); hexachlorobenzene (HCB); hexachlorohexane (HCH); perfluorooctanoic acid (PFOA); perfluorooctane sulfonic acid (PFOS); perfluorohexane sulfonate (PFHxS); perfluorononanoic acid (PFNA); perfluoroundecanoic acid (PFUA); interquartile range (IQR).

Figure I.2 Odds ratios and 95% confidence intervals for studies of drinking water contaminants in relation to preterm birth

Exposure	Reference	Exposure corresponding to OR
THMs	Kramer et al. 1992	1-9 µg/L vs. nondetect (chloroform)
		≥10 µg/L vs. nondetect (chloroform)
	Savitz et al. 1995	63-83 vs. 41-63 ppb
		83-169 vs. 41-63 ppb
	Gallagher et al. 1998	21-40 vs. <20 ppb
		41-60 vs. <20 ppb
		≥61 vs. <20 ppb
	Dodds et al. 1999	50-74 vs. <50 µg/L
		75-99 vs. <50 µg/L
		>100 vs. <50 µg/L
	Wright et al. 2003	60-80 vs. <60 µg/L
		>80 vs. <60 µg/L
	Wright et al. 2004	33-74 vs. <33 µg/L
		74-163 vs. <33 µg/L
	Lewis et al. 2007	40-60 vs. <40 µg/L
		≥60 vs. <40 µg/L
	Yang et al. 2007	4.9-13 vs. <4.9 µg/L
		>13 vs. <4.9 µg/L
	Hoffman et al. 2008	33-55 vs. 2.2-4.6 µg/L
		55-66 vs. vs. 2.2-4.6 µg/L
		66-75 vs. 2.2-4.6 µg/L
		75-109 vs. 2.2-4.6 µg/L
	Horton et al. 2011	50th-75th vs. <50th percentile
		75th-90th vs. <50th percentile
		>90th vs. <50th percentile
		50th-75th vs. <50th percentile[†]
		75th-90th vs. <50th percentile[†]
		>90th vs. <50th percentile[†]
	Patelarou et al. 2011	3rd tertile vs. 1st tertile

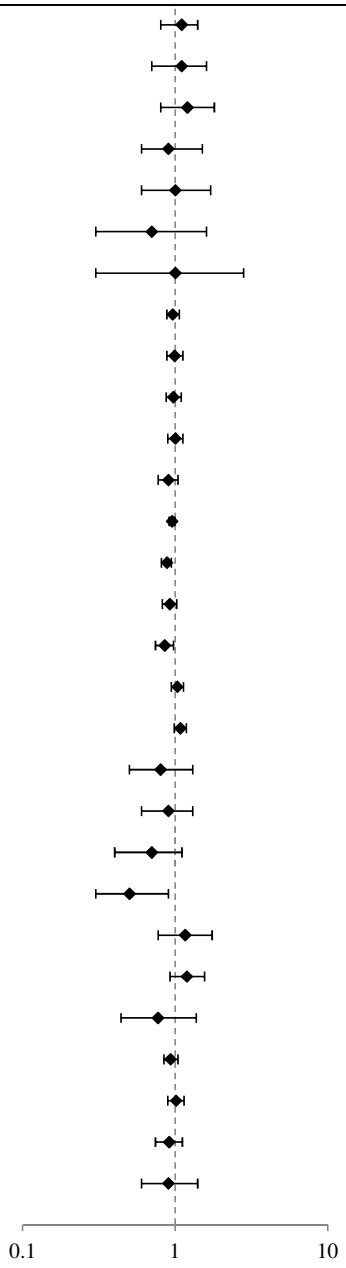
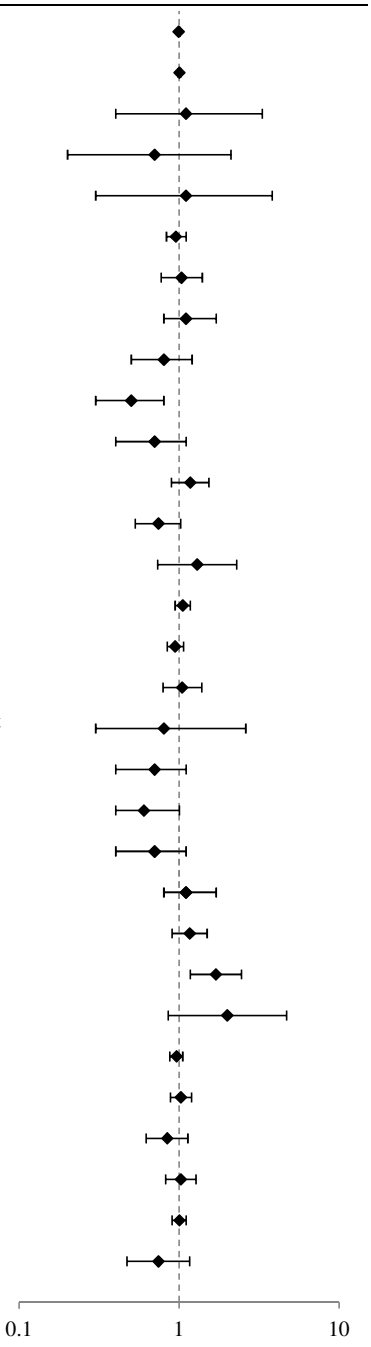


Figure I.2 *(Continued)*

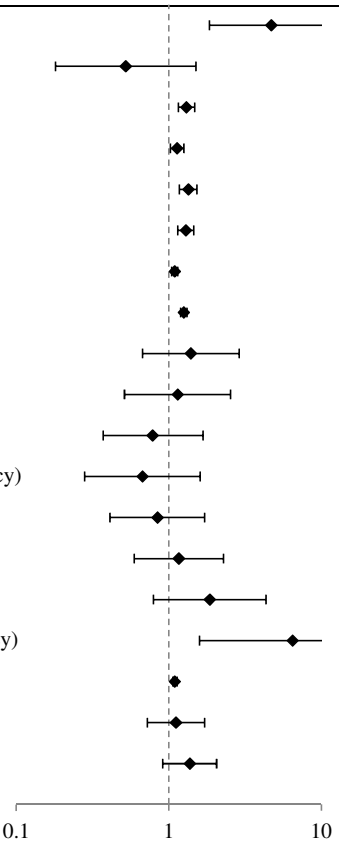
Exposure	Reference	Exposure corresponding to OR
THMs	Villanueva et al. 2011	10% increase in chloroform
		10% increase in brominated THM
	Costet et al. 2012	0-<0.01 vs. 0 µg/day
		0.01-<0.03 vs. 0 µg/day
		≥0.03 vs. 0 µg/day
HAAs	Wright et al. 2004	>30-49 vs. ≤30 µg/L
		>49-58 vs. ≤30 µg/L
	Hoffman et al. 2008	18-22 vs. 0-0.9 µg/L
		22-32 vs. 0-0.9 µg/L
		32-40 vs. 0-0.9 µg/L
		41-53 vs. 0-0.9 µg/L
	Horton et al. 2011	50th-75th vs. <50th percentile
		75th-90th vs. <50th percentile
		>90th vs. <50th percentile
		50th-75th vs. <50th percentile†
		75th-90th vs. <50th percentile†
		>90th vs. <50th percentile†
	Costet et al. 2012	Urinary TCAA detect vs. non-detect
TOX	Hoffman et al. 2008	137-170 vs. 14.3-22.4 µg/L
		170-178 vs. 14.3-22.4 µg/L
		178-193 vs. 14.3-22.4 µg/L
		193-235 vs. 14.3-22.4 µg/L
	Horton et al. 2011	50th-75th vs. <50th percentile
		75th-90th vs. <50th percentile
		>90th vs. <50th percentile
		50th-75th vs. <50th percentile†
		75th-90th vs. <50th percentile†
		>90th vs. <50th percentile†
TCE	Forand et al. 2011	Exposed vs. unexposed
PCE	Sonnenfeld et al. 2001	Exposed vs. unexposed
	Forand et al. 2011	Exposed vs. unexposed



Note. Adjusted odds ratios are presented where available. Results for Dodds et al. (1999), Hoffman et al. (2008), and Forand et al. (2011) are risk ratios. Results for Lewis et al. (2007) are hazard ratios. Results for Horton et al. 2011 are presented for both brominated and chlorinated (†) contamination sites. When OR were given for more than one window of exposure, average exposure across duration of pregnancy was preferentially presented. Abbreviations: Trihalomethanes (THMs); haloacetic acids (HAAs); trichloroacetic acid (TCAA); total organic halides (TOX); trichloroethylene (TCE); tetrachloroethylene (PCE).

Figure I.3 Odds ratios and 95% confidence intervals for studies of atmospheric pollutants in relation to preterm birth

Exposure	Reference	Exposure corresponding to OR
∑PAHs	Choi et al. 2008	Ln-unit increase (African American)
		Ln-unit increase (Dominican)
	Wilhelm et al. 2011	IQR increase
Benzo(*a*)pyrene	Wilhelm et al. 2011	IQR increase
Benzo(*g,h,i*)perylene	Wilhelm et al. 2011	IQR increase
Napthalene	Wilhelm et al. 2011	IQR increase
POM	Vassilev et al. 2001	Medium vs. low pollution
		High vs. low pollution
Benzene	Llop et al. 2010	1 µg/m^3 (≤2.7 µg/m3; 1st trimester)
		1 µg/m^3 (≤2.7 µg/m3; 2nd trimester)
		1 µg/m^3 (≤2.7 µg/m3; 3rd trimester)
		1 µg/m^3 (≤2.7 µg/m3; entire pregnancy)
		1 µg/m^3 (>2.7 µg/m3; 1st trimester)
		1 µg/m^3 (>2.7 µg/m3; 2nd trimester)
		1 µg/m^3 (>2.7 µg/m3; 3rd trimester)
		1 µg/m^3(>2.7 µg/m3; entire pregnancy)
	Wilhelm et al. 2011	IQR increase
Formaldehyde	Maroziene and Grazuleviciene 2009	Tertile 2 vs. tertile 1
		Tertile 3 vs. tertile 1



Note. Abbreviations: Polycyclic aromatic hydrocarbons (PAHs);interquartile range (IQR).

Figure I.4 Odds ratios and 95% confidence intervals for studies of metals and metalloids in relation to preterm birth

Exposure	Reference	Exposure corresponding to OR
Lead	Landgren 1996	> vs. < mean municipality exposure
	Torres-Sanchez et al. 1999	5.1-9.0 vs. <5.1 µg/dL
		9.1-14.9 vs. <5.1 µg/dL
		>14.9 vs. <5.1 µg/dL
	Jelliffe-Pawlowski et al. 2006	≥10 vs. <10 µg/dL
	Cantonwine et al. 2010a	SD increase (2nd trimester blood lead)
	Vigeh et al. 2011	Ln-unit increase
	Zhu et al. 2010	1.1-2.0 vs. ≤1 µg/dL
		2.1-3.0 vs. ≤1 µg/dL
		3.1-9.9 vs. ≤1 µg/dL
Arsenic	Landgren 1996	> vs. < mean municipality exposure
	Yang et al. 2003	Exposed area vs. unexposed area
	Myers et al. 2010	>50 vs. ≤50 µg/L
Mercury	Landgren 1996	> vs. < mean municipality exposure
	Xue et al. 2007	≥90th vs. <90th percentile
Cadmium	Landgren 1996	> vs. < mean municipality exposure

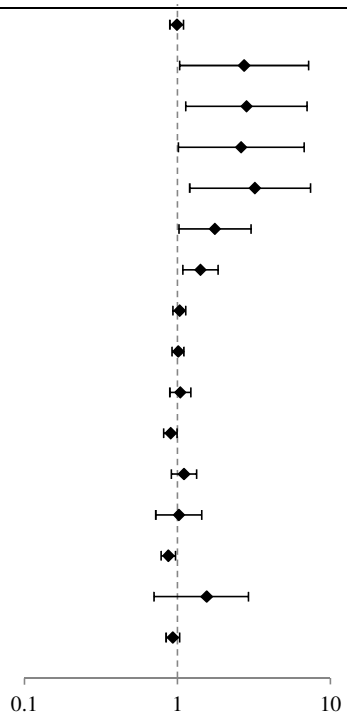
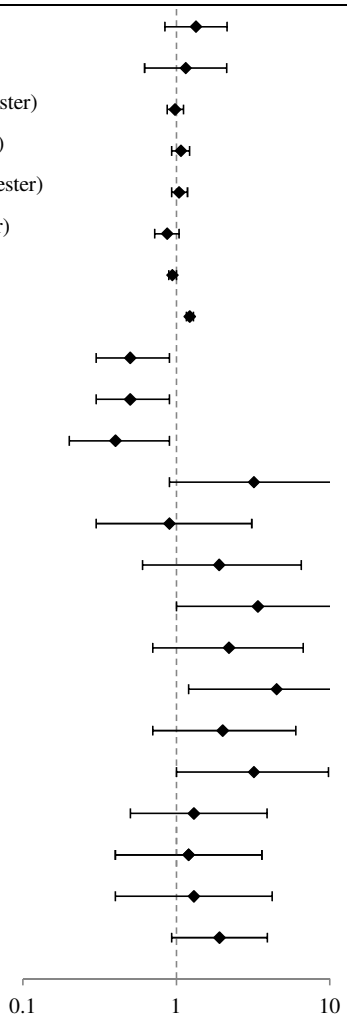


Figure I.5 Odds ratios and 95% confidence intervals for other environmental contaminants in relation to preterm birth

Exposure	Reference	Exposure corresponding to OR
Atrazine	Villanueva et al. 2005	0.05-0.08 vs. <0.05 µg/L
		>0.08 vs. <0.05 µg/L
	Ochoa-Acuña et al. 2009	0.06-0.44 vs. <0.06 µg/L (first trimester)
		>0.44 vs. <0.06 µg/L (first trimester)
		0.06-0.44 vs. <0.06 µg/L (third trimester)
		>0.44 vs. <0.06 µg/L (third trimester)
	Rinsky et al. 2012	0.01-0.08 vs. 0 µg/L
		>0.08 vs. 0 µg/L
Phthalates	Adibi et al. 2009	Log-unit increase (MEHP)
		Log-unit increase (MEHHP)
		Log-unit increase (MEOHP)
	Meeker et al. 2009	>Median vs. ≤Median (MEHP)
		>Median vs. ≤Median (MEHHP)
		>Median vs. ≤Median (MEOHP)
		>Median vs. ≤Median (MECPP)
		>Median vs. ≤Median (MBzP)
		>Median vs. ≤Median (MBP)
		>Median vs. ≤Median (MiBP)
		>Median vs. ≤Median (MCPP)
		>Median vs. ≤Median (MCOP)
		>Median vs. ≤Median (MCNP)
		>Median vs. ≤Median (MEP)
BPA	Cantonwine et al. 2010b	Log-unit increase



Note. Adjusted odds ratios are presented where available. Abbreviations: 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); monobenzyl phthalate (MBzP); mono-*n* -butyl phthalate (MBP); mono-isobutyl phthalate (MiBP); mono(3-carboxypropyl) phthalate (MCPP); monocarboxyisooctyl phthalate (MCOP); monocarboxyisononyl phthalate (MCNP); monoethyl phthalate (MEP); bisphenol-A (BPA).

CHAPTER II

ENVIRONMENTAL PHTHALATE EXPOSURE AND PRETERM BIRTH

INTRODUCTION

Prematurity is a leading cause of neonatal mortality and can lead to an array of adverse health effects in the lives of those who survive. The contribution of environmental exposures to preterm birth is understudied; however, identification of potential contributing factors offers significant hope for combating preterm birth for several reasons: (1) Pregnant women are unintentionally exposed to many chemicals throughout gestation, some of which have demonstrated reproductive toxicities (Woodruff et al. 2011); (2) Increased exposure to some chemicals over past decades correlates strongly with increased rates of preterm birth, which may be the result of various confounders but may also indicate a real association; and (3) Exposure to environmental contaminants may be largely modifiable, opportune for interventions at the individual, clinical and population levels.

Phthalates are a class of chemicals used in innumerable products worldwide, and exposure in humans and more specifically pregnant women is ubiquitous in many countries (Adibi et al. 2003; Berman et al. 2009; Irvin et al. 2010; Lin et al. 2011). Di-(2-ethylhexyl) phthalate (DEHP) exposure occurs primarily from the consumption of contaminated food and water, while exposure to other phthalates, including benzylbutyl phthalate (BzBP), dibutyl phthalate (DBP), and diethyl phthalate (DEP), occurs commonly through contact with personal care products, such as lotions, perfumes, and deodorants. Exposure in women has been linked to disrupted thyroid hormone levels, increased systemic levels of oxidative stress and inflammation, and adverse health endpoints such as endometriosis and breast cancer (Cobellis et al. 2003; Ferguson et al. 2012; Huang et al. 2007; Lopez-Carrillo et al. 2010). Previous epidemiologic studies of the relationship between phthalate exposure and gestation length or preterm birth have

been limited by sample size and exposure assessment methods, and results have been suggestive but not fully conclusive (Adibi et al. 2009; Meeker et al. 2009; Suzuki et al. 2010; Whyatt et al. 2009; Wolff et al. 2008). The present study utilized a powerful nested case-control design to assess the relationship between gestational phthalate exposure and preterm birth.

Additionally, we took advantage of the large number of cases in our study to investigate the link between phthalate exposure and spontaneous preterm birth. Delineating preterm births by obstetric presentation may help to elucidate specific mechanistic pathways (McElrath et al. 2008). Previously, McElrath and colleagues hypothesized that preterm births resulting from spontaneous preterm labor or preterm premature rupture of the membranes (pPROM) are likely to be the consequence of intrauterine inflammation, whereas medically indicated preterm births, typically subsequent to preeclampsia or intrauterine growth restriction, may result from aberrant placentation (McElrath et al. 2008). Toxicological studies suggest a role for phthalates in the inflammatory cascade leading up to preterm parturition. Hence, we additionally examined in this study the effects of phthalates on preterm birth in mothers who experienced spontaneous preterm birth.

METHODS

From 2006-2008 women from the Boston area who planned to deliver at the Brigham and Women's Hospital were recruited for participation in a large prospective cohort study designed to identify predictors of preeclampsia. In the first trimester (median ten weeks gestation) subjects completed demographic questionnaires providing information on Race/Ethnicity, tobacco and alcohol use, etc., and supplied urine and blood samples for biomarker analysis. First trimester ultrasound was used to validate and establish gestational age. During the three subsequent study visits, additional biological samples were collected in tandem with clinically relevant pregnancy characteristics. Delivery complications and neonate anthropomorphic measurements were recorded at birth. All specimens were stored at -80° C. In 2011 we selected from this population the 130 mothers who delivered prior to 37 weeks gestation and 352 randomly selected mothers who delivered at or after 37 weeks. Multiple births were excluded from our

study. Within the group of mothers who delivered preterm, we also examined a subset who delivered with clinical presentation by either spontaneous preterm labor and/or PPROM (N=57). These were considered spontaneous preterm births for our analysis.

Urinary phthalate metabolites

Urine samples were collected from up to four visits per subject during pregnancy. For visit 1 (median=9.71 weeks gestation), 479 samples were available (N=129 for cases, N=350 for controls). For visit 2 (median=17.9 weeks gestation), 422 samples were available (N=118 for cases, N=304 for controls). For visit 3 (median=26.0 weeks gestation), 412 samples were available (N=111 for cases, N=301 for controls). For visit 4 (median=35.1 weeks gestation), 380 samples were available (N=66 for cases, N=314 for controls). By the time of visit 4, many cases had already delivered, causing a disproportionately small number of samples for cases at that visit. Hence, levels measured in visit 4 samples were excluded for this analysis.

Nine phthalate metabolites were measured in each urine sample by NSF International (Ann Arbor, MI, USA) using the CDC method described elsewhere (Silva et al. 2007). Briefly, this entails enzymatic deconjugation of glucuronidated metabolites, solid-phase extraction, separation via high performance liquid chromatography, and detection by tandem mass spectrometry. Levels below the limit of detection (LOD) were kept as is if a numerical value was reported and otherwise were replaced with the LOD divided by the square root of two (Hornung and Reed 1990). Because concentration can vary with urine dilution, levels were corrected for urinary specific gravity (SG) using the following formula: $P_c=P[(1.015 - 1)]/SG - 1)]$, where P_c represents the specific gravity-corrected phthalate concentration (micrograms per liter), P represents the measured concentration in urine, 1.015 is the median SG of all samples measured, and SG represents the specific gravity of the individual sample (Meeker et al. 2009). Both unadjusted and adjusted metabolite levels were log-normally distributed and were ln-transformed for statistical analysis.

Phthalate metabolite levels may fluctuate over time as their half-lives are short and sources of exposure are variable. Hence, we used the geometric mean of levels from visit 1-3 to estimate each woman's average exposure throughout pregnancy. In addition to examining associations with individual phthalate metabolite averages, we also

examined the average of molar sums of DEHP metabolites (∑DEHP; nanomoles per milliliter) for each woman across pregnancy.

Statistical analysis

Population characteristics were tabulated for demographic and pregnancy-related variables of interest, including maternal age, Race/Ethnicity, education, health insurance provider, body mass index (BMI) measured at the first study visit, smoking status, alcohol use, parity, use of assisted reproductive technology (ART), and gender of infant. Each of these was considered a potential covariate for multivariate logistic regression models.

Urinary phthalate metabolite distributions were examined for all subjects combined and by case status. Differences in phthalate concentrations in preterm cases compared to controls were investigated with T-tests of ln-transformed and SG-corrected phthalate metabolite means. Next, using multivariate logistic regression we examined odds of preterm birth in association with phthalate metabolite concentrations in crude models adjusting for urinary specific gravity only and in full models, adjusting for *a priori* covariates, including maternal age, race/ethnicity, and education level (an indicator of socioeconomic status). Additional covariates were added to models in a forward stepwise procedure and were finally included if they altered the association between phthalate metabolite and preterm birth by greater than 10%. Similar regression models were created examining the subset of preterm births with clinical presentation of preterm labor or pPROM to explore relationships within spontaneous preterm births.

To examine effects of exposure at higher levels, and the potential for non-linear relationships, we divided average phthalate metabolite concentrations into quartiles using non-standardized values from the entire population. Adjusted odds ratios were calculated for each of the top three quartiles in comparison to the lowest quartile of exposure in models adjusting for the same sets of covariates used in models with continuous exposure. Tests for trend were conducted by modeling quartiles as a single ordinal variable, again using the same covariates. Quartile analysis and tests for trend were performed for the subset of spontaneous preterm births as well. All analysis was performed using R version 2.13.1.

RESULTS

Distributions of categorical covariates are presented in Table II.1 for the population overall and by preterm status. Overall the women enrolled in this study were predominantly white, well-educated, non-smokers. Few consumed alcohol during pregnancy and nearly half were nulliparous. Approximately 10% of the population utilized ART and 44% of infants were male, and proportions of these variables were equal among cases and controls. Consistent with previous US studies, elevated levels of mono-benzyl phthalate (MBzP), mono-*n*-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), and mono-ethyl phthalate (MEP) were observed in African American compared to Caucasian mothers (data not shown).

Each phthalate metabolite was detected in at least 95% of urine samples. As expected, distributions were log-normally distributed and levels were ln-transformed for statistical analysis. Spearman correlation coefficients between average phthalate levels in the overall population were modest to strong (Table II.2). Geometric means and 25th and 75th percentiles of SG-adjusted exposures overall and in cases and controls separately are presented in Table II.3. Significantly ($p<0.05$) elevated levels of mono-(2-ethyl)-hexyl phthalate (MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), ∑DEHP and MBP were observed in preterm cases compared to controls. Suggestively ($p<0.10$) elevated levels of mono-(3-carboxypropyl) phthalate (MCPP) were also noted.

Stepwise addition to incorporate covariates into logistic regression models demonstrated two sets of covariates. Full models for DEHP metabolites included SG, age, Race/Ethnicity, and education level as covariates. For other metabolites insurance provider was also included in full models, as addition of this variable altered effect estimates by greater than 10%. Odds ratios and 95% confidence intervals are presented in Table II.4. We observed significantly elevated odds of preterm birth with ln-unit increases in MEHP, MECPP, and ∑DEHP, and suggestively elevated odds for MBP. Interestingly, odds ratios for all associations were greater in magnitude for the subset of spontaneous preterm births and were statistically significant despite the much smaller sample size. Results were similar in crude models, adjusting only for urinary specific gravity (Table II.5).

Assessment of urinary phthalate metabolite quartiles showed dose-related relationships with odds of preterm birth. Significant positive trends were observed for MEHP, MECPP, ∑DEHP, and MBP (Figure II.1). When quartiles of exposure were examined within spontaneous preterm births alone, trends became stronger for all phthalate metabolites, and odds ratios associated with the top quartile of exposure were markedly increased (Figure II.2).

DISCUSSION

Our results demonstrate a robust increase in the odds of preterm birth in association with urinary phthalate metabolite concentrations during pregnancy. Specifically, maternal levels of DEHP metabolites, including MEHP (the putative toxic metabolite of DEHP), MECPP (the most stable marker of DEHP exposure), and summed DEHP metabolites showed the strongest and most clearly dose-dependent relationships with odds of birth before 37 weeks gestation. Additional suggestive relationships were observed for MBP. When spontaneous preterm births, those with clinical presentation of spontaneous preterm labor or pPROM, were examined alone, odds ratios became greater for all phthalate metabolites, and significant relationships emerged for mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), MBzP, MBP, and MCPP.

Associations observed in the present study are consistent with results from previous studies of preterm birth or gestation length. Early prospective cohort studies found that exposure to DEHP metabolites was associated with decreased gestational age at delivery (Whyatt et al. 2009; Latini et al. 2003). Latini and colleagues reported that infants who had detectable levels of MEHP in cord blood had significantly shorter gestational length compared to those who did not (N=84, preterm=11) (Latini et al. 2003). In another study where metabolites were measured during the third trimester maternal urine, gestational age similarly decreased with increasing DEHP metabolite exposure (N=311, preterm=10) (Whyatt et al. 2009). More recently, a small nested case-control study in Mexico City revealed significantly increased odds of preterm birth in association with third trimester levels of MECPP, MBP, and MCPP in models adjusted for urinary specific gravity and maternal factors (N=60, preterm=30) (Meeker et al. 2009).

Other authors, however, have reported contrary or null results. Adibi and colleagues found that odds of preterm birth were decreased with increasing exposure to the DEHP metabolites MEHP, MEOHP, and mono-(2-ethyl-5-hycroxyhexyl) phthalate (MEHHP) measured in maternal urine during the third trimester (N=283, preterm=14) (Adibi et al. 2009). Likewise, a 2008 study noted a positive association between third trimester low-molecular-weight phthalate (e.g., MEP) exposure and gestational age at delivery (N=404) (Wolff et al. 2008). Finally, in a study of women in Tokyo, Japan, Suzuki and colleagues were unable to detect an association between gestational length and any of nine urinary phthalate metabolites taken any time between the 9th and 40th weeks of gestation (N=149, preterm=2) (Suzuki et al. 2010).

Limitations in previous studies of phthalate exposure and prematurity potentially account for the differences in these findings, and highlight the advantages in our analysis. First, exposure assessment in prior studies utilized a single spot urine phthalate metabolite concentration from the third trimester. This is problematic since a single measurement, particularly late in pregnancy, is only loosely correlated with long-term exposure (Adibi et al. 2008). Second, prior work used self-recalled and reported dates of last menstrual period to calculate gestational age at delivery. Finally, sample size, either overall or in proportion of preterm births, lacked power to detect real associations. The present study is the first to use multiple urine samples collected longitudinally across gestation in order to more accurately integrate overall exposure. Additionally, the present work utilizes clinically and first trimester ultrasound validated gestational dates. These aspects greatly reduced the potential for exposure and outcome misclassification. This power was heightened by the case-control design and large sample size. Lastly, the sample size in the present study afforded more than adequate power.

We further leveraged the parent cohort study's strengths by examining the relationship between gestational phthalate exposure and spontaneous preterm birth. Relationships within this subset have not been previously explored, likely because of already limited numbers of preterm births in earlier studies. However, the results from this analysis both strengthened the overall findings and highlighted a potential mechanism for the connection between phthalate exposure and prematurity. As mentioned previously, McElrath and colleagues suggested that spontaneous preterm birth

is associated with intrauterine inflammation. The strong link observed between phthalate exposure and spontaneous preterm births found in the present study suggests phthalate exposure is associated with increased intrauterine inflammation. This is consistent with both *in vitro* data that have demonstrated the pro-inflammatory activity of phthalates, and limited cross-sectional human studies, which have shown that phthalate exposure is associated with increased systemic markers of inflammation (Ferguson et al. 2012; Latini et al. 2006).

Reducing rates of preterm birth is unlikely to occur by identification of one or two obvious causes; rather, detailed investigation of many component contributors is necessary. A recent study predicted that current interventions to prevent preterm birth will decrease rates by only 5% or less by 2015, making identification of preventable exposures crucial (Chang et al. 2012). In our study women with average gestational MEHP exposure in the top quartile had 4 times the odds of preterm birth compared to women in the bottom quartile. As over two thirds of preterm births every year are spontaneous, the subset of the population susceptible to these effects may be quite large (Goldenberg et al. 2008).

Also, importantly, phthalate exposure may be preventable with behavioral modification. A recent dietary intervention study demonstrated that when subjects altered their diets to consume only "fresh foods" that were not packaged in cans or plastic, urinary levels of DEHP metabolites decreased markedly (Rudel et al. 2011). However, another recent study with a similar dietary intervention did not observe the same effects (Sathyanarayana et al. 2013). Low-molecular weight phthalates, including DBP and DEP, are used frequently as solvents in cosmetic products such as fragrances, hair spray, nail polish, deodorants, and body lotions. Women who refrain from using such products may have lower levels of exposure (Buckley et al. 2012; Romero-Franco et al. 2011; Martina et al. 2012).

While we cannot rule out the role of unmeasured confounders in our study (e.g. dietary patterns that may be associated with both phthalate exposure and preterm birth), we did examine the potential for confounding among known predictors of preterm birth in our analysis. Another potential limitation in our study may be the measurement of phthalate metabolites in excreted urine. Although this is the best method to measure

cumulative exposure to phthalates to date, differences in individual metabolism and excretion patterns may affect urinary phthalate metabolite concentrations. However, these individual patterns would likely contribute non-differential measurement error which would shift measured associations toward the null.

Our results indicate a significant association between exposure to phthalates during pregnancy and preterm birth, which solidifies prior laboratory and epidemiologic evidence. Furthermore, as exposure to phthalates is widespread, and because the prevalence of preterm birth among women in our study cohort was similar to that in the general population, our results are generalizable to women in the US and elsewhere. These data provide strong support for taking action in the prevention or reduction of phthalate exposure during pregnancy.

Table II.1 Distribution of population characteristics by cases status: N (%) or median (25^{th}, 75^{th} percentiles).

Demographic characteristics		Overall (N=482)	Cases (N=130)	Controls (N=352)
Maternal age (years)		32.7 (29.0, 35.7)	32.8 (29.3, 35.8)	32.7 (28.7, 35.7)
Race/Ethnicity	Caucasian	282 (58.5)	75 (57.7)	207 (58.8)
	African-American	77 (16.0)	22 (16.9)	55 (15.6)
	Other	123 (25.5)	33 (25.4)	90 (25.6)
Education	High school	68 (14.1)	21 (16.2)	47 (13.4)
	Technical school	77 (16.0)	25 (19.2)	52 (14.8)
	Junior college or some college	139 (28.8)	38 (29.2)	101 (28.7)
	College graduate	187 (38.8)	45 (34.6)	142 (40.3)
	Missing	11 (2.30)	1 (0.80)	10 (2.80)
Health insurance	Private insurance/HMO/Self-pay	385 (79.9)	108 (83.1)	277 (78.7)
	Medicaid/SSI/MassHealth	85 (17.6)	20 (15.4)	65 (18.5)
	Missing	12 (2.50)	2 (1.50)	10 (2.80)
Body mass index (BMI)	Less than 25 kg/m^2 (underweight to normal weight)	250 (51.9)	62 (47.7)	188 (53.4)
	25 to less than 30 kg/m^2 (overweight)	126 (26.1)	32 (24.6)	94 (26.7)
	Greater than 30 kg/m^2 (obese to morbidly obese)	102 (21.2)	36 (27.7)	66 (18.8)
	Missing	4 (0.80)	0 (0)	4 (1.10)
Tobacco use	Smoked during pregnancy	31 (6.40)	11 (8.50)	20 (5.7)
	No smoking during pregnancy	445 (92.3)	119 (91.5)	326 (92.6)
	Missing	6 (1.20)	0 (0)	6 (1.70)
Alcohol use	Alcohol use during pregnancy	20 (4.10)	1 (0.80)	19 (5.40)
	No alcohol use during pregnancy	452 (93.8)	126 (96.9)	326 (92.6)
	Missing	10 (2.10)	3 (2.30)	7 (2.00)
Parity	Nulliparous	215 (44.6)	55 (42.3)	160 (45.5)
	Non-nulliparous	267 (55.4)	75 (57.7)	192 (54.5)

Table II.2 Spearman correlation coefficients (p-values) between urinary phthalate metabolite averages across pregnancy. Metabolite measures standardized to urinary specific gravity.

	MEHP	MEHHP	MEOHP	MECPP	∑DEHP	MBzP	MBP	MiBP	MEP	MCPP
MEHP	1.00	0.68 (<0.01)	0.78 (<0.01)	0.74 (<0.01)	0.83 (<0.01)	0.09 (0.04)	0.15 (<0.01)	0.16 (<0.01)	0.05 (0.30)	0.35 (<0.01)
MEHHP		1.00	0.91 (<0.01)	0.71 (<0.01)	0.84 (<0.01)	0.11 (0.02)	0.19 (<0.01)	0.14 (<0.01)	0.01 (0.85)	0.37 (<0.01)
MEOHP			1.00	0.89 (<0.01)	0.97 (<0.01)	0.11 (0.01)	0.20 (<0.01)	0.21 (<0.01)	0.05 (0.07)	0.43 (<0.01)
MECPP				1.00	0.96 (<0.01)	0.10 (0.03)	0.15 (<0.01)	0.20 (<0.01)	0.08 (0.07)	0.46 (<0.01)
∑DEHP					1.00	0.11 (0.02)	0.18 (<0.01)	0.21 (<0.01)	0.06 (0.16)	0.45 (<0.01)
MBzP						1.00	0.59 (<0.01)	0.46 (<0.01)	0.24 (<0.01)	0.25 (<0.01)
MBP							1.00	0.62 (<0.01)	0.36 (<0.01)	0.32 (<0.01)
MiBP								1.00	0.32 (<0.01)	0.28 (<0.01)
MEP									1.00	0.14 (<0.01)
MCPP										1.00

Table II.3 Average phthalate levels across gestation in overall population and in cases compared to controls.

		Adjusted geometric mean (25^{th}, 75^{th} percentiles) (µg/L)		
Parent phthalate compound	Phthalate metabolite	Overall (N=482)	Cases (N=130)	Controls (N=352)
Di-2-ethylhexyl phthalate (DEHP)	Mono-(2-ethyl)-hexyl phthalate (MEHP)	10.5 (5.51, 18.1)	12.3 (6.91, 23.7)	9.91 (5.17, 16.9)**
	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	31.9 (17.2, 55.3)	32.3 (15.0, 56.6)	31.7 (17.5, 54.4)
	Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)	16.9 (9.33, 29.7)	18.3 (9.33, 35.5)	16.3 (9.43, 28.9)
	Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)	41.3 (20.6, 73.8)	52.1 (25.1, 93.4)	37.9 (19.3, 67.1)**
	Summed DEHP metabolites (∑DEHP)	36.5 (20.2, 63.2)	42.6 (21.3, 80.2)	34.5 (19.7, 67.1)**
Benzyl butyl phthalate (BzBP)	Mono-benzyl phthalate (MBzP)	6.47 (3.25, 11.6)	6.85 (3.21, 13.4)	6.34 (3.27, 10.9)
Dibutyl phthalate (DBP)	Mono-*n*-butyl phthalate (MBP)	16.7 (10.5, 23.2)	18.9 (10.8, 26.0)	15.9 (10.2, 22.5)**
	Mono-isobutyl phthalate (MiBP)	6.75 (4.48, 10.3)	6.85 (4.63, 10.5)	6.71 (4.42, 10.27)
Diethyl phthalate (DEP)	Mono-ethyl phthalate (MEP)	134 (55.7, 276)	150 (64.3, 293)	129 (55.5, 273)
Di-*n*-octyl phthalate (DOP) et al.	Mono-(3-carboxypropyl) phthalate (MCPP)	2.02 (1.09, 3.09)	2.27 (1.25, 3.36)	1.93 (1.07, 2.95)*

Note. Summed DEHP levels in nanomoles/milliliter. *Suggestively increased levels of phthalate metabolites in cases compared to controls ($p<0.10$). **Statistically significant ($p<0.05$) increased levels of phthalate metabolites in cases compared to controls.

Table II.4 Odds ratios from fully adjusted logistic models of preterm birth (<37 weeks) and spontaneous preterm birth.

Phthalate metabolite	Preterm birth		Spontaneous preterm birth	
	Odds ratio (95% confidence interval)	p value	Odds ratio (95% confidence interval)	p value
MEHP	1.34 (1.07, 1.68)**	0.012	1.65 (1.20, 2.26)**	0.002
MEHHP	1.03 (0.82, 1.30)	0.784	1.27 (0.91, 1.78)	0.154
MEOHP	1.16 (0.91, 1.47)	0.235	1.47 (1.04, 2.08)**	0.030
MECPP	1.40 (1.13, 1.74)**	0.002	1.56 (1.15, 2.13)**	0.005
∑DEHP	1.33 (1.04, 1.70)**	0.021	1.63 (1.15, 2.31)**	0.006
MBzP	1.09 (0.86, 1.38)	0.492	1.41 (1.02, 1.95)**	0.037
MBP	1.27 (0.99, 1.63)*	0.063	1.49 (1.08, 2.06)**	0.014
MiBP	0.98 (0.72, 1.34)	0.906	1.52 (0.97, 2.38)*	0.066
MEP	1.11 (0.93, 1.32)	0.247	1.22 (0.96, 1.56)	0.101
MCPP	1.19 (0.95, 1.49)	0.127	1.36 (1.02, 1.81)**	0.038

Note. Models for MEHP, MEHHP, MEOHP, MECPP, and ∑DEHP adjusted for average specific gravity, maternal age at first visit, race/ethnicity, and education. Models for other metabolites additionally adjusted for insurance provider. Spontaneous preterm births limited to those with presentation of spontaneous preterm labor or pPROM. *Suggestively increased OR of preterm birth in association with ln-unit increase in phthalate level ($p<0.10$). **Statistically significant ($p<0.05$) increase.

Table II.5 Odds ratios from crude logistic models of preterm birth (<37 weeks) and spontaneous preterm birth.

Phthalate metabolite	Preterm birth		Spontaneous preterm birth	
	Odds ratio (95% confidence interval)	p value	Odds ratio (95% confidence interval)	p value
MEHP	1.34 (1.07, 1.68)**	0.012	1.65 (1.20, 2.26)**	0.002
MEHHP	1.03 (0.82, 1.30)	0.784	1.27 (0.91, 1.78)	0.154
MEOHP	1.16 (0.91, 1.47)	0.235	1.47 (1.04, 2.08)**	0.030
MECPP	1.40 (1.13, 1.74)**	0.002	1.56 (1.15, 2.13)**	0.005
∑DEHP	1.33 (1.04, 1.70)**	0.021	1.63 (1.15, 2.31)**	0.006
MBzP	1.09 (0.86, 1.38)	0.492	1.41 (1.02, 1.95)**	0.037
MBP	1.27 (0.99, 1.63)*	0.063	1.49 (1.08, 2.06)**	0.014
MiBP	0.98 (0.72, 1.34)	0.906	1.52 (0.97, 2.38)*	0.066
MEP	1.11 (0.93, 1.32)	0.247	1.22 (0.96, 1.56)	0.101
MCPP	1.19 (0.95, 1.49)	0.127	1.36 (1.02, 1.81)**	0.039

Note. Models adjusted for average urinary specific gravity only. *Suggestively increased OR of preterm birth in association with ln-unit increase in phthalate level ($p<0.10$).
**Statistically significant ($p<0.05$) increase.

Figure II.1 Odds of preterm birth and 95% confidence levels by quartile of average phthalate metabolite measuremed during pregnancy.

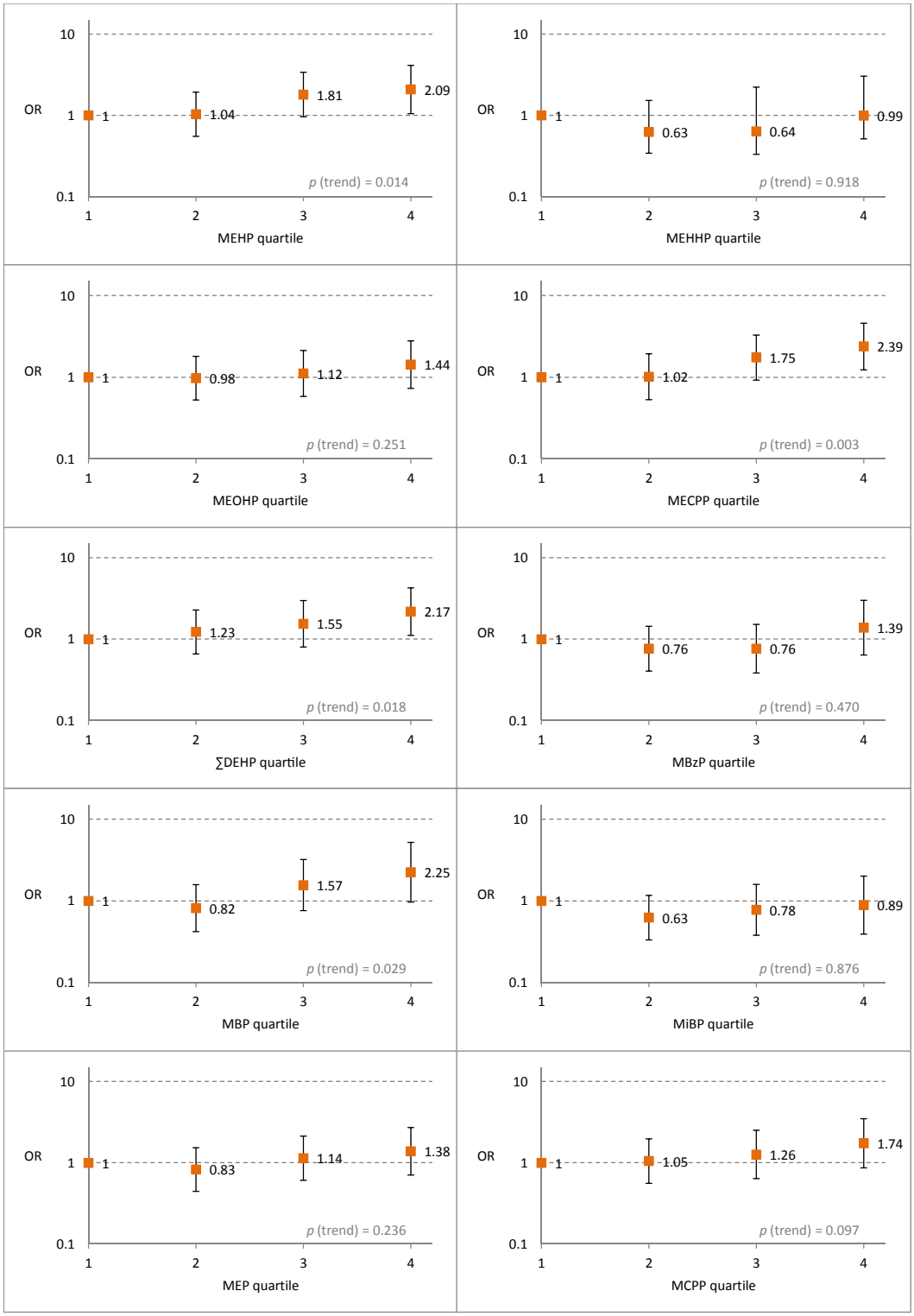
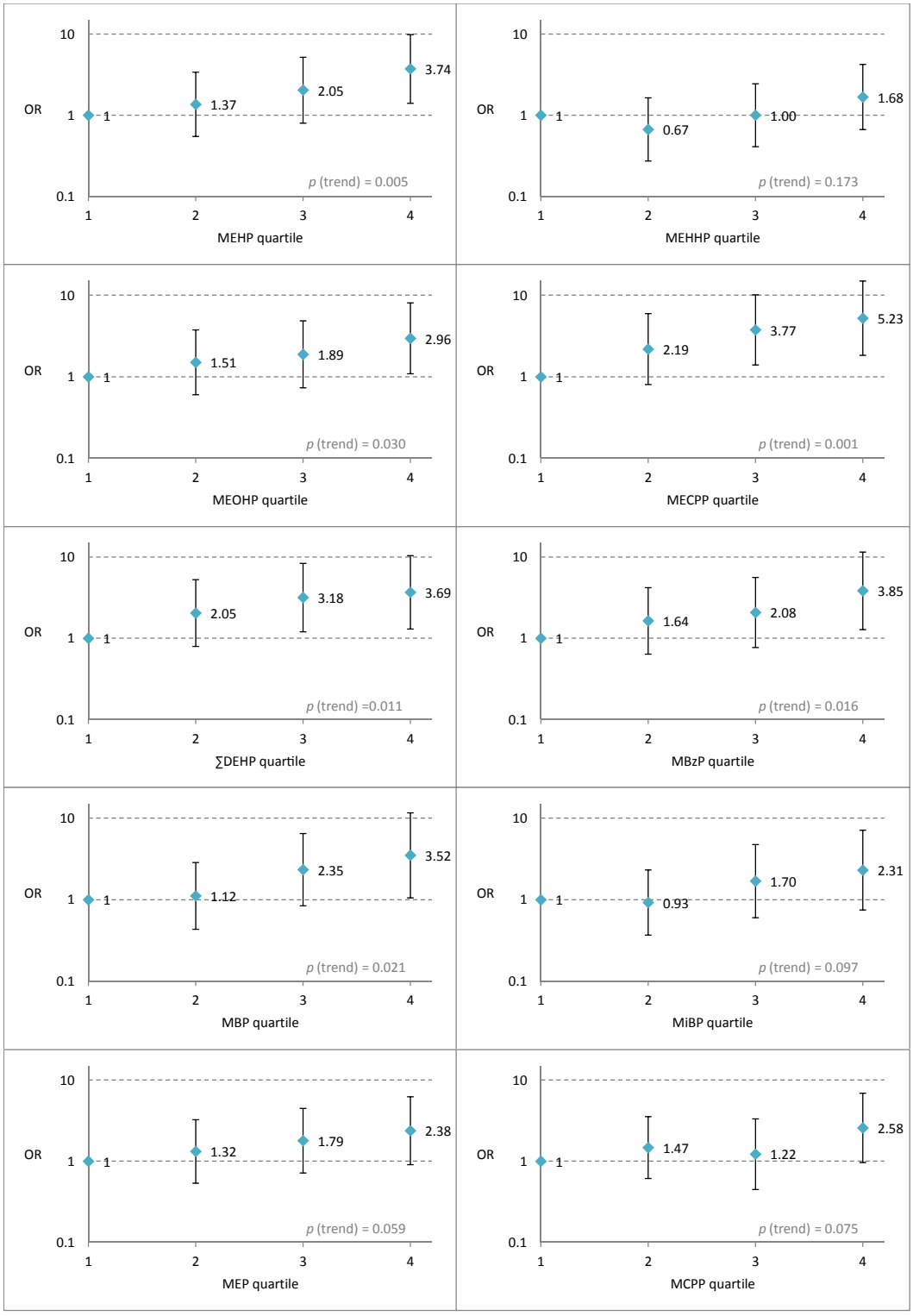


Figure II.2 Odds of spontaneous preterm birth and 95% confidence levels by quartile of average phthalate metabolite measured during pregnancy.



CHAPTER III

VARIABILITY IN URINARY PHTHALATE METABOLITE LEVELS ACROSS PREGNANCY AND SENSITIVE WINDOWS OF EXPOSURE FOR THE RISK OF PRETERM BIRTH

INTRODUCTION

Phthalate diesters are produced in large quantities yearly in the US for use in everyday products such as polyvinyl flooring, shower curtains, food packaging plastics, and personal care products. Exposure occurs through contact with these products as well as the consumption of contaminated food and drinking water (ATSDR 1995, 2001, 2002). While phthalates may not persist or accumulate in the human body, even transient exposures, because of their high frequency, have been linked to an array of adverse health outcomes in humans, including altered thyroid and reproductive hormone levels (Meeker and Ferguson 2011; Mendiola et al. 2012), decreased semen quality in males (Hauser et al. 2006), and asthma and allergic symptoms (Bornehag and Nanberg 2010). Exposure to phthalates *in utero* has been linked to adverse birth outcomes as well, including altered reproductive tract development in male infants (Swan et al. 2005), neurodevelopment in both sexes (Engel et al. 2010; Engel et al. 2009), and both prematurity and small size at birth (Meeker 2012; Meeker et al. 2009; Whyatt et al. 2009; Ferguson et al. 2014).

Preterm birth, defined as delivery before 37 weeks completed gestation, is a particularly important endpoint of interest due to: 1) its contribution to fetal mortality and morbidity and consequent cost to society; 2) the apparent increase in rates over the last three decades; and 3) poorly understood causes and lack of effective interventions (Behrman and Butler 2007). Research to uncover contributing causes, particularly those in connection with environmental contaminant exposures, is a public health priority (Ashton et al. 2009). We recently demonstrated clear associations between maternal

exposure to phthalate levels averaged from multiple time points during pregnancy and increased odds of preterm birth in a nested case-control study of women who delivered at the Brigham and Women's Hospital in Boston (Ferguson et al. 2014). Due to the short-half life of phthalates in the human body, and the consequent variability in exposure measures, a geometric mean of measures from multiple time points provides the most robust estimate of exposure over the course of pregnancy. However, the availability of multiple exposure measures additionally allows investigation of windows during gestation that may be particularly sensitive to the effects of phthalates. In the present analysis we examined variability in phthalate levels across pregnancy and attempted to identify any patterns in levels by gestational age. Also, we assessed associations between phthalate exposure at individual time points during pregnancy and preterm birth in order to identify windows of vulnerability.

METHODS

Study population

Participants were part of an ongoing prospective cohort study of pregnant women with initial prenatal visits at five clinics in the Boston area. All women who wished to participate were included if they planned to deliver at the Brigham and Women's Hospital and if their initial visit was prior to 15 weeks gestation. Subjects were followed throughout the course of pregnancy and provided information (e.g., health status, weight) and urine samples at up to four visits. Samples were stored at -30 degrees C for future use. At delivery, birth outcome characteristics such as mode of delivery and fetal measurements were recorded. From 2006 to 2008 approximately 1600 women were recruited, and 1181 delivered live singleton infants. From this population, the present nested case-control study includes all 130 mothers who delivered preterm, as well as 352 controls selected randomly from subjects who had a urine sample from visit 1 and from at least one additional visit. Gestational ages at individual visits and at delivery were calculated based on last menstrual period (LMP) and confirmed by first trimester ultrasound. Study participants provided written informed consent and institutional review board approval was obtained from Brigham and Women's Hospital as well as the University of Michigan.

Within this study, visit 1 urine samples were taken at median 9.71 weeks gestation (range 4.71 to 16.1 weeks), visit 2 at median 17.9 weeks (range 14.9 to 21.9 weeks), visit 3 at median 26.0 weeks (range 22.9 to 29.3 weeks), and visit 4 at median 35.1 weeks (range 33.1 to 38.3 weeks). The number of subjects with samples available decreased slightly with increasing visit, with the fourth visit having the smallest number of samples. Visit 4 also had a smaller proportion of cases with urine samples, since some had delivered by this time point.

Overall preterm birth was defined using the clinical definition of birth before 37 weeks postmenstrual gestation. However, further classifying preterm birth by clinical presentation may provide cleaner subpopulations with more homogenous etiologies. A study of placental histology of women who delivered very preterm (<32 weeks gestation) showed that women who presented with spontaneous preterm labor or preterm premature rupture of the membranes (PPROM) had distinct patterns of placental inflammation (McElrath et al. 2008). On the other hand, women who delivered preterm as a result of preeclampsia or intrauterine growth restriction (IUGR) showed placental aberrations. Because these differences may be indicative of different mechanistic precursors of preterm birth, we additionally examined prematurity by categories of clinical presentation including 1) spontaneous preterm birth, defined as delivery with presentation of spontaneous preterm labor or PPROM (N=52), and 2) preterm birth of placental origin (placental preterm birth), defined as preterm births following preeclampsia or IUGR (N=35). A third group were considered medically indicated preterm births, with non-spontaneous delivery or C-section preterm due to maternal complications (e.g., placental abruption, cervical insufficiency, etc.; N=38). These cases were not analyzed separately, as they have no known unifying etiology. Five cases had characteristics of both spontaneous and placental preterm birth, and were examined more carefully to determine the root of prematurity. Four were determined to be the result of spontaneous preterm labor, and one resulted from placental previa (final N for spontaneous preterm=56, final N for placental preterm=35, final N for medically indicated=39).

Phthalate exposure

Nine phthalate metabolites were measured in each available urine sample (N=1,693) by NSF International in Ann Arbor, MI, following methods developed by the Centers for Disease Control (CDC), described in detail elsewhere (Silva et al. 2007). Two urine samples (both from control subjects) from visit 1 had insufficient volume for analysis. The final number of samples analyzed for all phthalate metabolites were as follows by visit (cases, controls): Visit 1 (129, 350); Visit 2 (118, 304); Visit 3 (111, 301); and Visit 4 (66, 314). Phthalate measurements below the limit of detection (LOD) were replaced with the LOD divided by √2 (Hornung and Reed 1990).

To adjust for urinary dilution, specific gravity (SG) levels were also measured in each urine sample using a digital handheld refractometer (ATAGO Company Ltd., Tokyo, Japan). For univariate analyses phthalate levels were corrected for urinary SG using the following formula: $P_c=P[(1.015 - 1)]/SG - 1)]$, where P_c represents the SG-corrected phthalate concentration (micrograms per liter), P represents the measured concentration in urine, 1.015 is the median SG of all samples measured, and SG represents the SG of the individual sample (Meeker et al. 2009). For regression models, unadjusted phthalate levels were used and urinary SG was included as a covariate, since modeling adjusted phthalate levels may incur bias (Barr et al. 2005). In addition to analysis of individual phthalate metabolites, a summed measure of di(2-ethylhexyl) phthalate (DEHP) metabolites (∑DEHP; nanomoles/liter) was also examined. All individual metabolites and ∑DEHP were log-normally distributed and ln-transformed for analysis.

Statistical analysis

Analysis was performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) and R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria). To assess variability in phthalate levels across pregnancy, we examined changes in levels by visit both in the population overall and in cases and controls separately. Geometric means and standard deviations of phthalates at individual visits were calculated and differences in SG-corrected concentrations from visits 2-4 compared to visit 1 were tested using linear mixed models (LMM) with random intercepts to adjust for intra-individual

correlation. Spearman correlations between measures of the same phthalate metabolite from different visits were calculated using SG-corrected values.

To examine temporal variability in phthalate levels by subject, intraclass correlation coefficients (ICC) and 95% confidence intervals were calculated using uncorrected and SG-corrected phthalate levels, both in the overall population and by in cases and controls separately (Hankinson et al. 1995). The ICC measure represents the ratio of intra-individual variability to intra plus inter-individual variability, and ranges from zero to one with a value of one indicating no intra-individual variability (Rosner 2011).

Finally, we fit generalized additive mixed models (GAMM) using the R *mgcv* package to examine predicted phthalate metabolite levels across pregnancy in relation to gestational age at sample collection (Wood 2011). The GAMM framework was used to study repeated measures of phthalate levels across pregnancy as a smooth function of gestational age, after accounting for within-subject correlation in the phthalate measures. GAMM were adjusted for the same covariates as cross-sectional models and, as a second step, additional models were created with an interaction between gestational age at sample collection and the indicator variable for preterm birth, to assess whether the pattern of phthalate levels across gestation depended on case-control status. Observations without a complete set of covariates were excluded. Predicted values from models with interaction terms were calculated based on reference levels for each covariate; SG and gestational age were centered prior to modeling for interpretability. Predicted values were plotted to visually present deviations in urinary phthalate metabolite levels in cases compared to controls across gestation.

Windows of vulnerability for preterm birth were assessed in two ways. First, we fit logistic regression models with preterm birth as the outcome and obtained odds ratios corresponding to phthalate levels from individual visits. SG-corrected models only included urinary SG from the corresponding visit as a covariate. In full models, maternal age, race/ethnicity, and education level were included *a priori*, and additional covariates were added in a forward step-wise model selection procedure with inclusion in final models if they altered effect estimates by greater than 10 percent. Variables that were considered included health insurance category, pre-pregnancy body mass index (BMI),

smoking status, alcohol use during pregnancy, parity, gender of infant, use of assisted reproductive technology (ART), and time of day of urine sample collection (before vs. after 1pm). The same sets of covariates were included in models for each visit for consistency.

The second method we used to assess windows of sensitivity to phthalate exposure involved modeling subject-specific patterns of exposure in relation to preterm birth (Sanchez et al. 2011). Note that this is not a standard repeated measures outcome analysis, as we have a single binary outcome (preterm birth) and repeated measures of the exposure. To assess if change in phthalate level over pregnancy (i.e., individual exposure curve) was significantly associated with preterm birth, we created two-step models. In the first step we fit linear mixed models with phthalate levels as the outcome predicted by gestational age at sample collection with random intercepts and slopes for each subject, adjusting for exposure-specific covariates including urinary SG and time of day of sample collection (before vs. after 1pm). In the second step, the best linear unbiased predictors of the slopes for each subject obtained from this model were used as predictors of preterm birth with adjustment for the additional confounders used in other models. The two-step strategy allows one to extract features of the exposure trajectory over gestation (in this case the estimated random slopes) to use as a single summary predictor in the subsequent case-control analysis using logistic regression.

Finally, we repeated the steps for subtypes of preterm birth, including placental and spontaneous preterm birth. For these models, preterm cases that did not fit into the subtype were excluded from analysis as opposed to being recoded as controls. Covariates for full models were kept the same as for models of overall preterm birth for comparison.

RESULTS

Population characteristics are described in detail elsewhere (Ferguson, et al., 2014). As expected, phthalate levels were highly detectable in our population. All metabolites were detected in over 99% of samples with the exceptions of mono(2-ethylhexyl) phthalate (MEHP; 95.3%) and mono(2-carboxypropyl) phthalate (MCPP; 96.8%).

Variability in phthalate levels across pregnancy

Total population phthalate metabolite geometric means and standard deviations by individual visit are presented in Table III.1 (corrected for urinary SG). Significantly decreased levels compared to Visit 1 were detected with LMM for all DEHP metabolites, including MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), and ∑DEHP as well as MCPP. Significantly increased levels of mono-benzyl phthalate (MBzP), mono-*n*-butyl phthalate (MBP), and mono-iso-butyl phthalate (MiBP) were observed at Visit 4. Levels of urinary SG were relatively constant across pregnancy with means (standard deviations) as follows for each visit: Visit 1: 1.017 (0.008); Visit 2: 1.014 (0.008); Visit 3: 1.014 (0.008); and Visit 4: 1.015 (0.007).

Spearman correlations of individual phthalate metabolites within visit (e.g., between visit 1 MEHP, visit 2 MEHP, visit 3 MEHP, and visit 4 MEHP) were low to moderate (Table III.2). ICC for SG-corrected phthalate metabolites showed the lowest temporal reliability for DEHP metabolites and MCPP, and the highest reliability for MBzP and MBP (Table III.1). SG levels showed moderate reproducibility across pregnancy (ICC=0.38, 95% CI=0.33 to 0.43). When ICC were examined in cases and controls separately, coefficients were consistently, although slightly higher for all phthalate metabolites within mothers who delivered preterm, except for MiBP (Table III.3).

Predicted values from GAMM models were calculated using reference levels of included covariates. Smoothed plots of predicted phthalate levels across gestation showed a slight decrease in the total population as pregnancy progressed, similar to the patterns demonstrated in Table III.1 (data not shown). However, stratification by case status showed that while levels in controls remained relatively stable or even decreased across gestation, in spontaneous preterm cases many metabolite levels began to increase at about 20 weeks gestation and continued in an upward trend until delivery. Interaction terms between gestational age and spontaneous preterm birth were significant ($p<0.05$) or marginally significant for MEHP, MECPP, MBzP, and MBP (Figure III.1). In the analysis of placental preterm births alone, the only significant interaction detected was for MECPP, and in this plot cases had higher levels of MECPP early in pregnancy and

later had levels closer to those observed in controls (Figure III.2). For other metabolites, no significant interactions were observed between gestational age at sample collection and placental preterm, and levels were relatively flat across pregnancy.

Windows of susceptibility for preterm birth

Odds ratios (OR) from individual visits indicated that phthalate levels from visit 3 had the strongest associations with overall preterm birth (Table III.4). Models created for visit 4 may be biased because many preterm cases had already delivered by that time point, however results are presented for completeness. SG-corrected models showed significantly increased OR in association with MECPP and ∑DEHP levels at visits 1 and 3. Adjusted models for DEHP metabolites included urinary SG, maternal age at visit 1, race/ethnicity, education level, and time of day of urine sample collection (before vs. after 1pm) as covariates. Adjusted models for all other metabolites included urinary SG, maternal age, race/ethnicity, education level, and category of health insurance provider. OR from adjusted models were slightly attenuated compared to estimates from models adjusting for SG only. In both models OR for phthalate metabolites measured at visits 2 and 4 were generally null, although several OR for visit 4 were less than one.

Associations between phthalate exposure and odds of placental or spontaneous preterm births are presented in Tables III.5 and III.6, respectively. For placental preterm birth, the only relationship observed was in association with MECPP levels at visit 1, although OR for most other metabolites were somewhat higher at visit 1 compared to visits later in pregnancy. For spontaneous preterm birth, higher OR were observed for phthalate metabolite levels measured at visit 3 compared to other visits. OR for MECPP and ∑DEHP were elevated at visit 3, which was consistent with our observation for overall preterm birth, and relationships with spontaneous preterm birth emerged for MBzP and MBP at visit 3.

We additionally examined whether individual exposure curves across gestation were associated with preterm birth. Linear mixed models with random slopes and intercepts by subject were created, predicting phthalate level by gestational age at sample collection and adjusting for SG and time of day of sample collection (before vs. after 1pm) as covariates. Subject-specific random slopes from these models were then

examined in association with preterm birth, with and without adjustment for the covariates listed above. However, individual slopes were all close to zero and no meaningful associations were apparent (data not shown).

DISCUSSSION

In a nested case-control study from a large prospective birth cohort, we analyzed variability in urinary phthalate metabolite levels across pregnancy and identified vulnerable windows for the relationship between exposure and preterm birth. Furthermore, to address the mechanism for this relationship, we examined these associations within subsets of preterm birth which were classified based on clinical presentation.

Variability in phthalate levels across pregnancy

Urinary phthalate metabolite levels showed moderate individual and population-wide variability during pregnancy. DEHP metabolites and MCPP, all high molecular-weight phthalates, showed the most intra-individual variability; levels of low molecular-weight phthalates, including MBzP, MBP, MiBP, and MEP, were more stable. Additionally, most phthalate metabolites and particularly DEHP metabolites demonstrated a slightly downward sloping trend across gestation. Variability over pregnancy within women who later delivered prematurely was similar compared to women who carried to term, but trends in metabolite levels across pregnancy between these groups were different. In women who had a spontaneous preterm birth, metabolite levels decreased over the first half of pregnancy but began to slope upward at approximately 20 weeks gestation and continued to rise until parturition.

These conclusions support findings from smaller studies examining variability in urinary phthalate metabolites across the course of pregnancy. ICC for uncorrected DEHP metabolites were similar to those from a population of women from New York City with two to four urinary phthalate measurements taken between 33 weeks gestation and delivery (N=28 women) (Adibi et al. 2008). ICC for MBzP, MBP, and MiBP were somewhat lower in our population, which may have been due to the increased time in our study between sample collections or collection of samples earlier in gestation. In another

study of Boston women with at least two (median three) phthalate measures per woman from a wider window (3-38 weeks gestation), ICC for SG-corrected DEHP metabolites, MBzP, MBP, and MiBP were lower than in the present study but still conveyed the same trend, with low molecular weight metabolite measures being much more reliable over time compared to DEHP metabolite measures (N=113 women) (Braun et al. 2012).

The ICC for MEP levels in our study (ICC for SG-corrected MEP=0.47) was slightly higher compared to the ICC observed in a population of pregnant women in New York City (ICC=0.30) (Adibi et al. 2008) However our findings are consistent with the study in Boston women, where the highest ICC of all metabolites was observed for MEP (ICC for SG-corrected MEP=0.50), and with other studies of phthalate variability in adults over longer time periods, although those studies were in men (Frederiksen et al. 2013; Hauser et al. 2004).

In controls, DEHP metabolite and MCPP levels decreased slightly over the course of pregnancy, while levels of other metabolites showed little change. These findings are consistent with results from the study of pregnant women from Boston, where DEHP metabolite levels showed a significant decline across pregnancy (Braun et al. 2012). Such a decline may indicate that women are altering behavioral patterns during gestation that result in lower phthalate levels, or that pharmacokinetic changes occur during pregnancy that results in decreased excretion of metabolites (Braun et al. 2012). The latter seems less likely given the present data, as levels of non-DEHP metabolites remain largely unchanged for the duration of pregnancy.

Differences in variability by metabolites across pregnancy may be due to changes in exposure sources. Exposure to high molecular-weight phthalates may occur more prominently through ingestion of contaminated food and drinking water, and it seems highly likely that diet would change considerably across the course of pregnancy. Exposure to low molecular-weight phthalates, on the other hand, may be occurring more through use of personal care products, which could be more consistent across pregnancy. Sources and contributors to phthalate variability during pregnancy deserve more attention in future studies.

Windows of susceptibility for preterm birth

We previously demonstrated that average exposures to MEHP, MECPP, and ∑DEHP metabolites across the duration of pregnancy are associated with increased odds of preterm birth (Ferguson et al. 2014). In the present analysis, we expanded on these results, showing that odds ratios for overall preterm birth were strongest at visit 3, at approximately the beginning of the third trimester, for MECPP and ∑DEHP. Notably, odds ratios were not as strong as with average measurements, suggesting that a geometric average of multiple phthalate measurements over pregnancy may provide a more robust measure of exposure for assessing relationships with health outcomes.

Other studies examining the association between single measures of phthalate exposure during pregnancy and preterm birth or length of gestation have reported conflicting results, potentially due to the use of only one urine or blood sample for assessing exposure. The evidence from the present analysis strongly supports the conclusion that phthalate exposure at the beginning of the third trimester is related to increased odds of preterm birth. However, some metabolites measured at our visit 4 (median 35.1 weeks) were associated with reduced odds of preterm birth. As other studies may have observed similar effects due to sample collection later in pregnancy, these results may not be spurious. One possible explanation is that in those studies and in our subset with visit 4 measures available a statistical phenomenon similar to what is sometimes referred to as "harvesting" in air pollution epidemiology exists (Schwartz 2001). Under this scenario, women already at elevated risk of delivering prematurely are pushed to do so earlier by phthalate exposure, thereby decreasing the number of women in the high risk pool later in pregnancy. In this way, the already protective effect of survival until visit 4 against prematurity would be related to phthalate exposure. An alternative explanation is that women who carry until visit 4 or to sample collection in these other studies may be more likely to have a genetic polymorphism related to phthalate and other toxicant metabolism that is protective against preterm birth.

An advantage to the present analysis was our ability to stratify preterm cases based on clinical presentation at delivery, creating more homogenous subsets based on etiology of preterm birth. For placental preterm birth, odds ratios were generally null but were slightly elevated for MECPP measured at visit 1. Contrastingly, for spontaneous

preterm birth, odds ratios for nearly all metabolites were highest at visit 3, and the relationships with MECPP, ∑DEHP, MBzP, and MBP were particularly strong.

Two distinct mechanisms could explain the relationship between phthalate exposure and preterm birth. First, phthalate exposure early in pregnancy could cause impaired placentation early in pregnancy via induction of oxidative stress. MEHP and other phthalate metabolites have been shown to create oxidative stress in cellular studies (Fan et al. 2010; Tetz et al. 2013) and have been associated with oxidative stress biomarkers in cross-sectional studies of human populations (Ferguson et al. 2012; Hong et al. 2009). The intrauterine environment in early stages of placentation is highly sensitive. Increases in circulating maternal levels of reactive oxygen species can cause apoptosis and alter cytotrophoblast turnover rate in the developing placenta, leading to impaired invasion (Burton et al. 2009; Heazell and Crocker 2008). This impaired placentation can cause preeclampsia or IUGR which are characteristic of placental preterm birth. We observed some evidence to support this hypothesis, as MECPP measured during the first trimester was associated with increased odds of placental preterm birth. However, as one of many comparisons in this analysis, the association could have been spurious.

Spontaneous preterm birth results primarily from inappropriate initiation of an intrauterine inflammatory cascade that leads to a sequence of events, encompassing preterm rupture of membranes, cervical ripening, and parturition (Challis et al. 2009). Some phthalates have also been shown to induce proinflammatory cytokine release in cell lines (Jepsen et al. 2004; Nishioka et al. 2012) and have been linked to increased systemic levels of inflammatory markers such as C-reactive protein in humans (Ferguson et al. 2011). Our data support this mechanism, as for spontaneous preterm births we saw the strongest associations with phthalate metabolites measured in third trimester urine samples. Alternative pathways, for example via phthalate disruption of reproductive hormones during development and maintenance of pregnancy, are plausible as well, and further investigation will be necessary before drawing any firm conclusions.

The primary strengths of our study were the large number of subjects and repeated time points from which we collected phthalate measurements. This enabled us to examine the relationship between phthalate exposures at more than one time point during

pregnancy in relation to preterm birth, which has not been done previously. When phthalate metabolite levels from individual time points during pregnancy were modeled in relation to subtypes of preterm birth, we observed that MECPP exposure early in pregnancy may be related to a modest but significant increase in odds of placental preterm birth. MECPP, ∑DEHP, MBzP, and MBP levels measured near the beginning of the third trimester were associated with increased odds of spontaneous preterm birth. These data support previous evidence of a relationship between phthalate exposure and prematurity, and highlight potential mechanisms that deserve further exploration in toxicology and population studies.

Table III.1 Specific gravity-corrected urinary phthalate metabolite geometric means (standard deviations) by study visit.

	Visit 1	Visit 2	Visit 3	Visit 4	ICC (95% CI)
N (cases, controls)	129, 350	118, 304	111, 301	66, 314	129, 349
MEHP (ug/L)	12.7 (3.78)	11.3 (3.33)	9.83 (3.27) *	9.94 (3.44) *	0.30 (0.25, 0.35)
MEHHP (ug/L)	40.8 (3.69)	34.1 (3.13)*	27.1 (3.42) *	34.9 (3.37) *	0.21 (0.17, 0.27)
MEOHP (ug/L)	20.1 (3.62)	18.2 (3.05)	15.8 (3.38) *	20.1 (3.27)	0.19 (0.15, 0.25)
MECPP (ug/L)	51.8 (3.53)	43.0 (3.25)*	38.5 (3.47) *	48.7 (3.39)	0.31 (0.26, 0.36)
∑DEHP (µmol/L)	0.46 (3.38)	0.39 (3.00)*	0.33 (3.13) *	0.41 (3.17)	0.23 (0.18, 0.28)
MBzP (ug/L)	6.95 (3.19)	6.95 (3.05)	6.89 (2.95)	7.86 (2.97) *	0.61 (0.56, 0.65)
MBP (ug/L)	17.9 (2.57)	18.3 (2.62)	17.4 (2.75)	19.9 (2.33) *	0.57 (0.53, 0.62)
MiBP (ug/L)	7.28 (2.25)	7.17 (2.33)	7.30 (2.35)	9.04 (2.21) *	0.52 (0.48, 0.57)
MEP (ug/L)	140 (4.42)	147 (4.85)	140 (4.67)	147 (5.00)	0.47 (0.42, 0.52)
MCPP (ug/L)	2.27 (3/46)	2.30 (3.35)	1.95 (3.02) *	2.11 (2.89)	0.36 (0.31, 0.41)

Note. *Significant difference ($p<0.05$) in urinary phthalate metabolite concentration compared to Visit 1 (reference).

Table III.2 Spearman correlation coefficients between specific gravity-corrected urinary phthalate metabolite concentrations from individual study visits.

	MEHP_1	MEHP_2	MEHP_3
MEHP_2	0.31		
MEHP_3	0.27	0.49	
MEHP_4	0.24	0.31	0.35

	MEHHP_1	MEHHP_2	MEHHP_3
MEHHP_2	0.24		
MEHHP_3	0.25	0.31	
MEHHP_4	0.85	0.23	0.28

	MEOHP_1	MEOHP_2	MEOHP_3
MEOHP_2	0.20		
MEOHP_3	0.24	0.27	
MEOHP_4	0.81	0.80	0.23

	MECPP_1	MECPP_2	MECPP_3
MECPP_2	0.30		
MECPP_3	0.34	0.38	
MECPP_4	0.28	0.26	0.33

	∑DEHP_1	∑DEHP_2	∑DEHP_3
∑DEHP_2	0.23		
∑DEHP_3	0.26	0.32	
∑DEHP_4	0.21	0.21	0.27

	MBzP_1	MBzP_2	MBzP_3
MBzP_2	0.61		
MBzP_3	0.59	0.63	
MBzP_4	0.52	0.57	0.59

	MBP_1	MBP_2	MBP_3
MBP_2	0.46		
MBP_3	0.43	0.43	
MBP_4	0.37	0.50	0.44

	MiBP_1	MiBP_2	MiBP_3
MiBP_2	0.51		
MiBP_3	0.51	0.57	
MiBP_4	0.51	0.49	0.62

	MEP_1	MEP_2	MEP_3
MEP_2	0.60		
MEP_3	0.47	0.50	
MEP_4	0.43	0.44	0.55

	MCPP_1	MCPP_2	MCPP_3
MCPP_2	0.33		
MCPP_3	0.35	0.36	
MCPP_4	0.27	0.30	0.37

Table III.3 Urinary specific gravity-corrected phthalate metabolite intraclass correlation coefficients (95% confidence intervals) by case control status (N=129 cases, N=349 controls).

Metabolite	Cases	Controls
MEHP	0.33 (0.23, 0.44)	0.29 (0.23, 0.35)
MEHHP	0.24 (0.15, 0.36)	0.20 (0.15, 0.27)
MEOHP	0.20 (0.12, 0.33)	0.19 (0.13, 0.25)
MECPP	0.38 (0.28, 0.48)	0.27 (0.22, 0.34)
∑DEHP	0.26 (0.17, 0.38)	0.21 (0.16, 0.27)
MBzP	0.63 (0.55, 0.71)	0.60 (0.55, 0.65)
MBP	0.74 (0.67, 0.79)	0.43 (0.37, 0.49)
MiBP	0.48 (0.39, 0.58)	0.54 (0.48, 0.59)
MEP	0.50 (0.41, 0.60)	0.46 (0.40, 0.52)
MCPP	0.38 (0.28, 0.49)	0.34 (0.28, 0.40)

Table III.4 Odds ratios (95% CI) of overall preterm birth in association with ln-unit increase in urinary phthalate metabolite concentration.

	OR (95% CI) of preterm birth			
	Visit 1	Visit 2	Visit 3	Visit 4
N (cases, controls)	129, 350	118, 304	111, 301	66, 314
MEHP	1.11 (0.95, 1.29)	1.13 (0.94, 1.35)	1.17 (0.97, 1.40)	1.08 (0.87, 1.34)
MEHHP	1.03 (0.89, 1.20)	0.91 (0.75, 1.10)	1.06 (0.89, 1.26)	0.80 (0.64, 1.00)
MEOHP	1.09 (0.94, 1.28)	0.97 (0.80, 1.17)	1.14 (0.95, 1.35)	0.87 (0.69, 1.08)
MECPP	1.27 (1.08, 1.49)	1.08 (0.91, 1.30)	1.30 (1.09, 1.54)	1.10 (0.89, 1.37)
∑DEHP	1.18 (1.00, 1.39)	1.04 (0.86, 1.26)	1.24 (1.03, 1.49)	1.00 (0.80, 1.26)
MBzP	1.02 (0.86, 1.22)	1.02 (0.84, 1.24)	1.12 (0.92, 1.36)	0.94 (0.74, 1.20)
MBP	1.19 (0.97, 1.47)	1.17 (0.93, 1.46)	1.21 (0.98, 1.49)	1.13 (0.84, 1.50)
MiBP	1.04 (0.81, 1.33)	1.07 (0.83, 1.39)	0.97 (0.77, 1.24)	0.86 (0.63, 1.19)
MEP	1.05 (0.92, 1.20)	1.07 (0.94, 1.23)	1.06 (0.92, 1.21)	0.93 (0.79, 1.10)
MCPP	1.18 (1.01, 1.38)	1.00 (0.84, 1.19)	1.19 (0.98, 1.44)	1.11 (0.87, 1.41)

Note. Models adjusted for urinary specific gravity of respective visit.

Table III.5 Adjusted odds ratios (95% CI) of placental preterm birth in association with ln-unit increase in urinary phthalate metabolite.

	Visit 1	Visit 2	Visit 3	Visit 4
N (cases, controls)	35, 336	31, 297	32, 289	15, 303
MEHP	1.12 (0.85, 1.48)	1.03 (0.75, 1.41)	1.07 (0.78, 1.48)	1.02 (0.64, 1.63)
MEHHP	1.14 (0.86, 1.52)	0.99 (0.71, 1.39)	1.19 (0.86, 1.63)	0.74 (0.42, 1.29)
MEOHP	1.18 (0.88, 1.57)	1.03 (0.74, 1.45)	1.20 (0.87, 1.66)	0.91 (0.52, 1.56)
MECPP	1.46 (1.10, 1.95)	1.22 (0.90, 1.67)	1.32 (0.98, 1.78)	1.29 (0.79, 2.11)
∑DEHP	1.33 (0.99, 1.78)	1.14 (0.82, 1.60)	1.26 (0.91, 1.74)	1.04 (0.61, 1.78)
N (cases, controls)	33, 334	29, 295	30, 288	13, 300
MBzP	1.02 (0.73, 1.43)	1.07 (0.73, 1.55)	1.00 (0.68, 1.48)	1.02 (0.57, 1.84)
MBP	0.97 (0.62, 1.50)	1.23 (0.79, 1.93)	1.15 (0.77, 1.72)	0.94 (0.40, 2.22)
MiBP	0.92 (0.57, 1.47)	0.88 (0.54, 1.41)	0.75 (0.50, 1.13)	0.66 (0.28, 1.55)
MEP	0.92 (0.71, 1.19)	0.96 (0.74, 1.26)	0.99 (0.77, 1.27)	0.88 (0.60, 1.29)
MCPP	1.20 (0.90, 1.60)	0.91 (0.64, 1.28)	1.18 (0.83, 1.69)	1.24 (0.74, 2.09)

Note. Adjusted models include specific gravity of respective visit, maternal age, Race/Ethnicity, and education level. DEHP metabolite models additionally adjusted for time of day of sample collection (before vs. after 1pm). Other metabolite models additionally adjusted for health insurance category.

Table III.6 Adjusted odds ratios (95% CI) of spontaneous preterm birth in association with ln-unit increase in phthalate metabolite.

	Visit 1	Visit 2	Visit 3	Visit 4
N (cases, controls)	56, 336	52, 297	47, 289	25, 303
MEHP	1.17 (0.94, 1.46)	1.12 (0.88, 1.43)	1.28 (0.99, 1.66)	1.25 (0.90, 1.74)
MEHHP	1.09 (0.87, 1.36)	0.89 (0.68, 1.16)	1.19 (0.92, 1.54)	1.06 (0.76, 1.48)
MEOHP	1.14 (0.90, 1.45)	0.94 (0.71, 1.23)	1.28 (0.98, 1.66)	1.12 (0.79, 1.58)
MECPP	1.26 (0.99, 1.60)	1.01 (0.79, 1.30)	1.33 (1.04, 1.70)	1.27 (0.90, 1.78)
∑DEHP	1.21 (0.95, 1.55)	0.99 (0.75, 1.29)	1.33 (1.02, 1.73)	1.22 (0.85, 1.73)
N (cases, controls)	56, 334	52, 295	47, 288	25, 300
MBzP	1.16 (0.88, 1.52)	1.20 (0.90, 1.60)	1.43 (1.05, 1.95)	1.24 (0.83, 1.85)
MBP	1.32 (0.99, 1.77)	1.19 (0.87, 1.63)	1.45 (1.08, 1.96)	1.56 (0.99, 2.46)
MiBP	1.14 (0.78, 1.68)	1.36 (0.94, 1.97)	1.28 (0.88, 1.86)	1.26 (0.76, 2.10)
MEP	1.11 (0.91, 1.35)	1.20 (0.98, 1.46)	1.16 (0.94, 1.42)	0.95 (0.73, 1.24)
MCPP	1.18 (0.96, 1.47)	1.15 (0.91, 1.44)	1.25 (0.96, 1.64)	1.33 (0.93, 1.90)

Note. Adjusted models include specific gravity of respective visit, maternal age, Race/Ethnicity, and education level. DEHP metabolite models additionally adjusted for time of day of sample collection (before vs. after 1pm). Other metabolite models additionally adjusted for health insurance category.

Figure III.1 Predicted urinary phthalate metabolite concentrations (95% confidence intervals) from generalized additive mixed models in mothers with spontaneous preterm (light gray) compared to term (dark gray) births. N=180 obervations for cases, N=1211 observations for controls.

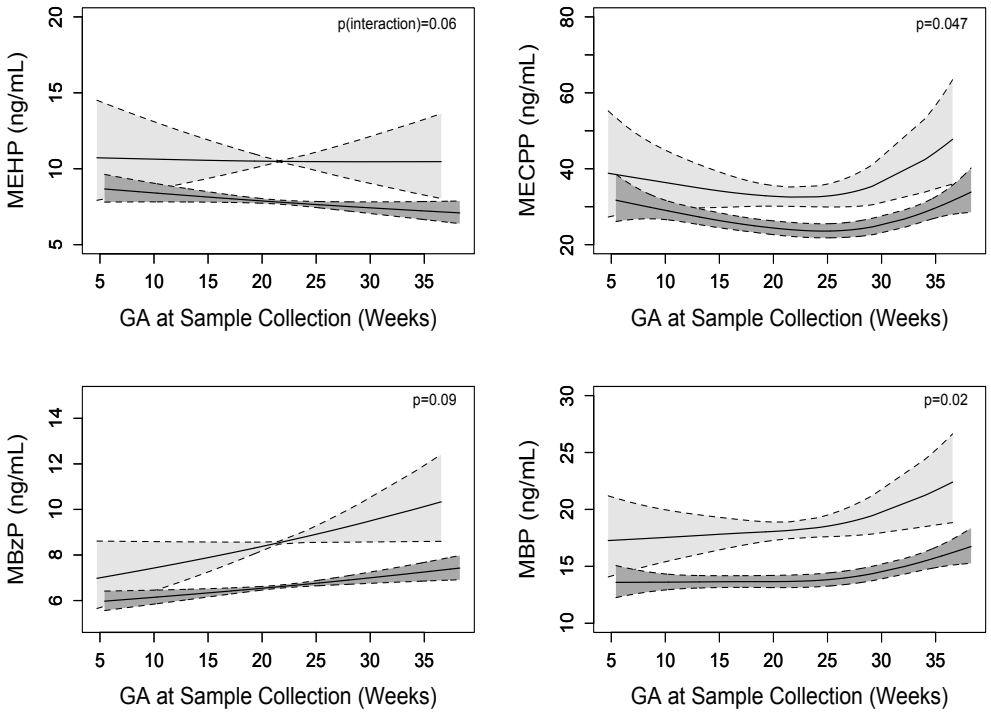
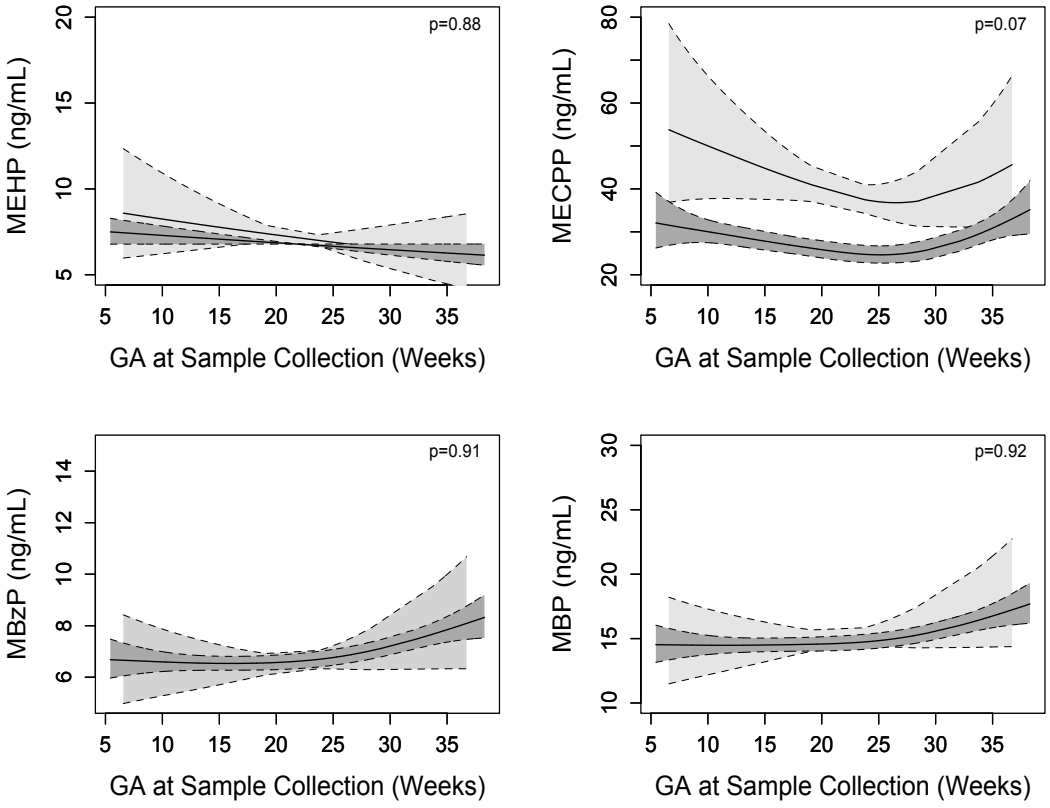


Figure III.2 Predicted urinary phthalate metabolite concentrations (95% confidence intervals) from generalized additive mixed models in mothers with placental preterm (light gray) compared to term (dark gray) births. N=104 obervations for cases, N=1211 observations for controls.



CHAPTER IV

URINARY PHTHALATE METABOLITES ARE ASSOCIATED WITH INCREASED OXIDATIVE STRESS BIOMARKERS IN PREGNANT WOMEN

INTRODUCTION

Phthalate diesters are used as plasticizers and solvents in a variety of consumer products, and can readily enter human systems through ingestion, inhalation, and dermal absorption (ATSDR 2001, 2002). Although diesters are metabolized and excreted quickly, constant contact results in daily exposures for the majority of the US population. Metabolites are consistently detected in urine of pregnant women in populations worldwide (Cantonwine et al. 2013; Lin et al. 2011; Woodruff et al. 2011; Zeman et al. 2013).

While phthalates are best known for their action as endocrine disruptors, there is also evidence from cellular and animal studies that mono (2-ethyl-hexyl) phthalate (MEHP) can cause oxidative stress by inducing release of reactive oxygen species (ROS) and/or impairing antioxidant defenses (Erkekoglu et al. 2010; Kasahara et al. 2002; Tetz et al. 2013; Zhao et al. 2012). However, few studies have examined this association in humans. Three cross-sectional studies have observed associations between some phthalate metabolites and serum levels of bilirubin, a potent antioxidant, and systemic markers of oxidative stress including serum gammaglutamyl transferase, and urinary malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) (Ferguson et al. 2012, 2011; Hong et al. 2009). A more recent longitudinal study in elderly subjects also observed a strong association between summed di (2-ethylhexyl) phthalate (DEHP) metabolites and MDA (Kim et al. 2013).

To our knowledge, no studies have examined the relationship between urinary phthalate metabolites and biomarkers of oxidative stress during pregnancy, when these possible effects represent particular concern given the gestational vulnerability of the

developing fetus. Increases in oxidative stress biomarkers in pregnant women have been associated with risk of pregnancy loss, preeclampsia, preterm birth, and fetal growth restriction (Agarwal et al. 2012; Stein et al. 2008). Additionally, these changes are relevant to a number of other outcomes in the general population, such as infertility, various cancers, and type 2 diabetes. In the present study we examined longitudinal associations between urinary phthalate metabolites and biomarkers of oxidative stress in pregnant women.

METHODS

Study population

Pregnant women were recruited prior to 15 weeks gestation at Brigham and Women's Hospital in Boston from 2006-2008 as part of a large prospective cohort study. Participants were included if they had a singleton pregnancy that resulted in a live birth. Women were followed throughout the duration of pregnancy and provided demographic and anthropometric data, urine samples from up to 4 study visits (targeted for 10, 18, 26, and 35 weeks gestation), as well as birth outcome data at delivery. Gestational age was calculated from last menstrual period and validated with first trimester ultrasound; if gestational ages calculated by the two methods differed by greater than 8% ultrasound dating was used. For the present study, 130 women who delivered preterm and 352 random controls were selected and their urine samples extracted from -80 degrees C storage for laboratory analysis (N=482 subjects total).

The nested-case control study was designed with the intention of examining associations between urinary phthalate metabolite across gestation and preterm birth (Ferguson et al. 2014). The present analysis examining the relationship between urinary phthalate metabolites and biomarkers of oxidative stress was a secondary aim of this study to help inform potential biological mechanisms involved. Our goal was to characterize these associations in a population that would be generalizable to the overall cohort. Therefore, the analyses was weighted using inverse probability weightings calculated based on selection probabilities for cases (90.1 percent) and controls (33.9 percent) from the parent cohort population (Jiang et al. 2006).

Urinary phthalate metabolites

All available urine samples (N=1693) were assayed for concentrations of 9 phthalate metabolites using high performance liquid chromatography and tandem mass spectrometry by NSF International in Ann Arbor, MI (Lewis et al. 2013; Silva et al. 2007). All metabolites were highly detectable (>95 percent); levels below the limit of detection (LOD) were replaced with the LOD/√2 (Hornung and Reed 1990). All distributions were right-skewed and natural log-transformed to meet normality assumptions for statistical analysis. To adjust for urine dilution, specific gravity (SG) was measured at the time of phthalate analysis using a handheld refractrometer (Atago Co., Ltd., Tokyo, Japan). For bivariate analysis we created SG-corrected concentrations using the following formula: $P_c=P[(1.015-1)/(SG-1)]$, where P_c is the corrected phthalate concentration, P is the raw concentration, 1.015 is the median SG for the study population, and SG is the specific gravity for the sample (Meeker et al. 2009). In regression models uncorrected phthalate metabolite levels were used and models were adjusted for SG as a covariate.

In addition to examining individual urinary phthalate metabolites we created a summed measure of DEHP metabolites (∑DEHP). MEHP, mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono (2-ethyl-5-carboxypentyl) phthalate (MECPP) concentrations were converted from µg/L to µmol/L using molecular weights (278, 294, 292, and 308 g/mol) and summed to create ∑DEHP.

Oxidative stress biomarkers

All urine samples with sufficient volume remaining following phthalate measurement (N=1678) were assayed for levels of 8-OHdG and 8-isoprostane using enzyme immunoassay by Cayman Chemical (Ann Arbor, MI). For 8-isoprostane measurement, samples were first hydrolyzed and passed through columns for affinity purification. Levels were detected to 3.9 pg/mL. For 8-OHdG measurement, samples were assayed without purification and levels were detected to 10.3 pg/mL. As with phthalate metabolites, undetected oxidative stress measures were replaced with the LOD/√2 (Hornung and Reed 1990). For calculation of distributions overall and by

categorical covariates biomarkers were corrected for urinary SG using the formula applied to phthalate measures above. Uncorrected biomarker concentrations were used for multivariate models with SG as a covariate, and SG-corrected levels were examined in a sensitivity analysis.

Statistical analysis

All statistical analysis was performed in R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria) and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Unless stated otherwise, all analysis was performed with inverse probability weightings. Overall distributions of urinary phthalate metabolites and oxidative stress biomarkers were assessed using SG-corrected concentrations. Variability in oxidative stress biomarker concentrations across gestation was examined using intraclass correlation coefficients (ICC), which represent a ratio of within to between individual variability (Rosner 2011). ICC for urinary phthalate metabolites have been examined previously (unpublished data), and were similar to other studies observing greater reproducibility of mono-benzyl phthalate (MBzP), mono-*n*-butyl phthalate (MBP), and mono-iso-butyl phthalate (MiBP) compared to DEHP metabolites across pregnancy (Adibi et al. 2008; Braun et al. 2012). Geometric means and standard deviations of corrected concentrations were created by categorical covariate groups and compared using subject-specific random intercepts in linear mixed models (LMM) to adjust for intra-individual correlation of measurements at multiple time points (*nlme* package in R) (Pinheiro et al. 2013). Covariates examined included race/ethnicity, education level, health insurance provider, body mass index (BMI) at the initial visit, tobacco and alcohol use during pregnancy, parity, infant gender, and use of assisted reproductive technology. We also examined the relationship between exposure and outcome biomarkers with time-varying covariates, including BMI and time of day of urine sample collection, which was dichotomized into before vs. after 1PM based on histograms which displayed a nadir in urinary phthalate metabolite concentrations at that time of day. These associations were also examined using LMM with random intercepts only.

Associations between uncorrected urinary phthalate metabolites and oxidative stress biomarkers were measured using LMM to adjust for intra-individual correlation,

and relationships were assessed by examining fixed effects only. For all statistical models very concentrated urine samples (SG>1.04) were excluded because markers measured in those samples may be inaccurate (N=4) (Boeniger et al. 1993; Braun et al. 2012). One oxidative stress biomarker was regressed on one phthalate metabolite per model. Crude models were adjusted for gestational age and urinary SG. Full models additionally included covariates that were significantly ($p<0.05$) associated with one or both oxidative stress biomarkers as well as one or more urinary phthalate metabolites.

Several sensitivity analyses were performed. First we examined associations in a stratified analysis of cases and controls separately. Second, we examined associations after excluding mothers who used alcohol and tobacco during pregnancy, as number of users was too small to include these as covariates. Third, we created generalized additive mixed models (GAMM) to investigate the possibility that the relationships between oxidative stress biomarkers and urinary phthalate metabolites were non-linear. Fourth, to examine sensitive windows of vulnerability for the relationship between phthalate exposure and oxidative stress, we examined interaction terms between urinary metabolites and gestational age at sample collection, also in LMM with random intercepts. Finally, we examined the effect of including multiple urinary phthalate metabolites in a single LMM.

RESULTS

Population demographics were presented elsewhere for the case control study population (Ferguson et al. 2014) and are presented in Table IV.1 for the weighted population examined in the present analysis. Maternal age at visit 1 was 32.0 years on average, and most women were White (59%), well educated (71% with junior college, some college, or above), did not use tobacco (94%) or alcohol (95%) during pregnancy, and did not used assisted reproductive technology to get pregnant (91%). More than half of infants were female (55%). Approximately half of the participants had normal to low BMI at visit 1 (53%), and as expected this proportion decreased across pregnancy (visit 2=47%, visit 3=28%, visit 4=15%). After weighting, deliveries in the study population were approximately 12% preterm (<37 weeks gestation).

Urine samples were collected from study participants at up to four visits (mean 3.52 samples per subject; N=3 subjects with one urine sample; N=38 subjects with 2 samples; N=148 subjects with 3 samples; N=293 subjects with 4 samples). Samples for visit 1 (median 9.95 weeks gestation) were collected for 474 subjects (range 4.17-19.1 weeks); for visit 2 (median 18.0 weeks) samples were collected for 421 subjects (range 14.9-32.1 weeks); for visit 3 (median 26.0 weeks) samples were collected for 409 subjects (range 22.9-36.3 weeks); and for visit 4 (median 35.2 weeks) samples were collected for 374 subjects (range 33.1-38.3 weeks). The proportion of cases with samples available was consistent for visits 1-3 (86-100 percent) but low for visit 4 (51 percent) as many had already delivered by that time point. Most urine samples were collected before 1PM at visits 1 (60%), 2 (66%), 3 (70%), and 4 (69%).

Urinary phthalate metabolite and oxidative stress biomarker distributions are presented in Table IV.2. Phthalate metabolites were detected in 95 to 100% of all samples measured, 8-OHdG was detected in all samples, and 8-isoprostane was below the LOD in 67 (4.0%) samples. As reported previously, correlations between phthalate metabolites were strongest within DEHP metabolites, as expected (Spearman R=0.68 to 0.91), were moderate between MBzP, MBP, and MiBP (R=0.46 to 0.62) and between mono (3-carboxypropyl) phthalate (MCPP) and DEHP metabolites (R=0.35 to 0.46) and were weak between all other metabolites (R=0.01 to 0.21) (Ferguson et al. 2014). Correlations between the two oxidative stress markers at each visit were weak but statistically significant (R=0.10 to 0.20, $p<0.05$). 8-OHdG concentrations were more variable across pregnancy (ICC=0.32, 95% confidence interval [CI]=0.27-0.38) compared to 8-isoprostane (ICC=0.60, 95% CI=0.56-0.64).

When oxidative stress biomarker concentrations were compared by categorical covariates, we observed different patterns for each marker (Table IV.3). Levels of 8-isoprostane were lowest in mothers who were white, had higher levels of education and better health insurance, were of lower BMI at visit 1, and who did not use tobacco or alcohol. Few differences were observed by categorical covariates for 8-OHdG, although mothers with better health insurance had significantly lower levels. Associations with covariates were also different for urinary phthalate metabolites. MEHP concentrations were higher in African American compared to white mothers but no other significant

differences were observed (Table IV.3). Likewise, few differences were observed for other DEHP metabolites, although concentrations were higher in mothers who used alcohol during pregnancy (Table IV.4). Patterns for MBzP, MiBP, and mono-ethyl phthalate (MEP) were similar to MBP (Table IV.3, Table IV.4); higher concentrations were observed in mothers who were African American or other race/ethnicity compared to white, in mothers with lower education levels, in mothers with Medicaid/social security income/MassHealth compared to Private insurance/health maintenance organization/self-pay, and in mothers with higher BMI at visit 1. Urinary MCPP concentrations did not differ by categorical covariates except that levels were slightly lower in mothers who did not smoke during pregnancy (Table IV.4). No differences in oxidative stress biomarkers and few differences in urinary phthalate metabolites were observed by fetus gender (lower MiBP concentrations in mothers of female vs. male fetus) or use of assisted reproductive technology (higher MEHHP concentrations and lower MBzP and MiBP concentrations in mothers who used assisted reproductive technology compared to those who did not).

For time-varying covariates, significantly higher oxidative biomarkers concentrations were observed in samples collected before vs. after 1PM, and significantly lower urinary phthalate metabolite concentrations were observed in samples collected before vs. after 1PM for all metabolites except MiBP and MEP which were slightly higher in the morning. When BMI was examined as a time-varying covariate, both oxidative stress biomarkers were positively associated with increasing BMI category. MEHP was inversely associated with the highest BMI category, and MBzP was positively associated with highest BMI category, but otherwise associations with urinary phthalate metabolites were null. Because longitudinal BMI measures may more accurately capture confounding by this variable, time-varying BMI was included as a covariate in fully adjusted models.

LMM modeling the relationship between one exposure and one outcome biomarker were created using random intercepts only as addition of random slopes did not significantly improve model fit, based on Akaike information criterion. Crude models were created with adjustment for gestational age at sample collection and urinary SG. Adjusted models included these covariates as well as maternal race/ethnicity, education

level, health insurance provider, time-varying BMI, and time of day of urine sample collection (before vs. after 1pm). Tobacco and alcohol use were excluded from adjusted models because of the small number of subjects who used either during pregnancy (N=31 and 20, respectively).

Effect estimates from adjusted models were similar to those from crude models; adjusted results alone are presented in Table IV.5. Fixed effect results are presented in the form of percent increase in oxidative stress biomarker with an interquartile range (IQR) increase in untransformed phthalate metabolite. All phthalate metabolites were associated with increases in 8-OHdG levels; the highest coefficients observed were for MBzP (percent change with IQR increase [%Δ]=20.7, 95% CI=15.6-26.1), MBP (%Δ=18.1, 95% CI=13.5-22.9), and MiBP (%Δ=30.3, 95% CI=24.4-36.5). All metabolites were associated with significant increases in 8-isoprostane levels, and coefficients were of greater magnitude compared to those observed in models predicting 8-OHdG. However, as with 8-OHdG, the largest changes in 8-isoprostane were in association with MBzP (%Δ=42.7, 95% CI=31.8-54.4), MBP (%Δ=42.0, 95% CI=32.0-52.7), and MiBP (%Δ=56.4, 95% CI=43.9-69.9).

Sensitivity analyses

LMM were replicated when stratifying by preterm birth case status. Effect estimates were similar with some exceptions (Tables IV.6 and IV.7, respectively). In cases alone, associations between 8-OHdG and MBP (%Δ=8.92, 95% CI=2.03, 16.3), MiBP (%Δ=20.3, 95% CI=10.2, 31.3), and MEP (%Δ=19.2, 95% CI=10.3, 28.8), and also between 8-isoprostane and MBP (%Δ=30.9, 95% CI=15.8, 48.0) had smaller effect estimates compared to associations in weighted models. Effect estimates from models of controls alone were similar to those from weighted models.

We also examined the effect of removing alcohol and tobacco users from the population; effect estimates to similar to those observed in the overall population. GAMM models were created to examine deviation of the oxidative stress-phthalate metabolite relationships from linearity. Models included the same covariates as in LMM. Based on visual inspection of smooth plots, relationships were largely linear (data not shown).

To identify potentially sensitive time points for the relationship between phthalate exposure and maternal oxidative stress, we additionally examined interaction terms in fully adjusted LMM between phthalate metabolite and gestational age at sample collection. These models were created in preterm cases and controls separately, because of the difference in proportions of cases compared to controls with measurements available at each time point, particularly at visit 4. No significant interactions were observed for 8-OHdG or 8-isoprostane models (data not shown).

Finally, we examined ∑DEHP metabolites as well as MiBP in the same model, as these measures were weakly correlated but represented important predictors for both oxidative stress responses. In the model for 8-OHdG, effect estimates remained significant for both phthalate variables but were slightly lower for ∑DEHP (%Δ=3.40, 95% CI=0.19, 6.17) and slightly higher for MiBP (%Δ=34.5, 95% CI=27.2, 42.1). The same was true in models for 8-isoprostane (%Δ for ∑DEHP=13.0, 95% CI=7.12, 19.3; %Δ for MiBP=60.9, 95% CI=46.0, 77.2).

DISCUSSION

We examined the longitudinal association between urinary phthalate metabolites and 8-OHdG and 8-isoprostane as biomarkers of oxidative stress during pregnancy. We observed that all phthalate metabolites were associated with increases in both biomarkers. Effect estimates were greater for associations with 8-isoprostane compared to 8-OHdG, and, among phthalates, MBzP, MBP, and MiBP, showed strongest associations with both outcome measures.

Many different biomarkers have been used in environmental and other epidemiologic studies to indicate systemic levels of oxidative stress. These can have very different specificities, both in terms of mechanism and downstream physiologic effect. The long time goal of the National Institute of Environmental Health Sciences Biomarkers of Oxidative Stress Study has been to identify sensitive and specific markers of oxidative injury, and best methods for measuring these markers in animal and eventually human matrices (Kadiiska et al. 2013; NIEHS 2012). However this task remains difficult, because of the numerous available markers and assays for detection, the long list of mechanisms that can cause oxidative stress, and the temporal instability of

some biomarkers, among other reasons. We selected 8-OHdG and 8-isoprostane for measurement in this study due to their well documented usefulness as systemic biomarkers of oxidative stress (Il'yasova et al. 2012), but also their representation of different cellular reactions to ROS exposure and their potential downstream effects.

As evidenced by their low correlation in this and other studies (Stein et al. 2008), urinary levels of 8-OHdG and 8-isoprostane represent two distinct cellular processes. 8-OHdG is a DNA adduct formed in the presence of excess ROS (e.g., hydroxyl radicals) (Wu et al. 2004). Via repair mechanisms, oxidized nucleotides are excised from DNA and excreted in the urine, making repair capabilities an important factor in urinary concentrations (Wu et al. 2004). 8-isoprostane is formed in a non-enzymatic reaction between ROS and arachidonic acid and is advantageous because it is very specific to lipid oxidation, yet is not affected by dietary lipid intake, and is highly detectable in urine samples (Roberts and Morrow 2000). Measurement of specific isomers (e.g., the biologically active 8-iso-$PGF_{2\alpha}$) with liquid chromatography/mass spectrometry is preferable to enzyme immunoassay, however multiple isomers can be representative of oxidative lipid damage and the liquid chromatography/mass spectrometry method in a study of this size is cost-prohibitive (Il'yasova et al. 2012; Smith et al. 2011).

In addition to indicating oxidative stress, these products may have direct physiologic consequences for pregnancy. Oxidative DNA damage in the intrauterine compartment could result in apoptosis at the maternal-fetal interface (Heazell et al. 2007) which can lead to poor vascularization of the placenta and consequently preeclampsia and/or intrauterine growth restriction (Potdar et al. 2009). Increased levels of prostaglandins such as 8-isoprostane may be particularly dangerous later in pregnancy because of their involvement in the preterm parturition pathway (Challis et al. 2009). This study illustrates that phthalates are associated with increases in both responses in pregnant women, which suggests that exposure to phthalates could play a role in downstream pregnancy outcomes via multiple mechanisms.

In a number of cellular studies phthalates have been shown to cause increases in ROS and various markers of oxidative stress, potentially via activation of peroxisome proliferator activated receptors or by increasing permeability of mitochondrial membranes (Hurst and Waxman 2003; Rosado-Berrios et al. 2011). These studies have

been performed using DEHP and/or MEHP in a number of cell types, including placental cells (Tetz et al. 2013), Leydig cells (Erkekoglu et al. 2010; Zhou et al. 2013), neutrophils (Vetrano et al. 2010), and Kupffer cells (Rusyn et al. 2001). Other phthalates and their metabolites have been studied less frequently, although there is also evidence that they may be capable of inducing oxidative stress (O'Brien et al. 2001; Zhou et al. 2010). Relative capacities for different phthalate metabolites to induce oxidative stress have not been examined.

In humans, studies of phthalates in relation to oxidative stress biomarkers have been limited. Two reports have examined the relationship in National Health and Nutrition Examination Survey, using gammaglutamyl transferase and bilirubin as markers of oxidative stress and a panel of phthalate metabolites similar to those measured in the present study. In the study of gammaglutamyl transferase, positive associations were observed in association with MEHP only, although C-reactive protein, a systemic marker of inflammation which may also be indicative of oxidative stress, was positively associated with MiBP and MBzP (Ferguson et al. 2011). In the study of bilirubin, a potent antioxidant which may be inversely related to oxidative stress levels, DEHP and dibutyl phthalate (DBP) metabolites as well as MCPP were found to be associated with significantly decreased bilirubin, although the strongest associations appeared to be for DEHP metabolites and MCPP (Ferguson et al. 2012).

Another cross-sectional study of urban-dwelling adults examined the relationship between phthalate metabolites and MDA as well as 8-OHdG (N=960) (Hong et al. 2009). The results from this analysis demonstrated significant and positive associations with DEHP metabolites as well as MBP and both oxidative stress biomarkers, although associations with 8-OHdG lost significance in adjusted models. Regression coefficients were higher for MDA compared to 8-OHdG in DEHP metabolite models, but lower for MBP models. Regression coefficients were also higher for DEHP metabolites compared to MBP for both outcomes. Finally, one longitudinal study in elderly subjects with measurements taken up to 5 times over three years observed a positive relationship between summed DEHP metabolites and MDA levels (N=560 subjects) (Kim et al. 2013). MBP levels were measured in the study but associations with MDA were not reported.

Our findings are somewhat consistent with these prior studies. As reported by Hong and colleagues, we also observed positive associations between phthalates and 8-OHdG. Consistent with the studies of MDA, we observed increased levels of 8-isoprostane in association with urinary phthalate metabolites that appeared to be stronger than the associations observed with 8-OHdG. However, contrary to these studies, we observed the strongest associations for MBzP, MBP, and MiBP compared to DEHP metabolites for both outcome measures. This disparity could be due to differences in diet, product use, toxicant metabolism, and/or other differences between study populations. Alternatively, it could be a result of lower temporal variability in those metabolites across pregnancy (Adibi et al. 2008; Braun et al. 2012). This would result in less measurement error and stronger associations, even if the true relationships between oxidative stress biomarkers and different phthalate metabolites were similar. Finally, it is possible that the associations observed are an effect of unknown confounders.

In conclusion, we report large and precise increases in oxidative stress biomarkers in association with urinary phthalate metabolites during pregnancy. Our ability to detect these relationships may be largely attributable to our longitudinal study design, with measurement of both urinary phthalate metabolites and oxidative stress biomarkers at up to 4 time points per subject across gestation. These changes in association with phthalate exposure may be important for pregnancy outcomes that are mediated by oxidative stress mechanisms. Additional exploration of these associations in other populations, particularly in non-pregnant women as well as men of reproductive age, children, and the elderly, may be of great importance for a range of other health outcomes that have been linked to phthalates in epidemiologic studies.

Table IV.1 Demographic characteristics in weighted study population (N=482).

Characteristic		Percent
Race/ethnicity (N=482)	White	59
	African American	16
	Other	26
Education (N=471)	High school	14
	Technical school	16
	Junior college or some college	30
	College graduate	41
Health insurance (N=470)	Private/HMO/self-pay	81
	Medicaid/SSI/MassHealth	19
Body mass index (N=478)	<25 kg/m^2 (underweight to normal)	53
	25-30 kg/m^2 (overweight)	27
	≥30 kg/m^2 (obese to morbidly obese)	20
Smoking during pregnancy (N=476)	Some	6
	None	94
Alcohol use during pregnancy (N=472)	Some	5
	None	95
Parity (N=482)	Nulliparous	45
	Parous	55

Note. HMO, health maintenance organization, SSI, supplmenetal security income, ART, assisted reproductive technology. Distributions of demographic characteristics created from inverse probability weightings for case-control status. Table adapted from Ferguson et al. (2014).

Table IV.2 Distributions of phthalate metabolites and oxidative stress biomarkers measured in urine samples collected from up to four time points during pregnancy in all samples measured from weighted population.

				Percentile					
Biomarker	LOD	%<LOD	Geometric mean (Geometric SD)	25th	50th	75th	90th	95th	Max.
MEHP (µg/L)	1.0	4.7	10.6 (3.49)	4.63	9.07	21.0	56.3	106	1555
MEHHP (µg/L)	0.1	0.0	34.2 (3.41)	14.9	27.5	70.3	182	305	2850
MEOHP (µg/L)	0.1	0.1	18.3 (3.32)	8.33	15.3	37.6	93.0	152	1128
MECPP (µg/L)	0.2	0.0	43.5 (3.40)	17.7	34.9	99.4	231	391	3713
∑DEHP (µmol/L)			0.39 (3.17)	0.17	0.31	0.78	2.00	3.01	21.1
MBzP (µg/L)	0.2	1.1	7.07 (3.05)	3.47	6.38	13.2	29.0	55.8	465
MBP (µg/L)	0.5	0.3	17.8 (2.40)	10.9	16.5	27.8	46.0	61.5	24879
MiBP (µg/L)	0.1	0.4	7.61 (2.29)	4.74	7.57	12.0	19.6	27.4	351
MEP (µg/L)	1.0	0.1	141 (4.68)	47.3	121	383	1084	2307	48130
MCPP (µg/L)	0.2	3.2	2.10 (3.14)	1.03	1.68	3.50	8.60	19.6	848
8-OHdG (ng/mL)	0.01	0.0	130 (1.66)	98.4	130	173	233	288	1339
8-Isoprostane (pg/mL)	3.9	4.0	180 (2.64)	130	210	320	460	574	2784

Note. 8-OHdG, 8-hydroxydeoxyguanosine, LOD, limit of detection, SD, standard deviation. All biomarkers corrected for urinary specific gravity. N=1693 samples, 482 subjects for urinary phthalate metabolites. N=1678 samples, 482 subjects for urinary oxidative stress biomarkers.

Table IV.3 Oxidative stress and exposure biomarker concentrations (geometric mean and geometric standard deviation) by categorical demographic characteristics in all samples measured from weighted population.

Characteristic		OHdG (ng/mL)	Iso (pg/mL)	MEHP (µg/L)	MBP (µg/L)
Race/ethnicity	White (Ref.)	130 (1.14)	153 (1.69)	10.1 (2.20)	15.4 (1.37)
	African-American	133 (1.11)	277 (1.33)*	13.5 (2.34)*	23.6 (1.45)*
	Other	129 (1.15)	205 (1.43)*	10.4 (2.02)	21.2 (1.63)*
Education	High school (Ref.)	146 (1.11)	289 (1.41)	9.20 (2.11)	27.8 (1.29)
	Technical school	133 (1.11)	217 (1.51)	9.91 (2.13)	19.6 (1.48)*
	Junior college or some college	124 (1.17)*	173 (1.65)*	9.57 (2.24)	16.7 (1.62)*
	College graduate	128 (1.13)	143 (1.57)*	12.0 (2.11)	15.6 (1.37)*
Health insurance	Private insurance/HMO/Self-pay (Ref.)	126 (1.14)	162 (1.64)	10.6 (2.19)	16.0 (1.47)
	Medicaid/SSI/MassHealth	151 (1.09)*	271 (1.33)*	9.74 (2.05)	29.1 (1.28)*
BMI at initial visit	Less than 25 kg/m^2 (Ref.)	128 (1.13)	155 (1.64)	10.8 (2.19)	16.7 (1.37)
	25 to less than 30 kg/m^2	132 (1.12)	181 (1.59)	10.5 (2.27)	16.5 (1.54)
	Greater than 30 kg/m^2	136 (1.17)	270 (1.34)*	10.6 (2.10)	23.6 (1.56)*
Tobacco use	Smoked during pregnancy (Ref.)	150 (1.11)	320 (1.47)	9.87 (1.83)	26.4 (1.34)
	No smoking during pregnancy	129 (1.14)	173 (1.60)*	10.6 (2.19)	17.5 (1.47)*
Alcohol use	Alcohol use during pregnancy (Ref.)	138 (1.06)	201 (1.29)	12.3 (2.27)	15.0 (1.19)
	No alcohol use during pregnancy	130 (1.14)	178 (1.63)	10.5 (2.16)	18.1 (1.49)
Parity	Nulliparous (Ref.)	129 (1.13)	166 (1.57)	11.4 (2.23)	17.0 (1.39)
	Parous	131 (1.14)	192 (1.62)*	10.1 (2.14)	18.6 (1.53)

Note. OHdG, 8-hydroxydeoxyguanosine. Ref., reference category. HMO, Health Maintenance Organization. SSI, Supplemental Security Income. BMI, body mass index. *p<0.05, **p<0.01 for significant difference in biomarker concentration from reference category, estimated from linear mixed model with random intercepts for subject ID. All biomarkers adjusted for urinary specific gravity. N=1693 samples, 482 subjects for urinary phthalate metabolites. N=1678 samples, 482 subjects for urinary oxidative stress biomarkers.

Table IV.4 Urinary phthalate metabolite concentrations (geometric mean and geometric standard deviation) by categorical demographic characteristics in all samples measured from weighted population (N=1678 samples, N=482 subjects).

Characteristic		MEHHP (µg/L)	MEOHP (µg/L)	MECPP (µg/L)	∑DEHP (µmol/L)
Race/ethnicity	White (Ref.)	35.8 (2.11)	18.9 (2.03)	43.9 (2.02)	0.39 (1.91)
	African-American	35.8 (2.13)	18.4 (2.16)	42.8 (2.22)	0.40 (2.05)
	Other	29.9 (2.11)	16.9 (2.06)	42.9 (2.27)	0.37 (1.98)
Education	High school (Ref.)	27.8 (1.76)	15.2 (1.79)	37.0 (1.94)	0.32 (1.74)
	Technical school	30.0 (2.10)	15.5 (2.07)	34.7 (2.11)	0.32 (1.98)
	Junior college or some college	33.2 (2.29)	17.9 (2.21)	44.1 (2.23)	0.38 (2.01)
	College graduate	38.7 (2.10)	20.9 (2.00)	49.5 (2.06)	0.44 (1.93)
Health insurance	Private insurance/HMO/Self-pay (Ref.)	34.6 (2.21)	18.7 (2.13)	45.0 (2.18)	0.40 (2.00)
	Medicaid/SSI/MassHealth	30.7 (1.76)	16.1 (1.78)	36.4 (1.85)	0.33 (1.73)
BMI at visit 1	Less than 25 kg/m^2 (Ref.)	33.7 (2.09)	18.3 (1.99)	43.5 (2.14)	0.38 (1.95)
	25 to less than 30 kg/m^2	34.0 (2.34)	17.7 (2.34)	41.4 (2.19)	0.38 (2.04)
	Greater than 30 kg/m^2	36.3 (1.94)	19.4 (1.90)	46.8 (1.96)	0.41 (1.82)
Tobacco use	Smoked during pregnancy (Ref.)	38.4 (1.71)	19.6 (1.65)	46.9 (1.64)	0.40 (1.64)
	No smoking during pregnancy	33.7 (2.15)	18.1 (2.08)	43.2 (2.15)	0.38 (1.97)
Alcohol use	Alcohol use during pregnancy (Ref.)	58.3 (1.84)	31.2 (1.78)	81.1 (2.05)	0.65 (1.87)
	No alcohol use during pregnancy	32.9 (2.11)*	17.6 (2.05)*	41.8 (2.09)*	0.37 (1.93)
Parity	Nulliparous (Ref.)	36.4 (2.14)	19.7 (2.08)	46.3 (2.13)	0.41 (1.96)
	Parous	32.4 (2.10)	17.2 (2.03)	41.3 (2.09)	0.36 (1.93)

Note. Ref., reference category. HMO, Health Maintenance Organization. SSI, Supplemental Security Income. BMI, body mass index. *p<0.05 for significant difference in biomarker concentration from reference category, estimated from weighted linear mixed model with random intercepts for subject ID. All biomarkers adjusted for urinary specific gravity.

Table IV.4 (continued)

Characteristic		MBzP (µg/L)	MiBP (µg/L)	MEP (µg/L)	MCPP (µg/L)
Race/ethnicity	White (Ref.)	5.67 (1.63)	6.28 (1.33)	111 (3.06)	2.00 (1.78)
	African-American	10.5 (1.90)*	11.2 (1.36)*	285 (3.04)*	2.49 (2.38)
	Other	9.31 (2.15)*	9.44 (1.47)*	165 (3.26)*	2.13 (2.02)
Education	High school (Ref.)	15.6 (2.30)	10.7 (1.25)	286 (3.22)	2.52 (2.04)
	Technical school	8.18 (1.97)*	9.45 (1.44)	197 (3.01)	2.10 (1.90)
	Junior college or some college	6.07 (1.72)*	6.43 (1.58)*	133 (3.10)*	1.99 (1.98)
	College graduate	5.73 (1.59)*	7.05 (1.30)*	101 (3.04)*	2.06 (1.88)
Health insurance	Private insurance/HMO/Self-pay (Ref.)	5.93 (1.66)	6.99 (1.43)	120 (3.20)	2.02 (1.92)
	Medicaid/SSI/MassHealth	15.9 (2.12)*	11.4 (1.24)*	295 (2.79)*	2.39 (1.75)
BMI at visit 1	Less than 25 kg/m^2 (Ref.)	6.58 (1.86)	7.08 (1.30)	116 (2.90)	1.95 (1.82)
	25 to less than 30 kg/m^2	6.08 (1.69)	7.89 (1.56)	162 (3.96)*	2.19 (2.06)
	Greater than 30 kg/m^2	10.1 (1.88)*	8.88 (1.51)*	202 (3.18)*	2.38 (1.97)
Tobacco use	Smoked during pregnancy (Ref.)	15.4 (1.75)*	9.06 (1.24)	177 (3.94)	3.55 (1.94)
	No smoking during pregnancy	6.76 (1.83)	7.56 (1.42)	140 (3.26)	2.03 (1.91)*
Alcohol use	Alcohol use during pregnancy (Ref.)	5.33 (1.41)	8.38 (1.53)	138 (2.88)	2.79 (2.72)
	No alcohol use during pregnancy	7.23 (1.89)*	7.61 (1.41)	143 (3.32)	2.07 (1.89)
Parity	Nulliparous (Ref.)	6.21 (1.79)	7.26 (1.43)	149 (3.16)	2.04 (1.90)
	Parous	7.87 (1.89)*	7.91 (1.39)	136 (3.40)	2.15 (1.95)

Note. Ref., reference category. HMO, Health Maintenance Organization. SSI, Supplemental Security Income. BMI, body mass index. *p<0.05 for significant difference in biomarker concentration from reference category, estimated from weighted linear mixed model with random intercepts for subject ID. All biomarkers adjusted for urinary specific gravity.

Table IV.5 Percent change (95% confidence intervals) in oxidative stress biomarker in association with interquartile range increase in phthalate metabolite level. Estimates from adjusted linear mixed effect models with random intercepts for subject ID (N=1578 samples, N=466 subjects).

	OHdG		Isoprostane	
Metabolite	% change (95% CI)	p	% change (95% CI)	p
MEHP	2.74 (-0.47, 6.05)	0.09	14.1 (8.06, 20.5)	<0.001
MEHHP	8.40 (4.93, 12.0)	<0.001	15.8 (9.53, 22.4)	<0.001
MEOHP	7.34 (4.01, 10.8)	<0.001	15.9 (9.87, 22.3)	<0.001
MECPP	6.53 (2.96, 10.2)	<0.001	23.0 (16.0, 30.4)	<0.001
∑DEHP	6.67 (3.23, 10.2)	<0.001	19.1 (12.7, 25.9)	<0.001
MBzP	20.7 (15.6, 26.1)	<0.001	42.7 (31.8, 54.4)	<0.001
MBP	18.1 (13.5, 22.9)	<0.001	42.0 (32.0, 52.7)	<0.001
MiBP	30.3 (24.4, 36.5)	<0.001	56.4 (43.9, 69.9)	<0.001
MEP	11.5 (7.32, 15.9)	<0.001	19.7 (11.8, 28.2)	<0.001
MCPP	7.23 (3.83, 10.7)	<0.001	20.2 (13.7, 27.1)	<0.001

Note. OHdG, 8-hydroxydeoxyguanosine. Adjusted for urinary specific gravity, gestational age at sample collection, Race/Ethnicity, education level, health insurance provider, body mass index (time-varying), time of day of urine sample collection (before vs. after 1pm, time-varying), and parity of infant. Models include inverse probability weights to adjust for case-control study design.

Table IV.6 Percent change (95% confidence intervals) in oxidative stress biomarker in association with interquartile range increase in phthalate metabolite level. Estimates from adjusted linear mixed effect models with random intercepts for subject ID. Cases only (N=401 samples, N=128 subjects).

	OHdG		Isoprostane	
Metabolite	% change (95% CI)	p	% change (95% CI)	p
MEHP	4.11 (-2.13, 10.8)	0.202	11.8 (1.27, 23.3)	0.028
MEHHP	6.90 (0.02, 14.3)	0.050	11.8 (0.69, 24.1)	0.038
MEOHP	6.22 (-0.33, 13.2)	0.064	10.9 (0.44, 22.5)	0.042
MECPP	6.14 (-0.76, 13.5)	0.083	14.8 (3.02, 28.0)	0.013
∑DEHP	6.08 (-0.61, 13.2)	0.077	12.9 (1.92, 25.0)	0.021
MBzP	18.5 (7.60, 30.5)	0.001	50.3 (27.4, 77.4)	<0.001
MBP	8.92 (2.03, 16.3)	0.011	30.9 (15.8, 48.0)	<0.001
MiBP	20.3 (10.2, 31.3)	<0.001	52.5 (32.2, 76.0)	<0.001
MEP	19.2 (10.3, 28.8)	<0.001	15.2 (0.70, 31.8)	0.040
MCPP	5.28 (-0.73, 11.6)	0.087	19.2 (8.46, 31.1)	<0.001

Note. 8-OHdG, 8-hydroxydeoxyguanosine. Adjusted for urinary specific gravity, gestational age at sample collection, Race/Ethnicity, education level, health insurance provider, body mass index (time-varying), time of day of urine sample collection (before vs. after 1pm, time-varying), and parity of infant.

Table IV.7 Percent change (95% confidence intervals) in oxidative stress biomarker in association with interquartile range increase in phthalate metabolite level. Estimates from adjusted linear mixed effect models with random intercepts for subject ID. Controls only (N=1177 samples, N=338 subjects).

	OHdG		Isoprostane	
Metabolite	% change (95% CI)	p	% change (95% CI)	p
MEHP	2.77 (-0.47, 6.11)	0.10	14.3 (8.15, 20.7)	<0.001
MEHHP	8.07 (4.74, 11.5)	<0.001	15.2 (9.15, 21.5)	<0.001
MEOHP	7.15 (3.91, 10.5)	<0.001	15.5 (9.61, 21.7)	<0.001
MECPP	4.90 (2.23, 7.64)	<0.001	16.9 (11.9, 22.2)	<0.001
∑DEHP	6.65 (3.22, 10.2)	<0.001	19.1 (12.6, 25.9)	<0.001
MBzP	19.5 (14.6, 24.5)	<0.001	39.8 (29.8, 50.6)	<0.001
MBP	17.5 (13.1, 22.2)	<0.001	40.7 (31.0, 51.0)	<0.001
MiBP	30.3 (24.4, 36.5)	<0.001	56.4 (43.9, 69.9)	<0.001
MEP	11.2 (7.12, 15.4)	<0.001	19.1 (11.5, 27.3)	<0.001
MCPP	7.11 (3.77, 10.6)	<0.001	19.9 (13.5, 26.6)	<0.001

Note. 8-OHdG, 8-hydroxydeoxyguanosine. Adjusted for urinary specific gravity, gestational age at sample collection, Race/Ethnicity, education level, health insurance provider, body mass index (time-varying), time of day of urine sample collection (before vs. after 1pm, time-varying), and parity of infant.

CHAPTER V

REPEATED MEASURES OF URINARY OXIDATIVE STRESS BIOMARKERS DURING PREGNANCY IN RELATION TO PRETERM BIRTH

INTRODUCTION

Preterm birth, commonly defined as birth before 37 weeks completed gestation, is a leading cause of neonatal mortality and morbidity, and occurs in approximately 1 in 10 births in the US (Martin et al. 2011). Despite the significance of this public health problem, mechanisms for preterm birth are poorly understood (Goldenberg et al. 2008). Many risk factors, such as maternal age, race/ethnicity, and tobacco use have been linked to prematurity but underlying pathways for these relationships remain unclear. This may be attributable in part to the difficulties in examining preterm birth in a small animal model; preterm birth has been induced in rodents in some studies but generally requires gene knockouts or high doses of lipopolysaccharides injection making translation of results to humans difficult (Cha et al. 2013; Kaga et al. 1996). Alternatively, screening of mechanistic biomarkers in humans may be helpful for identifying mechanisms contributing to preterm birth.

Inflammation and infection at the maternal-fetal interface are among the most well-established precursors to preterm birth, and justifiably much of the research on predictive biomarkers has focused on inflammatory cytokines and other indicators of inflammation (Wei et al. 2010). While predictive value of these biomarkers for use by clinicians is limited, these data provide *in vivo* evidence for a causative role of inflammation for some cases of preterm birth (Wei et al. 2010).

Oxidative stress, defined as an imbalance between antioxidant capacity and reactive oxygen species (ROS) generation, is another pathway, potentially related to inflammation, which has received less attention in the study of preterm birth. A number of biomarkers exist to measure oxidative stress, and each is formed through a unique

mechanism and has the potential for differing downstream physiologic effects. In the present study we measured two biomarkers in maternal urine samples collected from up to four time points during pregnancy, including 8-hydroxydeoxyguanosine (8-OHdG), which is representative of oxidative DNA damage (Wu et al. 2004), as well as 8-isoprostane, a prostaglandin formed by arachadonic acid peroxidation (Roberts and Morrow 2000). We examined changes in these markers over pregnancy as well as the relationship between these markers and risk of preterm birth.

METHODS

Study population

Subjects for this nested case-control study were selected from a longitudinal birth cohort of pregnant women who delivered at Brigham and Women's Hospital in Boston, MA, between 2006 and 2008. Participants were recruited early in pregnancy (median 10 weeks gestation) and completed demographic and medical history questionnaires and provided informed consent at enrollment. Participants additionally provided urine samples from up to a total of four visits across gestation (median 10, 18, 26, and 35 weeks gestation). At delivery, detailed birth outcome and infant data was recorded. From this parent population we selected all 130 women who delivered live singleton infants preterm, as well as 352 random control women who delivered live singleton term infants (Ferguson et al. 2014). This study was approved by institutional review boards at the University of Michigan and Brigham and Women's Hospital.

Women who delivered preterm were divided into subgroups based on physician-assessed origin of preterm birth. Women who presented at delivery with spontaneous preterm labor or preterm premature rupture of the membranes (pPROM) were combined into a single group, as previous research shows that women with these delivery precursors show similar patterns of placental inflammation (McElrath et al. 2008). These births were considered spontaneous preterm (N=56). A second category included women whose preterm deliveries were determined to be a result of preeclampsia or IUGR, since a prior study of very preterm births suggested these groups should be combined because the etiology for both is likely to be related to abnormal placentation (McElrath et al. 2008). These births were considered placental preterm for analysis (N=35). The

remaining preterm births (N=39) did not fall into either etiology-based subset (e.g., repeat C-section) and were not examined separately due to the lack of a hypothesized shared mechanism for these preterm births.

Oxidative stress biomarker analysis

Urine samples (N=1678 samples) were stored at -80C after collection until the time oxidative stress biomarkers were measured. Both 8-OHdG and total 8-isoprostane were measured by Cayman Chemical (Ann Arbor, MI). For total 8-isoprostane, urine samples were hydrolyzed to deconjugate 8-isoprostane esterified to phospholipids and were passed through affinity columns for purification. Eluted samples were dried and resuspended in a buffer before measurement with enzyme immunoassay (EIA). The lower limit of detection was 3.9 pg/mL. For 8-OHdG, samples were diluted directly into buffer without purification. Concentrations were also measured using EIA with a detection limit of 10.3 pg/mL. Levels of biomarkers below the limit of detection (LOD) were replaced with the LOD divided by the square root of 2 (Hornung and Reed 1990). Distributions of concentrations for both biomarkers were log-normal and were ln-transformed for data analysis.

To account for urine dilution, specific gravity was measured in urine samples using a digital handheld refractrometer (Atago Co., Ltd., Tokyo, Japan). For examining biomarker distributions, concentrations were corrected for specific gravity using the following formula: $OS_c=OS[(1.015-1)/(SG-1)]$ (Meeker et al. 2009). OS_c represents the corrected biomarker concentration; OS is the uncorrected urinary concentration; 1.015 is the median specific gravity in all samples; and SG is the specific gravity of the sample. In regression analyses uncorrected biomarker concentrations were used and specific gravity was included as a covariate, as modeling corrected concentrations can incur additional measurement error and subsequent bias (Barr et al. 2005). Extremely concentrated (specific gravity >1.04) samples were excluded from all analyses (N=4).

Statistical analysis

Statistical analysis was performed using R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) as well as SAS version 9.2 (SAS Institute, Inc.,

Cary, NC). Distributions of specific gravity-corrected urinary concentrations of oxidative stress biomarkers were examined by creating geometric means for each visit. Differences across gestation were tested using linear mixed models with random intercepts only to adjust for intra-individual correlation, with specific gravity-corrected urinary biomarker concentration regressed on visit of sample collection. To visually depict any non-linear trends in levels across gestation, generalized additive mixed models (GAMM; *mgcv* package in R) (Wood 2011) were created with specific gravity-corrected urinary biomarker concentration regressed on a smooth term for gestational age at urine sample collection, also with random intercepts only. As an additional measure of variability in biomarker concentrations over pregnancy we calculated intraclass correlation coefficients (ICC), which represent the ratio of within to between individual variability (Rosner 2011).

Associations between urinary oxidative stress markers and preterm birth were examined for overall preterm birth and also for the subtypes defined above. Odds ratios were calculated from logistic regression models in two ways. First, we created a geometric average level for each subject from levels measured at visits 1-3. Visit 4 concentrations were excluded from the average measure, as the proportion of cases with a measure at this time point was low, as many had already delivered (100% of cases had samples available at visit 1; 91% at visit 2; 86% at visit 3; 51% at visit 4). Averages were modeled in relation to preterm birth with adjustment for average urinary specific gravity, and adjusted models included covariates associated with oxidative stress biomarkers in bivariate analysis that have been linked to preterm birth in previous studies. Covariates that were considered included maternal age, race/ethnicity, education level, health insurance provider, body mass index (BMI) at visit 1, use of tobacco or alcohol during pregnancy, parity and gender of infant, and use of assisted reproductive technology.

In addition to models using averages, individual logistic regression models examining the association between urinary oxidative stress biomarkers at each visit in relation to preterm birth were constructed separately to investigate whether oxidative stress levels at a particular time point were more predictive of overall, spontaneous, or placental preterm delivery. These models were created using the same covariates included in average models.

RESULTS

Characteristics of the overall study population have been described elsewhere (Ferguson et al. 2014) and distributions in controls, cases, and spontaneous and placental cases separately are presented in Table V.1. Mothers were median 32.7 years of age at the first study visit and were predominantly white (58.5%), well educated (38.8% with a college education), had private (private, HMO, or self-paying, 79.9%) rather than public (Medicaid, Supplemental Security Income, MassHealth) health insurance (Ferguson et al. 2014). About half of the population was nulliparous (44.6%), and few mothers used tobacco (6.4%) or alcohol (4.1%) during pregnancy. Most women were underweight to normal weight at visit 1(BMI <25 kg/m^2, 51.9%), although 26.1% were overweight (25-<30 kg/m^2) and 21.2% were obese (BMI≥30 kg/m^2) (Ferguson et al. 2014).

In general, 8-isoprostane levels were more strongly associated with covariates than 8-OHdG levels (see Chapter IV). Specific gravity corrected urinary concentrations of 8-isoprostane were higher in African American women (geometric mean=274 pg/mL, geometric standard deviation [SD]=2.17) and women of other race/ethnicity (geometric mean=210 pg/mL, geometric SD=2.26) compared to white women (geometric mean=165 pg/mL, geometric SD=2.73). 8-isoprostane concentrations were also significantly higher in women with lower education levels, poor health insurance, high BMI (≥30 kg/m^2), and in women who smoked during pregnancy. Maternal age at visit 1 was inversely correlated with 8-isoprostane average over pregnancy (Spearman R=-0.23, p<0.01). For 8-OHdG, the only significant difference observed with categorical covariates was that women with private health insurance had lower levels (123 ng/mL compared to 146 ng/mL in women with Medicaid, supplemental security income, or MassHealth). As with 8-isoprostane, 8-OHdG average over pregnancy was inversely correlated with maternal age (Spearman R=-0.13, p<0.01).

Geometric mean specific gravity-corrected urinary oxidative stress biomarker concentrations by study, as well as predicted levels smoothed over gestational age at sample collection, are plotted in Figure V.1 for the overall population, as no significant differences in trends were observed in cases compared to controls. For 8-isoprostane, levels decreased slightly and linearly across pregnancy, and levels at visits 2, 3, and 4 were significantly lower than levels at visit 1. For 8-OHdG, levels increased in a

quadratic form as gestation progressed, and levels at visit 3 and 4 were significantly higher than levels at visit 1. ICC for 8-isoprostane (ICC=0.60, 95% confidence interval [CI]=0.56, 0.64) was stronger than for 8-OHdG (ICC=0.32, 95% CI=0.27, 0.38). An ICC ranges from 0 to 1, with 0 indicating no reproducibility in measures and 1 indicating perfect reproducibility (Rosner 2011). ICC for both urinary oxidative stress biomarkers were good (between 0.40 and 0.75), but 8-OHdG was less reliable over time (Rosner 2011). Biomarkers showed weak but statistically significant correlations with one another at each study visit (Spearman R=0.10-0.20, $p<0.05$).

In models with geometric average urinary oxidative stress biomarker concentrations from visits 1-3, 8-isoprostane was significantly associated with increased odds of overall preterm birth (adjusted odds ratio [aOR]=2.22, 95% confidence interval [CI]=1.47, 3.36). Effect estimates were similar in models adjusted for urinary specific gravity only (Table V.2, Model 1) and in models additionally adjusted for maternal age, race/ethnicity, education level, health insurance provider, and pre-pregnancy BMI (Table V.2, Model 2). Tobacco use was associated with 8-isoprostane levels but was not included in fully adjusted models due to the low number of tobacco users (N = 31); however, the addition of this variable did not alter effect estimates (data not shown). The association between 8-isoprostane and overall preterm birth was driven by associations with spontaneous preterm birth; analysis by subtypes showed large odds ratios for spontaneous preterm birth (aOR=6.25, 95%CI=2.86, 13.7) and null associations for placental preterm birth (aOR=0.94, 95%CI=0.52, 1.70). Cross-sectional logistic regression models for each study visit (Table V.3) showed that the odds for spontaneous preterm birth were most elevated at visits 2-4 compared to visit 1.

In models using biomarker geometric average over visits 1-3, 8-OHdG was associated with reduced odds of overall preterm birth (aOR=0.19, 95%CI=0.10, 0.34), and the relationship was slightly stronger in placental preterm births only (aOR=0.11, 95%CI=0.04, 0.32) (Table V.2). As with models of 8-isoprostane, effect estimates were similar in models adjusted for specific gravity only (model 1) and in models with all covariates (model 2). When odds ratios were calculated for each individual visit, we observed more protective odds ratios for spontaneous preterm birth when 8-OHdG levels

were measured early in pregnancy. For placental preterm birth, the most protective odds ratio was in association with levels measured at visit 2 (median 18 weeks gestation).

DISCUSSION

While much research has focused on the role of inflammation in the pathway to preterm birth, there are plausible mechanisms for a role of oxidative stress as well. ROS could a) act as a precursor to inflammatory responses that may prematurely initiate parturition processes (Challis et al. 2009); b) damage collagen in the myometrial membranes resulting in pPROM (Woods 2001); or c) cause apoptosis of the syncytiotrophoblast early in pregnancy, impairing spiral arteriole invasion of the myometrial wall and resulting in dysfunctional placentation that can lead to preterm birth (Burton et al. 2009; Heazell et al. 2007). There is some prior evidence supporting a relationship between oxidative stress and preterm birth in studies measuring maternal biomarkers.

Also known as 8-iso prostaglandin $F_{2\alpha}$, 8-isoprostane is a useful biomarker of oxidative stress in humans because of its stability, sensitivity to oxidant injury, and specificity to arachadonic acid peroxidation by ROS (Roberts and Morrow 2000). Urinary concentrations are preferred over plasma concentrations because plasma samples may be susceptible to auto-oxidation during storage, while urine samples, with low lipid concentrations, are not (Morrow et al. 1990). In addition to use in studies of adverse pregnancy outcomes, they are commonly used as markers of oxidative damage in the study of cardiovascular disease (Dalle-Donne et al. 2006).

One prospective study examined 8-isoprostane levels in urine collected from 5-16 weeks gestation and observed a slight but significant trend for decreased gestational duration with increasing quintiles of 8-isoprostane (N=508) (Peter Stein et al. 2008). Odds of preeclampsia were also slightly elevated among women in the highest compared to lowest quintile (Peter Stein et al. 2008). Another study measured maternal 8-isoprostane in plasma samples collected from 24-26 weeks gestation and observed higher levels in mothers who developed preeclampsia or had small for gestational age infants (N=503) (Hsieh et al. 2012). Neither study observed an association with preterm birth, though the number of cases in each study was small (N=48 and 37, respectively). Other

studies have examined 8-isoprostane levels in amniotic fluid and found higher levels in women with term PROM (Kwiatkowski et al. 2009) and pPROM (Longini et al. 2007). Finally, results from studies of preterm birth measuring maternal plasma levels of malondialdehyde (MDA), another marker of lipid peroxidation, have been conflicting (Pathak et al. 2010; Weber et al. 2013).

Our results provide strong evidence for an association between urinary 8-isoprostane levels and spontaneous preterm birth in a large nested case-control study examining urinary concentrations at multiple time points during pregnancy. We also examined urinary excretion patterns and intra-individual variability across gestation which have not been previously explored, although a study of levels in plasma collected at three time points (6-8 weeks, 15-20 weeks, and 26-30 weeks gestation) showed slight increases in the third trimester (Hung et al. 2010). In our study urinary concentrations decreased slightly across pregnancy but generally showed good intra-individual reproducibility, suggesting that future studies examining this marker may only need to do so at one time point. However, by measuring levels at multiple time points, we were able to indicate that associations with spontaneous preterm birth were strongest when 8-isoprostane was measured near the end of pregnancy (median 35 weeks gestation). This supports the hypothesis that oxidative stress levels later in pregnancy may cause preterm birth by weakening membranes or initiating a series of events leading to spontaneous preterm labor.

The aforementioned study in which 8-isoprostane was measured in urine also examined urinary 8-OHdG between 5-16 weeks gestation and found slight but significant associations with decreased gestational length (38.1 weeks in upper quintile compared to 39.0 weeks in lowest quintile, p=0.014) and decreased birth weight, but not with preeclampsia or preterm birth (Peter Stein et al. 2008). A previous smaller study by the same group (N=18 cases of low birth weight, growth restriction, or preterm; N=34 controls) reported significantly higher 8-OHdG concentrations measured in urine from the third trimester in cases compared to controls (Scholl and Stein 2001); however those results were not specific to preterm birth. In another study, where 8-OHdG was measured in urine collected between 24-26 weeks gestation, concentrations were higher in women who went on to deliver a low birth weight infant, but no association was observed with

preeclampsia or preterm birth (Hsieh et al. 2012). Finally, one study which measured 8-OHdG in umbilical cord serum found elevated levels in samples from babies born both preterm and with low birth weight (Negi et al. 2012).

Unexpectedly, we observed that women with higher urinary concentrations of 8-OHdG had reduced odds of preterm birth. This was true for both spontaneous and placental preterm birth, and odds ratios were most protective for spontaneous preterm birth at visit 1 (median 10 weeks gestation) and for placental preterm birth at visit 2 (median 18 weeks gestation). We hypothesized that 8-OHdG, like isoprostane, would be associated with increased risk of preterm birth because 8-OHdG is also utilized commonly as a marker of oxidative stress generated upon repair of oxidative DNA damage (Wu et al. 2004). Despite the common understanding that these markers both represent oxidative stress, levels of the two markers were lowly correlated with one another in this and a previous study (Peter Stein et al. 2008), indicating that they are truly representing different physiologic processes.

One explanation for the protective effect of 8-OHdG levels is that they are associated with unmeasured confounders in this population that strongly reduce the risk of preterm birth. For example, 8-OHdG levels increase with exercise (Okamura et al. 1997) and ferritin levels (Peter Stein et al. 2008). Current literature shows that association between these factors and preterm birth is generally null (Scholl 2005; Weissgerber et al. 2006); however, previous studies are not definitive and these or other prenatal factors could be confounders that reduce the risk of preterm birth. An alternative hypothesis hinges on the fact that 8-OHdG not only tracks oxidative DNA damage but also the successful execution of the excision repair process (Wu et al. 2004). It is possible that in mothers where this process is impaired, through a DNA polymorphism or other unknown factor, there is increased risk of preterm birth. Under this scenario, 8-OHdG is not a marker of oxidative stress but instead the mother's ability to repair oxidative damage. This hypothesis has been suggested in another study in which women who delivered infants with birth defects had lower urinary 8-OHdG levels in urine samples taken early in pregnancy (Peter Stein et al. 2008). This possibility deserves further exploration in future research.

More consistent with prior studies, we observed that 8-OHdG levels were moderately variable within-woman across gestation, and that levels were higher in later compared to early pregnancy. Another study that examined urinary 8-OHdG concentrations at 6-8 weeks, 15-20 weeks, and 26-30 weeks gestation also observed that levels were elevated later in pregnancy (Hung et al. 2010). A third study measuring levels at 20 and 30 weeks gestation and also at delivery showed that levels were higher in urine collected at delivery compared to earlier in pregnancy, although these results could have been complicated by laboring processes (Shoji et al. 2006).

Our study was limited in part by the measurement of 8-isoprostane via EIA; liquid chromatography-mass spectrometry (LC/MS) is preferable because of its improved specificity for the isomer. However, the correlation between levels measured with Cayman Chemical EIA and LC/MS is strong, specificity is improved in EIA following affinity purification which was used in this study, and other 8-isoprostane isomers may also be produced by exposure to ROS (Smith et al. 2011). Levels may not be comparable with those measured in other studies, but that was not the goal of this analysis. Also, LC/MS methods would have been cost-prohibitive in this large study; we would not have been able to analyze samples from as many subjects and time points, which was a strength of our study. 8-OHdG analysis via EIA is preferred to MS and correlation between results from the two methods is generally strong (Wu et al. 2004).

Despite these limitations, our study had many strengths. For example, it benefited from the measurement of 8-isoprostane and 8-OHdG in maternal urine samples from repeated time points across pregnancy, which has not been done previously in the study of preterm birth. Additionally, it represents one of the largest studies to date to examine oxidative stress markers in relation to preterm birth, and included by far the largest number of preterm cases.

In conclusion, we observed that increased 8-isoprostane levels were associated with increased risk of spontaneous but not placental preterm birth, and odds ratios were highest in association with levels measured later in pregnancy. These findings support the hypotheses that oxidative stress levels near the end of pregnancy could be related to weakened gestational membranes and pPROM, and/or could prematurely initiate inflammatory pathways leading to spontaneous preterm parturition. Conversely, we

found that 8-OHdG levels were significantly associated with decreased risk of both spontaneous and placental preterm subtypes, particularly for measures taken early in pregnancy. These results were unexpected and are more difficult to interpret, but could provide valuable information about heretofore unidentified factors that are preventative of preterm birth.

Table V.1 Distributions of demographic characteristics in study population.

Characteristic		N (%)			
		Term	Preterm	Spontaneous preterm	Placental preterm
Race/ethnicity (missing = 0)	White	207 (58.8)	75 (57.7)	30 (53.6)	20 (57.1)
	African American	55 (15.6)	22 (16.9)	8 (14.3)	8 (22.9)
	Other	90 (25.6)	33 (25.4)	18 (32.1)	7 (20.0)
Education (missing = 11)	High school	47 (13.7)	21 (16.3)	7 (12.5)	10 (28.6)
	Technical school	52 (15.2)	25 (19.4)	12 (21.4)	5 (14.3)
	Junior college/some college	101 (29.5)	38 (29.5)	16 (28.6)	12 (34.3)
	College graduate	142 (41.5)	45 (34.9)	21 (37.5)	8 (22.9)
Health insurance (missing = 12)	Private/HMO/self-pay	277 (81.0)	108 (84.4)	48 (85.7)	26 (78.8)
	Medicaid/SSI/MassHealth	65 (19.0)	20 (15.6)	8 (14.3)	7 (21.2)
BMI (missing = 4)	<25 kg/m^2	188 (54.0)	62 (47.7)	30 (53.6)	8 (22.9)
	25-30 kg/m^2	94 (27.0)	32 (24.6)	15 (26.8)	9 (25.7)
	≥30 kg/m^2	66 (19.0)	36 (27.7)	11 (19.6)	18 (51.4)
Tobacco use (missing =6)	Yes	20 (5.8)	11 (8.5)	4 (7.2)	7 (20.0)
	No	326 (94.2)	119 (91.5)	52 (92.8)	28 (80.0)
Alcohol use (missing = 10)	Yes	19 (5.5)	1 (0.8)	0 (0)	0 (0)
	No	326 (94.5)	126 (99.2)	54 (100)	35 (100)
Parity (missing = 0)	Nulliparous	160 (45.5)	55 (42.3)	24 (42.9)	20 (57.1)
	Parous	192 (54.5)	75 (57.7)	32 (57.1)	15 (42.9)
Gender (missing = 0)	Male	158 (44.9)	56 (43.1)	27 (48.2)	16 (45.7)
	Female	194 (55.1)	74 (56.9)	29 (51.8)	19 (54.3)
Use of ART (missing = 0)	Yes	33 (9.4)	12 (9.2)	4 (7.1)	4 (11.4)
	No	319 (90.6)	118 (90.8)	42 (92.9)	31 (88.6)

Note. BMI, body mass index, HMO, health maintenance organization, SSI, supplemental security income, ART, assisted reproductive technology.

Table V.2 Odds ratios (95% confidence intervals) for preterm birth in association with interquartile range increase in geometric average (visits 1-3) urinary oxidative stress biomarkers.

Overall preterm birth						
	Model 1			Model 2		
Biomarker	N (cases, controls)	OR (95% CI)	p value	N (cases, controls)	OR (95% CI)	p value
OHdG	129, 349	0.19 (0.11, 0.34)	<0.001	126, 331	0.19 (0.10, 0.34)	<0.001
Isoprostane	129, 349	2.17 (1.48, 3.20)	<0.001	126, 331	2.22 (1.47, 3.36)	<0.001
Spontaneous preterm birth						
	Model 1			Model 2		
Biomarker	N (cases, controls)	OR (95% CI)	p value	N (cases, controls)	OR (95% CI)	p value
OHdG	56, 349	0.21 (0.10, 0.42)	<0.001	56, 331	0.18 (0.09, 0.40)	<0.001
Isoprostane	56, 349	4.25 (2.21, 8.15)	<0.001	56, 331	6.25 (2.86, 13.7)	<0.001
Placental preterm birth						
	Model 1			Model 2		
Biomarker	N (cases, controls)	OR (95% CI)	p value	N (cases, controls)	OR (95% CI)	p value
OHdG	35, 349	0.17 (0.07, 0.41)	<0.001	33, 331	0.11 (0.04, 0.32)	<0.001
Isoprostane	35, 349	1.45 (0.79, 2.66)	0.24	33, 331	0.94 (0.52, 1.70)	0.84

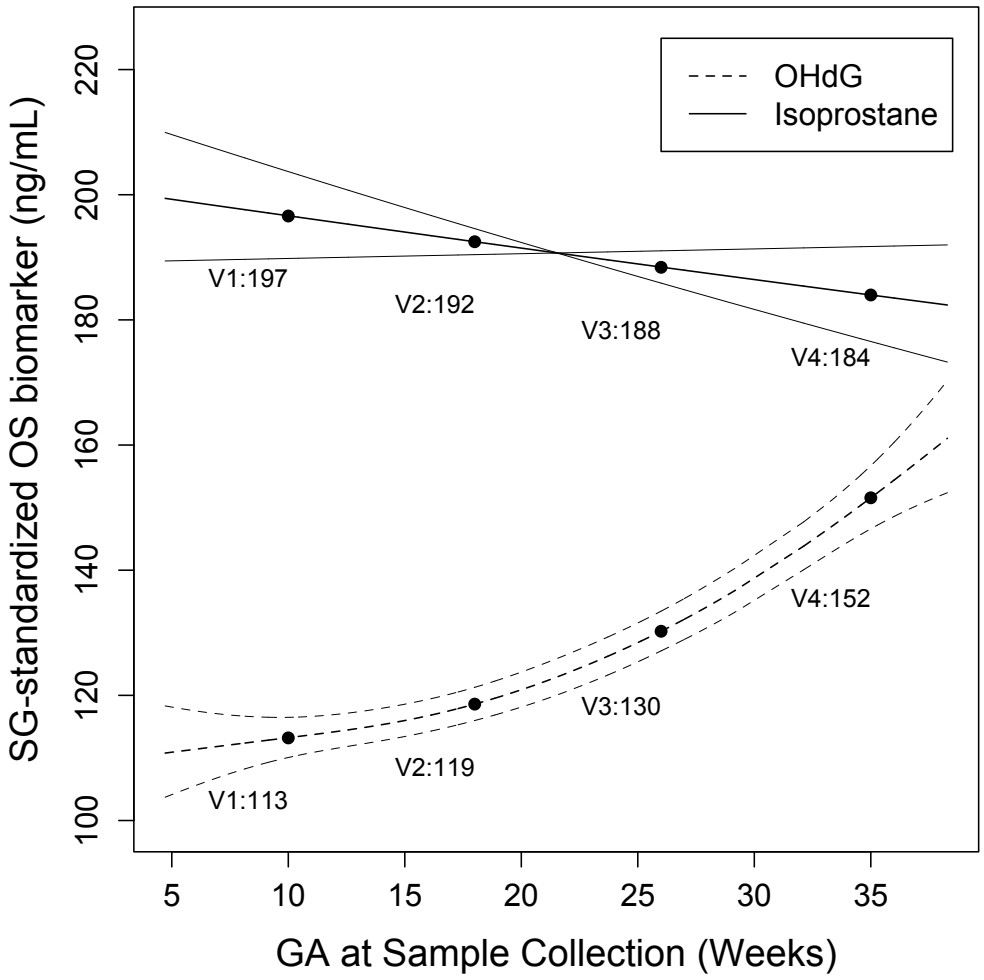
Note. Model 1 adjusted for urinary specific gravity only. Model 2 adjusted for urinary specific gravity, maternal age, race/ethnicity, education level, health insurance provider, and pre-pregnancy body mass index (BMI).

Table V.3 Odds ratios (95% confidence intervals) for preterm birth in association with interquartile range increase in urinary oxidative stress biomarkers by study visit.

	Overall preterm birth					
	OHdG			Isoprostane		
	N (cases, controls)	OR (95% CI)	p value	N (cases, controls)	OR (95% CI)	p value
Visit 1	123, 326	0.25 (0.14, 0.46)	<0.01	123, 326	1.72 (1.18, 2.51)	0.01
Visit 2	114, 289	0.21 (0.10, 0.42)	<0.01	114, 289	2.33 (1.44, 3.77)	<0.01
Visit 3	107, 282	0.44 (0.24, 0.81)	<0.01	107, 282	2.05 (1.31, 3.19)	<0.01
Visit 4	59, 294	0.45 (0.23, 0.90)	0.02	59, 294	1.76 (1.08, 2.88)	0.02
	Spontaneous preterm birth					
	OHdG			Isoprostane		
	N (cases, controls)	OR (95% CI)	p value	N (cases, controls)	OR (95% CI)	p value
Visit 1	54, 326	0.26 (0.12, 0.56)	<0.01	54, 326	2.72 (1.46, 5.06)	<0.01
Visit 2	52, 289	0.30 (0.13, 0.71)	<0.01	52, 289	7.10 (2.80, 18.0)	<0.01
Visit 3	47, 282	0.33 (0.14, 0.74)	<0.01	47, 282	4.45 (1.96, 10.1)	<0.01
Visit 4	24, 294	0.75 (0.28, 2.01)	0.57	24, 294	5.27 (1.82, 15.3)	<0.01
	Placental preterm birth					
	OHdG			Isoprostane		
	N (cases, controls)	OR (95% CI)	p value	N (cases, controls)	OR (95% CI)	p value
Visit 1	33, 326	0.21 (0.07, 0.58)	<0.01	33, 326	1.02 (0.62, 1.69)	0.93
Visit 2	29, 289	0.12 (0.04, 0.40)	<0.01	29, 289	1.19 (0.60, 2.34)	0.62
Visit 3	30, 282	0.40 (0.17, 0.94)	0.04	30, 282	0.79 (0.44, 1.43)	0.44
Visit 4	12, 294	0.19 (0.04, 0.85)	0.03	12, 294	0.59 (0.29, 1.19)	0.14

Note. Models adjusted for urinary specific gravity, maternal age, race/ethnicity, education level, health insurance provider, and pre-pregnancy body mass index (BMI).

Figure V.1 Predicted values (95% confidence intervals) of specific gravity-corrected urinary oxidative stress biomarker concentrations by gestational age at sample collection from generalized additive mixed models adjusted for random intercepts only. Geometric mean biomarker concentrations at visits 1 (median 10 weeks gestation), 2 (median 15 weeks gestation), 3 (median 26 weeks gestation), and 4 (median 35 weeks gestation) plotted for reference.



CHAPTER VI

MEDIATION OF THE PHTHALATE-PRETERM RELATIONSHIP BY MATERNAL OXIDATIVE STRESS

INTRODUCTION

Mediation analysis has been used in social research for decades in efforts to determine causality in more nebulous relationships. A good example comes from a psychology study investigating the relationship between individual expectations about alcohol in relation to drinking behaviors, e.g., drunkenness, in which authors determined that the relationship was mediated almost completely by the use of drinking as a coping mechanism (Catanzaro and Laurent 2004). More recently mediation analysis has been used in epigenetic research; one study found that the relationship between black carbon exposure and decreased fibrinogen levels was mediated in part by decreased tissue factor 3 methylation (Bind et al. 2014). Finally, a recent study utilized these methods to examine gestational age at delivery as a mediator between biological determinants of preterm birth, e.g., abnormal placentation, and adverse neonatal outcomes (Brown et al. 2013; Shapiro-Mendoza 2014). In this case health effects typically linked to preterm birth were determined to be a direct effect of the biological determinants of preterm birth.

Use of these statistical techniques to examine mechanistic pathways may be particularly useful in the study of preterm birth because of the limited ability to study this health outcome in a small animal model. Mice and rats rarely deliver preterm, and models that successfully induce prematurity have limited interpretability because they necessitate gene knock-outs (Hirota et al. 2010) and/or large doses of lipopolysaccharides (LPS) injection (Kaga et al. 1996). Additionally, such models may be inappropriate for studying the effects of more subtle factors that may lead to preterm birth, such as environmental pollutants. As an alternative, it may be more useful to screen human populations for

biomarkers of exposures as well as mechanistic intermediates and assess relationships with mediation analyses to aid in establishing causality. We recently observed that urinary concentrations of phthalate metabolites were associated with increased risk of preterm and particularly spontaneous preterm birth (Ferguson et al. 2014). Phthalate metabolites are indicative of exposure to phthalate diesters which are found ubiquitously in the environment in plastics, personal care products, and medications (ATSDR 2001, 2002). Exposure has been linked to preterm birth in other previous studies as well (Adibi et al. 2009; Meeker et al. 2009; Whyatt et al. 2009). We hypothesized that this relationship may be mediated by phthalate-induced maternal oxidative stress. This hypothesis is supported by data showing that 1) phthalates cause increased reactive oxygen species (ROS) production in various cell types (Rusyn et al. 2001; Tetz et al. 2013); 2) urinary phthalate metabolites have been associated with increased oxidative stress biomarkers in human populations (Ferguson et al. 2011, 2012; Hong et al. 2009); 3) oxidative stress may plausibly lead to preterm birth via several pathways; and 4) oxidative stress biomarkers have been linked to preterm birth in several epidemiologic studies (Pathak et al. 2010; Peter Stein et al. 2008; Scholl and Stein 2001).

In addition to identifying a relationship between phthalate exposure and preterm birth, we found that phthalates were associated with an increase in urinary 8-isoprostane, a biomarker of arachadonic acid peroxidation which results from increased cellular ROS levels, and that 8-isoprostane was associated with increased preterm and especially spontaneous preterm birth. This evidence circumstantially indicates that phthalate-induced preterm birth is mediated by changes in 8-isoprostane levels. However, in the present study we employ statistical mediation analysis in a more rigorous approach to examining this hypothesis.

METHODS

Study population

The study population has been described in detail previously in the studies of phthalate exposure and preterm birth (Ferguson et al. 2014), the study of 8-isoprostane and phthalate metabolites (Chapter IV), and in the study of 8-isoprostane levels and preterm birth (Chapter V). Briefly, mothers included in this nested case-control study

were selected from a prospective birth cohort examining predictors of preeclampsia in women who delivered at the Brigham and Women's Hospital in Boston, MA. The present study, designed with the primary purpose of examining phthalate exposure in relation to preterm birth, included 130 women who delivered preterm as well as 352 randomly selected controls. Urine samples were available for analysis of phthalate and oxidative stress biomarkers from up to 4 visits per subject (mean 3.52 visits per subject) across gestation. Demographic characteristics that were included in regression models in previous analyses within this population included maternal age at visit 1, race/ethnicity, education level (high school, technical school, junior college/some college, college graduate), health insurance provider (private/health maintenance organization/self-pay vs. Medicaid/supplemental security income/MassHealth), and pre-pregnancy body mass index (BMI; <25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2). These covariates were included in each model for the present analysis.

Urinary biomarkers

Nine urinary phthalate metabolites were measured by NSF International in Ann Arbor, MI, by high performance liquid chromatography and tandem mass spectrometry as reported previously (Ferguson et al. 2014; Lewis et al. 2013). Detection limits were in the low ng/mL range and levels below the limit of detection (LOD) were replaced by the LOD divided by the square root of 2 (Hornung and Reed 1990). In addition to individual metabolites, a summed measure of di-(2-ethylhexyl) phthalate (DEHP) metabolites, including mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), was created based on nanomolar concentrations for a more stable estimate of DEHP exposure. Because DEHP metabolites are highly correlated, and the strongest associations observed with preterm birth were for MEHP and MECPP, MEHHP and MEOHP were not examined separately in this analysis. Cayman Chemical (Ann Arbor, MI) measured total 8-isoprostane in affinity purified urine samples using enzyme immunoassay (EIA), and detection was to 3.9 pg/mL. Levels below the LOD were treated the same as phthalate metabolites. In addition, to adjust for urinary dilution, specific gravity was measured in all samples at the

time of phthalate analysis with a digital handheld refractometer (Atago Co., Ltd., Tokyo, Japan).

Both phthalate and 8-isoprostane concentrations were measured in up to four samples per subject, at median 10, 18, 26, and 35 weeks gestation. For the present analysis, we created subject-specific geometric averages of urinary phthalate metabolite and 8-isoprostane levels from visits 1-3 to indicate individual exposures. Visit 4 was excluded from averages because the proportion of cases with samples available at this time point was low, as many had already delivered. Subject-specific averages for all biomarkers exhibited log-normal distributions and were ln-transformed for statistical modeling. For specific gravity an arithmetic average was created from visits 1-3 and included as a covariate in regression models.

Statistical methods

Distributions of demographic characteristics of biomarkers were presented previously and are not reexamined here. Prior to investigation of mediation, several tenets of the relationships between exposure, outcome, and mediator must hold true (Baron and Kenny 1986). In reference to Figure 1, there must first be an established association between the exposure X, in this case urinary phthalate metabolite, and the outcome Y, in this case preterm birth.

Model 1. Preterm birth ~ log(urinary phthalate metabolite) + covariates

Second, there must be an established relationship between the exposure X, urinary phthalate metabolite, and the mediator M, in this case urinary 8-isoprostane (Association A).

Model 2. Log (urinary 8-isoprostante) ~ log (urinary phthalate metabolite) + covariates

Third, there must be an association between the mediator M, urinary 8-isoprostane, and the outcome Y, preterm birth, after adjustment for the exposure X, urinary phthalate metabolite (Association B).

Model 3. Preterm birth ~ log(urinary 8-isoprostane) + log(urinary phthalate metabolite) + covariates

Each of these relationships has been established previously (Ferguson et al. 2014), however sets of covariates differed slightly. Therefore, each model was rerun in the

current analysis using the consistent set of covariates listed above. Models were first created for the total population, including all cases of preterm birth as well as controls, and subsequently within a subset that included only cases with spontaneous preterm delivery, characterized by presentation with spontaneous preterm labor and/or preterm premature rupture of the membranes (pPROM), and controls (Ferguson et al. 2014). We performed a mediation analysis if there was a significant direct effect of urinary phthalate metabolite on preterm birth ($p<0.10$, Model 1).

Mediation analysis was performed by comparing effect estimates in the above models. Because effect estimates from logistic and linear regression models cannot be compared directly, effect estimates from logistic regression were standardized to linear regression coefficients using formulas proposed by MacKinnon and Dwyer (Mackinnon and Dwyer 1993). This involves a standardization factor calculated from the standard error of the residuals from the logistic regression model with an assumed error variance of $(0, \pi^2/_3)$. Standardized logistic regression coefficients are compared to linear coefficients to estimate the proportion of the effect of the exposure on the outcome variable that is indirect, i.e., occurs by way of the mediator.

RESULTS

For overall preterm birth, beta coefficients and standard errors for each regression model are presented in Table VI.1. As observed previously, MEHP, MECPP, ∑DEHP, and mono-*n*-butyl phthalate (MBP) metabolites were all associated with preterm birth after adjustment for covariates (Model 1) (Ferguson et al. 2014). All urinary phthalate metabolites measured were significantly associated with increased 8-isoprostane in the total population (Model 2). Finally, in logistic models in which preterm birth was regressed on phthalate metabolite and 8-isoprostane the beta estimates for 8-isoprostane were all statistically significant (Model 3). Beta coefficients for phthalate metabolites in those models decreased slightly, which would be expected in the case of mediation of the phthalate-preterm relationship by 8-isoprostane. Model 3 was additionally examined with an interaction term between phthalate metabolite and 8-isoprostane but no significant interactions were identified for any phthalate metabolite (data not shown). Mediation analysis was performed for MEHP, MECPP, ∑DEHP and MBP, as these metabolites

were significantly associated with increased odds of overall preterm birth, and showed that 21-35 percent of the association between urinary phthalate metabolite and preterm birth was mediated by maternal 8-isoprostane concentrations or oxidative stress (Table VI.2).

Results for Models 1-3 for the spontaneous preterm birth subset are presented in Table VI.3. Relationships with metabolites and spontaneous preterm birth were all statistically significant; therefore mediation analysis was performed for all metabolites. The proportion of the phthalate-preterm relationship mediated by maternal 8-isoprostane levels was higher for spontaneous preterm compared to overall preterm birth; the proportion mediated ranged from 24 to 48 percent (Table VI.4).

DISCUSSION

We previously observed that average urinary phthalate metabolite excretion during pregnancy was associated with urinary biomarkers of oxidative stress, and that both biomarkers were associated with increased risk of overall preterm birth, and even more strongly with spontaneous preterm birth. In the present mediation analysis we demonstrate that the relationship between phthalate exposure and preterm birth is mediated in part by phthalate-induced oxidative stress, which has not been elucidated clearly in human or animal studies previously.

A major assumption in this analysis is that phthalates cause oxidative stress. If oxidative stress causes an increase in urinary phthalate metabolite excretion, or if the connection between the two is not causal but instead a result of unmeasured confounding, then the interpretation of these results would be different. However, based on animal and cellular studies, there is good reason to believe that phthalate exposure during pregnancy causes an increase in ROS release which can be measured by urinary excretion of 8-isoprostane (Rusyn et al. 2001; Tetz et al. 2013).

There are also several plausible mechanisms by which increased maternal ROS could lead to preterm birth. First, increased circulating or tissue (placenta, myometrium) ROS could lead to increased recruitment of inflammatory mediators (IL-6, TNF-α, etc.). These factors precipitate a cascade of events resulting in premature cervical ripening, contractions, and parturition (Challis et al. 2009). Additionally, prostaglandins such as 8-

isoprostane are important factors in this cascade; thus ROS peroxidation of lipid to form 8-isoprostane could feed more directly into this pathway as well. A second mechanism may be that oxidative stress in the myometrial membranes weakens the collagen in that tissue leading directly to pPROM (Kwiatkowski et al. 2009). Third, recent evidence from an animal knock-out model suggests that senescence of the decidua may be a factor in prematurity (Hirota et al. 2010), and oxidative stress and consequent DNA damage is known to contribute to senescence (Serrano and Blasco 2001). Each of these potential mechanisms would require phthalate-induced oxidative stress in the maternal fetal compartment. Although in the present study we do not measure tissue-specific oxidative stress, 8-isoprostane generated in all tissues would be excreted in the urine. Additionally, although only excreted phthalates are measured in this study, previous studies have detected phthalates in the maternal-fetal compartment in matrices such as amniotic fluid (Jensen et al. 2012) and umbilical cord blood (Latini et al. 2003) and concentrations have been correlated to levels in urine (Lin et al. 2011; Wittassek et al. 2009).

Each of these mechanisms would be more sensitive to oxidative stress levels in the end of pregnancy; however it is possible that phthalate exposure and associated oxidative stress early in pregnancy are relevant as well. For example, oxidative stress in the placenta early in pregnancy could cause apoptosis of the syncitotrophoblast, impaired myometrial invasion, and poor placentation (Burton et al. 2010; Heazell et al. 2007). These changes have been linked to adverse pregnancy conditions like preeclampsia and intrauterine growth restriction which are associated with preterm birth. However, our data demonstrate that both phthalate and oxidative stress levels later in pregnancy may be more important for preterm birth, which points toward some of the previously described mechanisms.

We observed significant mediation of the phthalate-preterm relationship by maternal 8-isoprostane levels; however the proportion mediated was less than 50 percent for all phthalate metabolites. As we have no reason to believe that the remainder of the relationship is a direct effect of phthalate exposure on preterm birth, other indirect pathways may exist. One possibility is action through endocrine disruption; phthalates are well established endocrine disrupting compounds, acting largely through anti-androgen activity, and they may also adversely impact thyroid hormone signaling (Fisher 2004;

Meeker and Ferguson 2011). These changes may be connected to preterm birth. A second possibility is that phthalates, through non-oxidative stress mechanisms, are inducing inflammation which is leading to prematurity. Several cellular studies have demonstrated that phthalates may cause recruitment of inflammatory cytokines and other mediators of inflammation, and these factors have been well established as contributors to preterm birth. Exploration of these relationships using mediation analyses similar to those employed here will aid in establishing the importance of alternative pathways in the relationship between phthalate exposure and preterm birth.

Several aspects of our study as well as mediation analysis more generally are limited. In regards to our study design, we measured circulating markers of oxidative stress, though levels of exposures and mediators at the maternal-fetal interface may be more relevant. However, as mentioned previously, markers in urine may be indicative at least in part of activity in the uterine compartment, and collection of urine is much more feasible (i.e., less invasive) than tissue or fluid samples from the uterus during pregnancy. A second limitation is that 8-isoprostane is not a direct measure of ROS production but only a proxy. While we attempted to examine the mediation of the phthalate-preterm birth by oxidative stress, in reality we only examined the mediation of the relationship by 8-isoprostane, or the mediation by oxidative stress *detected* by 8-isoprostane production. Other factors may influence the relationship between phthalate exposure and 8-isoprostane levels, making 8-isoprostane incompletely representative of the oxidative stress that phthalates produce. Thus the mediation by total oxidative stress may be underestimated in this analysis.

Additionally, our mediation analysis may be incomplete because we do not fully utilize the power of having multiple measurements of urinary phthalate metabolites and 8-isoprostane levels measured across pregnancy. As compared to the associations with average measures presented here, we observed much stronger associations between urinary phthalate metabolites and 8-isoprostane levels when data was analyzed using linear mixed models examining associations between repeated biomarker measures. Methods for performing mediation analysis combining a binary outcome and longitudinal exposure and mediators have not been well-established, but future analyses in this vein

may provide more precise estimates of mediation by maximizing the use of the data available.

Despite these limitations our study had many strengths, namely the ability to examine these associations in a case-control population with a large number of subjects and biomarkers of both exposure and outcome. It is also the first analysis to our knowledge that attempts to identify through an epidemiologic study the mediators of relationships between an environmental contaminant exposure and preterm birth. A number of studies examine relationships between other environmental chemicals and prematurity, but are limited by the inability to establish causality. While mediation analysis still does not concretely establish a causal pathway, it provides an additional step that none of these previous studies have been able to take.

Table VI.1 Beta coefficients and standard errors (SE) for Models 1) preterm birth regressed on phthalate metabolite; 2) preterm birth regressed on phthalate metabolite and 8-isoprostane in the same model; and 3) 8-isoprostane regressed on phthalate metabolite. Estimates created for population with all preterm births and controls. N=461.

	Model 1		Model 2				Model 3	
	Phthalate metabolite		Phthalate metabolite		8-Isoprostane		Phthalate metabolite	
	B (SE)	p	B (SE)	p	B (SE)	p	B (SE)	p
MEHP	0.30 (0.12)	0.01	0.24 (0.12)	0.04	0.62 (0.17)	<0.01	0.11 (0.04)	<0.01
MECPP	0.32 (0.11)	<0.01	0.25 (0.12)	0.03	0.60 (0.17)	<0.01	0.16 (0.04)	<0.01
∑DEHP	0.28 (0.13)	0.03	0.21 (0.13)	0.11	0.62 (0.17)	<0.01	0.15 (0.04)	<0.01
MBzP	0.08 (0.12)	0.51	0.01 (0.13)	0.91	0.65 (0.17)	<0.01	0.10 (0.04)	0.02
MBP	0.24 (0.13)	0.06	0.16 (0.13)	0.21	0.63 (0.17)	<0.01	0.14 (0.05)	<0.01
MiBP	-0.01 (0.16)	0.94	-0.10 (0.17)	0.54	0.67 (0.17)	<0.01	0.16 (0.06)	<0.01
MEP	0.11 (0.09)	0.21	0.07 (0.09)	0.43	0.64 (0.17)	<0.01	0.07 (0.03)	0.02
MCPP	0.18 (0.12)	0.12	0.09 (0.12)	0.45	0.63 (0.17)	<0.01	0.15 (0.04)	<0.01

Note: All models adjusted for the same covariates, including average specific gravity, maternal age, Race/Ethnicity, education level, health insurance provider, and pre-pregnancy body mass index.

Table VI.2 Effect estimates (95% confidence intervals) and percent mediated calculated from regression estimates and standard errors generated from Models 1-3 (Table VI.1). Estimates created for population with all preterm births and controls. N=461.

	Total standardized effect (95% CI)	Mediated effect (95% CI)	Direct effect (95% CI)	Percent mediated
MEHP	0.162 (0.036, 0.288)	0.034 (0.002, 0.066)	0.128 (0.004, 0.252)	21
MECPP	0.176 (0.056, 0.295)	0.046 (0.008, 0.084)	0.130 (0.011, 0.249)	26
∑DEHP	0.153 (0.018, 0.288)	0.044 (0.005, 0.083)	0.109 (-0.024, 0.242)	29
MBP	0.131 (-0.007, 0.270)	0.046 (0.006, 0.085)	0.086 (-0.048, 0.220)	35

Note: All models adjusted for the same covariates, including average specific gravity, maternal age, Race/Ethnicity, education level, health insurance provider, and pre-pregnancy body mass index.

Table VI.3 Beta coefficients and standard errors (SE) for Models 1) spontaneous preterm birth regressed on phthalate metabolite; 2) spontaneous preterm birth regressed on phthalate metabolite and 8-isoprostane in the same model; and 3) 8-isoprostane regressed on phthalate metabolite. Estimates created for population with all spontaneous preterm births and controls. N=390.

	Model 1		Model 2				Model 3	
	Phthalate metabolite		Phthalate metabolite		8-Isoprostane		Phthalate metabolite	
	B (SE)	p	B (SE)	p	B (SE)	p	B (SE)	p
MEHP	0.49 (0.17)	<0.01	0.44 (0.17)	0.01	1.45 (0.32)	<0.01	0.10 (0.04)	0.03
MECPP	0.40 (0.16)	0.01	0.26 (0.17)	0.13	1.42 (0.32)	<0.01	0.19 (0.04)	<0.01
∑DEHP	0.46 (0.18)	0.01	0.34 (0.19)	0.08	1.43 (0.32)	<0.01	0.17 (0.05)	<0.01
MBzP	0.37 (0.17)	0.03	0.24 (0.18)	0.18	1.39 (0.32)	<0.01	0.10 (0.05)	0.03
MBP	0.40 (0.17)	0.01	0.29 (0.16)	0.08	1.41 (0.32)	<0.01	0.15 (0.05)	<0.01
MiBP	0.40 (0.23)	0.08	0.29 (0.24)	0.24	1.44 (0.32)	<0.01	0.15 (0.06)	0.02
MEP	0.23 (0.13)	0.07	0.14 (0.13)	0.28	1.43 (0.32)	<0.01	0.09 (0.03)	0.01
MCPP	0.34 (0.15)	0.02	0.22 (0.16)	0.17	1.41 (0.32)	<0.01	0.14 (0.04)	<0.01

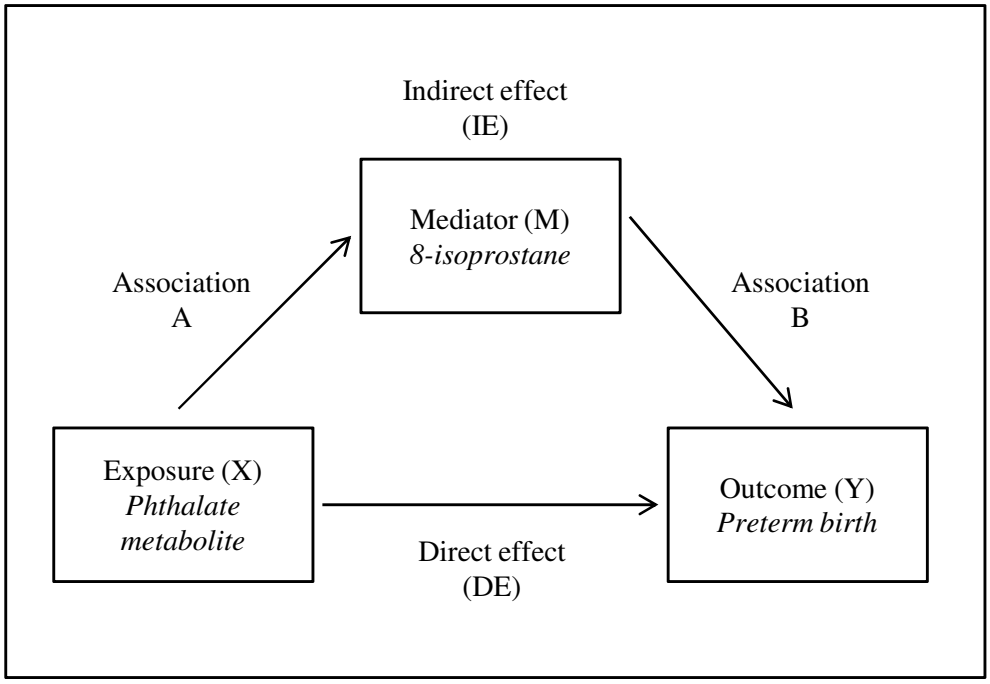
Note: All models adjusted for the same covariates, including average specific gravity, maternal age, Race/Ethnicity, education level, health insurance provider, and pre-pregnancy body mass index.

Table VI.4 Effect estimates (95% confidence intervals) and percent mediated calculated from regression estimates and standard errors generated from Models 1-3 (Table VI.5). Estimates created for population with spontaneous preterm births only and controls. N=390.

	Total standardized effect (95% CI)	Mediated effect (95% CI)	Direct effect (95% CI)	Percent mediated
MEHP	0.262 (0.089, 0.436)	0.063 (-0.0001, 0.126)	0.199 (0.045, 0.353)	24
MECPP	0.217 (0.048, 0.386)	0.099 (0.021, 0.178)	0.118 (-0.034, 0.269)	46
∑DEHP	0.246 (0.056, 0.437)	0.091 (0.011, 0.172)	0.155 (-0.016, 0.326)	37
MBzP	0.202 (0.022, 0.381)	0.091 (0.025, 0.158)	0.110 (-0.051, 0.271)	45
MBP	0.220 (0.044, 0.396)	0.086 (0.006, 0.167)	0.134 (-0.014, 0.282)	39
MiBP	0.219 (-0.028, 0.466)	0.086 (-0.007, 0.179)	0.133 (-0.088, 0.354)	39
MEP	0.126 (-0.008, 0.261)	0.061 (0.009, 0.113)	0.065 (-0.052, 0.183)	48
MCPP	0.185 (0.026, 0.345)	0.082 (0.012, 0.153)	0.103 (-0.043, 0.249)	44

Note: All models adjusted for the same covariates, including average specific gravity, maternal age, Race/Ethnicity, education level, health insurance provider, and pre-pregnancy body mass index.

Figure VI.1 Mediation of the relationship between urinary phthalate metabolites and preterm birth by 8-isoprostane.



CHAPTER VII

CONCLUSIONS

RESEARCH SUMMARY

This dissertation provides strong evidence for a relationship between phthalate exposure during pregnancy and increased risk of preterm birth, and also suggests that the relationship may be mediated in part by phthalate-induced oxidative stress. Previous research in this area has been limited. Studies examining phthalate exposure in relation to preterm birth have had small sample sizes and very small numbers of preterm deliveries, and any evidence for mechanism was piecemeal (Adibi et al. 2008; Meeker et al. 2009; Whyatt et al. 2009).

The relationship between phthalate exposure and preterm birth was evidenced in Chapter II, with a basic analysis of average urinary phthalate metabolites excreted over pregnancy in association with preterm birth (Ferguson et al. 2014). Odds ratios were strongest for mono-(2-ethylheyxl) phthalate (MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), and mono-*n*-butyl phthalate (MBP). When spontaneous preterm births were examined alone, odds ratios were higher and relationships for mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-benzyl phthalate (MBzP), and mono-(3-carboxypropyl) phthalate (MCPP) became significant as well. An examination of odds ratios in association with quartile of phthalate exposure demonstrated a dose-response effect, with significant trends for MEHP, MECPP, and MBP in relation to overall preterm birth, and strong and significant trends for MEHP, MEOHP, MECPP, MBzP, and MBP for spontaneous preterm birth with odds ratios as high as 5.23. This analysis utilized the power of multiple exposure measurements during pregnancy only by creating a more stable measure of exposure over pregnancy for each subject; however even with this simplistic analysis associations observed were quite strong.

Chapter III delved into this relationship further by examining the variability and patterns in urinary phthalate metabolite excretion patterns over pregnancy, and also by scrutinizing potential windows of vulnerability to phthalate exposure for the risk of preterm birth. As has been observed in previous studies (Braun et al. 2012), phthalate metabolites were moderately variable across gestation and metabolites of low molecular weight phthalates, such as MBP, were slightly more stable. There was generally no difference in stability measures in cases compared to controls; however when trends in phthalate metabolites were examined across pregnancy, some differences were observed. For MEHP, MECPP, MBzP, and MBP, levels in spontaneous preterm cases were similar to those compared to controls early in pregnancy, and as gestation progressed levels diverged so that they were most disparate near the last visit (median 35 weeks gestation). These patterns were reflected in cross sectional odds ratios by study visit; odds of spontaneous preterm birth were highest in association with urinary phthalate metabolite concentrations measured at visits 3 and 4. When trajectories were examined for placental preterm births, few differences were observed in cases compared to controls, although for MECPP levels were suggestively elevated compared to controls early in pregnancy. Odds ratios by visit for MECPP levels were highest at visit 1; however many comparisons were examined in this analysis and this one association may have been spurious, especially as no associations were observed with other di-(2-ethylhexyl) phthalate (DEHP) metabolites.

These associations between phthalate exposure and prematurity improve on previous studies in this vein by using a large study population, the largest number of preterm births which further enabled novel examination of preterm births by etiological subtypes, and the largest number of exposure measurements per subject during pregnancy. However, one of most significant contributions of this work to the literature on this subject is the investigation of a mechanistic intermediate and its role in mediating the relationship between phthalate exposure and preterm birth. This was investigated in Chapters IV through VI.

First, Chapter IV illustrated the relationship between phthalate exposure and oxidative stress, which has been demonstrated in a number of different cell types (Erkekoglu et al. 2010; Kasahara et al. 2002; Tetz et al. 2013) and also to a limited extent

in cross-sectional epidemiologic studies (Ferguson et al. 2011, 2012; Hong et al. 2009). Using repeated measures of all biomarkers, each urinary phthalate metabolite measured was associated with increases in DNA (8-hydroxydeoxyguanosine; 8-OHdG) and lipid (8-isoprostane) markers of reactive oxygen species (ROS) production. Interquartile range increases in urinary phthalate metabolite concentrations were associated with up to 30 percent and 56 percent increases in 8-OHdG and 8-isoprostane, respectively. This is by far the strongest evidence for an association between phthalate exposure and oxidative stress in humans and for the first time provides evidence of this relationship in pregnant women.

The second step in establishing oxidative stress as a mediator in the relationship between phthalate exposure and preterm birth was to add to the current body of literature showing that increased oxidative stress precipitates preterm birth. This data had been limited, likely due to limitations in previous study designs, including use of small study populations, small number of cases, single measures of oxidative stress during pregnancy (Hsieh et al. 2012; Peter Stein et al. 2008; Scholl and Stein 2001). In Chapter V biomarkers of oxidative stress were strongly associated with increased risk of preterm birth. However, the direction of association differed by biomarker. For 8-isoprostane, associations were positive; increased geometric urinary concentrations from visits 1-3 were associated with twice the odds of overall preterm birth and 4 times the odds of spontaneous preterm birth. These associations were strongest for levels measured later in pregnancy. On the other hand, 8-OHdG associations were inverse, or protective of preterm birth. These odds ratios were quite strong as well. A potential explanation for this relationship was that urinary concentrations of 8-OHdG in urine might not only indicate increased oxidative DNA lesions but also rate of nucleotide excision repair processes. Dysfunctional repair processes would lead to decreased levels of 8-OHdG in urine, and could reasonably be linked to preterm birth. This hypothesis, though tangential to this dissertation, may be of high interest in the future study of preterm birth and deserves further exploration.

The final step in establishing oxidative stress as a mediator in the phthalate-preterm continuum was to perform a mediation analysis, which was described in Chapter VI. Only 8-isoprostane was used in this analysis because 8-OHdG was unexpectedly

protective of preterm birth in Chapter V. For overall preterm birth, the mediation analysis demonstrated that oxidative stress, indicated by 8-isoprostane, mediated between 20 and 35 percent of the relationship between MEHP, MECPP, and MBP phthalate metabolites and preterm birth. In spontaneous preterm birth, oxidative stress mediated up to 50 percent of the relationship in all metabolites examined. These data provide evidence that phthalate exposure may be related to preterm birth by induction of oxidative stress.

In summary, this dissertation demonstrates that phthalate exposure during pregnancy is associated with increased risk of preterm birth, which may be mediated in part by phthalate-induced oxidative stress. This body of work improves significantly on previous research that has addressed this question by utilizing a powerful nested case-control design with cases and controls selected from a large prospective birth cohort. The number of preterm cases was larger in this study than any others to date, and also larger than most studies that have examined other environmental chemical exposures in relation to preterm birth (Ferguson et al. 2013). This additionally allowed investigation of effects in subpopulations of preterm birth, including spontaneous preterm birth and preterm birth of placental origin. This more targeted analysis is novel both to the study of phthalate exposure and prematurity and is also rare in studies of chemical exposures and preterm birth. Thus, these data provide a strong argument for a role of phthalate exposure in the risk of preterm birth and illustrate how a strong study design can aid in identifying some of the more subtle factors that contribute to preterm birth.

LIMITATIONS

In the overall study there were some limitations that should be addressed in future research in this vein. First, although the design of the study included a large number of preterm births, it became clear upon analysis that phthalate exposure may be most relevant for spontaneous prematurity. This study was important for establishing this relationship; until the present analysis it was unclear whether phthalates were impacting placental development that could cause prematurity via preeclampsia or intrauterine growth restriction, or if exposure more directly impacted the parturition process later in gestation. However, now that we have gained evidence for a more specific pathway by

which phthalates may cause prematurity future studies should include a larger number of specifically spontaneous preterm births.

A second limitation involves the use of urinary biomarkers to indicate exposure to phthalates and levels of maternal oxidative stress. Utilizing urinary biomarkers may inaccurately represent this exposure and outcome for several reasons: 1) levels may be complicated by differences in individual xenobiotic metabolism or excretion patterns; 2) urine concentration must be accounted for, but specific gravity or other markers of urine dilution may also be representative of kidney function; and 3) compounds measured in urine may not be representative of the exposure that reaches the targets of interest, the fluids and tissues in the fetal compartment. While using urinary markers is very useful in large-scale epidemiologic studies, an improved understanding of the transfer of phthalates from maternal circulation to the fetal interface would be useful. Additionally, examining the relationship between those phthalate levels and tissue-specific levels oxidative stress would provide more direct evidence for a role for this mechanism in the promotion of preterm birth.

A third overarching limitation to this dissertation is the use of the nested case-control study design. While this design was very powerful for addressing the relationship between exposure during pregnancy and preterm birth, it is also limited by the fact that there is no information about exposure in subjects who miscarried, delivered a stillbirth, or carried multiple fetuses during pregnancy. Examination of the role of phthalates and oxidative stress in relation to these outcomes was not the primary goal of this dissertation; however a role could exist and it would have been interesting and informative to investigate these questions.

FUTURE DIRECTIONS

Future research in this vein should address the aforementioned limitations and also aim to shed light on several new areas. First, as this study suggests that phthalate exposure may be a contributor to preterm birth, additional research is necessary to determine if and how women can avoid exposure to phthalates during pregnancy. A number of studies have attempted to identify exposure sources in female populations (Buckley et al. 2012; Parlett et al. 2013) and some have done so in pregnant women

(Braun et al. 2013; Cantonwine et al. 2014); however results from these studies are conflicting. In regards to personal care product use, no study has demonstrated that avoiding specific products in daily life can decrease individual exposure levels. Studies of women in communities where product use is low demonstrate lower levels of urinary phthalate metabolites (Martina et al. 2012); however other exposure routes may be limited in these populations as well. Exposure through dietary routes is also poorly understood, and it is unclear whether personal modification of diet can effectively decrease exposure levels (Rudel et al. 2011; Sathyanarayana et al. 2013). Additional study of effectiveness of such measures would determine if this was a possible way for women to reduce exposure to phthalates during pregnancy; if not, alternative policy modifications may be necessary.

For future studies of phthalate exposure and preterm birth, it will be important to explore additional mechanisms. In the present body of work the relationship between phthalate exposure and prematurity was only mediated at a maximum of 50 percent by oxidative stress as indicated by 8-isoprostane. Phthalate action via sex or thyroid hormone disruption, induction of inflammation, and/or epigenetic changes could play a role in this pathway as well. The role of these potential mechanisms should be investigated more closely, and the model established in the present work using maternal biomarkers and a mediation analysis may serve as a useful tool for this future work.

These research findings also provide motivation for future work investigating adverse health effects associated with phthalates that may be due to oxidative stress. While this dissertation demonstrates the important role of this mediator in preterm birth, oxidative stress stimulated by exposure to phthalates may contribute to known health outcomes associated with phthalate exposure, including other birth outcomes such as fetal growth (Philippat et al. 2012) as well effects in children and adults such as allergies and asthma (Kimber and Dearman 2010), male sperm quality (Hauser et al. 2006; Hauser et al. 2007), and female uterine leiyomyata and endometriosis (Buck Louis et al. 2013; Weuve et al. 2010). Other health outcomes that have been explored minimally in relation to phthalate exposure may also be targeted in future studies if there development is associated with oxidative stress. Cardiovascular disease and carcinogenesis have been explored in relation to phthalate exposure in some small and limited studies (Lind and

Lind 2011; Lopez-Carrillo et al. 2010) but with knowledge of a potential mechanism these associations may deserve exploration in expanded populations.

Another important step in future studies investigating environmental contributors to preterm birth will be to explore the effects of mixtures of chemicals. It is well understood in the field of environmental health that investigating the impact of not single chemicals but the subject-specific milieu will be important in the future. However less attention is given to the potential value of mechanistic intermediates in understanding how multiple chemicals interact to cause a multi-factorial outcome. Oxidative stress has been long studied in regards to a number of health outcomes but recognizing it as a lynchpin in the study of preterm birth and other health outcomes may be extremely valuable. The use of human biomarkers of oxidative stress in epidemiologic studies will become very important to this end as well, as the biological relevance of multiple exposures is less easily understood in data from animal models.

PUBLIC HEALTH IMPACT

This work adds to a growing body of literature indicating toxic effects of phthalate exposure, and it provides evidence for a mechanism that links exposure to preterm birth and potentially other adverse health outcomes. The public health impact of this and other studies vilifying phthalates has several faces. First is the promotion of individual awareness of environmental toxicants, exposure sources, and potential health consequences. This promotion may be undertaken in part by clinicians. A recent statement by the American College of Obstetricians and Gynecologists recognized the importance of environmental chemical exposures in the role of reproductive health. It encouraged 1) future study to examine toxic effects of chemicals found ubiquitously in the environment, and 2) recognition of the importance of these factors by clinicians so that they may communicate this knowledge to patients (ACOG 2013). However, communication of research findings in regards to phthalate exposure and preterm birth may be difficult because the ability to reduce exposure through behavioral modifications remains in question. These research findings might also impact policy initiatives to remove phthalates from some products that are clear routes of exposure. This, too, is problematic. Identification of these products is difficult; while phthalates have been

banned from use in children's toys since 2008, the greater concern remains for prenatal, not childhood, exposure. Even after successful phase-out, replacement chemicals may have similar or worse health effects. A final potential impact of this work may be to influence change in the process through which a chemical comes into use in our society. It suggests the value of testing effects before full implementation. Although such a change is unlikely at present, continued research demonstrating adverse effects of universal chemical use may slowly build toward this end. Thus, this research can contribute part to public health action in the study of chemicals and preterm birth, in the study of phthalates and an array of adverse health effects, and, finally, to any long-term campaign against use of compounds without evidence of safety.

REFERENCES

ACOG (American College of Obstetricians and Gynecologists). 2013. Exposure to toxic environmental agents. Fertil Steril 100(4): 931-934.

Adibi JJ, Hauser R, Williams PL, Whyatt RM, Calafat AM, Nelson H, et al. 2009. Maternal urinary metabolites of di-(2-ethylhexyl) phthalate in relation to the timing of labor in a US multicenter pregnancy cohort study. Am J Epidemiol 169(8): 1015-1024.

Adibi JJ, Perera FP, Jedrychowski W, Camann DE, Barr D, Jacek R, et al. 2003. Prenatal exposures to phthalates among women in New York City and Krakow, Poland. Environ Health Perspect 111(14): 1719-1722.

Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, et al. 2008. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. Environ Health Perspect 116(4): 467-473.

Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. 2012. The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol 10: 49.

Aggazzotti G, Righi E, Fantuzzi G, Biasotti B, Ravera G, Kanitz S, et al. 2004. Chlorination by-products (CBPs) in drinking water and adverse pregnancy outcomes in Italy. J Water Health 2(4): 233-247.

Ahmad SA, Sayed MH, Barua S, Khan MH, Faruquee MH, Jalil A, et al. 2001. Arsenic in drinking water and pregnancy outcomes. Environ Health Persp 109(6): 629-631.

Andrews KW, Savitz DA, Hertz-Picciotto I. 1994. Prenatal lead exposure in relation to gestational age and birth weight: a review of epidemiologic studies. Am J Ind Med 26(1): 13-32.

Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, et al. 2007. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect 115(11): 1670-1676.

Arbuckle TE, Kubwabo C, Walker M, Davis K, Lalonde K, Kosarac I, et al. 2012. Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants. Int J Hyg Environ Health.

Aschengrau A, Weinberg J, Rogers S, Gallagher L, Winter M, Vieira V, et al. 2008. Prenatal exposure to tetrachloroethylene-contaminated drinking water and the risk of adverse birth outcomes. Environ Health Perspect 116(6): 814-820.

Ashton DM, Lawrence HC, 3rd, Adams NL, 3rd, Fleischman AR. 2009. Surgeon General's Conference on the prevention of preterm birth. Obstet Gynecol 113(4): 925-930.

ATSDR. 1995a. Toxicological profile for polycyclic aromatic hydrocarbons (PAHs). Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 1995b. Toxicological Profile for Diethyl phthalate (DEP).

ATSDR. 1997a. Toxicological profile for trichloroethylene. Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 1997b. Toxicological profile for tetrachloroethylene. Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 1997c. Toxicological Profile for di-n-octylphthalate. . Agency for Toxic Substances and Disease Registry.

ATSDR. 1998. Toxicological profile for chlorinated dibenzo-p-dioxins (CDDs). Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2000. Toxicological Profile for Polychlorinated Biphenyls (PCBs). Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2001a. Toxicological profile for di-n-butyl phthalate (DBP). Agency for Toxic Substances and Disease Registry.

ATSDR. 2001b. Toxicological Profile for Di-n-butyl Phthalate. Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2002a. Toxicological profile for aldrin/dieldrin.

ATSDR. 2002b. Toxicological profile for Hexachlorobenzene. Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2002c. Toxicological profile for aldrin/dieldrin. Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2002d. Toxicological Profile for Di(2-ethylhexyl)phthalate (DEHP). Agency for Toxic Substances and Disease Registry.

ATSDR. 2002e. Toxicological Profile for Di(2-ethylhexyl)phthalate (DEHP). Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2003. Toxicological profile for atrazine. Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2005. Toxicological profile for alpha-, beta-, gamma-, and delta-hexachlorocyclohexane. Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2007a. Toxicological profile for heptachlor and heptachor epoxide. Atlanta, GA:US Department of Health and Human Services.

ATSDR. 2007b. Toxicological Profile for Lead (*Update*). Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2008. Toxicological Profile for Cadmium. Atlanta, GA:US Department of Health and Human Services, Public Health Service.

ATSDR. 2009. Toxicological profile for perfluoroalkyls (draft for public comment). Atlanta, GA:US Department of Health and Human Services, Public Health Service.

Aylward LL, Lorber M, Hays SM. 2011. Urinary DEHP metabolites and fasting time in NHANES. Journal of exposure science & environmental epidemiology 21(6): 615-624.

Baron RM, Kenny DA. 1986. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 51(6): 1173-1182.

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environmental health perspectives 113(2): 192-200.

Bartell SM, Calafat AM, Lyu C, Kato K, Ryan PB, Steenland K. 2010. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environ Health Perspect 118(2): 222-228.

Behrman RE, Butler AS, eds. 2007. Preterm birth: causes, consequences, and prevention. Washington, DC:The National Academies Press.

Bergonzi R, De Palma G, Specchia C, Dinolfo M, Tomasi C, Frusca T, et al. 2011. Persistent organochlorine compounds in fetal and maternal tissues: evaluation of their potential influence on several indicators of fetal growth and health. Sci Total Environ 409(15): 2888-2893.

Berkowitz GS, Lapinski RH, Wolff MS. 1996. The role of DDE and polychlorinated biphenyl levels in preterm birth. Arch Environ Contam Toxicol 30(1): 139-141.

Berman T, Hochner-Celnikier D, Calafat AM, Needham LL, Amitai Y, Wormser U, et al. 2009. Phthalate exposure among pregnant women in Jerusalem, Israel: results of a pilot study. Environ Int 35(2): 353-357.

Bind MA, Lepeule J, Zanobetti A, Gasparrini A, Baccarelli A, Coull BA, et al. 2014. Air pollution and gene-specific methylation in the Normative Aging Study: Association, effect modification, and mediation analysis. Epigenetics 9(3).

Bloom MS, Buck Louis GM, Schisterman EF, Liu A, Kostyniak PJ. 2007. Maternal serum polychlorinated biphenyl concentrations across critical windows of human development. Environ Health Persp 115(9): 1320-1324.

Boeniger MF, Lowry LK, Rosenberg J. 1993. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. American Industrial Hygiene Association journal 54(10): 615-627.

Bornehag CG, Nanberg E. 2010. Phthalate exposure and asthma in children. Int J Androl 33(2): 333-345.

Bornehag CG, Sundell J, Weschler CJ, Sigsgaard T, Lundgren B, Hasselgren M, et al. 2004. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. Environmental health perspectives 112(14): 1393-1397.

Bove FJ, Fulcomer MC, Klotz JB, Esmart J, Dufficy EM, Savrin JE. 1995. Public drinking water contamination and birth outcomes. Am J Epidemiol 141(9): 850-862.

Boyle EM, Poulsen G, Field DJ, Kurinczuk JJ, Wolke D, Alfirevic Z, et al. 2012. Effects of gestational age at birth on health outcomes at 3 and 5 years of age: population based cohort study. Br Med J 344: e896.

Braun JM, Just AC, Williams PL, Smith KW, Calafat AM, Hauser R. 2013. Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. Journal of exposure science & environmental epidemiology.

Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, et al. 2012. Variability of Urinary Phthalate Metabolite and Bisphenol A Concentrations before and during Pregnancy. Environmental health perspectives.

Brown HK, Speechley KN, Macnab J, Natale R, Campbell MK. 2013. Neonatal morbidity associated with late preterm and early term birth: the roles of gestational age and biological determinants of preterm birth. Int J Epidemiol.

Buck Louis GM, Peterson CM, Chen Z, Croughan M, Sundaram R, Stanford J, et al. 2013. Bisphenol A and phthalates and endometriosis: the Endometriosis: Natural History, Diagnosis and Outcomes Study. Fertil Steril 100(1): 162-169 e161-162.

Buckley JP, Palmieri RT, Matuszewski JM, Herring AH, Baird DD, Hartmann KE, et al. 2012. Consumer product exposures associated with urinary phthalate levels in pregnant women. J Expo Sci Environ Epidemiol 22(5): 468-475.

Burton GJ, Charnock-Jones DS, Jauniaux E. 2009a. Regulation of vascular growth and function in the human placenta. Reproduction 138(6): 895-902.

Burton GJ, Jauniaux E, Charnock-Jones DS. 2010. The influence of the intrauterine environment on human placental development. Int J Dev Biol 54(2-3): 303-312.

Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. 2009b. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. Placenta 30 Suppl A: S43-48.

Bustamante-Montes LP, Hernandez-Valero MA, Flores-Pimentel D, Garcia-Fabila M, Amaya-Chavez A, Barr DB, et al. 2013. Prenatal exposure to phthalates is associated with decreased anogenital distance and penile size in male newborns. J Dev Orig Health Dis 4(4).

Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environ Health Persp 116(1): 39-44.

Cantonwine D, Hu H, Sanchez BN, Lamadrid-Figueroa H, Smith D, Ettinger AS, et al. 2010a. Critical windows of fetal lead exposure: Adverse impacts on length of gestation and risk of premature delivery. J Occup Environ Med 52(11): 1106-1111.

Cantonwine D, Meeker JD, Hu H, Sanchez BN, Lamadrid-Figueroa H, Mercado-Garcia A, et al. 2010b. Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study. Environ Health 9: 62.

Cantonwine DE, Cordero JF, Rivera-Gonzalez LO, Anzalota Del Toro LV, Ferguson KK, Mukherjee B, et al. 2013. Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: Distribution, temporal variability, and predictors. Environ Int 62C: 1-11.

Cantonwine DE, Cordero JF, Rivera-Gonzalez LO, Anzalota Del Toro LV, Ferguson KK, Mukherjee B, et al. 2014. Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors. Environment international 62: 1-11.

Catanzaro SJ, Laurent J. 2004. Perceived family support, negative mood regulation expectancies, coping, and adolescent alcohol use: evidence of mediation and moderation effects. Addict Behav 29(9): 1779-1797.

CDC. 2009. Fourth national report on human exposure to environmental chemicals.

Cha J, Bartos A, Egashira M, Haraguchi H, Saito-Fujita T, Leishman E, et al. 2013. Combinatory approaches prevent preterm birth profoundly exacerbated by gene-environment interactions. J Clin Invest 123(9): 4063-4075.

Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, 3rd, Petraglia F. 2009. Inflammation and pregnancy. Reproductive sciences 16(2): 206-215.

Chang HH, Larson J, Blencowe H, Spong CY, Howson CP, Cairns-Smith S, et al. 2012. Preventing preterm births: analysis of trends and potential reductions with interventions in 39 countries with very high human development index. Lancet.

Chen MH, Ha EH, Wen TW, Su YN, Lien GW, Chen CY, et al. 2012. Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS One 7(8): e42474.

Chevrier C, Petit C, Philippat C, Mortamais M, Slama R, Rouget F, et al. 2012. Maternal urinary phthalates and phenols and male genital anomalies. Epidemiology 23(2): 353-356.

Cho SC, Bhang SY, Hong YC, Shin MS, Kim BN, Kim JW, et al. 2010. Relationship between environmental phthalate exposure and the intelligence of school-age children. Environmental health perspectives 118(7): 1027-1032.

Choi H, Rauh V, Garfinkel R, Tu Y, Perera FP. 2008. Prenatal exposure to airborne polycyclic aromatic hydrocarbons and risk of intrauterine growth restriction. Environ Health Persp 116(5): 658-665.

Christensen KL, Lorber M, Koch HM, Kolossa-Gehring M, Morgan MK. 2012. Population variability of phthalate metabolites and bisphenol A concentrations in spot urine samples versus 24- or 48-h collections. Journal of exposure science & environmental epidemiology.

Citron M. 1995. Perplexing peroxisome proliferators. Environmental health perspectives 103(3): 232-235.

Clark SL, Miller DD, Belfort MA, Dildy GA, Frye DK, Meyers JA. 2009. Neonatal and maternal outcomes associated with elective term delivery. Am J Obstet Gynecol 200(2): 156 e151-154.

Cobellis L, Latini G, De Felice C, Razzi S, Paris I, Ruggieri F, et al. 2003. High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. Hum Reprod 18(7): 1512-1515.

Cole FS, Alleyne C, Barks JD, Boyle RJ, Carroll JL, Dokken D, et al. 2011. NIH Consensus Development Conference statement: inhaled nitric-oxide therapy for premature infants. Pediatrics 127(2): 363-369.

Colon I, Caro D, Bourdony CJ, Rosario O. 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environmental health perspectives 108(9): 895-900.

Costet N, Garlantezec R, Monfort C, Rouget F, Gagniere B, Chevrier C, et al. 2012. Environmental and Urinary Markers of Prenatal Exposure to Drinking Water Disinfection By-Products, Fetal Growth, and Duration of Gestation in the PELAGIE Birth Cohort (Brittany, France, 2002-2006). Am J Epidemiol 175(4): 263-275.

Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. 2006. Biomarkers of oxidative damage in human disease. Clin Chem 52(4): 601-623.

de Rosa CT, El-Masri HA, Pohl H, Cibulas W, Mumtaz MM. 2004. Implications of chemical mixtures in public health practice. J Toxicol Environ Health B 7(5): 339-350.

Dodds L, King W, Woolcott C, Pole J. 1999. Trihalomethanes in public water supplies and adverse birth outcomes. Epidemiology 10(3): 233-237.

Durmaz E, Ozmert EN, Erkekoglu P, Giray B, Derman O, Hincal F, et al. 2010. Plasma phthalate levels in pubertal gynecomastia. Pediatrics 125(1): e122-129.

Duty SM, Calafat AM, Silva MJ, Ryan L, Hauser R. 2005. Phthalate exposure and reproductive hormones in adult men. Human reproduction 20(3): 604-610.

Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen Z, et al. 2003a. Phthalate exposure and human semen parameters. Epidemiology 14(3): 269-277.

Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, Ryan L, et al. 2003b. The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. Environmental health perspectives 111(9): 1164-1169.

Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, et al. 2010. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environmental health perspectives 118(4): 565-571.

Engel SM, Zhu C, Berkowitz GS, Calafat AM, Silva MJ, Miodovnik A, et al. 2009. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. Neurotoxicology 30(4): 522-528.

Erkekoglu P, Rachidi W, Yuzugullu OG, Giray B, Favier A, Ozturk M, et al. 2010. Evaluation of cytotoxicity and oxidative DNA damaging effects of di(2-ethylhexyl)-phthalate (DEHP) and mono(2-ethylhexyl)-phthalate (MEHP) on MA-10 Leydig cells and protection by selenium. Toxicol Appl Pharmacol 248(1): 52-62.

Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, et al. 2004. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Persp 112(10): 1116-1124.

Eskenazi B, Mocarelli P, Warner M, Chee WY, Gerthoux PM, Samuels S, et al. 2003. Maternal serum dioxin levels and birth outcomes in women of Seveso, Italy. Environ Health Persp 111(7): 947-953.

Fagher U, Laudanski T, Schutz A, Sipowicz M, Akerlund M. 1993. The relationship between cadmium and lead burdens and preterm labor. Int J Gynaecol Obstet 40(2): 109-114.

Falcon M, Vinas P, Luna A. 2003. Placental lead and outcome of pregnancy. Toxicology 185(1-2): 59-66.

Fan J, Traore K, Li W, Amri H, Huang H, Wu C, et al. 2010. Molecular mechanisms mediating the effect of mono-(2-ethylhexyl) phthalate on hormone-stimulated steroidogenesis in MA-10 mouse tumor Leydig cells. Endocrinology 151(7): 3348-3362.

Farhang L, Weintraub JM, Petreas M, Eskenazi B, Bhatia R. 2005. Association of DDT and DDE with birth weight and length of gestation in the Child Health and Development Studies, 1959-1967. Am J Epidemiol 162(8): 717-725.

Fei C, McLaughlin JK, Tarone RE, Olsen J. 2007. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Persp 115(11): 1677-1682.

Fenster L, Eskenazi B, Anderson M, Bradman A, Harley K, Hernandez H, et al. 2006. Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect 114(4): 597-602.

Ferguson KK, Loch-Caruso R, Meeker JD. 2011a. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999-2006. Environmental research 111(5): 718-726.

Ferguson KK, Loch-Caruso R, Meeker JD. 2011b. Exploration of oxidative stress and inflammatory markers in relation to urinary phthalate metabolites: NHANES 1999-2006. Environ Sci Technol 46(1): 477-485.

Ferguson KK, Loch-Caruso R, Meeker JD. 2012. Exploration of oxidative stress and inflammatory markers in relation to urinary phthalate metabolites: NHANES 1999-2006. Environ Sci Technol 46(1): 477-485.

Ferguson KK, McElrath TF, Meeker JD. 2013a. Environmental Phthalate Exposure and Preterm Birth. JAMA Pediatr.

Ferguson KK, McElrath TF, Meeker JD. 2014. Environmental phthalate exposure and preterm birth. JAMA Pediatr 168(1): 61-67.

Ferguson KK, O'Neill MS, Meeker JD. 2013b. Environmental contaminant exposures and preterm birth: a comprehensive review. J Toxicol Environ Health B Crit Rev 16(2): 69-113.

Fisher JS. 2004. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. Reproduction 127(3): 305-315.

Forand SP, Lewis-Michl EL, Gomez MI. 2011. Maternal exposure to tetrachloroethylene and trichloroethylene through soil vapor intrusion and adverse birth outcomes in New York State. Environ Health Persp 120: 616-621.

Foster PM, Cattley RC, Mylchreest E. 2000. Effects of di-n-butyl phthalate (DBP) on male reproductive development in the rat: implications for human risk assessment. Food Chem Toxicol 38(1 Suppl): S97-99.

Frederiksen H, Kranich SK, Jorgensen N, Taboureau O, Petersen JH, Andersson AM. 2013. Temporal variability in urinary phthalate metabolite excretion based on spot, morning, and 24-h urine samples: considerations for epidemiological studies. Environ Sci Technol 47(2): 958-967.

Gallagher MD, Nuckols JR, Stallones L, Savitz DA. 1998. Exposure to trihalomethanes and adverse pregnancy outcomes. Epidemiology 9(5): 484-489.

Gaspari L, Paris F, Jandel C, Kalfa N, Orsini M, Daures JP, et al. 2011. Prenatal environmental risk factors for genital malformations in a population of 1442 French male newborns: a nested case-control study. Human reproduction 26(11): 3155-3162.

Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D. 2004. Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. Epidemiology 15(1): 36-45.

Glynn A, Larsdotter M, Aune M, Darnerud PO, Bjerselius R, Bergman A. 2011. Changes in serum concentrations of polychlorinated biphenyls (PCBs), hydroxylated PCB metabolites and pentachlorophenol during pregnancy. Chemosphere 83(2): 144-151.

Goldenberg RL, Culhane JF, Iams JD, Romero R. 2008. Epidemiology and causes of preterm birth. Lancet 371(9606): 75-84.

Govarts E, Nieuwenhuijsen M, Schoeters G, Ballester F, Bloemen K, de Boer M, et al. 2012. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ Health Perspect 120(2): 162-170.

Gray LE, Jr., Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L. 2000. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicological sciences : an official journal of the Society of Toxicology 58(2): 350-365.

Grellier J, Bennett J, Patelarou E, Smith RB, Toledano MB, Rushton L, et al. 2010. Exposure to disinfection by-products, fetal growth, and prematurity: a systematic review and meta-analysis. Epidemiology 21(3): 300-313.

Guellec I, Lapillonne A, Renolleau S, Charlaluk ML, Roze JC, Marret S, et al. 2011. Neurologic outcomes at school age in very preterm infants born with severe or mild growth restriction. Pediatrics 127(4): e883-891.

Guo Y, Huo X, Wu K, Liu J, Zhang Y, Xu X. 2012. Carcinogenic polycyclic aromatic hydrocarbons in umbilical cord blood of human neonates from Guiyu, China. Sci Total Environ 427-428: 35-40.

Hamel A, Mergler D, Takser L, Simoneau L, Lafond J. 2003. Effects of low concentrations of organochlorine compounds in women on calcium transfer in human placental syncytiotrophoblast. Toxicol Sci 76(1): 182-189.

Hamm MP, Cherry NM, Chan E, Martin JW, Burstyn I. 2010. Maternal exposure to perfluorinated acids and fetal growth. J Expo Sci Environ Epidemiol 20(7): 589-597.

Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. 1995. Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. Cancer Epidemiol Biomarkers Prev 4(6): 649-654.

Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, et al. 2008. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. Environmental health : a global access science source 7: 27.

Hauser R. 2008. Urinary phthalate metabolites and semen quality: a review of a potential biomarker of susceptibility. International journal of andrology 31(2): 112-117.

Hauser R, Calafat AM. 2005. Phthalates and human health. Occup Environ Med 62(11): 806-818.

Hauser R, Meeker JD, Duty S, Silva MJ, Calafat AM. 2006. Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiology 17(6): 682-691.

Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. 2004. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environmental health perspectives 112(17): 1734-1740.

Hauser R, Meeker JD, Singh NP, Silva MJ, Ryan L, Duty S, et al. 2007. DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. Human reproduction 22(3): 688-695.

Heazell AE, Crocker IP. 2008. Live and let die - regulation of villous trophoblast apoptosis in normal and abnormal pregnancies. Placenta 29(9): 772-783.

Heazell AE, Moll SJ, Jones CJ, Baker PN, Crocker IP. 2007. Formation of syncytial knots is increased by hyperoxia, hypoxia and reactive oxygen species. Placenta 28 Suppl A: S33-40.

Hinckley AF, Bachand AM, Nuckols JR, Reif JS. 2005. Identifying public water facilities with low spatial variability of disinfection by-products for epidemiological investigations. Occup Environ Med 62(7): 494-499.

Hirota Y, Daikoku T, Tranguch S, Xie H, Bradshaw HB, Dey SK. 2010. Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. J Clin Invest 120(3): 803-815.

Hoffman CS, Mendola P, Savitz DA, Herring AH, Loomis D, Hartmann KE, et al. 2008. Drinking water disinfection by-product exposure and duration of gestation. Epidemiology 19(5): 738-746.

Hong YC, Park EY, Park MS, Ko JA, Oh SY, Kim H, et al. 2009. Community level exposure to chemicals and oxidative stress in adult population. Toxicology letters 184(2): 139-144.

Hoppin JA, Ulmer R, London SJ. 2004. Phthalate exposure and pulmonary function. Environmental health perspectives 112(5): 571-574.

Hornung RW, Reed L. 1990. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 5: 46-51.

Hornung RW, Reed LD. 1990. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 5(1): 46-51.

Horton BJ, Luben TJ, Herring AH, Savitz DA, Singer PC, Weinberg HS, et al. 2011. The effect of water disinfection by-products on pregnancy outcomes in two southeastern US communities. J Occup Environ Med 53(10): 1172-1178.

Hsieh TT, Chen SF, Lo LM, Li MJ, Yeh YL, Hung TH. 2012. The association between maternal oxidative stress at mid-gestation and subsequent pregnancy complications. Reprod Sci 19(5): 505-512.

Huang PC, Kuo PL, Chou YY, Lin SJ, Lee CC. 2009. Association between prenatal exposure to phthalates and the health of newborns. Environment international 35(1): 14-20.

Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. 2007. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum Reprod 22(10): 2715-2722.

Huang PC, Tsai EM, Li WF, Liao PC, Chung MC, Wang YH, et al. 2010. Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. Human reproduction 25(4): 986-994.

Hung TH, Lo LM, Chiu TH, Li MJ, Yeh YL, Chen SF, et al. 2010. A longitudinal study of oxidative stress and antioxidant status in women with uncomplicated pregnancies throughout gestation. Reprod Sci 17(4): 401-409.

Hurst CH, Waxman DJ. 2003. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. Toxicol Sci 74(2): 297-308.

Il'yasova D, Scarbrough P, Spasojevic I. 2012. Urinary biomarkers of oxidative status. Clin Chim Acta 413(19-20): 1446-1453.

Irvin EA, Calafat AM, Silva MJ, Aguilar-Villalobos M, Needham LL, Hall DB, et al. 2010. An estimate of phthalate exposure among pregnant women living in Trujillo, Peru. Chemosphere 80(11): 1301-1307.

Jaakkola JJ, Ieromnimon A, Jaakkola MS. 2006. Interior surface materials and asthma in adults: a population-based incident case-control study. American journal of epidemiology 164(8): 742-749.

Jaakkola JJ, Magnus P, Skrondal A, Hwang BF, Becher G, Dybing E. 2001. Foetal growth and duration of gestation relative to water chlorination. Occup Environ Med 58(7): 437-442.

Jelliffe-Pawlowski LL, Miles SQ, Courtney JG, Materna B, Charlton V. 2006. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. J Perinatol 26(3): 154-162.

Jensen MS, Norgaard-Pedersen B, Toft G, Hougaard DM, Bonde JP, Cohen A, et al. 2012. Phthalates and perfluorooctanesulfonic acid in human amniotic fluid: temporal trends and timing of amniocentesis in pregnancy. Environ Health Perspect 120(6): 897-903.

Jepsen KF, Abildtrup A, Larsen ST. 2004. Monophthalates promote IL-6 and IL-8 production in the human epithelial cell line A549. Toxicol In Vitro 18(3): 265-269.

Ji K, Lim Kho Y, Park Y, Choi K. 2010. Influence of a five-day vegetarian diet on urinary levels of antibiotics and phthalate metabolites: a pilot study with "Temple Stay" participants. Environmental research 110(4): 375-382.

Jiang Y, Scott AJ, Wild CJ. 2006. Secondary analysis of case-control data. Stat Med 25(8): 1323-1339.

Joshi SR, Mehendale SS, Dangat KD, Kilari AS, Yadav HR, Taralekar VS. 2008. High maternal plasma antioxidant concentrations associated with preterm delivery. Annals of nutrition & metabolism 53(3-4): 276-282.

Kadiiska MB, Basu S, Brot N, Cooper C, Saari Csallany A, Davies MJ, et al. 2013. Biomarkers of oxidative stress study V: Ozone exposure of rats and its effect on lipids, proteins, and DNA in plasma and urine. Free Radic Biol Med 61C: 408-415.

Kaga N, Katsuki Y, Obata M, Shibutani Y. 1996. Repeated administration of low-dose lipopolysaccharide induces preterm delivery in mice: a model for human preterm parturition and for assessment of the therapeutic ability of drugs against preterm delivery. Am J Obstet Gynecol 174(2): 754-759.

Kallen BA, Robert E. 2000. Drinking water chlorination and delivery outcome-a registry-based study in Sweden. Reprod Toxicol 14(4): 303-309.

Kambia N, Dine T, Gressier B, Frimat B, Cazin JL, Luyckx M, et al. 2011. Correlation between exposure to phthalates and concentrations of malondialdehyde in infants and children undergoing cyclic parenteral nutrition. JPEN Journal of parenteral and enteral nutrition 35(3): 395-401.

Kanitz S, Franco Y, Patrone V, Caltabellotta M, Raffo E, Riggi C, et al. 1996. Association between drinking water disinfection and somatic parameters at birth. Environ Health Persp 104(5): 516-520.

Kasahara E, Sato EF, Miyoshi M, Konaka R, Hiramoto K, Sasaki J, et al. 2002. Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl)phthalate. Biochem J 365(Pt 3): 849-856.

Kay VR, Chambers C, Foster WG. 2013. Reproductive and developmental effects of phthalate diesters in females. Crit Rev Toxicol 43(3): 200-219.

Kim BN, Cho SC, Kim Y, Shin MS, Yoo HJ, Kim JW, et al. 2009. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol Psychiatry 66(10): 958-963.

Kim H, Haltmeier P, Klotz JB, Weisel CP. 1999. Evaluation of biomarkers of environmental exposures: urinary haloacetic acids associated with ingestion of chlorinated drinking water. Environ Res 80(2 Pt 1): 187-195.

Kim JH, Park HY, Bae S, Lim YH, Hong YC. 2013. Diethylhexyl phthalates is associated with insulin resistance via oxidative stress in the elderly: a panel study. PLoS One 8(8): e71392.

Kimber I, Dearman RJ. 2010. An assessment of the ability of phthalates to influence immune and allergic responses. Toxicology 271(3): 73-82.

Klebanoff MA, Shiono PH. 1995. Top down, bottom up and inside out: Reflections on preterm birth. Paediatr Perinat Epidemiol 9(2): 125-129.

Kochanek KD, Kirmeyer SE, Martin JA, Strobino DM, Guyer B. 2012. Annual summary of vital statistics: 2009. Pediatrics 129(2): 338-348.

Kolarik B, Naydenov K, Larsson M, Bornehag CG, Sundell J. 2008. The association between phthalates in dust and allergic diseases among Bulgarian children. Environmental health perspectives 116(1): 98-103.

Kramer MD, Lynch CF, Isacson P, Hanson JW. 1992. The association of waterborne chloroform with intrauterine growth retardation. Epidemiology 3(5): 407-413.

Kwiatkowski S, Torbe A, Dolegowska B, Blogowski W, Czajka R, Chlubek D, et al. 2009. Isoprostanes 8-iPF2alpha-III: risk markers of premature rupture of fetal membranes? Biomarkers 14(6): 406-413.

Landgren O. 1996. Environmental pollution and delivery outcome in southern Sweden: a study with central registries. Acta Paediatr 85(11): 1361-1364.

Lapinskas PJ, Brown S, Leesnitzer LM, Blanchard S, Swanson C, Cattley RC, et al. 2005. Role of PPARalpha in mediating the effects of phthalates and metabolites in the liver. Toxicology 207(1): 149-163.

Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. 2003. In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. Environ Health Perspect 111(14): 1783-1785.

Latini G, Del Vecchio A, Massaro M, Verrotti A, De Felice C. 2006. In utero exposure to phthalates and fetal development. Curr Med Chem 13(21): 2527-2534.

Le TN, Johansson A. 2001. Impact of chemical warfare with agent orange on women's reproductive lives in Vietnam: a pilot study. Reprod Health Matters 9(18): 156-164.

Lewis C, Suffet IH, Hoggatt K, Ritz B. 2007. Estimated effects of disinfection by-products on preterm birth in a population served by a single water utility. Environ Health Persp 115(2): 290-295.

Lewis RC, Meeker JD, Peterson KE, Lee JM, Pace GG, Cantoral A, et al. 2013. Predictors of urinary bisphenol A and phthalate metabolite concentrations in Mexican children. Chemosphere.

Lin CM, Li CY, Mao IF. 2006. Birth outcomes of infants born in areas with elevated ambient exposure to incinerator generated PCDD/Fs. Environ Int 32(5): 624-629.

Lin S, Ku HY, Su PH, Chen JW, Huang PC, Angerer J, et al. 2011. Phthalate exposure in pregnant women and their children in central Taiwan. Chemosphere 82(7): 947-955.

Lind PM, Lind L. 2011. Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly. Atherosclerosis 218(1): 207-213.

Llop S, Ballester F, Estarlich M, Esplugues A, Rebagliato M, Iniguez C. 2010. Preterm birth and exposure to air pollutants during pregnancy. Environ Res 110(8): 778-785.

Longini M, Perrone S, Vezzosi P, Marzocchi B, Kenanidis A, Centini G, et al. 2007. Association between oxidative stress in pregnancy and preterm premature rupture of membranes. Clin Biochem 40(11): 793-797.

Longnecker MP, Klebanoff MA, Brock JW, Guo X. 2005. Maternal levels of polychlorinated biphenyls in relation to preterm and small-for-gestational-age birth. Epidemiology 16(5): 641-647.

Longnecker MP, Klebanoff MA, Zhou H, Brock JW. 2001. Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet 358(9276): 110-114.

Lopez-Carrillo L, Hernandez-Ramirez RU, Calafat AM, Torres-Sanchez L, Galvan-Portillo M, Needham LL, et al. 2010. Exposure to phthalates and breast cancer risk in northern Mexico. Environ Health Perspect 118(4): 539-544.

Lorber M, Koch HM, Angerer J. 2011. A critical evaluation of the creatinine correction approach: can it underestimate intakes of phthalates? A case study with di-2-ethylhexyl phthalate. Journal of exposure science & environmental epidemiology 21(6): 576-586.

MacKinnon DP, Dwyer JH. 1993. Estimating mediated effects in prevention studies. Evaluation Review 17:144-158.

Makri A, Goveia M, Balbus J, Parkin R. 2004. Children's susceptibility to chemicals: a review by developmental stage. J Toxicol Environ Health B 7(6): 417-435.

Maloney EK, Waxman DJ. 1999. trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. Toxicol Appl Pharmacol 161(2): 209-218.

Maroziene L, Grazuleviciene R. 2002. Maternal exposure to low-level air pollution and pregnancy outcomes: a population-based study. Environ Health 1(1): 6.

Martin JA, Centers for Disease C, Prevention. 2011. Preterm births - United States, 2007. MMWR Surveill Summ 60 Suppl: 78-79.

Martina CA, Weiss B, Swan SH. 2012. Lifestyle behaviors associated with exposures to endocrine disruptors. Neurotoxicology 33(6): 1427-1433.

McElrath TF, Hecht JL, Dammann O, Boggess K, Onderdonk A, Markenson G, et al. 2008. Pregnancy disorders that lead to delivery before the 28th week of gestation: an epidemiologic approach to classification. Am J Epidemiol 168(9): 980-989.

Meeker JD. 2012. Exposure to environmental endocrine disruptors and child development. Arch Pediatr Adolesc Med 166(10): 952-958.

Meeker JD, Calafat AM, Hauser R. 2007. Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. Environmental health perspectives 115(7): 1029-1034.

Meeker JD, Calafat AM, Hauser R. 2009a. Urinary metabolites of di(2-ethylhexyl) phthalate are associated with decreased steroid hormone levels in adult men. J Androl 30(3): 287-297.

Meeker JD, Calafat AM, Hauser R. 2012. Urinary phthalate metabolites and their biotransformation products: predictors and temporal variability among men and women. Journal of exposure science & environmental epidemiology 22(4): 376-385.

Meeker JD, Ferguson KK. 2011. Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007-2008. Environmental health perspectives 119(10): 1396-1402.

Meeker JD, Hu H, Cantonwine DE, Lamadrid-Figueroa H, Calafat AM, Ettinger AS, et al. 2009b. Urinary phthalate metabolites in relation to preterm birth in Mexico city. Environ Health Persp 117(10): 1587-1592.

Mehendale S, Kilari A, Dangat K, Taralekar V, Mahadik S, Joshi S. 2008. Fatty acids, antioxidants, and oxidative stress in pre-eclampsia. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 100(3): 234-238.

Mendiola J, Meeker JD, Jorgensen N, Andersson AM, Liu F, Calafat AM, et al. 2012. Urinary concentrations of di(2-ethylhexyl) phthalate metabolites and serum reproductive hormones: pooled analysis of fertile and infertile men. J Androl 33(3): 488-498.

Mieritz MG, Frederiksen H, Sorensen K, Aksglaede L, Mouritsen A, Hagen CP, et al. 2012. Urinary phthalate excretion in 555 healthy Danish boys with and without pubertal gynaecomastia. International journal of andrology 35(3): 227-235.

Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ, 2nd. 1990. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci U S A 87(23): 9383-9387.

Mouritsen A, Frederiksen H, Sorensen K, Aksglaede L, Hagen C, Skakkebaek NE, et al. 2013. Urinary phthalates from 168 girls and boys measured twice a year during a 5-year

period: associations with adrenal androgen levels and puberty. J Clin Endocrinol Metab 98(9): 3755-3764.

Mukherjee SC, Saha KC, Pati S, Dutta RN, Rahman MM, Sengupta MK, et al. 2005. Murshidabad--one of the nine groundwater arsenic-affected districts of West Bengal, India. Part II: dermatological, neurological, and obstetric findings. Clin Toxicol (Phila) 43(7): 835-848.

Myers SL, Lobdell DT, Liu Z, Xia Y, Ren H, Li Y, et al. 2010. Maternal drinking water arsenic exposure and perinatal outcomes in inner Mongolia, China. J Epidemiol Community Health 64(4): 325-329.

Negi R, Pande D, Kumar A, Khanna RS, Khanna HD. 2012. In vivo oxidative DNA damage and lipid peroxidation as a biomarker of oxidative stress in preterm low-birthweight infants. J Trop Pediatr 58(4): 326-328.

NIEHS. 2012. Biomarkers of Oxidative Stress Study. Available: http://www.niehs.nih.gov/research/resources/databases/bosstudy/.

Nishijo M, Nakagawa H, Honda R, Tanebe K, Saito S, Teranishi H, et al. 2002. Effects of maternal exposure to cadmium on pregnancy outcome and breast milk. Occup Environ Med 59(6): 394-396; discussion 397.

Nishioka J, Iwahara C, Kawasaki M, Yoshizaki F, Nakayama H, Takamori K, et al. 2012. Di-(2-ethylhexyl) phthalate induces production of inflammatory molecules in human macrophages. Inflamm Res 61(1): 69-78.

Nolan LA, Nolan JM, Shofer FS, Rodway NV, Emmett EA. 2009. The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reprod Toxicol 27(3-4): 231-238.

O'Brien ML, Cunningham ML, Spear BT, Glauert HP. 2001. Effects of peroxisome proliferators on glutathione and glutathione-related enzymes in rats and hamsters. Toxicol Appl Pharmacol 171(1): 27-37.

Ochoa-Acuña H, Frankenberger J, Hahn L, Carbajo C. 2009. Drinking-water herbicide exposure in Indiana and prevalence of small-for-gestational-age and preterm delivery. Environ Health Persp 117(10): 1619-1624.

Okamura K, Doi T, Hamada K, Sakurai M, Yoshioka Y, Mitsuzono R, et al. 1997. Effect of repeated exercise on urinary 8-hydroxy-deoxyguanosine excretion in humans. Free Radic Res 26(6): 507-514.

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. 2007. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Persp 115(9): 1298-1305.

Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, et al. 2000. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal

testosterone synthesis during sexual differentiation in the male rat. Toxicological sciences : an official journal of the Society of Toxicology 58(2): 339-349.

Parlett LE, Calafat AM, Swan SH. 2013. Women's exposure to phthalates in relation to use of personal care products. Journal of exposure science & environmental epidemiology 23(2): 197-206.

Patelarou E, Kargaki S, Stephanou EG, Nieuwenhuijsen M, Sourtzi P, Gracia E, et al. 2011. Exposure to brominated trihalomethanes in drinking water and reproductive outcomes. Occup Environ Med 68(6): 438-445.

Pathak R, Ahmed RS, Tripathi AK, Guleria K, Sharma CS, Makhijani SD, et al. 2009. Maternal and cord blood levels of organochlorine pesticides: association with preterm labor. Clin Biochem 42(7-8): 746-749.

Pathak R, Suke SG, Ahmed T, Ahmed RS, Tripathi AK, Guleria K, et al. 2010. Organochlorine pesticide residue levels and oxidative stress in preterm delivery cases. Human & experimental toxicology 29(5): 351-358.

Peter Stein T, Scholl TO, Schluter MD, Leskiw MJ, Chen X, Spur BW, et al. 2008. Oxidative stress early in pregnancy and pregnancy outcome. Free radical research 42(10): 841-848.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, et al. 2012. Exposure to Phthalates and Phenols during Pregnancy and Offspring Size at Birth. Environmental health perspectives 120(3): 464-470.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team. 2013. nlme: Linear and Nonlinear Mixed Effects Models. R package version 31-111.

Pirkle JL, Brody DJ, Gunter EW, Kramer RA, Paschal DC, Flegal KM, et al. 1994. The decline in blood lead levels in the United States. The National Health and Nutrition Examination Surveys (NHANES). J Am Med Assoc 272(4): 284-291.

Potdar N, Singh R, Mistry V, Evans MD, Farmer PB, Konje JC, et al. 2009. First-trimester increase in oxidative stress and risk of small-for-gestational-age fetus. BJOG 116(5): 637-642.

Preau JL, Jr., Wong LY, Silva MJ, Needham LL, Calafat AM. 2010. Variability over 1 week in the urinary concentrations of metabolites of diethyl phthalate and di(2-ethylhexyl) phthalate among eight adults: an observational study. Environmental health perspectives 118(12): 1748-1754.

Procianoy RS, Schvartsman S. 1981. Blood pesticide concentration in mothers and their newborn infants. Relation to prematurity. Acta Paediatr Scand 70(6): 925-928.

Reddy JK, Rao MS. 1989. Oxidative DNA damage caused by persistent peroxisome proliferation: its role in hepatocarcinogenesis. Mutat Res 214(1): 63-68.

Revich B, Aksel E, Ushakova T, Ivanova I, Zhuchenko N, Klyuev N, et al. 2001. Dioxin exposure and public health in Chapaevsk, Russia. Chemosphere 43(4-7): 951-966.

Ribas-Fitó N, Sala M, Cardo E, Mazon C, De Muga ME, Verdu A, et al. 2002. Association of hexachlorobenzene and other organochlorine compounds with anthropometric measures at birth. Pediatr Res 52(2): 163-167.

Rinsky JL, Hopenhayn C, Golla V, Browning S, Bush HM. 2012. Atrazine exposure in public drinking water and preterm birth. Public Health Rep 127(1): 72-80.

Rivera-Nunez Z, Meliker JR, Linder AM, Nriagu JO. 2010. Reliability of spot urine samples in assessing arsenic exposure. Int J Hyg Environ Health 213(4): 259-264.

Roberts LJ, Morrow JD. 2000. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. Free Radic Biol Med 28(4): 505-513.

Romero-Franco M, Hernandez-Ramirez RU, Calafat AM, Cebrian ME, Needham LL, Teitelbaum S, et al. 2011. Personal care product use and urinary levels of phthalate metabolites in Mexican women. Environ Int 37(5): 867-871.

Rosado-Berrios CA, Velez C, Zayas B. 2011. Mitochondrial permeability and toxicity of diethylhexyl and monoethylhexyl phthalates on TK6 human lymphoblasts cells. Toxicol In Vitro 25(8): 2010-2016.

Rosner B. 2011. Fundamentals of biostatistics. 7th edition ed. Boston, MA: Brooks/Cole.

Rudel RA, Gray JM, Engel CL, Rawsthorne TW, Dodson RE, Ackerman JM, et al. 2011. Food packaging and bisphenol A and bis(2-ethyhexyl) phthalate exposure: findings from a dietary intervention. Environ Health Perspect 119(7): 914-920.

Rusyn I, Kadiiska MB, Dikalova A, Kono H, Yin M, Tsuchiya K, et al. 2001. Phthalates rapidly increase production of reactive oxygen species in vivo: role of Kupffer cells. Mol Pharmacol 59(4): 744-750.

Salmasi G, Grady R, Jones J, McDonald SD. 2010. Environmental tobacco smoke exposure and perinatal outcomes: a systematic review and meta-analyses. Acta Obstet Gynecol Scand 89(4): 423-441.

Sanchez BN, Hu H, Litman HJ, Tellez-Rojo MM. 2011. Statistical methods to study timing of vulnerability with sparsely sampled data on environmental toxicants. Environ Health Perspect 119(3): 409-415.

Sathyanarayana S, Alcedo G, Saelens BE, Zhou C, Dills RL, Yu J, et al. 2013. Unexpected results in a randomized dietary trial to reduce phthalate and bisphenol A exposures. J Expo Sci Environ Epidemiol 23(4): 378-384.

Sathyanarayana S, Basso O, Karr CJ, Lozano P, Alavanja M, Sandler DP, et al. 2010. Maternal pesticide use and birth weight in the agricultural health study. J Agromedicine 15(2): 127-136.

Savitz DA. 2008. Invited commentary: Disaggregating preterm birth to determine etiology. Am J Epidemiol 168(9): 990-992; discussion 993-994.

Savitz DA, Andrews KW, Pastore LM. 1995. Drinking water and pregnancy outcome in central North Carolina: Source, amount, and trihalomethane levels. Environ Health Persp 103(6): 592-596.

Savitz DA, Blackmore CA, Thorp JM. 1991. Epidemiologic characteristics of preterm delivery: Etiologic heterogeneity. Am J Obstet Gynecol 164(2): 467-471.

Savitz DA, Stein CR, Bartell SM, Elston B, Gong J, Shin HM, et al. 2012a. Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community. Epidemiology 23(3): 386-392.

Savitz DA, Stein CR, Elston B, Wellenius GA, Bartell SM, Shin HM, et al. 2012b. Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the Mid-Ohio Valley. Environ Health Persp 120: 1201-1207.

Saxena MC, Siddiqui MK, Seth TD, Krishna Murti CR, Bhargava AK, Kutty D. 1981. Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature of full-term delivery. J Anal Toxicol 5(1): 6-9.

Schisterman EF, Whitcomb BW, Louis GM, Louis TA. 2005. Lipid adjustment in the analysis of environmental contaminants and human health risks. Environ Health Persp 113(7): 853-857.

Scholl TO. 2005. Iron status during pregnancy: setting the stage for mother and infant. Am J Clin Nutr 81(5): 1218S-1222S.

Scholl TO, Stein TP. 2001. Oxidant damage to DNA and pregnancy outcome. J Matern Fetal Med 10(3): 182-185.

Schwartz J. 2001. Is there harvesting in the association of airborne particles with daily deaths and hospital admissions? Epidemiology 12(1): 55-61.

Serrano M, Blasco MA. 2001. Putting the stress on senescence. Curr Opin Cell Biol 13(6): 748-753.

Shah PS, Balkhair T. 2011. Air pollution and birth outcomes: a systematic review. Environ Int 37(2): 498-516.

Shapiro-Mendoza CK. 2014. Commentary: Mediation and moderation analyses: a novel approach to exploring the complex pathways between maternal medical conditions, preterm birth and associated newborn morbidity risk. Int J Epidemiol.

Shoji H, Franke C, Campoy C, Rivero M, Demmelmair H, Koletzko B. 2006. Effect of docosahexaenoic acid and eicosapentaenoic acid supplementation on oxidative stress levels during pregnancy. Free Radic Res 40(4): 379-384.

Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, et al. 2004. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Environ Health Persp 112(3): 331-338.

Silva MJ, Samandar E, Preau JL, Jr., Reidy JA, Needham LL, Calafat AM. 2007. Quantification of 22 phthalate metabolites in human urine. J Chromatogr B Analyt Technol Biomed Life Sci 860(1): 106-112.

Singh VK, Singh J, Anand M, Kumar P, Patel DK, Krishna Reddy MM, et al. 2008. Comparison of polycyclic aromatic hydrocarbon levels in placental tissues of Indian women with full- and preterm deliveries. Int J Hyg Environ Health 211(5-6): 639-647.

Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. 2008. Meeting report: Atmospheric pollution and human reproduction. Environ Health Persp 116(6): 791-798.

Smith KA, Shepherd J, Wakil A, Kilpatrick ES. 2011. A comparison of methods for the measurement of 8-isoPGF(2alpha): a marker of oxidative stress. Ann Clin Biochem 48(Pt 2): 147-154.

Snijder CA, Roeleveld N, Te Velde E, Steegers EA, Raat H, Hofman A, et al. 2012. Occupational exposure to chemicals and fetal growth: the Generation R Study. Human reproduction 27(3): 910-920.

Sonnenfeld N, Hertz-Picciotto I, Kaye WE. 2001. Tetrachloroethylene in drinking water and birth outcomes at the US Marine Corps Base at Camp Lejeune, North Carolina. Am J Epidemiol 154(10): 902-908.

Sowers M, Jannausch M, Scholl T, Li W, Kemp FW, Bogden JD. 2002. Blood lead concentrations and pregnancy outcomes. Arch Environ Health 57(5): 489-495.

Sram RJ, Binkova B, Dejmek J, Bobak M. 2005. Ambient air pollution and pregnancy outcomes: a review of the literature. Environ Health Perspect 113(4): 375-382.

Stahlhut RW, van Wijngaarden E, Dye TD, Cook S, Swan SH. 2007. Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult U.S. males. Environmental health perspectives 115(6): 876-882.

Stein CR, Savitz DA, Dougan M. 2009. Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. Am J Epidemiol 170(7): 837-846.

Stillerman KP, Mattison DR, Giudice LC, Woodruff TJ. 2008. Environmental exposures and adverse pregnancy outcomes: a review of the science. Reprod Sci 15(7): 631-650.

Sugiyama S, Shimada N, Miyoshi H, Yamauchi K. 2005. Detection of thyroid system-disrupting chemicals using in vitro and in vivo screening assays in Xenopus laevis. Toxicological sciences : an official journal of the Society of Toxicology 88(2): 367-374.

Suzuki Y, Niwa M, Yoshinaga J, Mizumoto Y, Serizawa S, Shiraishi H. 2010. Prenatal exposure to phthalate esters and PAHs and birth outcomes. Environ Int 36(7): 699-704.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environmental health perspectives 113(8): 1056-1061.

Tetz LM, Cheng AA, Korte CS, Giese RW, Wang P, Harris C, et al. 2013. Mono-2-ethylhexyl phthalate induces oxidative stress responses in human placental cells in vitro. Toxicol Appl Pharmacol 268(1): 47-54.

Torres-Arreola L, Berkowitz G, Torres-Sanchez L, Lopez-Cervantes M, Cebrian ME, Uribe M, et al. 2003. Preterm birth in relation to maternal organochlorine serum levels. Ann Epidemiol 13(3): 158-162.

Torres-Sánchez LE, Berkowitz G, Lopez-Carrillo L, Torres-Arreola L, Rios C, Lopez-Cervantes M. 1999. Intrauterine lead exposure and preterm birth. Environ Res 81(4): 297-301.

Tuthill GF, Schwalm WA. 1992. Biased interacting self-avoiding walks on the four-simplex lattice. Phys Rev B Condens Matter 46(21): 13722-13734.

Upson K, Sathyanarayana S, De Roos AJ, Thompson ML, Scholes D, Dills R, et al. 2013. Phthalates and risk of endometriosis. Environmental research 126: 91-97.

Vassilev ZP, Robson MG, Klotz JB. 2001. Outdoor exposure to airborne polycyclic organic matter and adverse reproductive outcomes: a pilot study. Am J Ind Med 40(3): 255-262.

Vetrano AM, Laskin DL, Archer F, Syed K, Gray JP, Laskin JD, et al. 2010. Inflammatory effects of phthalates in neonatal neutrophils. Pediatr Res 68(2): 134-139.

Vigeh M, Yokoyama K, Seyedaghamiri Z, Shinohara A, Matsukawa T, Chiba M, et al. 2011. Blood lead at currently acceptable levels may cause preterm labour. Occup Environ Med 68(3): 231-234.

Villanueva CM, Durand G, Coutte MB, Chevrier C, Cordier S. 2005. Atrazine in municipal drinking water and risk of low birth weight, preterm delivery, and small-for-gestational-age status. Occup Environ Med 62(6): 400-405.

Villanueva CM, Gracia-Lavedan E, Ibarluzea J, Santa Marina L, Ballester F, Llop S, et al. 2011. Exposure to trihalomethanes through different water uses and birth weight, small for gestational age, and preterm delivery in Spain. Environ Health Persp 119(12): 1824-1830.

Vrijheid M, Casas M, Bergstrom A, Carmichael A, Cordier S, Eggesbo M, et al. 2012. European birth cohorts for environmental health research. Environ Health Persp 120(1): 29-37.

Wassermann M, Ron M, Bercovici B, Wassermann D, Cucos S, Pines A. 1982. Premature delivery and organochlorine compounds: polychlorinated biphenyls and some organochlorine insecticides. Environ Res 28(1): 106-112.

Weber D, Stuetz W, Bernhard W, Franz A, Raith M, Grune T, et al. 2013. Oxidative stress markers and micronutrients in maternal and cord blood in relation to neonatal outcome. Eur J Clin Nutr.

Wei SQ, Fraser W, Luo ZC. 2010. Inflammatory cytokines and spontaneous preterm birth in asymptomatic women: a systematic review. Obstet Gynecol 116(2 Pt 1): 393-401.

Weissgerber TL, Wolfe LA, Davies GA, Mottola MF. 2006. Exercise in the prevention and treatment of maternal-fetal disease: a review of the literature. Appl Physiol Nutr Metab 31(6): 661-674.

Weuve J, Hauser R, Calafat AM, Missmer SA, Wise LA. 2010. Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999-2004. Environmental health perspectives 118(6): 825-832.

Whitworth KW, Haug LS, Baird DD, Becher G, Hoppin JA, Skjaerven R, et al. 2012. Perfluorinated compounds in relation to birth weight in the Norwegian mother and child cohort study. Am J Epidemiol 175: 1209-1216.

Whyatt RM, Adibi JJ, Calafat AM, Camann DE, Rauh V, Bhat HK, et al. 2009. Prenatal di(2-ethylhexyl)phthalate exposure and length of gestation among an inner-city cohort. Pediatrics 124(6): e1213-1220.

Wigle DT, Arbuckle TE, Turner MC, Berube A, Yang Q, Liu S, et al. 2008. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J Toxicol Environ Health B 11(5-6): 373-517.

Wilhelm M, Ghosh JK, Su J, Cockburn M, Jerrett M, Ritz B. 2011. Traffic-related air toxics and preterm birth: a population-based case-control study in Los Angeles County, California. Environ Health 10: 89.

Wittassek M, Angerer J, Kolossa-Gehring M, Schafer SD, Klockenbusch W, Dobler L, et al. 2009. Fetal exposure to phthalates--a pilot study. Int J Hyg Environ Health 212(5): 492-498.

Wojtyniak BJ, Rabczenko D, Jonsson BA, Zvezday V, Pedersen HS, Rylander L, et al. 2010. Association of maternal serum concentrations of 2,2', 4,4'5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE) levels with birth weight, gestational age and preterm births in Inuit and European populations. Environ Health 9: 56.

Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, et al. 2008. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect 116(8): 1092-1097.

Wood SL, Jarrell JJ, Swaby C, Chan S. 2007. Endocrine disruptors and spontaneous premature labor: a case control study. Environ Health 6: 35.

Wood SN. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. Journal of the Royal Statistical Society (B) 73(1): 3-36.

Woodruff TJ, Zota AR, Schwartz JM. 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ Health Perspect 119(6): 878-885.

Woods JR, Jr. 2001. Reactive oxygen species and preterm premature rupture of membranes-a review. Placenta 22 Suppl A: S38-44.

Wright JM, Schwartz J, Dockery DW. 2003. Effect of trihalomethane exposure on fetal development. Occup Environ Med 60(3): 173-180.

Wright JM, Schwartz J, Dockery DW. 2004. The effect of disinfection by-products and mutagenic activity on birth weight and gestational duration. Environ Health Persp 112(8): 920-925.

Wu K, Xu X, Liu J, Guo Y, Li Y, Huo X. 2010. Polybrominated diphenyl ethers in umbilical cord blood and relevant factors in neonates from Guiyu, China. Environ Sci Technol 44(2): 813-819.

Wu K, Xu X, Peng L, Liu J, Guo Y, Huo X. 2012. Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes. Environ Int 48: 1-8.

Wu LL, Chiou CC, Chang PY, Wu JT. 2004. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta 339(1-2): 1-9.

Xue F, Holzman C, Rahbar MH, Trosko K, Fischer L. 2007. Maternal fish consumption, mercury levels, and risk of preterm delivery. Environ Health Perspect 115(1): 42-47.

Yang CY. 2004. Drinking water chlorination and adverse birth outcomes in Taiwan. Toxicology 198(1-3): 249-254.

Yang CY, Chang CC, Tsai SS, Chuang HY, Ho CK, Wu TN. 2003. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. Environ Res 91(1): 29-34.

Yang CY, Cheng BH, Tsai SS, Wu TN, Lin MC, Lin KC. 2000. Association between chlorination of drinking water and adverse pregnancy outcome in Taiwan. Environ Health Persp 108(8): 765-768.

Yang CY, Xiao ZP, Ho SC, Wu TN, Tsai SS. 2007. Association between trihalomethane concentrations in drinking water and adverse pregnancy outcome in Taiwan. Environ Res 104(3): 390-395.

Zeman FA, Boudet C, Tack K, Floch Barneaud A, Brochot C, Pery AR, et al. 2013. Exposure assessment of phthalates in French pregnant women: results of the ELFE pilot study. Int J Hyg Environ Health 216(3): 271-279.

Zhang X, Kramer MS. 2009. Variations in mortality and morbidity by gestational age among infants born at term. J Pediatr 154(3): 358-362, 362 e351.

Zhang YL, Zhao YC, Wang JX, Zhu HD, Liu QF, Fan YG, et al. 2004. Effect of environmental exposure to cadmium on pregnancy outcome and fetal growth: a study on healthy pregnant women in China. J Environ Sci Health A Tox Hazard Subst Environ Eng 39(9): 2507-2515.

Zhao Y, Ao H, Chen L, Sottas CM, Ge RS, Li L, et al. 2012. Mono-(2-ethylhexyl) phthalate affects the steroidogenesis in rat Leydig cells through provoking ROS perturbation. Toxicol In Vitro 26(6): 950-955.

Zhou D, Wang H, Zhang J, Gao X, Zhao W, Zheng Y. 2010. Di-n-butyl phthalate (DBP) exposure induces oxidative damage in testes of adult rats. Syst Biol Reprod Med 56(6): 413-419.

Zhou L, Beattie MC, Lin CY, Liu J, Traore K, Papadopoulos V, et al. 2013. Oxidative stress and phthalate-induced down-regulation of steroidogenesis in MA-10 Leydig cells. Reprod Toxicol 42C: 95-101.

Zhu M, Fitzgerald EF, Gelberg KH, Lin S, Druschel CM. 2010. Maternal low-level lead exposure and fetal growth. Environ Health Persp 118(10): 1471-1475.