Investigations into the Effects of Gestational Exposure to Environmental Phthalates on Maternal and Perinatal Outcomes and the Role of Inflammation Biomarkers as Potential Mediators

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ABSTRACT

Objectives
The aims of this thesis were to (1) investigate the association of gestational exposure to environmental phthalates with maternal and perinatal outcomes, and (2) explore phthalate-induced changes to maternal inflammatory responses as potential mediators of possible health effects.

Methods
A systematic review was performed to summarize existing evidence on the association of gestational exposure to phthalates with obstetrical outcomes, including pre-eclampsia (PE), pregnancy-induced hypertension (PIH), gestational diabetes mellitus (GDM), intrauterine growth restriction (IUGR), birth weight (BW), head circumference (HC), gestational age (GA), preterm birth (PB), and Apgar scores (AS). Additionally, a secondary analysis of data from the MIREC Study was conducted to evaluate the association of phthalate metabolites with clinical outcomes in the mother and infant using multiple linear and logistic regression, and with inflammatory biomarkers using multinomial logistic regression.

Results
The systematic review identified a total of 24 articles, and observed inconsistent evidence on BW, HC, GA, and PB, a paucity of research on IUGR, PE, GDM, and AS, and a lack of studies on PIH. However, among studies with statistically significant ($p<0.05$) results, most suggest an association of phthalates with decreased BW and GA, and increased HC and PB. Findings from the MIREC Study indicate a significant ($p<0.01$) positive association between MBP and HC among female infants; however, null results were identified for BW, GA, PB, AS, and PIH. In relation to the exposure to phthalates, general trends among suggestive associations ($p<0.05$) for head circumference showed consistent increases in females and decreases in males, and for gestational age displayed decreases in both stratums. Additionally, a significant positive association of MBzP and ∑DEHP was observed with high MMP-2 and low VCAM levels, respectively. Results approaching statistical significance demonstrated a positive association of ∑DEHP with low MCP-1 and ICAM levels, MCPP with low GMCSF levels, MBzP with low CRP and high ICAM levels, and MEP with high MMP-7 and IL-2 levels.

Conclusion
From the systematic review, the effects of phthalates on maternal and perinatal health remain unclear, possibly due to sources of heterogeneity and challenges in exposure assessment. In the MIREC Study cohort, phthalate levels were associated with GA and HC in infants in a sex-specific manner. Phthalates also appear to influence the circulating inflammatory marker levels, possibly explaining the observed adverse effects. Future research is needed to validate these findings.

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LIST OF ABBREVIATIONS

AS	Apgar Scores
BBP	Benzyl Butyl Phthalate / Butyl Benzyl Phthalate
BMI	Body Mass Index
BMPP	Bis (4-methyl-2-pentyl) Phthalate
BPA	Bisphenol A
BW	Birth Weight
CHMS	Canadian Health Measures Survey
CRP	C-Reactive Protein
DBEP	Bis (2-n-butoxyethyl) Phthalate
DBP	Dibutyl Phthalate
DCHP	Dicyclohexyl Phthalate
DEEP	Bis (2-ethoxyethyl) Phthalate
DEHP	Di-(2-ethylhexyl) Phthalate
DEP	Diethyl Phthalate
DiBP	Di-isobutyl Phthalate
DiNP	Di-isononyl Phthalate
DMEP	Bis (2-methoxyethyl) Phthalate
DMP	Dimethyl Phthalate
DnBP	Di(n-butyl) Phthalate
DNHP	Dihexyl Phthalate / Di-n-hexyl Phthalate
DNOP	Di-n-octyl Phthalate

DNP	Dinonyl Phthalate
DnPP	Di-n-propyl Phthalate
DPP	Diamyl Phthalate
GA	Gestational Age
GDM	Gestational Diabetes Mellitus
GM	Geometric Mean
G-CSF	Granulocyte-Colony-Stimulating Factor
GMCSF	Granulocyte-Macrophage Colony-Stimulating Factor
HC	Head Circumference
HMW	High Molecular Weight
HOMA	Homeostatic Model Assessment
ICAM	Intracellular Adhesion Molecule
IFN-γ	Interferon Gamma
IgE	Immunoglobulin E
IL-1β	Interleukin-1β
IL-2	Interleukin-2
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-33	Interleukin-33
INSPQ	Institut national de Santé Publique du Québec

IUGR	Intrauterine Growth Restriction
JEM	Job-Exposure Matrix
LBW	Low Birth Weight
LMW	Low Molecular Weight
LOD	Limit of Detection
MBP	Mono-butyl Phthalate
MBzP	Mono-benzyl Phthalate
MCHP	Mono-cyclo-hexyl Phthalate
MCiOP	Mono(4-methyl-7-carboxyheptyl) Phthalate
MCNP	Monocarboxyisononyl Phthalate
MCOP	Monocarboxyisooctyl Phthalate
MCP-1	Monocyte Chemoattractant Protein-1
MCPP	Mono-(3-carboxypropyl) Phthalate
MDP	Mono-3-methyl-7-methyloctyl Phthalate
MECPP	Mono-(2-ethyl-5-carboxypentyl) Phthalate
MEHHP	Mono-(2-ethyl-5-hydroxy-hexyl) Phthalate
MEHP	Mono-(2-ethylhexyl) Phthalate
MEOHP	Mono-(2-ethyl-5-oxo-hexyl) Phthalate
MEP	Mono-ethyl Phthalate
MHiNP	Mono(4-methyl-7-hydroxyloctyl) Phthalate
MiBP	Mono-isobutyl Phthalate
MiDP	Mono-iso-decyl Phthalate
MiNP	Mono-iso-nonyl Phthalate

MIP-1β	Human Macrophage Inflammatory Protein 1-Beta
MIREC	Maternal-Infant Research on Environmental Chemicals
MMP	Mono-methyl Phthalate
MMP-1	Matrix Metalloproteinase-1
MMP-2	Matrix Metalloproteinase-2
MMP-7	Matrix Metalloproteinase-7
MMP-9	Matrix Metalloproteinase-9
MMP-10	Matrix Metalloproteinase-10
MnBP	Mono-n-butyl Phthalate
MnOP	Mono-n-octyl Phthalate
MNP	Mono-3-methyl-5-dimethyl Phthalate
MOiNP	Mono(4-methyl-7-oxo-octyl) Phthalate
ND	Not Detected
NHANES	National Health and Nutrition Examination Survey
OR	Odds Ratio
p	p-value
PB	Preterm Birth
PE	Pre-eclampsia
PIH	Pregnancy-Induced Hypertension / Gestational Hypertension
pPROM	Preterm Premature Rupture of Membranes
PROM	Premature Rupture of Membranes
Q	Quartile
SD	Standard Deviation

SG	Specific Gravity
T	Tertile
TNF-α	Tumor Necrosis Factor Alpha
TSLP	Thymic Stromal Lymphopoietin
VCAM	Vascular Cell Adhesion Molecule
VEGF	Vascular Endothelial Growth Factor
95% CI	95% Confidence Interval

CHAPTER 1: INTRODUCTION

1.1. Sources of Exposure to Phthalates

Phthalates are synthetic chemicals with many industrial and commercial applications [1]. These chemical compounds have been incorporated into a variety of consumer products, as plasticizers, solvents, excipients, and fixatives of colour and scent [2-4]. Phthalates are present in a number of commercial and personal care products, and include plastic food containers, medical devices, pharmaceuticals, dietary supplements, fragrances, haircare products, lotions, baby-care products, and toys [3, 5-8]. These chemical compounds are ubiquitous in the environment because of their common usage. This is demonstrated by the widespread detection of phthalates in dietary sources, such as fruits, vegetables, dairy, and meat, as well as in the environment, including in air, dust, soil, rivers, and lakes [9-14]. The frequent use of phthalates and their omnipresence in the environment presents abundant opportunities for human exposure.

1.2. Routes of Exposure to Phthalates

Several possible modes by which phthalates can enter the human body have been suggested in the literature. The existing evidence demonstrates that dietary sources may be a major contributor of the overall body burden of high molecular weight (HMW) phthalates [15]. A study conducted by Koch et al. (2013) monitored urinary concentrations of phthalate metabolites over a 48-hour fasting period, and reported a substantial decrease in the metabolite levels of HMW phthalates; however, following the intake of food, subsequent increases in concentrations were observed [15]. Inhalation of phthalates is another exposure pathway that has also been investigated.

The study by Koch et al. (2013) also observed fluctuating increases and decreases in the metabolite concentrations of low molecular weight phthalates during the 48-hour fasting period, and suggests that sources including dust and indoor air, may be responsible [15]. A study consisting of workers from three polyvinyl chloride factories reported significant correlation coefficients of 0.71 for mono-2-ethylhexyl phthalate (MEHP), 0.78 for mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and 0.74 for mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) concentrations in post-shift urine samples from raw-materials workers with di(2-ethylhexyl) phthalate (DEHP) concentrations in air monitored during the work shift [16]. Furthermore, examination of a sample of pregnant women, indicated a significant positive correlation between indoor air concentrations of phthalates and urinary levels of corresponding metabolites. This study reported a correlation of 0.71 for butylbenzyl phthalate (BBP), 0.44 for di-isobutyl phthalate (DiBP), and 0.39 for diethyl phthalate (DEP) from exposure to indoor air, with monobenzyl phthalate (MBzP), monoisobutyl phthalate (MiBP), and monoethyl phthalate (MEP) in urine, respectively [17].

Exposure to phthalates through dermal absorption has also been suggested in the literature. A study conducted by Gong et al. (2015) measured the concentrations of phthalates on the hands of children using handwipes, and evaluated its relationship with metabolite levels in urine; during the summer sampling period, a significant correlation of 0.41 for DiBP, 0.50 for di(n-butyl) phthalate (DnBP), 0.48 for BBP, and 0.36 for DEHP, with respective urinary monoester metabolites were identified [18]. A study investigating the occupational exposure of manicurists to dibutyl phthalate, measured metabolite concentrations from urinary samples collected pre- and post-shifts [19]. The median cross-shift change in mono-n-butyl phthalate (MnBP) concentrations was reported to be significantly decreased among manicurists wearing gloves (-15.1 ng/mL), and increased among those not (20.5 ng/mL) [19]. In utero exposure to phthalates as a result of

maternal-fetal transmission has also been conveyed as a possible route of exposure. A study conducted by Wittassek et al. (2009), examined the concentrations of phthalate metabolites measured in amniotic fluid and maternal urine, and identified a strong significant positive correlation of 0.93 for MiBP between metabolite concentrations in amniotic fluid and maternal urine [20]. These and other studies demonstrate that various possible exposure pathways, including ingestion, inhalation, dermal absorption, and maternal-fetal transmission, may act as important contributors to the overall uptake of phthalates in humans.

1.3. Human Exposure to Phthalates

The exposure to phthalates among the general population has been explored through human biomonitoring, which involves the quantification of chemicals or related products in biological matrixes [21]. It is essential that critical information regarding the estimated total internal concentration of phthalates from various exposure sources and routes be identified [22]. The Canadian Health Measures Survey (CHMS) measured urinary concentrations of phthalate metabolites from individuals of the Canadian population between the ages of 6 to 49 years [23]. Results from the study show that more than 90% of Canadians were exposed to mono-3-carboxylpropyl phthalate (MCPP), and greater than 99% were exposed to MEP, MnBP, MBzP, MEHP, MEOHP, and MEHHP [23]. However, detection frequencies of less than 20% were also observed for monomethyl phthalate, monocyclohexyl phthalate, mono-n-octyl phthalate (MnOP), and monoisononyl phthalate (MiNP) [23].

The National Health and Nutrition Examination Survey (NHANES) is a nationally-representative study that evaluated levels of phthalate metabolites in urine among the general U.S. population [24]. Results from this study identified detection frequencies of greater than 89% for

MEOHP, MCPP, monocarboxyoctyl phthalate, and monocarboxynonyl phthalate, and 98% or more for MBzP, MnBP, and MEP [24]. The results of the Canadian Health Measures Survey and the National Health and Nutrition Examination Survey are comparable because they are representative of the respective populations: the results from these two population studies demonstrate the ubiquity of phthalates among individuals of the general population. In order to determine the potential toxicities associated with exposures to phthalates, an understanding of the biochemical responses in the human body is imperative.

1.4. Human Metabolism and Excretion of Phthalates

In the human body, phthalates– which are phthalic acid diesters–can undergo two stages of metabolism: (1) hydrolysis, and (2) conjugation [25]. These chemical compounds are initially broken down into monoesters through a hydrolysis reaction facilitated by lipases and esterases [25]. At this point in the metabolic pathway, primary metabolites of short-branched phthalates are often excreted [25]. However, prior to converting the products of long-branched phthalates into metabolites that are more readily excreted in urine via conjugation, monoesters are additionally altered through chemicals reactions, such as oxidation or hydroxylation [25].

The metabolism and elimination of phthalates in humans have been previously investigated. In a study performed by Koch et al. (2005), three different doses of deuterium-tagged DEHP were orally administered to a male participant on separate occasions, and with associated metabolites monitoring upon excretion [26]. After monitoring the low (4.7µg/kg bodyweight) and medium (28.7µg/kg bodyweight) doses for 24 hours, and the high dose (650µg/kg bodyweight) for 44 hours, the exposure concentration was not found to influence metabolite elimination in urine [26]. Of the oral dose ingested, approximately 67% was identified in urine following 24 hours, and

a total of 74.3% was detected after 44 hours and 20 minutes [26]. A similar study by Koch et al. (2012) administered doses of approximately 60µg/Kg bodyweight of DnBP, and DiBP orally to a male participant at different points in time [27]. Approximately 92.2% and 90.3% of the DnBP and DiBP doses, respectively, were identified in urine as corresponding metabolites following 24 hours [27]. As demonstrated by the two studies discussed, most of the administered doses were detected in urine after the first day of exposure; the results of these studies provide evidence of the relatively short biological half-life of phthalates.

1.5. Potential Adverse Health Effects of Phthalates

Although existing research shows that phthalates can be quickly metabolized and excreted from the human body, there remains concerns regarding the potential health risks of phthalates. Experimental studies literature have demonstrated the potential toxic effects of phthalates in animals. Male mice exposed to diisononyl phthalate (DINP) at 150 mg/kg/day exhibited adverse effects on learning and memory abilities, as well as signs of oxidative stress, inflammation, and apoptosis [28]. An experiment involving exposure of male rats to either 10,000 ppm of DEHP, 10,000 ppm of di-n-hexyl phthalate (DnHP), or a mixture of 10,000 ppm of DEHP and 10,000 ppm of DnHP, reported lower levels of serum cholesterol and triglyceride in each group that was statistically different from controls [29]. The potential adverse health effects of phthalates have also been investigated in humans. A study involving adults aged 20 years and over reported a significant positive association of urinary MnBP (OR: 1.19; 95% Cl: 1.01 – 1.41) and mono-n-methyl phthalate (OR: 1.16; 95% Cl: 1.03 – 1.32) with high blood pressure, defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg [30]. Also, the relationship of phthalates with abdominal obesity and insulin resistance were examined in adult males; the

study results showed significant associations of ln-transformed MBzP (1.09; SE: 0.36), MEP (0.66; SE: 0.31), MEHHP (1.65; SE: 0.50), and MEOHP (1.79; SE: 0.55) with waist circumference, and ln-transformed MBzP (0.061; SE: 0.022) and MEP (0.044; SE: 0.021) with ln-homeostatic model assessment (HOMA), which was employed to estimate insulin resistance [31].

Concerns about adverse health outcomes that may manifest in relation to the exposure to phthalates during gestation exist, as reflected by the increasing number of animal and human studies. For instance, a number of animal studies have been conducted to explore the potential toxicities of phthalates during pregnancy. Experiments involving the exposure of pregnant rats to DnHP and dicyclohexyl phthalate (DCHP) at doses of 250, 500, and 750mg kg^{-1}/day, and di-n-propyl phthalate (DnPP) at doses of 1.0 and 1.5g kg^{-1}/day reported a significant decrease in the anogenital distance of the male fetus [32, 33]. In addition, exposure of rats to 500mg/kg/day of DnBP during pregnancy was linked to reduced concentrations of testosterone in the fetal testis [34]. Pregnant rats exposed to diallyl phthalate at 200 and 250mg/kg/day, DnHP at 500 and 750mg kg^{-1}/day, DCHP at 750mg kg^{-1}/day, DnPP at 1.5g kg^{-1}/day, and DIBP at 500, 750, and 1,000mg/kg/day, displayed a significant decrease in fetal weight [32, 33, 35, 36]. The findings from these experimental animal studies demonstrate the detrimental effects of phthalates during pregnancy, and suggests a role of these environmental chemicals as potential reproductive and developmental toxicants.

Evidence supporting the relationship of gestational exposure to phthalates with multiple adverse health endpoints is plentiful in epidemiology. A study performed to evaluate the effects of phthalates on the development of genital anomalies in male infants reported significantly elevated odds of hydrocele per log-unit increase in the total concentration of DEHP metabolites measured in urine during the first trimester (OR: 3.0; 95% Cl: 1.2 – 7.6) [37]. Among pregnant women with

paid employment, a significant association of occupational exposure to phthalates with decreased weight (-0.01691 SD per gestational week) and length (-0.0185 SD per gestational week) growth rates were identified in the fetus [38]. Investigations of phthalates during the third trimester reported elevated odds of intrauterine growth restriction in association with MEHHP concentrations in tertile 3 compared to 1 (OR: 5.80; 95% Cl: 1.55 – 21.67), and decreased birth weight per log-10-unit increase in MEHHP (-0.393kg; 95% Cl: -0.740 – -0.046) and MEOHP (-0.434kg; 95% Cl: -0.762 – -0.107) among males but not females [39]. In contrast, research examining the relationship between cord plasma concentrations of phthalates and birth weight among males identified a 436.4g (95% Cl: 65.22 – 807.67) increase in association with MEHHP levels in tertile 2 compared to tertile 1, and a 504.3g (95% Cl: -974.83 – -33.84) decrease in association with mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) concentrations in tertile 3 compared to 1; no significant effects were observed among females [40].

Although a positive correlation of 0.43 (p-value=0.005) was observed between cord blood concentrations of monobutyl phthalate and head circumference in males, findings from another study evaluating the exposure using maternal urinary samples yielded results that were not statistically significant in a study population comprised of both sexes [41, 42]. Investigations into the effects of phthalates on pregnancy duration, demonstrated an increase in gestational age for each log-unit increase in urinary concentrations of MEHP (0.16 week; 95% Cl: 0.02 – 0.30), MEOHP (0.19 week; 95% Cl: 0.03 – 0.35), and MEHHP (0.16 week; 95% Cl: 0.01 – 0.31) [43]. However, evidence of an inverse association between the same phthalate metabolites and gestational age was also reported in the literature [44]. Overall, in agreement with the existing knowledge acquired from animal studies, current research in human participants demonstrate the potential of phthalates to interfere with normal development of the offspring. However, as some

opposing results were observed between studies, further research is imperative to clarify the inconsistencies presented in the literature.

1.6. Potential Mediating Role of Maternal Inflammation Biomarkers as one of the Effects of Exposure to Phthalates

Phthalate-induced inflammatory responses during gestation may act as key intermediates in the mechanism of action contributing to the incidence of adverse clinical outcomes in the mother and infant. Throughout the course of pregnancy, the maternal immune system plays a critical role in establishing a viable environment [45]. In particular, inflammation is strictly regulated through the dynamic adjustments of pro- and anti-inflammatory cytokines, which are secreted proteins with immunoregulatory properties that are involved with various activities, including the expression of adhesion molecules, and recruitment of cells [45, 46]. As an appropriate inflammatory response is important for certain gestational processes, such as embryo implantation and parturition, inflammation may be linked with a number of adverse pregnancy outcomes [45].

Multiple studies have investigated the association between biomarkers of inflammation and adverse endpoints during pregnancy. For instance, an analysis conducted using a subset of participants in Maternal-Infant Research on Environmental Chemicals (MIREC) Study, reported links of maternal plasma markers, including matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) with birth weight at $<25^{th}$ or $>75^{th}$ percentile, and matrix metalloproteinase-2 (MMP-2) and VEGF with gestational age [47]. Amniotic fluid concentrations of matrix metalloproteinase-8 above the 90^{th} percentile was significantly associated with elevated odds of preterm premature rupture of membranes (OR: 3.4; 95% Cl: 1.2 – 9.9) [48]. In addition, granulocyte colony-stimulating factor measured in maternal serum was associated with higher

odds of preterm birth for each increase in standard deviation (OR: 1.52; 95% Cl: 1.07 – 2.16) [49]. Women with severe pre-eclampsia displayed significantly greater plasma concentrations of interleukin-8 (IL-8) and C-reactive protein (CRP) compared to those with healthy pregnancies; specifically, the mean concentrations of IL-8 were 13.55pg/mL (SD: 25.73) and 3.43pg/mL (SD: 7.98), and CRP were 36.64mg/L (SD: 42.24) and 16.67mg/L (SD: 33.95) for women with severe pre-eclampsia, and healthy pregnancies, respectively [50]. Furthermore, in comparison to controls matched for gravidity, parity, maternal age, and gestational age, a significantly greater mean concentration of CRP in plasma measured during first trimester was observed among women with intrauterine growth restriction (0.0163g/l vs. 0.0062g/l) [51]. Overall, the study findings presented collectively show the potential of adverse health outcomes in the mother and infant in relation to altered levels of inflammation during pregnancy.

Research on the biochemical responses to phthalates have been conducted to evaluate the capacity of these chemicals to elicit an immune response. An experimental study involving the exposure of human epithelial cells to monophthalates reported observing IL-6 and IL-8 stimulatory effects of MEHP, MnOP, MINP, and monoisodecyl phthalate, which were followed by suppressive effects at higher doses [52]. A study consisting of pregnant women in Northern Puerto Rico analyzed the relationship of phthalate metabolites in urine, with inflammatory biomarkers in plasma, and observed statistically significant increases in interleukin-6 (IL-6) and interleukin-10 (IL-10) in association with monocarboxyisononyl phthalate and MECPP, respectively [53]. Research conducted on a study sample of pregnant women that delivered at a hospital in Boston examined exposures and outcomes up to four times during pregnancy, and identified significantly elevated plasma levels of IL-6 in association with urine concentrations of MCPP [54]. Overall, the current literature demonstrates a possible link between phthalates and inflammation, which

reinforces the potential role of inflammatory responses as critical mediators in the association of gestational exposure to phthalates with adverse outcomes in the mother and infant. However, as existing human studies on phthalate-induced maternal immunological changes during pregnancy are scarce, validation through further epidemiological research is necessary.

1.7. Overview of Master's Thesis

The review presented above demonstrates the current knowledge gaps in the literature. This serves as a rationale for further research, and thus a need for the current master's thesis. The objectives of this thesis are as follows.

1) To summarize the existing literature, a systematic review will be conducted on the effects of gestational exposure to environmental phthalates on perinatal and pregnancy outcomes. Multiple electronic databases, including MEDLINE, EMBASE, PubMed, CINAHL, and POPLINE, will be searched for relevant studies reporting on pre-eclampsia (PE), pregnancy-induced hypertension (PIH), gestational diabetes mellitus (GDM), intrauterine growth restriction (IUGR), birth weight (BW), head circumference (HC), gestational age (GA), preterm birth (PB), and Apgar scores (AS). To supplement the primary search, additional articles identified through citation tracking, hand-searching of reference lists, and recommendation, will be considered for inclusion.
2) To address the inconsistencies in the literature, secondary data-analysis will be conducted to investigate the association between gestational exposure to environmental phthalates and the observed clinical outcomes in the mother and infant using data from the MIREC Study. Using quartile 1 concentrations of urinary phthalate metabolites as

the reference, multiple linear regression will be used to estimate the change in BW, HC, and GA, and multiple logistic regression will be used to estimate the odds of PIH, PB, low AS at 1 minute (score < 7), and low AS at 5 minutes (score < 7).

3) To address the paucity of research in the literature, secondary data-analysis will be conducted to investigate the association between maternal exposure to environmental phthalates and inflammatory responses among pregnant women who had consented to participate in the MIREC Study. Using quartile 1 concentrations of urinary phthalate metabolites as the reference, multinomial logistic regression will be used to estimate the odds of low (≤ 10th percentile) and high (≥ 90th percentile) levels of plasma inflammation biomarkers, relative to levels considered normal (10th – 90th percentile).

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PREFACE TO CHAPTER 2

The objective of the first manuscript was to summarize the existing literature, and evaluate the potential risks associated with the exposure to phthalates during pregnancy, by performing a systematic review of observational studies that investigated the effects of gestational exposure to environmental phthalates on obstetrical outcomes, including birth weight, head circumference, gestational age, preterm birth, Apgar scores, intrauterine growth restriction, pregnancy-induced hypertension, pre-eclampsia, and gestational diabetes mellitus. The complete search strategy employed in this systematic review (Appendix 1), and a summary of study characteristics and reported results (Appendix 2) are provided in the appendix of this manuscript.

Methodological guidance on the systematic review was provided by Dr. Daniel Krewski and Dr. James Gomes. Content expertise for the selection of *a priori* maternal and perinatal outcomes was provided by Dr. James Gomes and Dr. Premkumari Kumarathasan. The random sample of articles for independent review, verification of data extraction, and independent appraisal, was completed by Dr. James Gomes.

CHAPTER 2: THE EFFECTS OF GESTATIONAL EXPOSURE TO ENVIRONMENTAL PHTHALATES ON MATERNAL AND PERINATAL OUTCOMES: A SYSTEMATIC REVIEW OF OBSERVATIONAL STUDIES (MANUSCRIPT 1)

2.1. ABSTRACT

Background: Phthalates are used in a variety of products, and are environmental contaminants that have been linked to several potential health concerns. The widespread distribution of phthalates in the environment, and their detection in the vast majority of the general population, confirm broad human exposure to this class of chemicals. To elucidate the risks of phthalates among vulnerable populations, such as pregnant women and the developing fetus, a systematic review was performed to evaluate the association between phthalate exposure during pregnancy and maternal and perinatal health outcomes.

Methods: MEDLINE, EMBASE, PUBMED, CINAHL, and POPLINE were searched to identify observational studies that reported on the following outcomes: birth weight (BW), head circumference (HC), gestational age (GA), preterm birth (PB), APGAR scores (AS), intrauterine growth restriction (IUGR), pregnancy-induced hypertension (PIH), pre-eclampsia (PE), and gestational diabetes mellitus (GDM). Articles were initially screened by title and abstract, and the full-text of potentially pertinent articles were retrieved. Hand-searching of reference lists and citation tracking were conducted to supplement the primary search. In addition, articles that were recommended were also considered. Studies included in this systematic review were evaluated using a modified Downs and Black quality assessment tool.

Results: Twenty-four articles met the inclusion criteria. Most studies retrieved reported on BW, HC, GA, and PB. One article reported on AS, IUGR, PE, and GDM. No studies investigating PIH were identified. Studies assessing BW, HC, GA, and PB yielded conflicting results; however, among the significant findings, the majority of the results suggest that phthalates may decrease BW and GA, and increase HC and PB. Studies evaluating AS, PE and GDM yielded nonsignificant findings, whereas the study on IUGR reported a significant positive association with phthalate exposure.

Conclusion: Overall, results suggest possible associations of phthalate exposure with decreases in BW and GA, and increases in HC and PB, although inconsistent findings among studies were observed, possibly due to heterogeneity in the study populations and exposure assessment methods used between studies. As well, a significant relationship between MEHHP and elevated odds of IUGR was observed; however, this was based on a single study.

2.2. INTRODUCTION

2.2.1. Background

Phthalates are a group of synthetic chemicals that have been used in industry as plasticizers, chemical stabilizers, and excipients [1, 2]. Phthalates are present in a wide range of commonly used products, such as lotions, haircare products, fragrances, toys, medical products, pharmaceuticals, supplements, packaging materials and more [1-6]. Due to the extensive use of phthalates, and the weak attachment of these chemicals to products, these contaminants have been detected ubiquitously in the environment [1]. In particular, studies have identified phthalates in both indoor and outdoor air, dust, soil, rivers, lakes, and food [4, 7-11]. The widespread distribution of phthalates in our immediate environment suggests that human exposure to these environmental contaminants is inevitable. In particular, studies have identified ingestion, inhalation, dermal absorption, and maternal-fetal transmission as possible routes of human exposure [12-14].

These human-made chemicals have been detected in the vast majority of the general population in biomonitoring studies. For example, a biomonitoring study conducted on the Canadian population reports that 7 of the 11 urinary phthalate metabolites measured were detected in more than 90% of the general population [15]. Further studies have detected these environmental pollutants in other biological specimens, such as blood, breast milk, saliva, sweat, semen, cord blood, meconium, and amniotic fluid [16-21]. In the human body, phthalates are quickly metabolized prior to excretion [22]. A study examining the metabolism of DEHP through the excretion of five urinary phthalate metabolites reported identifying 74.3% of an oral dose 2 days after its administration in urine [23]. Despite the fact that phthalates are considered as non-persistent chemicals, their ubiquitous presence in the environment indicates that humans may be

subject to ongoing environmental exposure, raising concerns regarding the potential effects of exposure to phthalates on human health.

Several phthalates are believed to be endocrine disrupting chemicals, and have exhibited effects that interfere with androgen synthesis [24-26]. In particular, both animal and human studies have linked this environmental toxicant to a decrease in testosterone levels [25, 26]. Further studies investigating the effects of phthalates have suggested a possible association with male infertility, reduced fetal growth, clinical pregnancy loss, hydrocele, reduced anogenital index in boys, and pubertal timing [16, 27-31]. The research findings reported by these studies demonstrate the potential role of phthalates as reproductive and developmental toxicants.

Interest in the manifestation of adverse health outcomes among vulnerable populations, such as pregnant women, have yielded studies examining the relationship between phthalates and pregnancy outcomes, such as pre-eclampsia and gestational diabetes mellitus [32, 33]. Furthermore, as the existing literature demonstrates that phthalates may be capable of crossing the placental barrier, concerns regarding the harm to fetus and infant health have resulted in a rising number of studies evaluating the effects of this toxicant on various birth outcomes, including birth weight, head circumference, gestational age, and preterm births [14, 34-41]. To elucidate the risks associated with this environmental toxicant on perinatal and maternal health, a systematic review was conducted to evaluate and synthesize the current body of scientific knowledge in this field.

2.2.2. Objective

The objective of this systematic review is to summarize the existing literature of observational studies investigating the effects of phthalates on maternal and perinatal outcomes,

and to evaluate the potential risks associated with this chemical exposure of vulnerable populations, such as pregnant women and their developing fetus.

2.3. METHODS

2.3.1. Eligibility Criteria

The eligibility criteria was predefined prior to performing the systematic review. Studies that met the criteria presented below were considered for inclusion.

- ***Population:*** Studies that recruited pregnant women or their offspring as the population of interest were included. Based on a preliminary search conducted, a large number of studies were not anticipated, and thus restrictions were not applied to maternal characteristics. As a result, participants of any age, race, or lifestyle were accepted.
- ***Exposure:*** Studies that estimated maternal and/or fetal phthalate exposures during gestation were included. No restrictions were applied to the methods used to determine the status or degree of phthalate exposure. Direct measures of parent phthalate compounds and/or respective metabolites through biomonitoring research were accepted. Alternatively, indirect measures of human exposure, such as via environmental monitoring or job-exposure matrixes (JEM), were also considered. No restrictions were applied to the magnitude, frequency, or time of exposure assessment.
- ***Outcome:*** Studies that investigated the effects of phthalates on the following maternal and perinatal outcomes were included: birth weight, head circumference, gestational age, preterm birth, APGAR scores, intrauterine growth restriction, pregnancy-induced hypertension, pre-eclampsia, and gestational diabetes mellitus. No restrictions were applied to the methods used to measure the outcomes of interest.

- ***Study Design:*** Observational study designs such as cohort studies, case-control studies, and cross-sectional studies were included.
- ***Language:*** Only studies published in English were considered in this systematic review

2.3.2. Search Development

Key concepts associated with the research question of interest were used to develop a comprehensive search, consisting of controlled vocabulary to identify studies that have been indexed, and keywords to identify articles that have not been assigned an indexed term. The implemented search included both exposure- and population-related terms. To implement a more rigorous search of the current body of literature, outcome-related terms were not included to further restrict the number of articles generated; instead, relevant articles evaluating the outcomes of interest were manually identified. The search strategy conducted for this systematic review was initially constructed in MEDLINE, and then adapted for use in other electronic databases. The searches performed for the current systematic review are presented in appendix 1.

2.3.3. Information Sources

To ensure an exhaustive search of the existing literature, multiple search methods were implemented to identify articles pertinent to this systematic review. In particular, the standard and specialized electronic databases that were systematically searched, consists of MEDLINE, EMBASE, PUBMED, CINAHL, and POPLINE. These searches were conducted on October 7th, 2015, and restricted to humans; all articles identified were considered up to this date. To supplement the primary search, the reference lists of included studies were searched to ensure the recovery of relevant studies that were not captured with the current search strategy. Citation

tracking via Scopus was also performed to identify potentially pertinent articles that had been subsequently published; this was conducted on February 4th, 2016. Any other articles that the investigators came across by any other means were also included if they were relevant.

2.3.4. Study Selection

Articles identified by the electronic databases were managed and compiled using a citation manager. Exact duplicates were immediately removed, and close duplicates were further assessed by comparing the study authors, titles, and dates of publication. The screening process was conducted in two phases. During phase 1 of screening, studies were evaluated by the title and abstract for relevancy according to the predetermined eligibility criteria of this systematic review. The full-texts of potentially pertinent articles were retrieved and assessed during phase 2 of screening; to be conservative, studies with unclear relevancy based on the title and abstract were also retained and evaluated at this stage. For greater reliability in the study selection process, a random sample of articles identified during phases 1 (N=80) and 2 (N=10) of screening were confirmed by an independent second reviewer. The screening process and justifications for exclusion were documented using the PRISMA flow diagram [42].

2.3.5. Data Collection

The information extracted from the articles included in this systematic review consists of the following: the study location and design, recruitment date and methods, sample size and characteristics, type of phthalates measured, methods (time and frequency) of exposure assessment, methods of adjusting for urinary dilution, magnitude of exposures, types of outcomes evaluated, methods of outcome assessment, adjusted confounders, and reported measures of health

outcome. For greater accuracy in the data extraction process, a random sample of five articles was selected and verified by a second study author.

2.3.6. Quality Assessment

A modified Downs and Black study quality assessment tool was implemented to appraise the quality of individual studies included in this systematic review [43]. This quality assessment tool consists of a 15-item checklist that appraises the internal and external validity of cohort and case-control studies. Study characteristics, such as the recruitment of study participants, methods of exposure and outcome assessment, and the adjustment of confounders, were considered when allocating a final quality score out of 20 [43]. Among the studies being evaluated, 5 articles were randomly selected and independently appraised by a second study author.

2.3.7. Presentation of Study Findings

Relevant sections from the PRISMA checklist were used in this systematic review [42]. The screening and study selection processes were documented and illustrated using the PRISMA flow diagram [44]. Details regarding the individual study characteristics, exposures, outcomes, and research findings were summarized in tables.

2.4. RESULTS

2.4.1. Study Selection

The electronic database searches of MEDLINE, EMBASE, PUBMED, CINAHL, and POPLINE yielded 1,704 articles. Five potentially relevant articles were identified through supplementary searches or recommendation. After removing duplicate studies, 807 articles were

examined by title and abstracts. The full texts of 70 articles that were potentially pertinent or were of indefinite relevancy were retrieved and further assessed; 46 articles did not meet the eligibility criteria mentioned before and were excluded during this stage of screening. A total of 24 articles were found to be eligible, and thus included in this systematic review. The PRISMA flow diagram in Figure 2.1 summarizes the screening and study selection processes, as well as the reasons for exclusion.

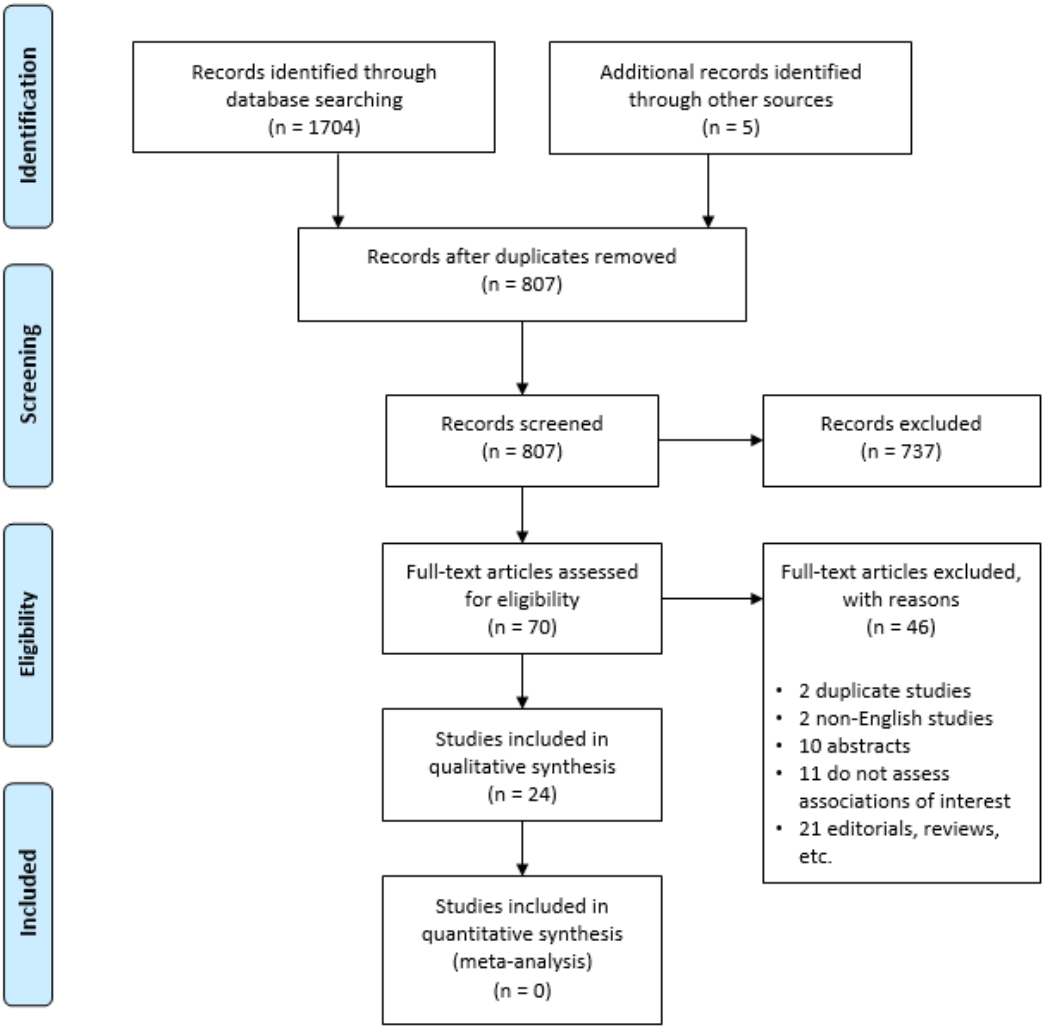

Figure 2.1. Summary of the screening and study selection process using the PRISMA flow diagram [42].

2.4.2. Study Characteristics

The current systematic review is composed of studies conducted in several countries including Canada [33], USA [36, 37, 40, 44-46], Mexico [47], Taiwan [19, 34], China [18, 35, 48, 49], Japan [38, 50, 51], France [52, 53], Greenland [54], Poland [54], Ukraine [54], and the Netherlands [32, 39, 55]. Among the 24 articles identified with the current search strategy, 14 were cohort studies [19, 32-34, 37-39, 44-46, 50, 51, 54, 55], 7 were case-control studies [18, 36, 40, 47-49, 53], and 3 were cross-sectional studies [35, 52, 56]. Studies were considered to have a cross-sectional design when exposures and outcomes were measured at approximately the same time. The sample size of each study differed, with the smallest consisting of 60 individuals [47], and the largest including 6,302 study participants [39]. Several differences in the sample characteristics were observed; specifically, 2 studies restricted their sample to consist only of male infants [52, 53], 20 studies examined a sample consisting only of singleton births [18, 32-38, 40, 44, 45, 47-55], 1 study selected study participants with indications for amniocentesis [19], and a single study intentionally selected study participants from a high-risk population of pregnant women [44].

The time of exposure ascertainment varied among studies. Among studies that conducted trimester-specific assessments of phthalate exposure, the most common period was during the third trimester [34, 46, 47, 49]. Only 1 study reported assessing the exposure solely during the first trimester [33]. Among studies that conducted non-time specific exposure assessments, 3 studies reported accepting different sample collection times between the first and third trimesters [38, 45, 53], whereas 3 studies only examined exposure measurements collected between the second and third trimesters [37, 50, 51]. The study by Lenters et al. (2016) reported the median sample collection times among study samples from three countries, which ranged between the second and third trimesters [54]. Most studies collected only one biological specimen; studies that did not

specify the number of biological samples collected were assumed to have collected only one sample. Only 2 articles reported collecting multiple samples from study participants, ranging from 3 to 4 that were included in their analyses; however, both of these articles involved participants enrolled in the same study [36, 40].

Both direct and indirect measures of gestational phthalate exposures were implemented among studies included in this review. Among the direct measures of phthalate exposure examined via biomonitoring analysis, the majority of the studies quantified the presence of phthalates in maternal urinary samples [33, 34, 36-38, 40, 44-47, 49, 53]. Methods of adjusting for urinary dilution varied between the use of creatinine [34, 37, 38, 45, 53] and specific gravity [33, 36, 40, 44, 46, 49]; only a single study presented phthalate concentrations corrected using both methods [47]. Other biological specimens used to estimate phthalate exposures include maternal blood [50, 51, 54], umbilical cord blood [18, 35, 52, 55, 56], amniotic fluid [19], and meconium [18, 48]. Among the few studies that employed indirect measures of estimating phthalate exposures, 2 studies used a JEM [32, 39], and 1 study measured levels detected in air samples [46]. In cases where the measured exposure levels were below the limit of detection (LOD), an approximately equal number of studies reported imputing concentrations as either "LOD/2" [33, 38, 46-49] or "LOD/√2" [18, 35, 36, 40, 45, 53, 55].

Most studies included in this systematic review reported on more than one of the outcomes of interest. Outcomes that were frequently reported on consists of birth weight (N=16) [18, 19, 34, 35, 37-39, 48-56], head circumference (N=7) [34, 35, 37, 38, 50, 52, 53], gestational age (N=10) [19, 34, 35, 37, 38, 44-46, 51, 56], and preterm birth (N=6) [35, 36, 39, 40, 45, 47]. Only a single study reported assessing Apgar scores [56], intrauterine growth restriction [49], pre-eclampsia [32], and gestational diabetes mellitus [33], respectively. No studies reporting on pregnancy-

induced hypertension were identified. A summary of the study characteristics is provided in Tables 2.1 to 2.3.

2.4.3. Quality Assessment

The quality of 21 cohort and case-control studies were critically appraised using a modified Downs and Black quality assessment tool. The studies evaluated yielded an average total score of 14.38 out of 20 (range: 9-19). The average subtotal score for external validity was 0.52 out of 2, internal validity in relation to bias was 4.10 out of 6, internal validity in relation to exposure measurement was 6.24 out of 8, and internal validity in relation to confounding was 3.52 out of 4. While variation in quality scores were observed across studies, this does not necessarily indicate significant issues with the conduct of the study, but rather that the reporting of studies may be insufficient, or lacking the essential details needed to appropriately evaluate its quality. Refer to Tables 2.1 to 2.3 for the quality score assigned to each study.

2.4.4. The Effects of Phthalates on Birth Weight

A summary of the reported study results on the association between phthalate exposure and birth weight is presented in Table 2.1. A total of 16 articles investigating the effects of phthalates on birth weight were identified with the current search strategy [18, 19, 34, 35, 37-39, 48-56]. It was observed that the results reported by those studies included in this review were fairly inconsistent. Among the 8 studies that identified results that were statistically significant, 7 presented research findings that supported an inverse association between phthalate exposure and birth weight [18, 35, 48, 49, 53-55].

The studies by Zhang et al. (2009) and Xie et al. (2015) similarly measured phthalate levels in meconium samples collected up to 48 hours after delivery and assessed its association with low birth weight (LBW), defined as weight less than 2500g. Both studies consistently demonstrated a positive association between MEHP and MBP or MnBP levels and the odds of LBW. The analysis by Zhang et al. (2009) which evaluated the effect of these phthalates in quartiles exhibited a significant exposure-response relationship; in comparison to phthalate levels in quartile 1 (Q1), the odds of LBW among those exposed to levels in Q2, Q3, and Q4 were 1.58 (95% Cl: 1.08, 2.46), 2.84 (95% Cl: 1.19, 4.82), and 4.68 (95% Cl: 2.14, 6.85) times greater for MBP, and 1.12 (95% Cl: 0.89, 2.03), 2.89 (95% Cl: 1.19, 5.02), and 3.23 (95% Cl: 1.31, 5.94) times greater for MEHP. Moderate associations of MEHP (OR: 4.4; 95% Cl: 2.0, 9.3) and MnBP (OR: 2.4; 95% Cl: 1.1, 4.8) with LBW were also reported by Xie et al. (2015).

Both studies conducted by Zhang et al. (2009), and Xie et al. (2015) conducted additional analyses which yielded results supporting the previously discussed study findings. The results reported by Zhang et al. (2009) demonstrates greater reliability, as the relationship between LBW and phthalates measured in cord serum supports that observed when assessing phthalates measured in meconium. In particular, a significant positive exposure-response relationship of LBW with MEHP and DBP, which is the parent compound of MBP, was also reported by the study authors [18]. Xie et al. (2015) examined the change in birth weight (g) in addition to LBW, and consistently reported a significant inverse association with MEHP (β: -0.62; 95% Cl:-1.09, -0.13) and MnBP (β: -0.92; 95% Cl: -2.09, -0.03). Although consistency has been demonstrated between the two studies, conflicting results have been reported in several other studies. Huang et al. (2009) conducted a stratified analysis, and reported an increase in birth weight in association with MBP among females, but not males. Furthermore, a number of studies have reported finding null

associations when assessing the effects of MEHP [19, 34, 37, 49-51, 53, 56], and MBP or MnBP [34, 37, 49, 52, 53] using other exposure matrixes, such as maternal urine, maternal blood, cord blood, and amniotic fluid.

The study by Zhao et al. (2014) assessed the effects of phthalates measured in maternal urinary samples collected during the third trimester, and reported a significant inverse association between MEHHP and MEOHP levels and changes in birth weight. The investigators also performed a stratified analysis based on the sex of the infant, and reported a greater decrease in birth weight (kg) among males for each log-10-unit increase in MEHHP (β: -0.393; 95% Cl: -0.740, -0.046) and MEOHP (β: -0.434; 95% Cl: -0.762, -0.107) [49]; however, no significant associations were observed among females [49]. A similar inverse association was observed among study results reported by Lenters et al. (2016), which demonstrated a 70.22g (95% Cl: -117.59, -22.85) decrease in birth weight following a 2-SD increase in ln-MEHHP [54]. Other investigators assessing the same metabolites have reported conflicting results. De Cock et al. (2016) measured phthalate levels using cord blood samples and reported an increase in birth weight in association with MEHHP among males, and non-significant results in association with MEOHP for both sexes. In addition, three other studies that examined both MEHHP and MEOHP exposures using maternal urinary samples have reported finding no significant results for the relationship with birth weight [34, 37, 53].

De Cock et al. (2016) additionally reported a decrease in birth weight for each tertile increase in cord blood MECPP concentrations among males but not females; compared to MECPP levels in the first tertile (T1), T2 and T3 were associated with a 287.7g (95% Cl: -755.26, 179.87) and 504.3g (95% Cl: -974.83, -33.84) decrease in birth weight. Similarly, the study by Philippat et al. (2012) reported a 141g (95% Cl: -277, -5) decrease in birth weight among those exposed to

MECPP levels in tertile 2 compared to tertile 1. These results were not consistent with those of Wolff et al. (2008), who reported finding no significant results. The study by Philippat et al. (2012) also reported changes in birth weight that were significantly different by MCPP tertiles (p=0.03); however, the non-significant p-value (p=.73) for trend suggests a non-monotonic exposure-response relationship. In particular, the study authors report a 198g (95% Cl: -343, -52) decrease in birth weight among those exposed to MCPP levels in the second tertile compared to the first [53]. However, these results were not consistent with those of Wolff et al. (2008) who reported null findings. Huang et al. (2014) measured phthalate levels in cord blood samples and reported an inverse association between DEEP levels and birth weight among females but not males: women who gave birth to female infants with detectable levels of DEEP demonstrated a 143g (95% Cl: -273, -14) decrease in birth weight, compared to those with non-detectable levels. However, as there is a lack of studies assessing DEEP, these results could not be compared.

Overall, the majority of the studies reporting significant results suggest that phthalate exposure may be associated with a decrease in birth weight. Additionally, 6 studies conducted a stratified analysis based on the sex of the infant [19, 34, 35, 49, 50, 55], with 2 of the 6 studies reporting null findings [34, 50]. Among the remaining 4 studies, effect modification is a possibility as the results varied between the stratums; however, the nature of the association is unclear as different stratified results were observed between studies [19, 35, 49, 55] (Table 2.1).

2.4.5. The Effects of Phthalates on Head Circumference

A summary of the reported study results on the association between phthalate exposure and head circumference is presented in Table 2.1. Seven articles investigating the effects of phthalates on head circumference were identified [34, 35, 37, 38, 50, 52, 53]. Conflicting results reported

between studies were observed. Among the 4 studies that reported results that were statistically significant at a p-value less than 0.05, 3 provided evidence suggesting a positive association between phthalate exposure and head circumference [34, 37, 52].

The study by Su et al. (2014) measured phthalate levels in maternal urinary samples collected during the third trimester of pregnancy. The study authors report an increase in head circumference (cm) expressed as z-scores for each unit increase in MEHHP (0.004; SE: 0.002), and MEOHP (0.002; SE: 0.001) among males but not females; while the p-values for these statistical tests are below 0.05, these results were not considered significant as the authors have set the p-value for statistical significance at less than 0.00625 [34]. Contradicting results were reported by 2 studies measuring MEHHP and MEOHP metabolites using the same exposure matrix; in particular, both studies reported null findings for the relationship between MEHHP and MEOHP exposure and head circumference [37, 53].

Wolff et al. (2008) assessed the effect of phthalates measured in maternal urinary sample collected between the second and third trimester, and reported a significant increase in head circumference with exposure to MEP. Specifically, a ln-unit increase in MEP was associated with a 0.12 cm (95% Cl: 0.01, 0.23) increase in head circumference [37]. In contrast, the studies by Philippat et al. (2012) and Su et al. (2014) reported null findings. The study by Brucker-Davis et al. (2010) assessed the effect of phthalates measured in cord blood samples among a population of male infants, and reported a positive correlation between MBP and head circumference. However, another study assessing the same exposure using maternal urinary samples reported results that were not significant [37]. The study by Huang et al. (2014) was the only study to report an inverse association between phthalates and head circumference. The authors performed a stratified analysis and reported a 11.79mm (95% Cl: -22.05, -1.52) decrease in head circumference among

females with detectable DNHP levels, compared to those without; however, no significant effects were observed among males [35].

Although the overall results were somewhat inconsistent, most studies yielding significant results suggest that phthalates may be associated with an increase in head circumference. In total, 3 studies did a stratified analysis based on the sex of the infant [34, 35, 50]. However, there is uncertainty as to whether or not the sex of the infant acts as an effect modifier, as the measured phthalate types yielding significant results did not overlap between studies (Table 2.1).

2.4.6. The Effects of Phthalates on Gestational Age and Preterm Birth

A summary of the reported study results on the association of phthalate exposure with gestational age and preterm birth is presented in Table 2.2. A total of 10 articles reporting on gestational age and 6 articles reporting on preterm births were identified [19, 34-40, 44-47, 51, 56]. A number of inconsistencies were observed among the reported results. Among the articles that reported significant findings, most presented evidence to support a decrease in gestational age (4 of 7 articles), and an increase in preterm birth (4 of 5 articles) [35, 36, 40, 44, 46, 47, 56].

The study by Whyatt et al. (2009) measured four DEHP metabolites in maternal urinary samples collected during the third trimester, and reported an inverse association between MEHP, MEHHP, MEOHP, and MECPP exposures and gestational age. Specifically, a 1-log-unit increase in MEHP, MEHHP, MEOHP, and MECPP was significantly associated with a 0.15 (95% Cl: -0.26, -0.03), 0.18 (95% Cl: -0.31, -0.05), 0.17 (95% Cl: -0.30, -0.03), and 0.16 (95% Cl: -0.31, -0.01) weeks decrease in gestational age [46]. Similar results were reported by several other studies [35, 44, 56]. In particular, the study by Huang et al. (2014) examined the effects of DEHP exposure using cord blood samples, and reported a 0.46 (95% Cl: -0.61, -0.31) week decrease in gestational

age for each unit-ln increase in DEHP (μg/L) levels. The study authors additionally evaluated the effects of DEHP exposure on preterm birth and identified consistent results that suggested a positive association [35]. The study conducted by Latini et al. (2003) also measured MEHP in cord blood and reported that for each weekly increase in gestational age, the odds of being MEHP-negative were 1.50 (95% Cl: 1.013, 2.21) times greater than those who were MEHP-positive; however, the effect of DEHP exposure did not yield significant results. The study by Weinberger et al. (2014) assessed the effects of MEHP, MEHHP, and MEOHP in maternal urine, and reported an inverse association between MEHHP and gestational age among males; however, null findings were reported for MEHP and MEOHP among both sexes. Consistently, non-significant results were observed among several other studies assessing the effects of MEHP on gestational age [19, 34, 51].

In contrast, conflicting results on the association between maternal urinary levels of DEHP metabolites and gestational age were reported in the 3 articles included in this review [34, 37, 45]. The study by Adibi et al. (2009) measured DEHP metabolites, and reported that a log-unit increase in MEHP, MEOHP, and MEHHP was significantly associated with a 0.16 (95% Cl: 0.02, 0.30), 0.19 (95% Cl: 0.03, 0.35), and 0.16 (95% Cl: 0.01, 0.31) week increase in gestational age; consistent results were presented for the analysis between the aforementioned metabolites and preterm birth which demonstrated an inverse association. Su et al. (2014), who also assessed third trimester phthalate levels, reported that for each unit increase in MEHHP and MEOHP (μg/L), a 0.0054 (SE: 0.0023), and 0.0025 (SE: 0.0011) week increase in gestational age were observed among males but not females. (Although these results had a p-value below 0.05, they were not considered statistically significant by the study authors who set their level of significance at a p-value below 0.00625.) Wolff et al. (2008) assessed DEHP metabolite levels predominantly during

the third trimester, and reported that for each unit-ln increase in MEHP (μg/L), a 0.15 (95% Cl: 0.02, 0.29) week increase in gestational age was observed; however, null results were reported when assessing the effects of MEHHP, MEOHP, and MECPP.

The study conducted by Huang et al. (2014) assessed a larger selection of phthalates, most of which were found to be inversely associated with gestational age. Specifically, the results indicate that women with detectable DMP, DEP, DEEP, DPP, BMPP, DNHP, BBP, and DNOP levels exhibited a 2.22 (95% Cl: -2.80, -1.65), 1.67 (95% Cl: -2.23, -1.12), 1.01 (95% Cl: -1.54, -0.48), 3.00 (95% Cl: -3.61, -2.40), 2.68 (95% Cl: -3.27, -2.09), 1.67 (95% Cl: -2.26, -1.09), 1.05 (95% Cl: -1.59, -0.51), and 1.89 (95% Cl: -2.45, -1.34) week decrease in gestational age compared to those with non-detectable phthalate levels [35]. In addition, the study authors also reported that a ln-unit increase in DMEP, DBP, DIBP, DBEP, and DNP is significantly associated with a 0.24 (95% Cl: -0.36, -0.12), 0.55 (95% Cl: -0.81, -0.30), 0.75 (95% Cl: -1.03, -0.46), 0.42 (95% Cl: -0.55, -0.28), and 0.49 (95% Cl: -0.62, -0.37) week decrease in gestational age [35]. Further analyses conducted to assess the association between these metabolites and preterm birth yielded consistent evidence of a positive association [35].

Ferguson et al. (2014a) estimated average phthalate exposure using maternal urinary samples collected at three time points during the first and second trimester of pregnancy, and identified a positive association with preterm births. In particular, a unit-ln increase in MEHP and MECPP (μg/L) concentrations was significantly associated with a 1.34 (95% Cl: 1.07, 1.68), and 1.40 (95% Cl: 1.13, 1.74) times greater odds of preterm birth [40]. The study authors additionally conducted a subgroup analysis, and reported that a unit-ln increase in MEHP, MEOHP, MECPP, MBzP, MnBP, and MCPP was associated with a 1.65 (95% Cl:1.20, 2.26), 1.47 (95% Cl: 1.04, 2.08), 1.56 (95% Cl: 1.15, 2.13), 1.41 (95% Cl: 1.02, 1.95), 1.49 (95% Cl: 1.08, 2.06), and 1.36

(95% Cl: 1.02, 1.81) times greater odds of spontaneous preterm births [40]. An approximately increasing exposure-response relationship was also demonstrated with MEHP, MECPP, ∑DEHP, and MnBP in association with preterm birth and spontaneous preterm birth, when the exposure was categorized into quartiles [40].

Using the same study but a different analytic approach, Ferguson et al. (2014b) investigated the effect of phthalates at four different time points between the first and third trimesters in association with preterm births, and additionally conducted two subgroup analyses assessing placental preterm births and spontaneous preterm births. The study authors reported an elevated odds of preterm birth with a ln-unit increase in MCPP exposure during the first study visit (OR: 1.19; 95% Cl: 1.01, 1.41), and MECPP exposure during the first (OR: 1.25; 95% Cl: 1.06, 1.48) and third (OR: 1.27; 95% Cl: 1.06, 1.52) visits [36]. However, an inverse association was observed between MEHHP and preterm birth at visit 4 (OR: 0.77; 95% Cl: 0.60, 0.98) [36]. The subgroup analyses indicated that a unit-ln increase in MECPP, MBzP, and MnBP measured during the third study visit was associated with a 1.33 (95% Cl: 1.04, 1.70), 1.43 (95% Cl: 1.05, 1.95), and 1.45 (95% Cl: 1.08, 1.96) times greater odds of spontaneous preterm births [36]. In addition, MECPP measured during the first visit was found to be positively associated with placental preterm births (OR: 1.46; 95% Cl: 1.10, 1.95) [36].

The study conducted by Meeker et al. (2009) examined a number of phthalate metabolites using maternal urinary samples collected during the third trimester. A significant positive association between MnBP and preterm birth (OR: 4.5; 95% Cl: 1.2, 16.6) was observed, and, consistent with the findings by Ferguson et al. (2014a) and Ferguson et al. (2014b), the assessment of MEP and MiBP yielded null results [36, 40, 47]. However, in contrast with the aforementioned

studies, the effects of MCPP, MBzP, MECPP, MEOHP, MEHHP, and MEHP were not significant [47].

Overall, a greater number of studies reporting significant results presented evidence that supports an inverse association with gestational age, and a positive association with preterm birth. Effect modification by infant sex in the association between phthalate exposure and gestational age appears to be a possibility as 2 of the 3 studies that conducted a stratified analysis yielded results that differed between strata [19, 34, 44]. However, the effect of phthalates between the sexes remains unclear, as different stratum-specific results were observed between the two studies [34, 44] **(**Table 2.2).

2.4.7. The Effects of Phthalates on Intrauterine Growth Restriction, Apgar Scores, Pre-eclampsia, and Gestational Diabetes Mellitus

A summary of the reported study results on the association of phthalate exposure with intrauterine growth restriction, Apgar scores, pre-eclampsia, and gestational diabetes mellitus is presented in Table 2.3. One article reported on the effects of phthalates on intrauterine growth restriction, Apgar scores, pre-eclampsia, and gestational diabetes mellitus, respectively [32, 33, 49, 56]. 3 of 4 studies identified by the current search strategy reported null associations between phthalates and the clinical outcome of interest [32, 33, 56].

The study conducted by Zhao et al. (2014), estimated phthalate exposure using maternal urinary samples collected during the third trimester, and assessed its association with cases of intrauterine growth restriction comprised of fetuses with an estimated weight below the 10^{th} centile for gestational age, as determined using sonographic measures. The research results demonstrate that the odds of intrauterine growth restriction among those exposed to MEHHP levels in the third

tertile was 5.80 (95% Cl: 1.55, 21.67) times greater compared to those exposed to levels in the first tertile [49]. Latini et al. (2003) measured DEHP and MEHP levels in cord blood samples, and assessed its association with the 1 and 5 minute Apgar test scores, but reported no significant results [56].

The study by Shapiro et al. (2015), measured MEP, MnBP, MBzP, MCPP, MEHP, MEHHP, and MEOHP in maternal urinary samples collected during the first trimester of pregnancy, and investigated the association between phthalates and gestational diabetes mellitus; no significant relationships were observed [33]. Finally, the study conducted by Nugteren et al. (2012), which examined phthalate exposure using a job-exposure matrix and assessed its association with pre-eclampsia, yielded non-significant findings [32].

Overall, as only one study assessed the effects of phthalates on each of these outcomes, the reported study findings could not be compared; consequently, the consistency of the results could not be determined (Table 2.3).

2.5. DISCUSSION

2.5.1. Summary of Evidence

A total of 24 articles that investigated the effects of phthalates on maternal and perinatal outcomes were identified and included in this systematic review. Birth weight was the most commonly reported outcome (N=16), followed by gestational age (N=10), head circumference (N=7), and preterm births (N=6). Only 1 study evaluated the effects of phthalates on intrauterine growth restriction, Apgar scores, pre-eclampsia, and gestational diabetes mellitus, respectively. No articles reporting on pregnancy-induced hypertension were identified.

Considering the effects of phthalates in general, a greater number of studies with significant findings suggest a possible association between these environmental contaminants, and a decrease in birth weight and gestational age, and an increase in head circumference and preterm births. The effect of phthalates on intrauterine growth restriction, Apgar scores, pre-eclampsia, and gestational diabetes mellitus is difficult to assess as an insufficient number of studies have investigated these outcomes. However, based on the studies identified, exposure to MEHHP was significantly associated with an increased odds of intrauterine growth restriction, whereas null findings were reported for the remaining outcomes. Overall, the impact of specific phthalates and their respective metabolites on the *a priori* selected maternal and perinatal outcomes often varied between studies; a contributing factor for the inconsistent research results reported could consist of the heterogeneous study populations and methods observed across studies.

2.5.2. Heterogeneity in Study Populations

Among the individual studies included in this review, the different eligibility criteria implemented for subject selection may yield study participants that are not comparable across studies. For instance, 2 articles reported restricting their study samples to consist only of male newborns [52, 53]. The existing literature demonstrates a possible relationship between fetal sex and perinatal outcomes. In particular, several studies indicate that males may be more susceptible to certain adverse outcomes, such as preterm births, higher birth weight, and lower Apgar scores at 5 minutes [57-59]. The research findings reported by these articles may be comparable to several other studies that conducted stratified analyses to address the possibility of effect modification according to sex of the offspring [19, 34, 35, 44, 49, 50, 55]. While most articles examined only singleton births, the study Latini et al. (2003), analyzed a sample consisting of both singletons and

twins [56]. Studies consisting of multiple births may not be comparable to those consisting solely of singleton births, as the former tends to yield a greater percentage of adverse delivery outcomes; in particular, an international study assessing populations from Canada, England and Wales, France, and the USA, reports a higher risk of preterm births, and low birth weight among women giving birth to two or more children at a time [60].

A number of other distinct population characteristics relating to high-risk pregnancies were observed among the individual studies included in this review. In particular, the study conducted by Huang et al. (2009), included women intending to have an amniocentesis completed due to older age or irregular serum test results. Studies indicate that women of older ages are more likely to experience adverse birth and pregnancy outcomes. In particular, advanced maternal age has been linked to greater odds of very preterm births, low birth weight, Apgar scores below 7 at 1 minute, and gestational diabetes mellitus [61, 62]. Additionally, to recruit a study population with more cases of preterm births, the study conducted by Weinberger et al. (2014), included study participants from an obstetric clinic consisting of high risk individuals: in particular, this study consisted of pregnant women with a history of preterm birth, pregnancy complications, congenital abnormalities, and more [44]. Overall, the different population characteristics as a result of the different eligibility criteria employed, is a source of heterogeneity that may be contributing to the inconsistent results that were observed between studies included in this systematic review.

2.5.3. Heterogeneity in Methods of Exposure Assessment

The various methods applied to estimate maternal and fetal exposures to environmental phthalates may in part, be responsible for the inconsistency of effects observed across studies. Among studies implementing biomonitoring methods to assess internal human exposures to

phthalates, most measured this chemical using maternal urinary samples [33, 34, 36-38, 40, 44-47, 49, 53]; however, others also evaluated maternal blood [50, 51, 54], cord blood [18, 35, 52, 55, 56], amniotic fluid [19], and meconium [18, 48]. The existing literature suggests that there is potential for contamination among studies measuring hydrolytic monoesters as biomarkers of exposures in biological specimens such as blood and meconium, due to the presence of enzymes that are capable of breaking down external phthalate contaminants post-sample collection [63-65]. As a result, the quantification of contaminants in addition to internal phthalate levels would yield an inaccurately higher concentration. This, however, is unlikely to affect analyses conducted using urinary samples, as they do not consist of such enzymes [21].

The use of urine as the biological matrix to assess phthalate exposure among humans has a number of advantages. In addition to the reduced risk of external contamination, high sample volume, and non-invasive method of collection, studies comparing multiple human specimens have reported higher concentrations and detection frequencies of phthalates in urine, relative to other biological specimens, such as serum, breast milk, and saliva [21, 66, 67]. For the reasons described, it may be more appropriate to use urinary phthalate metabolites as biomarkers of exposure, as opposed to other matrices.

Despite the benefits of quantifying the concentrations of phthalate metabolites in urinary samples, a challenge with the use of this exposure matrix consists of the influence of individual hydration. As urinary concentrations can be influenced by hydration, appropriate corrections must be made to ensure the validity of measurements used to estimate phthalate exposures [68]. Among the 12 included studies using maternal urinary samples for exposure assessment, the implemented normalization methods varied between the use of specific gravity [33, 36, 40, 44, 46, 47, 49], and creatinine levels [34, 37, 38, 45, 47, 53]. The correction of urinary dilution using both methods

demonstrated slight differences; in particular, the study by Meeker et al. (2009), reported consistently higher concentrations among measurements adjusted for creatinine [47]. A review summarizing the literature on the adjustment of chemical exposures using creatinine reported that creatinine elimination may be influenced by a number of factors, including age, diet, physical activity, and disease status [69]. Further research investigating a population of pregnant women reports greater within-person variability following the correction of urinary phthalate concentrations using creatinine levels, and proposes that the effect of physiological changes associated with late gestation may be responsible; the study authors additionally compared specific gravity- and creatinine-corrected DEHP, DEP, DnBP, and BBzP concentrations, and reported higher intra-class correlation coefficients among the former [70]. While these study findings suggest that specific gravity may provide a better basis to correct for variability in urinary dilution, more research is needed to understand this issue among pregnant women throughout gestation.

In addition to assessing the effects of phthalates using biological specimens, few studies also evaluated indirect measures of the exposure. In particular, two studies employed a JEM which evaluates the impact of phthalates as a broad category of chemically related compounds [32, 39]. Evaluating the effects of phthalates in general assumes that all phthalates have similar toxicities. This assumption may not be reasonable as research results reported by experimental studies suggests that the potency of phthalates may be dependent on the length of the chemical side chain, and that greater bioactivity may be observed among monoesters in comparison to parent diesters [71-73]. Therefore, investigating the general effects of phthalates in relation to adverse maternal and perinatal outcomes may yield an attenuated measure of association.

The frequency of exposure assessment is an essential factor to consider due to concerns associated with temporal consistency. As the majority of studies included in this review only

conducted a single assessment of the exposure, there is potential for non-differential exposure misclassification [18, 19, 32-35, 37-39, 44-56]. Research conducted on this potential toxicant has demonstrated that phthalates are non-persistent chemicals, and that the collection of urinary measurements at multiple time points during pregnancy has yielded concentrations with low to moderate reproducibility [22, 74, 75]. Specifically, a prospective cohort study comparing the urinary concentrations of 8 phthalate metabolites (MEP, MBzP, MiBP, MECPP, MEHHP, MEOHP, and MEHP) measured multiple times during pregnancy, reported intra-class correlation coefficients that ranged from 0.08 to 0.50 [75]. A possible explanation for the variability of urinary phthalate metabolite concentrations over time may be related to the dependence of these chemicals on factors relating to lifestyle and dietary habits. In particular, a biomonitoring study evaluating the changes in urinary phthalate metabolite concentrations over a 48 hour fasting period reported a decrease in concentrations of high molecular weight (HMW) phthalates, and fluctuations in concentrations of low molecular weight (LMW) phthalates; the study findings indicate that dietary sources may be largely responsible for HMW phthalate exposures, and that other sources may be contributing to LMW phthalate exposures [12]. Overall, the evidence presented in the existing literature suggests that multiple assessments of phthalates may be essential to acquire a more representative estimate of exposure during pregnancy.

Investigations into critical exposure time windows are important as the fetus may be more sensitive to the potential adverse effects of environmental phthalates during certain time periods during gestation relative to others. This was demonstrated by Ferguson et al. (2014b), who collected multiple urinary samples to assess the relationship between phthalate exposure and preterm births: the study authors reported an increased odds of placental preterm births following exposure to MECPP between 4.71 and 16.1 gestational weeks, and an elevated odds of

spontaneous preterm births following exposure to ∑DEHP, MECPP, MBzP, and MnBP between 22.9 and 29.3 gestational weeks [36]. Comparison of results in different trimesters, either within the same study or across different studies, can provide valuable information regarding time windows of vulnerability in relation to phthalate exposure. As the timing of exposure may influence the effects of environmental phthalates on adverse clinical outcomes, the use of phthalate measurements from samples collected at various times between participants (non-time-specific measurements) to estimate gestational exposure may yield less precise exposure estimates; as a consequence, the observed results may be biased towards the null hypothesis of no effect.

Overall, acquiring a reliable and valid measure of human phthalate exposures may be difficult as methods of exposure assessment have been linked to a number of challenges. Due to the lower risk of contamination, and the higher detection rates or greater concentration yields, the use of multiple urinary samples to determine the effects of phthalates may produce more accurate estimates, and additionally elucidate vulnerable periods of exposures. As a consequence of the differences in analytical methods used to characterize phthalate exposure, variability is introduced between studies; this may be partially responsible for the contradictory findings reported across studies.

2.5.4. Heterogeneity in Methods of Statistical Analysis

Additional sources of variability could be introduced through selected methods of statistical analyses. Among studies included in this review, several studies reported replacing concentrations below the LOD with "LOD/2" or "LOD/√2". Moreover, the covariates adjusted for in several articles were either non-exhaustive or unclear. The studies by Huang et al. (2014), and Su et al. (2014), only accounted for gestational age in their investigations into the effects of

phthalates on birth weight and head circumference. Unadjusted results were extracted from Huang et al. (2009), Burdorf et al. (2011), Araki et al. (2014), and Brucker-Davis et al. (2010). Additionally, the covariates adjusted for by Latini et al., (2003) were unclear, if any. This in turn raises concern for the potential of residual confounding, as the reported results may be influenced by risk factors that were not considered in the study.

2.5.5. Strengths and Limitations

A major strength among the included studies is the high degree of consistency of the research results reported within studies. The study conducted by Zhang et al. (2009), reported a positive exposure-response relationship between MEHP and DBP or MBP with LBW when using estimated exposure concentrations that were measured in both meconium and cord blood samples. These results demonstrate that the study findings were consistent regardless of the biological specimens selected for exposure assessment. The study by Ferguson et al. (2014a) reported greater odds of preterm birth and spontaneous preterm birth for each log-unit increase in MEHP, MECPP, and ∑DEHP; in support of these findings, positive exposure-response relationships were also observed when the exposure was categorized into quartiles. The study by Xie et al. (2015), assessed the effect of MEHP and MBP on both the odds of LBW and the change in birth weight, and yielded results that supported an inverse association between phthalates and birth weight. Moreover, the studies by Huang et al. (2014), and Adibi et al. (2009), exhibited within-study consistency in the results reported for the association of phthalate exposure with gestational age and preterm births, although between-study consistency was lacking. These studies demonstrate that the effects of phthalates did not differ when evaluating the outcome or exposure on different scales (continuous or categorical) within the same study.

Of equal importance, the limitations associated with this systematic review and the included studies should be considered when interpreting the findings of this review. In addition to reporting on the effects of individual phthalates, the effects of phthalates as a group of chemically related compounds were also examined in this systematic review. As previously discussed, summarizing the general effects of phthalates may not be reasonable as the toxicities may vary among different phthalate compounds [71-73]. While an overall assessment of individual phthalates would have been ideal, it was not feasible due to the lack of studies being replicated that investigated the same compounds, or the conflicting results reported between studies. Therefore, general trends for the effects of phthalates on maternal and perinatal outcomes were identified for exploratory purposes; this in turn may contribute knowledge that may be informative in the planning of prospective studies.

Indeed, the potential for human error is elevated, as duplicate screening, data extraction, and quality assessments were not implemented for the entire conduct of this review. This was partially addressed as a random sample of articles identified during title and abstract screening (N=80), and full-text screening (N=10) were independently verified by a second reviewer. In addition to this, 5 articles were randomly selected and checked for data extraction, and independently appraised for quality assessment by a second study author. Of these five studies, the quality scores assigned by the two reviewers were between plus or minus 1 to 2 points for the four studies that were either of a case control or cohort design; as the fifth study was a cross-sectional study, a quality score was not assigned. Finally, although heterogeneity among included studies presents the current systematic review with difficulties in assessing the risks associated with exposures to specific phthalates during pregnancy, it may also result in individual study weaknesses as certain methods of exposure assessment may be preferable to others.

2.6. CONCLUSION

Although inconsistent results were observed across the studies included in this systematic review, the majority of the results that were statistically significant suggest a possible association of phthalate exposure with a decrease in birth weight and gestational age, and an increase in head circumference and preterm birth. Presently, there is insufficient evidence to definitively conclude on the effects of phthalates on maternal and perinatal health, due to the inconsistent results reported between studies and the paucity of research investigating select outcomes or phthalate compounds. Therefore, before we can better understand the risks associated with this environmental toxicant, more research is needed. Currently, the existing literature appears to present evidence supporting the use of urine as the preferred exposure matrix for phthalate exposure assessment due to the lower risk of contamination, and the higher detection rates. To acquire a more accurate estimate of phthalate exposure, the collection of multiple urinary samples may be essential to counteract the low to moderate reproducibility of urinary phthalate levels over time. The use of time-specific measurements, such as during either early-, mid-, or late-pregnancy, may be of crucial importance to investigations into critical exposure windows. Although this review is suggestive of an association between exposure to phthalates during pregnancy and adverse maternal and perinatal health outcomes, additional research is needed before firm conclusions can be drawn.

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2.8. APPENDIX

2.8.1. Appendix 1: Search Strategy

The search was initially developed using MEDLINE, and then adapted for use in other electronic databases. The searches performed for the current systematic review are presented below.

MEDLINE

1. Dibutyl phthalate/
2. Diethylhexyl phthalate/
3. Phthalate*.tw.
4. 1 or 2 or 3
5. Pregnancy/
6. Gestat*.tw.
7. Pregnan*.tw.
8. Infant, newborn/
9. Infant/
10. Newborn*.tw.
11. Infant*.tw.
12. Fetus/
13. Fetus.tw.
14. Fetal.tw.
15. Pregnancy complications/
16. Pregnancy outcome/
17. (Pregnancy adj1 complication*).tw.

18. (Pregnancy adj1 outcome*).tw.
19. (Birth adj1 outcome*).tw.
20. 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19
21. 4 and 20
22. Limit 21 to humans

EMBASE

1. Phthalic acid dibutyl ester/
2. "phthalic acid bis(2 ethylhexyl) ester"/
3. Phthalate*.tw.
4. 1 or 2 or 3
5. Pregnancy/
6. Pregnan*.tw.
7. Gestat*.tw.
8. Infant/
9. Newborn/
10. Infant*.tw.
11. Newborn*.tw.
12. Fetus/
13. Fetus.tw.
14. Fetal.tw.
15. Pregnancy complication/
16. Pregnancy outcome/

17. (pregnancy adj1 complication*).tw.
18. (pregnancy adj1 outcome*).tw.
19. (birth adj1 outcome*).tw.
20. 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19
21. 4 and 20
22. Limit 21 to human

PUBMED

1. Dibutyl phthalate[MeSH Terms]
2. Diethylhexyl phthalate[MeSH Terms]
3. Phthalate*[Text Word]
4. 1 OR 2 OR 3
5. Pregnancy[MeSH Terms]
6. Pregnan*[Text Word]
7. Gestat*[Text Word]
8. Infant, newborn[MeSH Terms]
9. Infant[MeSH Terms]
10. Newborn*[Text Word]
11. Infant*[Text Word]
12. Fetus[MeSH Terms]
13. Fetus[Text Word]
14. Fetal[Text Word]
15. Pregnancy complications[MeSH Terms]

16. Pregnancy outcome[MeSH Terms]
17. Pregnancy complication*[Text Word]
18. Pregnancy outcome*[Text Word]
19. Birth outcome*[Text Word]
20. 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18
21. 4 AND 20
22. Limit 21 to humans

CINAHL

1. TI phthalate*
2. AB phthalate*
3. 1 OR 2
4. (MH "Pregnancy")
5. TI gestat*
6. AB gestat*
7. TI pregnan*
8. AB pregnan*
9. (MH "infant, newborn")
10. (MH "infant")
11. TI newborn*
12. AB newborn*
13. TI infant*

14. AB infant*
15. (MH "Fetus")
16. TI fetus
17. AB fetus
18. TI fetal
19. AB fetal
20. (MH "pregnancy complications")
21. (MH "pregnancy outcomes")
22. TI pregnancy complication*
23. AB pregnancy complication*
24. TI pregnancy outcome*
25. AB pregnancy outcome*
26. TI birth outcome*
27. AB birth outcome*
28. 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27
29. 3 AND 28

POPLINE

1. Phthalate*
2. Pregnan*
3. Gestat*
4. Newborn*

5. Infant*
6. Fetus
7. Fetal
8. “pregnancy complication*”
9. “pregnancy outcome*”
10. “birth outcome*”
11. 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10
12. 1 AND 11

2.8.2. Appendix 2: Summary of Study Characteristics and Reported Results

A summary of the study characteristics, reported results, and quality scores are presented in the tables below. Table 2.1 displays results reported on measures of infant growth (BW and HC), Table 2.2 displays results reported on measures of pregnancy duration (GA and PB), and Table 2.3 displays results reported on the remaining maternal and perinatal outcomes (AS, IUGR, PE, GDM).

Table 2.1. Summary of Study Characteristics and Results Reported on Measures of Infant Growth (BW & HC).

1st author, publication date & location	Study Design & Sample	Exposure	Outcome	Adjusted Covariates	Results	Quality Score
Su, 2014 (Taiwan)	• Cohort study • Time of recruitment: November 2001 • N_{SAMPLE}: 130 • Stratified analysis by baby sex: N_{BOYS}: 61 N_{GIRLS}: 69	• MU sample collected during 3rd trimester • GM of creatinine corrected concentrations in Mg/g (95% Cl): MEHP 17.15 (14.56, 20.19) MEHHP 7.6 (5.49, 10.53) MEOHP 13.29 (10.05, 17.58) ∑DEHP 50.71 (42.28, 60.83) MnBP 66 (55.65, 78.27) MBzP 15.72 (13.79, 17.93) MMP 53.51 (44.39, 64.5) MEP 61.15 (52.26, 71.56) • ∑DEHP = Sum of MEHP, MEHHP, & MEOHP	• BW and HC obtained from records.	• GA	• No significant association between PTH metabolites and change in BW. • No significant association between PTH metabolites and change in HC in unstratified analyses and in girls. • Change in head circumference (cm) expressed as z-scores for each unit increase in PTH (μg/L) in boys: MEHHP (0.004; SE: 0.002) p: 0.017 MEOHP (0.002; SE: 0.001) p: 0.020 ∑DEHP (0.001; SE: 0.0004) p: 0.034 • Authors selected p<0.00625 for statistical significance	12
Suzuki, 2010 (Japan)	• Cohort study • Time of recruitment: 2005-2008 • N_{SAMPLE}: 149 • Sample consisted of women with no obvious clinical symptoms • Mean maternal age (SD), years: 31.9 (4.5)	• MU sample collected between 9th and 40th gestational weeks • GM of creatinine corrected concentrations in Mg/g Cr: MMP (9.14) MEP (9.76) MnBP (51.6) MBzP (5.62) MEHP (5.45) MEHHP (10.6) MEOHP (11.3) MINP (0.031) MnOP (0.025)	• BW and HC determined by nurse using standard procedures.	• Maternal age • BMI • GA • Parity • Newborn sex • Smoking	• No significant association between the sum of log-PTH metabolites and change in BW. • No significant association between the sum of log-PTH metabolites and change in HC.	14

		• Concentration <LOD replaced by LOD/2				
Wolff, 2008 (USA)	• Cohort study • Time of recruitment: March 1998 - March 2002 • N_{SAMPLE}: 382 • Maternal age group (%), years: <20 (35); 20-29 (44); ≥30 (21) • Maternal race/ethnicity (%): White (21); Black (28); Hispanic (50); Other (1)	• MU sample collected mostly during 3rd trimester • Median concentrations in µg/L (25th, 75th percentile): MECPP 35 (16, 70) MEHHP 20 (9.5, 39) MEOHP 17 (8.3, 36) MEHP 6.0 (2.9, 14) MBzP 22 (8.8, 50) MCPP 3.2 (1.8, 6.0) MiBP 6.2 (2.7, 12) MBP 36 (16, 75) MEP 380 (137, 1010) MMP 1.6 (1, 3.8)	• BW and HC obtained from hospital perinatal database	• Race • Infant sex • GA • Ln-creatinine • Smoking • Education • Marital status • Pre-pregnancy BMI	• No significant association between PTH metabolites and change in BW • Change in HC (cm) for each ln-unit increase in PTH (µg/L): ∑LMW (0.13; 95% Cl: 0.01, 0.24) MEP (0.12; 95% Cl: 0.01, 0.23)	14
Philippat, 2012 (France)	• Nested case-control study • Time of recruitment:2002-2006 • N_{CASE}: 72 (males with undescended testis or hypospadias) • $N_{CONTROL}$: 215 (males with no genitalia congenital malformations) • Mean maternal age (5th, 95th percentile), years: 29.3 (22, 38)	• MU sample collected between 6 and 30 gestational weeks • Median concentrations in µg/L (5th, 95th percentiles): MEP 110.22 (24.9, 983.4) MnBP 48.1 (7.6, 398.0) MiBP 45.9 (10.9, 219.0) MBzP 17.7 (2.0, 116.6) MCPP 2.2 (0.4, 10.0) MEHP 7.1 (0.8, 40.7) MEHHP 32.3 (4.6, 147.0) MEOHP 25.0 (3.6, 112.0) MECPP 43.8 (11.6, 183.0) MCOP 2.7 (0.5, 17.2) MCNP 1.7 (0.6, 11.7) • ∑DEHP = Sum of MEHP, MEHHP, MEOHP & MECPP • Concentration <LOD replaced by LOD/√2	• BW and HC obtained from maternity records	• Gestational duration • Pre-pregnancy weight • Pre-pregnancy height • Smoking • Education • Parity • Recruitment center • Creatinine • Mode of delivery (HC model only)	• Change in BW (g) among those with PTH levels in T2 and T3 compared to T1 (µg/L): MCPP T1: 0 T2 (-198; 95% Cl: -343, -52) T3 (-95; 95% Cl: -243, 52) $p_{heterogeneity}$: 0.03; p_{trend}: 0.73 MECPP T1: 0 T2: -141 (95% Cl: -277, -5) T3: -20 (95% Cl: -162, 121) $p_{heterogeneity}$: 0.08; p_{trend}: 0.59 • No significant association between PTH metabolites and change in HC	16
Zhao, 2014 (China)	• Case-control study • Time of recruitment: March 2012 - January 2013 • N_{CASE}: 42 (fetus with intrauterine growth restriction) • $N_{CONTROL}$: 84 (2 fetus per case, and matched by maternal age)	• MU sample collected during 3rd trimester • Median concentrations in ng/mL (25th, 75th percentile): MnBP 21.6 (11.0, 37.6) MMP 8.9 (4.8, 15.9) MEHP 6.5 (1.5, 17.4) MEHHP 10.6 (3.9, 19.2) MEOHP 4.3 (1.7, 9.7) ∑DEHP 26.8 (13.6, 46.3)	• BW obtained from hospital records	• Maternal age • Education • GA • Passive smoking • Other PTH metabolites	• Change in BW (kg) for each log-10-unit increase in PTH (ng/mL) MEHHP (-0.213; 95% Cl: -0.401, -0.025) MEOHP (-0.233; 95% Cl: -0.411, -0.056) • Change in BW (kg) for each log-10-unit increase in PTH (ng/mL) in males: MEHHP (-0.393; 95% Cl: -0.740, -0.046) MEOHP (-0.434; 95% Cl: -0.762, -0.107) • No significant association between PTH metabolites and change in BW in females	16

	• Maternal morbidities (% case, control): pregnancy-induced hypertension (11.9, 7.1); pregnancy-induced diabetes (11.9, 8.3)	• ∑DEHP = Sum of MEHP, MEHHP, & MEOHP • Concentrations corrected using specific gravity levels • Concentration <LOD replaced by LOD/2				
Latini, 2003 (Italy)	• Cross-sectional study • N_{SAMPLE}: 84 newborns • Sample consisted of singletons and twins • Maternal age (range), years: 29.5 (18-42)	• CB sample collected • Mean concentrations in µg/mL (95% CI): DEHP 1.19 (0.93, 1.44) MEHP 0.52 (0.39, 0.66)	• BW	• Unknown	• No significant association between DEHP or MEHP and BW	N/A
Huang, 2009 (Taiwan)	• Cohort Study • Time of recruitment: 2005-2006 • N_{FEMALE}: 32 • N_{MALE}: 33 • Sample consisted of women intending to have amniocentesis conducted	• AF sample collected at the start of amniocentesis • Median concentrations in ng/mL (10^{th}, 90^{th} percentile) for females[a] and males[b]: MBP 85.5 (45.6, 134.6) [a] 81.3 (44.3, 127.8)[b] MEHP 24.0 (5.0, 91.1)[a] 22.1 (2.6, 100.6)[b] MEP ND (ND, 3.9)[a] ND (ND, 4.4)[b] MBzP ND (ND, 84.1)[a] ND (ND, 87.9)[b] MMP ND (ND, ND)[a] ND (ND, ND)[b]	• BW determined by same pediatrician and assistant	• None	• Comparing BW (g) between low-[a] and high-[b] PTH levels (ng/mL) measured in AF in females. PTH levels are categorized based on median concentrations. MBP (2810 ± 439)[a] vs. (3172± 398)[b] (p: 0.031) • No significant association between MBP or MEHP and BW in males.	9
Burdorf, 2011 (Netherlands)	• Cohort study • Recruited those with dates of delivery between April 2002 – January 2006 • N_{SAMPLE}: 6302 • Maternal age group (%), years: <25 (17), 25-30 (29), 30-35 (40), ≥35 (16)	• PTH exposure determined using a JEM • JEM applied the knowledge of occupational hygienists to link job titles to chemical exposures	• BW obtained from midwife/ hospital records. Decreased BW defined as <3000g.	• None	• No significant association between PTH & decreased birth weight	10
Jia, 2015 (Japan)	• Cohort Study • Time of recruitment: July 2002 – October 2005 • N_{SAMPLE}: 318	• MB sample collected between 23 gestational weeks and one week following birth (postnatal samples were not analyzed) • Mean concentration in nmol/mL (SD): MEHP: 0.049 (0.040)	• BW and HC obtained from medical records	• Maternal age • Height • Pre-pregnancy weight • Parity • Smoking	• No significant association between MEHP and BW in both unstratified and stratified analyses by baby sex • No significant association between MEHP and HC in both unstratified and stratified analyses by baby sex	15

	• Maternal age group (%), years: <30 (48.1); ≥30 (51.9)			• Alcohol use • Annual household income • GA • Blood sampling period • Delivery type (HC models only) • Infant sex (Unstratified models only)		
Brucker-Davis, 2010 (France)	• Cross-sectional study • Time of recruitment: 2002-2005 • N_{SAMPLE}: 86 • Median maternal age (range), years: 29.55 (18-40)	• CB sample collected • Median concentration in ng/mL; (25^{th}, 75^{th} percentile): DBP: 47.6 (32.4, 66) MBP: 2.9 (1.2, 4.9)	• BW and HC	• None	• No significant correlation between MBP & BW • Correlation between PTH (ng/mL) and HC (cm): MBP (0.43) p:0.005	N/A
Zhang, 2009 (China)	• Nested case-control study • Time of recruitment: 2005-2006 • N_{CASE}: 88 (term infants with birth weight < 2500g) • $N_{CONTROL}$: 113 (term infants with birth weight ≥ 2500g) • Mean maternal age (SD), years: Case: 28.4 (3.9); Control: 28.1 (3.3)	• CB collected after delivery • ME collected ≤ 48hrs after delivery • Median CB concentrations in mg/L (25^{th}, 75^{th} percentile) for cases[a] and controls[b]: DEP: 1.6 (1.3, 2.0)[a]; 2.0 (0.9, 2.4)[b] DBP: 2.7 (2.2, 3.0)[a]; 1.8 (1.2, 2.7)[b] DEHP: 0.6 (0.3, 1.0)[a]; 0.5 (0.1, 0.9)[b] MBP: ND MEHP: 2.5 (1.6, 3.4)[a]; 1.1 (0.9, 1.7)[b] • Median ME concentrations in mg/g; (25^{th}, 75^{th} percentile) for cases[a] and controls[b]: DEP, DBP, DEHP: ND MBP: 2.2 (1.6, 3.6)[a]; 1.7 (1.2, 2.4)[b] MEHP: 5.5 (3.4, 9.3)[a]; 2.9 (1.8, 4.4)[b] • Concentration <LOD replaced by LOD/√2	• LBW defined as BW < 2500g	• GA • Smoking at home • Socioeconomic level • Pre-pregnancy BMI • Other PTH	• Odds of LBW among infants with PTH measured in CB in 2^{nd}, 3^{rd}, and 4^{th} compared to 1^{st} quartile (mg/L) DBP (Q1: reference) p: 0.008 Q2: (OR: 0.54; 95% CI: 0.45, 1.47) Q3: (OR: 2.69; 95% CI: 1.30, 4.74) Q4: (OR: 3.54; 95% CI: 1.54, 6.15) MEHP (Q1: reference) p: 0.05 Q2: (OR: 0.53, 95% CI: 0.31, 1.61) Q3: (OR: 1.22; 95% CI: 0.90, 2.52) Q4: (OR: 2.05; 95% CI: 1.17, 3.70) • Odds of LBW among infants with PTH measured in ME in 2^{nd}, 3^{rd}, and 4^{th} compared to 1^{st} quartile (mg/g) MBP (Q1: reference) p: 0.000 Q2: (OR:1.58; 95% CI: 1.08, 2.46) Q3: (OR: 2.84; 95% CI: 1.19, 4.82) Q4: (OR: 4.68; 95% CI: 2.14, 6.85) MEHP (Q1: reference) p: 0.04 Q2: (OR: 1.12; 95% CI: 0.89, 2.03) Q3: (OR: 2.89; 95% CI: 1.19, 5.02) Q4: (OR: 3.23; 95% CI: 1.31, 5.94)	18
Xie, 2015 (China)	• Case-control study • Time of case recruitment: January 2011 - December 2011	• ME collected ≤ 48hrs after birth • Median concentrations in µg/g (25^{th}, 7^{th} percentile) for cases[a] and controls[b]: MnBP: 119.25 (93.2, 147.3)[a]; 101.70 (80.7, 171.9)[b] MEHP: 238.07 (158.50, 447.15)[a];	• BW obtained from hospital records. LBW defined as <2500g	• GA • Pregnancy complications • Pregnancy weight gain	• Change in BW (g) for each unit increase in PTH (µg/g): MEHP (-0.62; 95% CI: -1.09, -0.13) MnBP (-0.92; 95% CI: -2.09, -0.03) • Odds of LBW among infants with high (>median) vs. low (≤median) PTH exposure:	17

	• N_{CASE}: 74 (term infants with birth weight <2500g) • $N_{CONTROL}$: 111 (term infants with normal BW) • Mean maternal age (SD), years: Case: 28.0 (2.9); Control: 28.1 (2.3)	163.80 (90.91, 226.20)[b] • Concentration <LOD replaced by LOD/2			MEHP (OR: 4.4; 95% Cl: 2.0, 9.3) MnBP (OR: 2.4; 95% Cl: 1.1, 4.8)	
De Cock, 2016 (Netherlands)	• Cohort study • Time of recruitment: January 2011 – January 2013 • N_{SAMPLE}: 91 • Mean maternal age (SD), years: 30.9 (4.5)	• CB sample collected after delivery • Mean concentrations in ng/mL (range): MECPP: 0.31 (0.11-1.00) MEHHP: 0.33 (0.10-1.00) MEOHP: 0.29 (0.12-0.87) • Concentration <LOD replaced by LOD/√2	• BW obtained from birth records and determined by midwife/nurse using weighing scale.	• GA • Maternal BMI • Maternal height • Maternal age • Parity • Paternal BMI • Paternal height • Education • Fish intake	• Change in BW (g) among male infants with PTH exposure in tertile 2 and 3 compared to 1. MECPP (T1: reference) T2: (-287.7; 95% Cl: -755.26, 179.87) p:0.219 T3: (-504.3; 95% Cl: -974.83, -33.84) p:0.037 MEHHP (T1: reference) T2: (436.4; 95% Cl: 65.22, 807.67) p:0.023 T3: (273.8; 95% Cl: -93.98, 641.61) p:0.139 • No significant association between PTH metabolites and BW in unstratified analyses and in girls	16
Huang, 2014 (China)	• Cross-sectional study • Recruited those with dates of delivery between October 2011 – September 2012 • N_{SAMPLE}: 207 • Mean maternal age (SD): 28.06 (3.28)	• CB sample collected at ≤ 10 minutes of delivery • Mean concentrations in µg/L (25^{th}, 75^{th} percentile): DMP 6.69 (ND,ND) DEP 8.99 (ND, 0.73) DMEP 8.11 (ND, 7.08) DBP 68.14 (19.61, 72.03) DEEP 32.96 (ND, 9.17) DIBP 31.34 (11.08, 26.92) DPP 26.64 (ND, ND) BMPP 11.81 (ND, ND) DBEP 53.51 (ND, 4.64) DCHP 125.02 (5.83, 38.46) DNHP 8.08 (ND, 0.29) BBP 22.55 (ND, 0.99) DEHP 187.16 (9.18, 78.46) DNOP 27.66 (ND, 0.44) DNP 13.42 (0.23, 2.13) • If detection rate >60%, concentration <LOD replaced by LOD/√2	• BW and HC obtained from hospital perinatal database	• GA	• Change in BW (g) among females with detectable vs. non-detectable PTH level: DEEP (-143; 95% Cl: -273, -14) • Change in HC (mm) among females with detectable vs. non-detectable PTH level: DNHP (-11.79; 95% Cl:-22.05, -1.52) • No significant association between PTH metabolites and BW or HC among males	N/A
Araki, 2014 (Japan)	• Cohort study • Time of recruitment: July 2002 – October 2005	• MB sample collected between 23-35 gestational weeks • Median concentrations in ng/mL (Interquartile Range):	• BW obtained from medical records	• None	• No significant correlation between MEHP and BW	11

	• N_{SAMPLE}: 202 • Mean maternal age (SD), years: 29.8 (4.9)	MEHP 10.4 (5.88-15.3)				
Lenters, 2016 (Greenland, Poland, and Ukraine)	• Cohort study • Time of recruitment: June 2002 – May 2004 • N_{SAMPLE}: 1250 • Maternal age group (n), years: 18-24 (546), 25-29 (393), 30-34 (213), 35-45 (103) • Country (n): Greenland (513), Poland (180), Ukraine (557)	• MB sample collected between 23-33 gestational weeks (median between countries) • Median concentrations in ng/mL (5th, 95th percentile) in participants from Greenland[a], Poland[b], and Ukraine[c]: MEHHP: 0.68 (0.24, 2.36) [a] 0.41 (0.17, 1.07) [b] 0.46 (0.11, 2.94) [c] MEOHP: 0.12 (0.05, 0.28) [a] 0.10 (0.04, 0.23) [b] 0.11 (0.04, 0.38) [c] MECPP: 0.58 (0.25, 2.00) [a] 0.85 (0.34, 2.22) [b] 0.93 (0.35, 4.00) [c] MHiNP: 0.24 (0.07, 0.79) [a] 0.11 (0.02, 0.54) [b] 0.04 (<LOD, 0.48) [c] MOiNP: 0.02 (<LOD, 0.07) [a] 0.02 (0.005, 0.06) [b] 0.01 (<LOD, 0.24) [c] MCiOP: 0.23 (0.07, 3.25) [a] 0.25 (0.11, 0.88) [b] 0.21 (0.05, 4.57) [c] • Concentration <LOD replaced using log-normal probability distribution	• Term BW defined as birth ≥37 gestational weeks. • BW obtained from hospital records.	Multiple-exposure model: • ln-MEHHP • ln-MOiNP • ln-PFOA • ln-p'p'-DDE • Population • Maternal age • BMI • Parity • GA • Infant sex • Maternal height • Alcohol use • Cotinine • Vitamin D	• Change in term BW (g) for each 2-SD increase in ln-PTH (ng/mL): MEHHP (-70.22; 95% Cl: -117.59, -22.85)	14

- **Abbreviations:** GA: gestational age; PB: preterm birth; pPROM: preterm premature rupture of membranes; BW: birth weight; LBW: low birth weight; HC: head circumference; IUGR: intrauterine growth restriction; PE: pre-eclampsia; PIH: pregnancy-induced hypertension; GDM: gestational diabetes mellitus; MU: maternal urine; CB: cord blood; MB: maternal blood; AF: amniotic fluid; ME: meconium; JEM: job-exposure matrix; PTH: phthalate; DEHP: di(2-ethyl-hexyl) PTH; MEHP: mono-(2-ethyl)-hexyl PTH; MEHHP: mono-2-ethyl-5-hydroxyhexyl PTH; MEOHP: mono-2-ethyl-5-oxohexyl PTH; MECPP: mono-(2-ethyl-5-carboxypentyl) PTH; BBP: benzyl butyl PTH; MBzP: mono-benzyl PTH; DBP: dibutyl PTH; MBP: mono-butyl PTH; MnBP: mono-n-butyl PTH, DIBP: diisobutyl PTH; MiBP: mono-isobutyl PTH; MNP: mono-3-methyl-5-dimethyl PTH; DEP: diethyl PTH; MEP: mono-ethyl PTH; DiNP: di-iso-nonyl PTH; MiNP: mono-iso-nonyl PTH; MHiNP: mono(4-methyl-7-hydroxyloctyl) PTH; MOiNP: mono(4-methyl-7-oxo-octyl) PTH; MCiOP: mono(4-methyl-7-carboxyheptyl) PTH; MCOP: monocarboxyisooctyl PTH; DMP: dimethyl PTH; MMP: monomethyl PTH; DCHP: dicyclohexyl PTH; MCHP: monocyclohexyl PTH; DNOP: di-n-octyl PTH; MnOP: mono-n-octyl PTH; MCPP: mono-(3-carboxypropyl) PTH; MCNP: monocarboxyisononyl PTH; MDP: mono-3-methyl-7-methyloctyl PTH; DEEP: bis (2-ethoxyethyl) PTH; DPP: diamyl PTH; BMPP: bis (4-methyl-2-pentyl) PTH; DNHP: dihexyl PTH; DMEP: bis (2-methyoxyethyl) PTH; DBEP: bis(2-n-butoxyethyl) PTH; DNP: dinonyl PTH; LOD: limit of detection; ND: not detected; SD: standard deviation; 95% Cl: 95% confidence interval; p: p-value; Q: quartile; T: tertile
- **Note:** covariates listed by study were adjusted for in all analyses reported, unless otherwise specified. Exposure concentrations reported for Lenters, 2016 were acquired from the available supplementary data.

Table 2.2. Summary of Study Characteristics and Results Reported on Measures of Pregnancy Duration (GA & PB).

1st author, publication date & location	Study Design & Sample	Exposure	Outcome	Adjusted Covariates	Results	Quality Score
Adibi, 2009 (USA)	• Cohort study • Time of recruitment: 2000-2004 • N_{SAMPLE}: 283 • Mean maternal age (SD), years: 30.2 (6.0) • Maternal race (%): White (84); Hispanic (9); Other (6)	• MU sample collected at start of 3rd trimester (on average) • GM concentrations in ng/mL (95% Cl): MEHP 3.6 (3.1, 4.3), MEOHP 10.9 (9.3, 12.6) MEHHP 11.9 (10.1, 13.9) • Concentration <LOD replaced by LOD/√2	• GA obtained from birth records and determined by ultrasound, infant examination, and reported dates. PB defined as birth <37 gestational weeks	<u>GA model:</u> • Geographic center • Education • Job-related stress • Non-gestational diabetes • Thyroid disorders • Fibroids • Parity • Creatinine <u>PB model:</u> • High blood pressure • Non-gestational diabetes • Creatinine	• Change in GA (weeks) for each log-unit increase in PTH (ng/mL): MEHP (0.16; 95% Cl: 0.02, 0.30) MEOHP (0.19; 95% Cl: 0.03, 0.35) MEHHP (0.16; 95% Cl: 0.01, 0.31) • Odds of PB for each log-unit increase in PTH (ng/mL): MEHP: (OR: 0.5; 95% Cl: 0.3, 0.9) MEOHP (OR: 0.4; 95% Cl: 0.2, 0.9) MEHHP (OR: 0.5; 95% Cl: 0.3, 0.9)	16
Ferguson, 2014a (USA)	• Nested case-control study • Time of recruitment: 2006-2008 • N_{CASE}: 130 (women with preterm births) • $N_{CONTROL}$: 352 (women with term births) • Subgroup analysis: spontaneous preterm births (N_{CASE}: 57) • Median maternal age (25th-75th percentile), years: 32.7 (29.0-35.7) • Maternal race/ethnicity (%): White (58.5); African American (16.0); Other (25.5)	• 4 MU sample collected at (median gestational weeks): Visit 1 (9.71), visit 2 (17.9), visit 3 (26.0), & visit 4 (35.1) • 4th sample not included in analysis • GM concentrations in µg/L (25th, 75th percentile): MEHP 10.5 (5.51, 18.1) MEHHP 31.9 (17.2, 55.3) MEOHP 16.9 (9.33, 29.7) MECPP 41.3 (20.6, 73.8) ∑DEHP 36.5 (20.2, 63.2) MBzP 6.47 (3.25, 11.6) MnBP 16.7 (10.5, 23.2) MiBP 6.75 (4.48, 10.3) MEP 134 (55.7, 276) MCPP 2.02 (1.09, 3.09) • Concentrations corrected using specific gravity • ∑DEHP = Sum of MEHP, MEHHP, MEOHP, MECPP • Concentrations <LOD with no value were replaced by LOD/√2	• PB defined as birth < 37 gestational weeks. GA determined using ultrasonography • Spontaneous PB consisted of spontaneous preterm labour or pPROM	• Average specific gravity • Maternal age • Race/ethnicity • Education • Health insurance provider (non-DEHP metabolites models only)	• Odds of PB for each ln-unit increase in average PTH (µg/L) MEHP (OR: 1.34; 95% Cl: 1.07, 1.68) MECPP (OR: 1.40; 95% Cl: 1.13, 1.74) ∑DEHP (OR: 1.33; 95% Cl: 1.04, 1.70) • Odds of spontaneous PB for each ln-unit increase in average PTH (µg/L) MEHP (OR: 1.65; 95% Cl: 1.20, 2.26) MEOHP (OR: 1.47; 95% Cl: 1.04, 2.08) MECPP (OR: 1.56; 95% Cl: 1.15, 2.13) ∑DEHP (OR: 1.63; 95% Cl: 1.15, 2.31) MBzP (OR: 1.41; 95% Cl: 1.02, 1.95) MnBP (OR: 1.49; 95% Cl: 1.08, 2.06) MCPP (OR: 1.36; 95% Cl: 1.02, 1.81) • Odds of PB among those exposed to PTH levels in quartile 2, 3, and 4 compared to 1 MEHP (Trend P: 0.01) Q1 (OR: 1.00); Q2 (OR: 1.04); Q3 (OR: 1.81); Q4 (OR: 2.09) MECPP (Trend P: 0.003) Q1 (OR: 1.00); Q2 (OR: 1.02); Q3 (OR: 1.75); Q4 (OR: 2.39) ∑DEHP (Trend P: 0.02) Q1 (OR: 1.00); Q2 (OR: 1.23); Q3 (OR: 1.55); Q4 (OR: 2.17) MnBP (Trend P: 0.03)	18

					Q1 (OR: 1.00); Q2 (OR: 0.82); Q3 (OR: 1.57); Q4 (OR: 2.25) • Odds of spontaneous PB among those exposed to PTH levels in quartile 2, 3, and 4 compared to 1 MEHP (Trend P: 0.005) Q1 (OR: 1.00); Q2 (OR: 1.37); Q3 (OR: 2.05); Q4 (OR: 3.74) MECPP (Trend P: 0.001) Q1 (OR: 1.00); Q2 (OR: 2.19); Q3 (OR: 3.77); Q4 (OR: 5.23) ∑DEHP (Trend P: 0.01) Q1 (OR: 1.00); Q2 (OR: 2.05); Q3 (OR: 3.18); Q4 (OR: 3.69) MnBP (Trend P: 0.02) Q1 (OR: 1.00); Q2 (OR: 1.12); Q3 (OR: 2.35); Q4 (OR: 3.52)	
Ferguson, 2014b (USA)	• Nested case-control study • Time of recruitment: 2006-2008 • N_{CASE}: 130 (women with preterm births) • $N_{CONTROL}$: 352 (women with term births) • Subgroup analysis: 1. Spontaneous preterm births (N_{CASE}: 52) 2. Placental preterm births (N_{CASE}: 35)	• 4 MU sample collected at (median gestational weeks): Visit 1 (9.71), visit 2 (17.9); visit 3 (26.0), visit 4 (35.1) • GM concentrations in µg/L[a] or µmol/L[b] (SD) at visit 1 to 4: MEHP[a]: 12.7 (3.78), 11.3 (3.33), 9.83 (3.27), 9.94 (3.44) MEHHP[a]: 40.8 (3.69); 34.1 (3.13), 27.1 (3.42), 34.9 (3.37) MEOHP[a]: 20.1 (3.62), 18.2 (3.05), 15.8 (3.38), 20.1 (3.27) MECPP[a]: 51.8 (3.53), 43.0 (3.25), 38.5 (3.47), 48.7 (3.39) ∑DEHP[b]: 0.46 (3.38), 0.39 (3.00), 0.33 (3.13), 0.41 (3.17) MBzP[a]: 6.95 (3.19), 6.95 (3.05), 6.89 (2.95), 7.86 (2.97) MnBP[a]: 17.9 (2.57), 18.3 (2.62), 17.4 (2.75), 19.9 (2.33) MiBP[a]: 7.28 (2.25), 7.17 (2.33), 7.30 (2.35), 9.04 (2.21) MEP[a]: 140 (4.42), 147 (4.85), 140 (4.67), 147 (5.00) MCPP[a]: 2.27 (3.46), 2.30 (3.35), 1.95 (3.02), 2.11 (2.89) • ∑DEHP = Sum of MEHP, MEHHP, MEOHP, MECPP • Concentration <LOD replaced by LOD/√2	• PB defined as birth < 37 gestational weeks. GA determined using last menstrual period and ultrasonography • Spontaneous PB defined as PB relating to spontaneous preterm labour or pPROM • Placental PB defined as PB relating to PE or IUGR	• Specific gravity • Maternal age • Race/ethnicity • Educational • Time of sample collection (DEHP metabolite models only) • Health insurance category (non-DEHP metabolite models only)	• Odds of PB for each ln-unit increase in PTH (µg/L) Visit 1 MECPP (OR: 1.25; 95% Cl: 1.06, 1.48) MCPP (OR: 1.19; 95% Cl: 1.01, 1.41) Visit 3 MECPP (OR: 1.27; 95% Cl: 1.06, 1.52) Visit 4 MEHHP (OR: 0.77; 95% Cl: 0.60, 0.98) • Odds of placental PB for each ln-unit increase in PTH (µg/L): Visit 1: MECPP (OR: 1.46; 95% Cl: 1.10, 1.95) • Odds of spontaneous PB for each ln-unit increase in PTH (µg/L): Visit 3: MECPP (OR: 1.33; 95% Cl: 1.04, 1.70) ∑DEHP (OR: 1.33; 95% Cl: 1.02, 1.73) MBzP (OR: 1.43; 95% Cl: 1.05, 1.95) MnBP (OR: 1.45; 95% Cl: 1.08, 1.96)	19
Meeker, 2009 (Mexico)	• Nested case-control study	• MU sample collected during 3rd trimester	• PB defined as birth at < 37	• Marital status • Education	• Odds of high PTH levels (> median) among cases compared to controls	15

	• Time of recruitment: 2001-2003 • N_{CASE}: 30 (women with preterm births) • $N_{CONTROL}$: 30 (women with term births) • Median maternal age (25^{th}, 75^{th} percentile), years: Case: 27 (23, 32) Control: 27 (23, 30)	• GM concentrations in µg/L[a] or nmol/L[b] (25^{th}, 75^{th} percentile): MEHP[a] 1.9 (0.60, 4.40) MEHHP[a] 13.6 (6.20, 28.4) MEOHP[a] 10.4 (5.00, 24.5) MECPP[a] 29.7 (14.3, 53.8) MBzP[a] 2.3 (1.00, 5.20) MBP[a] 38.1 (21.3, 74.0) MiBP[a] 1.9 (0.80, 4.10) MCPP[a] 1.1 (0.50, 2.00) MCOP[a] NC (<LOD, 1.20) MCNP[a] NC (<LOD, 1.20) MEP[a] 112 (47.1, 224) ∑DEHP[b] 0.19 (0.09, 0.38) • ∑DEHP = Sum of MEHP, MEHHP, MEOHP, MECPP • Concentration <LOD replaced by LOD/2	gestational weeks. GA determined using last menstrual period date.	• Infant sex • GA at time of sample collection	Creatinine corrected value (µg/g Cr): MnBP (OR: 5.4; 95% Cl: 1.5, 19.3) Specific gravity corrected values (µg/L): MnBP (OR: 4.5; 95% Cl: 1.2, 16.6)	
Su, 2014 (Taiwan)	• Cohort study • Time of recruitment: November 2001 • N_{SAMPLE}: 130 • Stratified analysis by baby sex: N_{BOYS}: 61 N_{GIRLS}: 69	• MU sample collected during 3^{rd} trimester • GM of creatinine corrected concentrations in Mg/g (95% Cl): MEHP 17.15 (14.56, 20.19) MEHHP 7.6 (5.49, 10.53) MEOHP 13.29 (10.05, 17.58) ∑DEHP 50.71 (42.28, 60.83) MnBP 66 (55.65, 78.27) MBzP 15.72 (13.79, 17.93) MMP 53.51 (44.39, 64.5) MEP 61.15 (52.26, 71.56) • ∑DEHP = Sum of MEHP, MEHHP, & MEOHP	• GA obtained from records	• None	• No significant association between PTH metabolites and change in GA in unstratified analyses and in girls • Change in GA (weeks) for each unit increase in PTH (µg/L) in boys: MEHHP (0.0054; SE: 0.0023) p: 0.025 MEOHP (0.0025; SE: 0.0011) p: 0.026 ∑DEHP (0.0011; SE: 0.0005) p: 0.036 • Authors selected p<0.00625 for statistical significance	12
Weinberger, 2014 (USA)	• Cohort study • N_{SAMPLE}: 72 • Stratified analysis by baby sex: N_{MALE}: 40 N_{FEMALE}: 32 • Maternal age group (%), years: 18-23 (11), 24-29 (25), 30-35 (25), 36-41 (32), >41 (7) • Maternal race (%): White (32); Black (29); Hispanic (29); Other (10)	• MU sample collected at last obstetric visit • IQR concentrations in ng/mL: MEP 309.5 MEHHP 21.9 MEOHP 17.4 MCHP 18.6 MEHP 5.8 MBP 77.8 • MMP, MnOP, MNP, & MDP not analyzed due to low concentrations • Concentrations corrected using specific gravity	• GA obtained from medical records and determined using sonographic dating or implantation date.	• Parity • Race	• Change in GA (days) for each IQR increase in PTH (ng/mL): MEHHP (-4.2; 95% Cl: -7.9, -0.4) • Change in GA (days) for each IQR increase in PTH (ng/mL) in boys: MEHHP (-5.1; 95% Cl: -9.6, -0.6) • No significant association between PTH metabolites and change in GA in girls	13

Whyatt, 2009 (USA)	• Cohort study • N_{SAMPLE}: 331 • Mean maternal age (SD), years: 25.5 (4.8) • Maternal ethnicity (%): Black (28), Dominican or other Hispanic (72)	• Personal air sample collected during 3rd trimester to assess DEHP • MU sample collected during 3rd trimester to assess DEHP metabolites • GM concentrations in μg/m^3 for DEHP and ng/mL for metabolites (95% Cl): DEHP 0.20 (0.18, 0.21) MEHP 4.8 (4.1, 5.7) MEHHP 21.3 (18.4, 24.6) MEOHP 18 (15.6, 20.8) MECPP 38.9 (34.2, 44.3) • Urinary concentrations corrected using specific gravity • Concentration <LOD replaced by LOD/2	• GA obtained from medical records. When not available, GA determined using expected delivery date	• Maternal age • Ethnicity • Pre-pregnancy weight • Pre-pregnancy height • Smoking • Prenatal asthma, diabetes, and hypertension • Planned caesarean section • Premature rupture of membranes	• Change in GA (weeks) for each 1-log-unit increase in PTH (ng/mL) MEHP (-0.15; 95% Cl: -0.26, -0.03) MEHHP (-0.18; 95% Cl: -0.31, -0.05) MEOHP (-0.17; 95% Cl: -0.30, -0.03) MECPP (-0.16; 95% Cl: -0.31, -0.01) ∑PTH (-0.18; 95% Cl: -0.32, -0.03) • No significant association between air sample DEHP and GA	14
Wolff, 2008 (USA)	• Cohort study • Time of recruitment: March 1998 - March 2002 • N_{SAMPLE}: 382 • Maternal age group (%), years: <20 (35); 20-29 (44); ≥30 (21) • Maternal race/ethnicity (%): White (21); Black (28); Hispanic (50); Other (1)	• MU sample collected mostly during 3rd trimester • Median concentrations in μg/L (25th, 75th percentile): MECPP 35 (16, 70) MEHHP 20 (9.5, 39) MEOHP 17 (8.3, 36) MEHP 6.0 (2.9, 14) MBzP 22 (8.8, 50) MCPP 3.2 (1.8, 6.0) MiBP 6.2 (2.7, 12) MBP 36 (16, 75) MEP 380 (137, 1010) MMP 1.6 (1, 3.8)	• GA obtained from hospital perinatal database and determined using information on last menstrual period.	• Race • Infant sex • Ln-creatinine • Smoking • Education • Marital status • Pre-pregnancy BMI	• Change in GA (weeks) for each ln-unit increase in PTH (μg/L) ∑Low-MWP (0.14; 95% Cl: 0.01, 0.27) MEHP (0.15; 95% Cl: 0.02, 0.29)	14
Latini, 2003 (Italy)	• Cross-sectional study • N_{SAMPLE}: 84 newborns • Sample consisted of singletons and twins • Maternal age (range), years: 29.5 (18-42)	• CB sample collected • Mean concentrations in μg/mL (95% Cl): DEHP 1.19 (0.93, 1.44) MEHP 0.52 (0.39, 0.66)	• GA	• Unknown	• Odds of being PTH- compared to PTH+ for each unit increase in GA (weeks) MEHP (OR: 1.50; 95% Cl: 1.013, 2.21)	N/A
Suzuki, 2010 (Japan)	• Cohort study • Time of recruitment: 2005-2008 • N_{SAMPLE}: 149 • Sample consisted of women with no obvious clinical symptoms	• MU sample collected between 9th and 40th gestational weeks • GM of creatinine corrected concentrations in Mg/g Cr: MMP (9.14) MEP (9.76) MnBP (51.6) MBzP (5.62) MEHP (5.45)	• GA (weeks) estimated using information on last menstrual period	• Maternal age • BMI • Parity • Newborn sex • Smoking	• No significant association between the sum of Log-PTH metabolites and change in GA	14

	• Mean maternal age (SD), years: 31.9 (4.5)	MEHHP (10.6) MEOHP (11.3) MINP (0.031) MnOP (0.025) • Concentration <LOD replaced by LOD/2				
Huang, 2014 (China)	• Cross-sectional study • Recruited those with dates of delivery between October 2011 – September 2012 • N_{SAMPLE}: 207 • Mean maternal age (SD): 28.06 (3.28)	• CB sample collected at ≤ 10 minutes of delivery • Mean concentrations in µg/L (25^{th}, 75^{th} percentile): DMP 6.69 (ND,ND) DEP 8.99 (ND, 0.73) DMEP 8.11 (ND, 7.08) DBP 68.14 (19.61, 72.03) DEEP 32.96 (ND, 9.17) DIBP 31.34 (11.08, 26.92) DPP 26.64 (ND, ND) BMPP 11.81 (ND, ND) DBEP 53.51 (ND, 4.64) DCHP 125.02 (5.83, 38.46) DNHP 8.08 (ND, 0.29) BBP 22.55 (ND, 0.99) DEHP 187.16 (9.18, 78.46) DNOP 27.66 (ND, 0.44) DNP 13.42 (0.23, 2.13) • If detection rate >60%, concentration <LOD replaced by LOD/√2	• GA obtained from hospital perinatal database, and based on last menstrual period. PB defined as birth at < 37 gestational weeks.	• Maternal age • BMI • Prenatal examination frequency • Pregnancy history • Intravenous infusions therapy history (GA models only)	• Change in GA (weeks) among those with detectable vs. non-detectable PTH levels[a] or for each ln-unit increase in PTH (µg/L)[b] DMP[a] (-2.22; 95% CI: -2.80, -1.65) DEP[a] (-1.67; 95% CI: -2.23, -1.12) DEEP[a] (-1.01; 95% CI: -1.54, -0.48) DPP[a] (-3.00; 95% CI: -3.61, -2.40) BMPP[a] (-2.68; 95% CI: -3.27, -2.09) DNHP[a] (-1.67; 95% CI: -2.26, -1.09) BBP[a] (-1.05; 95% CI: -1.59, -0.51) DNOP[a] (-1.89; 95% CI: -2.45, -1.34) DMEP[b] (-0.24; 95% CI: -0.36, -0.12) DBP[b] (-0.55; 95% CI: -0.81, -0.30) DIBP[b] (-0.75; 95% CI: -1.03, -0.46) DBEP[b] (-0.42; 95% CI: -0.55, -0.28) DEHP[b] (-0.46; 95% CI: -0.61, -0.31) DNP[b] (-0.49; 95% CI: -0.62, -0.37) • Odds of PB among those with detectable vs. non-detectable PTH level[a] or for each ln-unit increase in PTH (µg/L)[b] DMP[a] (OR: 34.47; 95% CI: 11.32, 104.94) DEP[a] (OR: 16.13; 95% CI: 5.63, 46.21) DEEP[a] (OR: 15.14; 95% CI: 3.44, 66.66) DPP[a] (OR: 58.34; 95% CI: 18.78, 181.24) BMPP[a] (OR: 50.05; 95% CI: 15.62, 160.36) DNHP[a] (OR: 16.22; 95% CI: 5.93, 44.38) BBP[a] (OR: 9.97; 95% CI: 3.25, 30.53) DNOP[a] (OR: 20.20; 95% CI: 7.01, 58.18) DMEP[b] (OR: 1.99; 95% CI: 1.37, 2.89) DBP[b] (OR: 3.35; 95% CI: 2.05, 5.50) DIBP[b] (OR: 6.01; 95% CI: 3.24, 11.17) DBEP[b] (OR: 2.56; 95% CI: 1.87, 3.51) DEHP[b] (OR: 2.32; 95% CI: 1.71, 3.16) DNP[b] (OR: 2.34; 95% CI: 1.80, 3.04)	N/A
Huang, 2009 (Taiwan)	• Cohort Study • Time of recruitment: 2005-2006 • N_{FEMALE}: 32 • N_{MALE}: 33 • Sample consisted of women intending to	• AF sample collected at the start of amniocentesis • Median concentrations in ng/mL (10^{th}, 90^{th} percentile) for females[a] and males[b]: MBP 85.5 (45.6, 134.6) [a] 81.3 (44.3, 127.8)[b] MEHP 24.0 (5.0, 91.1)[a] 22.1 (2.6, 100.6)[b] MEP ND (ND, 3.9)[a]	• GA determined by same pediatrician and assistant.	• None	• No significant association between PTH metabolites and GA in females or males	9

	have amniocentesis conducted	ND (ND, 4.4)[b] MBzP ND (ND, 84.1)[a] ND (ND, 87.9)[b] MMP ND (ND, ND)[a] ND (ND, ND)[b]				
Burdorf, 2011 (Netherlands)	• Cohort study • Recruited those with dates of delivery between April 2002 – January 2006 • N_{SAMPLE}: 6302 • Maternal age group (%), years: <25 (17), 25-30 (29), 30-35 (40), ≥35 (16)	• PTH exposure determined using a JEM • JEM applied the knowledge of occupational hygienists to link job titles to chemical exposures	• PB defined as delivery <37 gestational weeks. GA determined by ultrasound.	• None	• No significant association between PTH and PB	10
Araki, 2014 (Japan)	• Cohort study • Time of recruitment: July 2002 – October 2005 • N_{SAMPLE}: 202 • Mean maternal age (SD), years: 29.8 (4.9)	• MB sample collected between 23-35 gestational weeks • Median concentrations in ng/mL (Interquartile Range): MEHP 10.4 (5.88-15.3)	• GA obtained from medical records	• None	• No significant correlation between MEHP and GA	11

- **Abbreviations:** GA: gestational age; PB: preterm birth; pPROM: preterm premature rupture of membranes; BW: birth weight; LBW: low birth weight; HC: head circumference; IUGR: intrauterine growth restriction; PE: pre-eclampsia; PIH: pregnancy-induced hypertension; GDM: gestational diabetes mellitus; MU: maternal urine; CB: cord blood; MB: maternal blood; AF: amniotic fluid; ME: meconium; JEM: job-exposure matrix; PTH: phthalate; DEHP: di(2-ethyl-hexyl) PTH; MEHP: mono-(2-ethyl)-hexyl PTH; MEHHP: mono-2-ethyl-5-hydroxyhexyl PTH; MEOHP: mono-2-ethyl-5-oxohexyl PTH; MECPP: mono-(2-ethyl-5-carboxypentyl) PTH; BBP: benzyl butyl PTH; MBzP: mono-benzyl PTH; DBP: dibutyl PTH; MBP: mono-butyl PTH; MnBP: mono-n-butyl PTH, DIBP: diisobutyl PTH; MiBP: mono-isobutyl PTH; MNP: mono-3-methyl-5-dimethyl PTH; DEP: diethyl PTH; MEP: mono-ethyl PTH; DiNP: di-iso-nonyl PTH; MiNP: mono-iso-nonyl PTH; MHiNP: mono(4-methyl-7-hydroxyloctyl) PTH; MOiNP: mono(4-methyl-7-oxo-octyl) PTH; MCiOP: mono(4-methyl-7-carboxyheptyl) PTH; MCOP: monocarboxyisooctyl PTH; DMP: dimethyl PTH; MMP: monomethyl PTH; DCHP: dicyclohexyl PTH; MCHP: monocyclohexyl PTH; DNOP: di-n-octyl PTH; MnOP: mono-n-octyl PTH; MCPP: mono-(3-carboxypropyl) PTH; MCNP: monocarboxyisononyl PTH; MDP: mono-3-methyl-7-methyloctyl PTH; DEEP: bis (2-ethoxyethyl) PTH; DPP: diamyl PTH; BMPP: bis (4-methyl-2-pentyl) PTH; DNHP: dihexyl PTH; DMEP: bis (2-methyoxyethyl) PTH; DBEP: bis(2-n-butoxyethyl) PTH; DNP: dinonyl PTH; LOD: limit of detection; ND: not detected; SD: standard deviation; 95% CI: 95% confidence interval; p: p-value; Q: quartile; T: tertile
- **Note:** covariates listed by study were adjusted for in all analyses reported, unless otherwise specified. Results reported by Ferguson, 2014b regarding the effects of PTH on PB were obtained from the available supplementary data.

Table 2.3. Summary of Study Characteristics and Results Reported on Pregnancy Outcomes (AS, IUGR, PE, & GDM).

1st author, publication date & location	Study Design & Sample	Exposure	Outcome	Adjusted Covariates	Results	Quality Score
Latini, 2003 (Italy)	• Cross-sectional study • N_{SAMPLE}: 84 newborns • Sample consisted of singletons and twins • Maternal age (range), years: 29.5 (18-42)	• CB sample collected • Mean concentrations in µg/mL (95% CI): DEHP 1.19 (0.93, 1.44) MEHP 0.52 (0.39, 0.66)	• 1 minute & 5 minute Apgar scores	• Unknown	• No significant association between DEHP or MEHP and 1 minute or 5 minute Apgar scores	N/A
Zhao, 2014 (China)	• Case-control study • Time of recruitment: March 2012 - January 2013 • N_{CASE}: 42 (fetus with intrauterine growth restriction) • $N_{CONTROL}$: 84 (2 fetus per case, and matched by maternal age) • Maternal morbidities (% case, control): pregnancy-induced hypertension (11.9, 7.1); pregnancy-induced diabetes (11.9, 8.3)	• MU sample collected during 3rd trimester • Median concentrations in ng/mL (25th, 75th percentile): MnBP 21.6 (11.0, 37.6) MMP 8.9 (4.8, 15.9) MEHP 6.5 (1.5, 17.4) MEHHP 10.6 (3.9, 19.2) MEOHP 4.3 (1.7, 9.7) ∑DEHP 26.8 (13.6, 46.3) • ∑DEHP = Sum of MEHP, MEHHP, & MEOHP • Concentrations corrected using specific gravity • Concentration <LOD replaced by LOD/2	• IUGR defined as weight <10th centile for GA. Determined using sonographic measures.	• GA • Infant sex • Passive smoking • Other PTH metabolites	• Odds of IUGR among those exposed to tertile 3 compared to tertile 1 MEHHP (OR: 5.80; 95% CI: 1.55, 21.67)	16
Nugteren, 2012 (Netherlands)	• Cohort study • N_{SAMPLE}: 4465 • Mean maternal age (SD), years: 31.09 (4.5) • Maternal ethnicity (%): Netherlands (63.7), Surinam and Dutch Antilles (8.1), Morocco and Turkey (7.2), & Other (19.0)	• PTH exposure determined using a JEM which applies expert knowledge to link job title & descriptions to chemical exposure	• PE obtained from medical records, & determined using the International Society for the Study of Hypertension in Pregnancy and the College of Obstetricians and Gynaecologists criteria	• Maternal age • Educational • Parity • Ethnicity • BMI	• No significant association between PTH and odds of PE	11
Shapiro, 2015 (Canada)	• Cohort study • Time of recruitment: 2008-2011 • N_{SAMPLE}: 1274	• MU sample collected during 1st trimester • GM concentration in µg/L (SD) for those with normal glucose[a], and gestational diabetes mellitus[b] MEP 38.8(4.1)[a], 34.5(4.0)[b]	• GDM determined using the glucose challenge test and oral glucose tolerance test, following the Canadian Diabetes	• Maternal age • Race • Pre-pregnancy BMI • Education	• No significant association between PTH metabolites and odds of GDM	14

	• Maternal age group (%), years: ≤29 (24.2), 30-34 (35.1), ≥35 (40.4) • Maternal race (%): White (84.5), Non-White (15.5)	MBP 13.3(2.2)[a], 12.3(1.9)[b] MBzP 5.8(2.7)[a], 6.3(2.9)[b] MCPP 1.0(3.0)[a], 0.8(3.2)[b] MEHP 2.6(2.5)[a], 2.7(2.9)[b] MEHHP 10.6(2.5)[a], 11.4(3.0)[b] MEOHP 7.4(2.3)[a], 7.8(2.7)[b] • Concentrations corrected using specific gravity • Concentration <LOD replaced by LOD/2	Association and the Society of Obstetrician and Gynaecologists of Canada guidelines	• Specific gravity		

- **Abbreviations:** GA: gestational age; PB: preterm birth; pPROM: preterm premature rupture of membranes; BW: birth weight; LBW: low birth weight; HC: head circumference; IUGR: intrauterine growth restriction; PE: pre-eclampsia; PIH: pregnancy-induced hypertension; GDM: gestational diabetes mellitus; MU: maternal urine; CB: cord blood; MB: maternal blood; AF: amniotic fluid; ME: meconium; JEM: job-exposure matrix; PTH: phthalate; DEHP: di(2-ethyl-hexyl) PTH; MEHP: mono-(2-ethyl)-hexyl PTH; MEHHP: mono-2-ethyl-5-hydroxyhexyl PTH; MEOHP: mono-2-ethyl-5-oxohexyl PTH; MECPP: mono-(2-ethyl-5-carboxypentyl) PTH; BBP: benzyl butyl PTH; MBzP: mono-benzyl PTH; DBP: dibutyl PTH; MBP: mono-butyl PTH; MnBP: mono-n-butyl PTH, DIBP: diisobutyl PTH; MiBP: mono-isobutyl PTH; MNP: mono-3-methyl-5-dimethyl PTH; DEP: diethyl PTH; MEP: mono-ethyl PTH; DiNP: di-iso-nonyl PTH; MiNP: mono-iso-nonyl PTH; MHiNP: mono(4-methyl-7-hydroxyloctyl) PTH; MOiNP: mono(4-methyl-7-oxo-octyl) PTH; MCiOP: mono(4-methyl-7-carboxyheptyl) PTH; MCOP: monocarboxyisooctyl PTH; DMP: dimethyl PTH; MMP: monomethyl PTH; DCHP: dicyclohexyl PTH; MCHP: monocyclohexyl PTH; DNOP: di-n-octyl PTH; MnOP: mono-n-octyl PTH; MCPP: mono-(3-carboxypropyl) PTH; MCNP: monocarboxyisononyl PTH; MDP: mono-3-methyl-7-methyloctyl PTH; DEEP: bis (2-ethoxyethyl) PTH; DPP: diamyl PTH; BMPP: bis (4-methyl-2-pentyl) PTH; DNHP: dihexyl PTH; DMEP: bis (2-methyoxyethyl) PTH; DBEP: bis(2-n-butoxyethyl) PTH; DNP: dinonyl PTH; LOD: limit of detection; ND: not detected; SD: standard deviation; 95% Cl: 95% confidence interval; p: p-value; Q: quartile; T: tertile
- **Note:** covariates listed by study were adjusted for in all analyses reported, unless otherwise specified.

BRIDGE TO CHAPTER 3

In the previous manuscript, a systematic review was performed to summarize the existing literature on the association of gestational exposure to environmental phthalates with maternal and perinatal outcomes. Among the statistically significant study findings, most suggested an association of phthalates with decreased birth weight and gestational age, and increased head circumference and preterm birth; however discrepancies were also observed. Furthermore, a scarcity of research on the role of phthalates on intrauterine growth restriction, pre-eclampsia, gestational diabetes mellitus, and Apgar scores, as well as a lack of studies on pregnancy-induced hypertension, was identified.

To address the inconsistencies in the literature and the paucity of research on the adverse effects of phthalates during pregnancy, a secondary analysis of data from the MIREC Study was conducted and presented in the following manuscript. Multiple linear and logistic regression models were constructed and employed to investigate the association of first trimester urinary phthalate metabolites with clinical outcomes in the mother and infant.

PREFACE TO CHAPTER 3

The objective of the second manuscript was to address the inconsistencies in the literature and paucity of research on the association between phthalates and maternal and perinatal health outcomes. This was accomplished by examining associations between gestational exposure to environmental phthalates and clinical outcomes among mother-infant pairs participating in the MIREC Study. Ethics approval for the secondary data-analysis performed was acquired from the Ottawa Health Science Network Research Ethics Board. A copy of the informed consent documents for the MIREC Study is provided in the appendix of this thesis (Appendix 1 and 2).

Dr. Premkumari Kumarathasan, Dr. Renaud Vincent, Dr. William Fraser, and Dr. Tye Arbuckle are main MIREC Study platform investigators that contributed to the establishment of the study. MIREC data handling for urinary phthalate levels was performed by Mandy Fisher. Erica Blais was involved with maternal blood biomarker analysis and biomarker data handling.

This study was a secondary data-analysis of the MIREC database to identify biomarkers of exposure and examine adverse maternal and perinatal outcomes from exposure to phthalates. Access to MIREC data, and content expertise on toxicology, inflammation biomarkers, and obstetrical outcomes was provided by Dr. Premkumari Kumarathasan. Expertise in statistics and SAS coding was provided by Dr. Sabit Cakmak. Guidance on development of this research and methodological guidance for statistical analysis was provided by Dr. Daniel Krewski, Dr. Premkumari Kumarathasan, and Dr. James Gomes.

CHAPTER 3: THE ASSOCIATION OF GESTATIONAL EXPOSURE TO ENVIRONMENTAL PHTHALATES WITH CLINICAL OUTCOMES IN THE MOTHER AND INFANT: THE MIREC STUDY (MANUSCRIPT 2)

3.1. ABSTRACT

Introduction: Phthalates are ubiquitous environmental contaminants that have been detected in biological specimens, primarily urine, in the general population. Although existing animal studies and epidemiological research have demonstrated the potential role of phthalates as reproductive and developmental toxicants, the effects of these compounds on several infant and maternal outcomes remain unclear due to inconsistent study findings or insufficient research. To address this knowledge gap, the current study was conducted to evaluate the association of gestational exposure to environmental phthalates with *a priori* chosen clinical outcomes in the mother and infant among participants in the MIREC Study.

Methods: Women in their first trimester of pregnancy were recruited from prenatal clinics between 2008 and 2011. Maternal urine samples from the first trimester were used to assess the exposure to phthalates. Details of maternal and infant clinical outcomes, including birth weight, head circumference, gestational age, preterm birth, Apgar scores, and gestational hypertension, were obtained from chart reviews. Questionnaires were administered to collect information on various participant characteristics. The current analysis consisted of 1,412 study participants with live singleton births, and complete information on exposures, outcomes, and relevant covariates.

Multiple linear and logistic regression models were employed to investigate the associations of interest.

Results: Quartile 3 of MBP was significantly ($p<0.01$) associated with an increase in head circumference among female infants (0.53cm; 95% Cl: 0.19 – 0.88). Initially, a significant inverse association between quartile 4 of MCPP and head circumference was identified among male infants (-0.48cm; 95% Cl: -0.83 – -0.12); however, this relationship only approached statistical significance ($p<0.05$) following adjustment for the effects of additional covariates included in the maximally-adjusted model (-0.48cm; 95% Cl: -0.84 – -0.11). Associations between maternal urinary concentrations of phthalate metabolites and birth weight, gestational age, preterm birth, Apgar scores, and gestational hypertension were not statistically significant.

Conclusion: A significant association of MBP with head circumference in females was identified. Non-significant findings included a possible effect of phthalates on head circumference which yielded consistent increases in females, and decreases in males, and gestational age, which demonstrated an inverse association among both male and female stratums. Although the current study yielded findings similar to others reported in the literature, inconsistencies were also observed. These differences may attributed in part to multiple sources of variability among studies. To more effectively evaluate the risk of phthalates using the existing evidence as a whole, more research is needed to characterize the potential risks associated with exposure to phthalates during pregnancy, and to identify and possibly minimize heterogeneity among studies being compared.

3.2. INTRODUCTION

Phthalates are a class of synthetic chemicals that have been used as plasticizers and solvents in the production of various consumer items [1]. Possible sources of exposure to these chemicals include, but are not limited to, polyvinyl chloride plastics, cosmetics, perfumes, food packaging materials, and adhesives [1]. As phthalates are ubiquitous environmental contaminants, opportunities for human exposure to these chemicals may be numerous [2-4]. In particular, a biomonitoring study conducted between 2007 and 2009, which was highly representative of Canadians between the ages of 6 to 49 years, reported that a vast majority of the general population were exposed to phthalate-based compounds (MEP, MnBP, MBzP, MCPP, MEHP, MEOHP, and MEHHP) [5].

Following exposure, phthalates can be metabolized by hydrolysis and conjugation [6]. In humans, these chemicals have been reported to have exhibited relatively short half-lives, and are largely excreted in urine [7, 8]. In particular, research investigating the metabolism of phthalates, reported detecting a substantial proportion of oral study doses of di-n-butyl phthalate (92.2%), diisobutyl phthalate (90.3%), and di(2-ethylhexyl) phthalate (67.0%) in urine following 24 hours after its administration [7, 8]. Although phthalates have been shown to be readily eliminated from the body, there are concerns associated with exposure in vulnerable population, such as pregnant women and the developing fetus. A study comparing phthalate metabolite levels measured in maternal urine and amniotic fluid, reported identifying a statistically significant correlation for monoisobutyl phthalate; this suggests that maternal exposure to phthalates may be capable of crossing the placenta, and consequently resulting in fetal exposure [9]. Other biomonitoring studies have also detected phthalates in umbilical cord blood and meconium samples [10, 11].

Phthalates are also considered as endocrine disrupting chemicals that have been linked to a number of adverse health endpoints [12]. Studies conducted in animals have demonstrated possible relationships of phthalates with a decrease in testosterone, anogenital distance, and fetal weight [13, 14]. Epidemiological studies suggest possible associations of phthalates with lower sperm concentrations, reduced sperm motility, male infertility, endometriosis, uterine leiomyoma, and hydrocele [15-19]. These results demonstrate the potential role of phthalates as reproductive and developmental toxicants. A systematic review summarizing the literature on the relationship of phthalates with perinatal outcomes and pregnancy complications reported possible associations of phthalates with decreased birth weight and gestational age, and increased head circumference and preterm births [20]. However, these findings are not conclusive since other studies have reported inconclusive or conflicting results [20]. Additionally, studies investigating maternal clinical outcomes were lacking, as no studies reporting on pregnancy-induced hypertension were identified, and only a single study reporting on gestational diabetes mellitus and pre-eclampsia, respectively, were captured in the review [20]. Overall, the existing literature demonstrates that the effects of phthalates on pregnancy outcomes remain unclear.

To address current knowledge gaps in the literature regarding the potential risks of phthalates among susceptible populations, notably pregnant women and the developing fetus, the present study was undertaken in order to investigate the association of gestational exposure to environmental phthalates with clinical outcomes in the mother and their infants. The study involves an analysis of relevant data collected through the Maternal-Infant Research on Environmental Chemicals (MIREC) Study within the Canadian population.

3.3. MATERIALS AND METHODS

3.3.1. Study Design

The MIREC Study recruited pregnant women in their first trimester of pregnancy from multiple prenatal clinics between 2008 and 2011. Women with fetal abnormalities, fetal chromosomal malformations, or a history of select medical conditions or illegal drug use were not included in the study. Of the 5,108 pregnant women that met the eligibility criteria of the study, a total of 2,001 individuals provided consent to participate. Information on various characteristics of the study participants were acquired through questionnaires. Medical charts were used to obtain details regarding the pregnancy and the fetus and the infant. Additionally, multiple biological specimens, such as maternal blood and urine, were collected for chemical analysis.

A sample of 1,983 study participants were retained following the withdrawal of 18 pregnant women who requested the destruction of their information and biological samples. The present investigation was restricted to study participants with live singleton births, and complete exposure history, outcome and adjusted covariate information: a total of 1,412 pregnant women and infant pairs were included in the current analysis. The MIREC Study was approved by the Research Ethics Board at Health Canada, and by more than 10 other academic and hospital ethics committees. In addition, ethics approval for the current analysis was acquired from the Ottawa Health Science Network Research Ethics Board. Further details regarding the MIREC Study has been previously described [21].

3.3.2. Phthalate Exposure

Phthalate concentrations were measured using maternal urinary samples collected during the first trimester of pregnancy. Chemical analyses were performed by the Centre de Toxicologie

du Québec, Institut national de Santé Publique du Québec. Laboratory analyses were conducted for 11 phthalate metabolites, including mono-butyl phthalate (MBP), mono-benzyl phthalate (MBzP), mono-cyclo-hexyl phthalate (MCHP), mono-(3-carboxypropyl) phthalate (MCPP), mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), mono-ethyl phthalate (MEP), mono-methyl phthalate (MMP), mono-isononyl phthalate (MiNP), and mono-n-octyl phthalate (MnOP). In brief, the assessment of these compounds included the following steps: (1) enzymatic deconjugation, (2) solid phase extraction, and (3) analysis using LC-MS/MS. The laboratory methods implemented for the quantification of phthalate metabolites has been more comprehensively described elsewhere [22].

3.3.3. Maternal and Infant Outcomes

The seven clinical outcomes of interest that were examined in the current analysis include: birth weight (g), head circumference (cm), gestational age at delivery (weeks), preterm birth, the Apgar score at 1 minute, the Apgar score at 5 minutes, and gestational hypertension. Information regarding these infant and maternal outcomes were acquired from chart reviews, and were available for pregnancies with 20 completed weeks of gestation or more. Preterm birth was defined as delivery at less than 37 weeks of gestation [23]. Following birth, newborns are evaluated on 5 components, including heart rate, respiration, muscle tone, reflexes, and colour, and assigned an Apgar score ranging from 0 to 10 [24]. Although the suggested interpretations of the Apgar scores consist of low for 0 to 3, intermediate for 4 to 6, and normal for 7 to 10, this clinical endpoint was dichotomized in the current analysis as low (<7) and normal (≥7), as few infants with scores of 3 or less at 1 minute (2.87%), and 5 minutes (0.33%) were observed [24]. Gestational hypertension

is defined as the development of high blood pressure in pregnant women following 20 gestational weeks [25]. This maternal outcome was derived from two separate variables regarding physician diagnosis of gestational hypertension on different occasions: (1) prior to delivery admission, and (2) at or after delivery admission. Study participants were considered as having gestational hypertension if their clinical charts indicated that they were diagnosed at either time points, or non-cases if they were not diagnosed with this condition at both time points.

3.3.4. Statistical Analysis

Statistical analysis was conducted using SAS 9.4 (Statistical Analysis System). Observations were visually inspected to identify extreme values, and outliers were retained in the analysis if determined to be biologically plausible. A descriptive analysis was performed to describe the distribution of maternal and infant characteristics in the study sample, as well as by categories of dichotomous outcomes. The descriptive statistics generated to summarize the distribution of categorical covariates include proportions and 95% confidence intervals, with means and standard deviations presented for continuous covariates. P-values for associations with continuous covariates were acquired using t-tests, with categorical covariates evaluated using chi-square tests. Fisher's exact tests were otherwise employed when the expected cell counts of categorical covariates were below 5.

Concentrations of phthalate metabolites below the limit of detection (LOD) were replaced with the LOD/2. As MCHP, MMP, MiNP, and MnOP consisted of a high proportion of observations below the LOD, ranging from 85.4% to 98.5%, these four metabolites were not analyzed in the present study. Non-parametric Spearman correlation analyses were conducted to evaluate the magnitude of association between MEHP, MEHHP, and MEOHP, which are

metabolites of di-(2-ethylhexyl) phthalate (DEHP); due to the high correlation among these phthalates (all pairwise Spearman correlation coefficients were greater than 0.90), the concentrations of these metabolites were summed to yield a single exposure variable (∑DEHP) [22]. Similar methods of handling phthalates as an exposure has been previously implemented in several other studies [26-28]. To adjust for the effects of urinary dilution, the following formula was employed to correct the measured concentrations of phthalate metabolites for specific gravity: Pc = Pi [(SGm – 1) / (SGi – 1)] [22]. In this equation, "Pc" refers to the metabolite concentration that was adjusted for specific gravity, "Pi" refers to the metabolite concentration that was measured, "SGm" refers to the median specific gravity measurement in the cohort, and "SGi" refers to the specific gravity measurement of the urine sample [22]. To account for the skewed distribution of phthalate concentrations in the study sample, geometric means and standard deviations were calculated, and presented for the entire sample, as well for the categories of dichotomous outcomes. As in previous studies, phthalate concentrations were categorized into quartiles, with the lowest exposure group used as the reference category [26, 28, 29].

Multiple linear regression models were constructed for continuous outcomes, including birth weight, head circumference, and gestational age. Alternatively, multiple logistic regression models were built for binary outcomes, including preterm birth (yes vs. no), a low Apgar score at 1 minute (yes vs. No), a low Apgar score at 5 minutes (yes vs. no), and gestational hypertension (yes vs. no). The minimally-adjusted models incorporated a theoretical approach to covariate selection by including *a priori* confounders selected from similar studies in the existing literature [10, 11, 23, 30-43]. In particular, the general criteria employed in the selection of potential confounders include the following: (1) the covariate was repeatedly reported as relevant in 3 or more studies, (2) the covariate can be added or removed from criteria 1 based on biological

plausibility, and (3) if reasonable, the covariate can be added to develop a common parsimonious model for all outcomes of interest. The confounders considered for inclusion in the minimally-adjusted models were: gestational age, maternal age, pre-pregnancy weight, pre-pregnancy BMI, maternal height, pregnancy weight gain, maternal BMI, chronic conditions (high blood pressure and nongestational diabetes), pregnancy complications (pregnancy-induced hypertension and diabetes), parity, active smoking, passive smoking, alcohol use, race/ethnicity, education, income, marital status, baby sex, premature rupture of membranes, and specific gravity.

As insufficient information regarding confounding factors was available for the effects of phthalates on the Apgar score at 1 minute, the Apgar score at 5 minutes, and gestational hypertension, the minimally-adjusted models for these clinical endpoints were constructed parallel to the other study outcomes of interest. The 7 covariates selected for inclusion in the minimally-adjusted models for birth weight, head circumference, the Apgar score at 1 minute, and the Apgar score at 5 minutes were: gestational age at delivery (weeks), maternal age (years), pre-pregnancy BMI (underweight / normal weight / overweight / obese), parity (0 / 1 / 2 / 3 or more live births), active smoking status (never / current / quit during pregnancy / former), maternal education (high school or less / some college or college diploma / university degree), and specific gravity (g/mL). Apart from excluding gestational age as a confounding factor, identical models were constructed for gestational age at delivery and preterm birth. As well, a similar set of covariates were selected for the maternal outcome, gestational hypertension, including maternal age, pre-pregnancy BMI, parity, active smoking status, maternal education, specific gravity, and baby sex (male / female). Maternal race/ethnicity was identified as potentially relevant among 3 studies evaluating gestational age as an outcome [39-41]. However, as the study sample was predominantly composed of women of white race (82.86%), and as this variable was not identified as a

confounding factor among the majority of studies assessing birth weight and head circumference, it was not retained in the minimally-adjusted models, but was further considered for inclusion in the maximally-adjusted models. As similar studies have presented research results that varied between males and females, the sex of the newborn was not included in models constructed for infant outcomes [10, 37, 39, 43]. Instead, a stratified analysis was performed to evaluate the possibility of effect modification.

The maximally-adjusted models were constructed using an empirical approach to covariate selection by consecutively adding and removing a single covariate from the minimally-adjusted logistic regression models, and calculating the percent change in the effect of the exposure on the outcome. Covariates that altered the odds ratios for the association of interest by 10% or more were highlighted and considered for inclusion in the final models. This percent change-in-estimate method of variable selection has been suggested as a more appropriate approach than the use of significance-testing algorithms [44]. To limit the overall false positive rate associated with multiple testing, results were considered statistically significant at a p-value of less than 0.01. However, to enable the identification of trends that are potentially meaningful, and comparability of results across studies in the literature, results with a p-value of less than 0.05 were also highlighted.

The variables considered in the analysis implemented to identify potential confounding factors were: maternal race (white / black / other), marital status (married / same partner for a year or more / widowed / divorced / separated / single / other), annual household income (≤$50,000 / $50,001 - $100,000, >$100,000 / don't know), chronic illness: high blood pressure (no / yes), chronic illness: diabetes (no / yes), chronic illness: other (no / yes), family history of maternal high blood pressure during pregnancy (no / yes / don't know), prenatal multivitamin preparation use

(no / yes), folic acid supplements use (no / yes), other supplements use (no / yes), second-hand smoke exposure (no / yes / don't know), alcohol consumption (no / yes / refuse to answer), average systolic blood pressure (SBP), average diastolic blood pressure (DBP), gestational diabetes (no / yes), spontaneous rupture of membranes prior to labour onset (no / yes), congenital abnormalities (no / yes), and Bisphenol A exposure in μg/L (<0.37 / 0.37–0.82 / 0.83–1.79 / >1.80). The rationale for the categorization of covariates and the selection of cut-offs have been previously described in greater detail elsewhere [45].

As a consequence of the inadequate number of study participants with low Apgar scores at 5 minutes (N=21), the distribution of several covariates according to outcome resulted in small cell counts, which yielded statistical models of questionable validity. As a consequence, the following statistical modifications were performed for this infant outcome: (1) DEHP was categorized into tertiles, and (2) only maternal age, gestational age at delivery, and specific gravity were retained in the minimally-adjusted models. Similarly, the low number of cases of preterm birth among female infants (N=34) was also an issue that raised concerns regarding the validity of the models; for this reason, results associated with this outcome were only presented for the pooled analysis, and for the stratum comprised of male infants.

3.4. RESULTS

Table 3.1 presents the distribution of maternal and infant characteristics among eligible participants sampled for the MIREC Study, as well as for each dichotomous outcome category. Of the 1,412 pregnant women included in the current analysis, most were of white race (82.86%), had a university education (63.46%), were of normal weight prior to pregnancy (61.54%), had never smoked (62.68%), had no previous live births (44.76%), and reported annual household income

between $50,001 and $100,000 (40.93%). Among this study sample, the average maternal age was 33.3 years, systolic blood pressure during the second trimester was 108.3 mmHg, and diastolic blood pressure during the second trimester was 66.0mmHg. Significant associations ($p<0.01$) were observed between preterm birth with pre-pregnancy BMI and average systolic blood pressure during the second trimester, between the Apgar score at 1 minute and parity, and between gestational hypertension and maternal race, pre-pregnancy BMI, and average systolic and diastolic blood pressures during the second trimester.

Table 3.2 displays the geometric means and standard deviations of specific gravity-corrected phthalate metabolite concentrations in the study sample, as well as for each dichotomous outcome category. The LOD for the urinary phthalate metabolites varied between values of 0.2µg/L to 0.5µg/L. Among the cohort of pregnant women with live singleton birth and available exposure data, the proportion of observations with concentration below the LOD ranged between 0.12% and 15.53%. The highest and lowest geometric mean concentrations in the sample were observed for MEP and MCPP, respectively. In particular, the sample geometric mean (SD) of MBP is 12.26µg/L (2.31), MBzP is 5.61µg/L (2.74), MEP is 34.32µg/L (3.97), MCPP is 0.93µg/L (3.01), MEHP is 2.42µg/L (2.49), MEHHP is 9.88µg/L (2.49), MEOHP is 6.90µg/L (2.35), and DEHP is 19.47µg/L (2.39).

The algorithm used to select potential confounding factors for the maximally-adjusted models identified 10 covariates that altered the odds ratios for the association of interest by 10% or more. These covariates were: chronic high blood pressure, chronic diabetes, other chronic conditions, average systolic blood pressure during the first trimester, average diastolic blood pressure during the first trimester, average systolic blood pressure during the second trimester, average diastolic blood pressure during the second trimester, premature rupture of membranes

(PROM), gestational diabetes, and bisphenol A exposure. To reduce bias related to modelling repeated measures, a single set of blood pressure measurements were selected for inclusion in the maximally-adjusted models. Compared to the blood pressure measurements taken during the first trimester which were only influential in 3 of 20 models each, measurements taken during the second trimester were considerably more influential, affecting a minimum of 14 models each. For this reason, the latter set of blood pressure measurements were retained. Gestational diabetes status was not selected for inclusion in the final models, as this covariate demonstrates a high level of missing observations ($N_{Missing}$=577).

The status of chronic high blood pressure and chronic diabetes were each identified as influential in a single model; for this reason, they were considered spurious, and thus were not retained. The covariate representing the status of having other chronic conditions may have consisted of illnesses that are associated with several other covariates already accounted for in the model, such as body mass index, systolic blood pressure, and diastolic blood pressure. Therefore to avoid the repeat adjustment of covariates, this variable was not selected for inclusion. Evidence in the literature demonstrates that phthalates may be capable of promoting an inflammatory response, elevated levels of pro-inflammatory cytokines among women with PROM, and that preterm PROM is a precursor to approximately 25% of preterm births [46-48]. For this reason, PROM was not retained in the final models as it may be an intermediate variable in the causal pathway. The maximally-adjusted models constructed for infant outcomes included the following additional covariates: average systolic blood pressure during the second trimester, average diastolic blood pressure during the second trimester, and bisphenol A exposure. For gestational hypertension, only bisphenol A was additionally included in the maximally-adjusted models, as second trimester blood pressure measurements may have been used in its diagnosis.

The effect estimates with 95% confidence intervals from the minimally- and maximally-adjusted models are reported in Tables 3.3 and 3.6, respectively. Additionally, the corresponding results from the stratified analysis can be found in Tables 3.4 and 3.7 for male infants, and in Tables 3.5 and 3.8 for female infants. Among results that were statistically significant ($p<0.01$), a positive association was observed between quartile 3 concentrations of MBP and head circumference among female infants (0.51cm; 95% Cl: 0.17 – 0.85); after the adjustment of additional covariates in the final model, a more pronounced significant increase in head circumference was observed among the female stratum (0.53cm; 95% Cl: 0.19 – 0.88). In contrast, a significant inverse association was initially observed between quartile 4 of MCPP and head circumference among male infants (-0.48cm; 95% Cl: -0.83 – -0.12); however, in the maximally-adjusted model, the association between MCPP and head circumference among male infants only approached statistical significance at $p<0.05$. Associations between maternal urinary concentrations of phthalate metabolites and maternal and perinatal outcomes, such as with birth weight, gestational age, preterm birth, Apgar scores, and gestational hypertension through the primary or stratified analyses conducted did not reach significance.

Study findings with a p-values of less than 0.05 were also noted in the regression analysis performed; unless otherwise specified, the results presented are from the maximally-adjusted models. Similar to the significant positive association between quartile 3 of MBP and head circumference among females, marginally significant ($p<0.05$) increases in head circumference were also observed in association with quartiles 2 of MBzP (0.33cm; 95% Cl: 0.04 – 0.63), and MCPP (0.31cm; 95% Cl: 0.02 – 0.60). In contrast, male infants demonstrated a decrease in head circumference in association with MEP concentrations in quartile 3 (-0.30cm; 95% Cl: -0.61 – -0.001), and MCPP concentrations in quartiles 3 (-0.33cm; 95% Cl: -0.65 – -0.01), and 4 (-0.48cm;

95% Cl: -0.84 – -0.11). Overall, the effect of phthalates on head circumference has demonstrated consistent increases in females and decreases in males.

Among the sample consisting of pregnant women with infants of both sexes, MBzP concentrations in quartile 3 were associated with an increase in birth weight (81.22g; 95% Cl: 10.32 – 152.12); following stratification of the study sample, a greater positive association was observed among male infants (103.34g; 95% Cl: 5.89 – 200.78). Additionally, an inverse relationship was demonstrated between quartile 4 of MCPP and birth weight (-82.40g; 95% Cl: -162.00 – -2.81), and a greater decrease was observed within the male stratum (-110.01g; 95% Cl: -216.53 – -3.49). However, following adjustment for additional covariates in the maximally-adjusted model, the effects of MCPP were no longer apparent, with no effect of phthalates on birth weight observed among female infants.

Maternal urinary concentrations of MBP in quartile 2 were associated with a decrease in gestational age (-0.28 weeks; 95% Cl: -0.54 – -0.02); following stratification, greater reductions were observed among male infants (-0.42 weeks; 95% Cl: -0.82 – -0.03). Similarly, an inverse association of MEP concentrations in quartile 2 with gestational age were also observed among female infants (-0.42 weeks; 95% Cl: -0.77 – -0.08). Additionally, while an association between quartile 3 concentrations of MCPP and elevated odds of preterm birth were initially observed among male infants (OR: 2.89; 95% Cl: 1.03 – 8.12), this relationship was not demonstrated in the maximally-adjusted model. Based on the study findings presented, a consistent decrease in pregnancy duration in relation to metabolite concentrations of phthalates was observed.

Reduced odds of a low Apgar score at 1 minute were observed in association with quartile 3 (OR: 0.44; 95% Cl: 0.23 – 0.84) and 4 (OR: 0.46; 95% Cl: 0.23 – 0.94) of MBzP among the unstratified sample, quartile 3 of ∑DEHP among males (OR: 0.31; 95% Cl: 0.12 – 0.81), and

quartiles 3 of MBzP (OR: 0.31; 95% Cl: 0.10 – 0.93) and MEP (OR: 0.31; 95% Cl: 0.11 – 0.94) among females. In addition, tertile 2 concentrations of ∑DEHP were positively associated with a low Apgar score at 5 minutes among the sample consisting of both male and female infants (OR: 4.44; 95% Cl: 1.21 – 16.24). Furthermore, while lower odds of gestational hypertension were observed with MBP concentrations in quartile 4 (OR: 0.38; 95% Cl: 0.15 – 0.92), greater odds of this maternal outcome were also observed with MBzP (OR: 2.50; 95% Cl: 1.17 – 5.38) and MEP (OR: 2.04; 95% Cl: 1.01 – 4.14) concentrations in the highest quartile. Overall, although the study findings presented only approached statistical significance at $p<0.05$, the general trends identified may warrant further investigation in future studies.

Table 3.1. Distribution of maternal and infant characteristics among participants sampled for the MIREC Study (N=1,412).[a]

Characteristics	N	(%)[b]	Preterm Birth (%)			Low AS at 1 minute (%)			Low AS at 5 minutes (%)			Gestational Hypertension (%)		
			Case	Control	p	Case	Control	p	Case	Control	p	Case	Control	p
Maternal Age (years)														
Mean (SD)	1,412	33.29 (4.99)	34.13 (5.55)	33.24 (4.96)	0.11	32.77 (5.16)	33.33 (4.98)	0.25	33.43 (4.42)	33.29 (5.00)	0.90	32.49 (5.43)	33.35 (4.96)	0.10
Maternal Race														
White	1,170	(82.86)	5.81	94.19	1.00	8.12	91.88	0.15	1.54	98.46	1.00	7.01	92.99	<0.01
Black	35	(2.48)	5.71	94.29		0.00	100.00		0.00	100.00		20.00	80.00	
Other	207	(14.66)	5.80	94.20		9.66	90.34		1.45	98.55		4.35	95.65	
Maternal Education														
High School or Less	116	(8.22)	8.62	91.38	0.25	9.48	90.52	0.68	0.86	99.14	0.84	11.21	88.79	0.047
College Diploma	400	(28.33)	6.50	93.50		7.25	92.75		1.50	98.50		8.25	91.75	
University Degree	896	(63.46)	5.13	94.87		8.37	91.63		1.56	98.44		5.80	94.20	
Household Income														
≤ $50,000	222	(15.72)	6.31	93.69	0.24	12.61	87.39	0.03	2.70	97.30	0.35	8.56	91.44	0.02
$50,001-$100,000	578	(40.93)	6.40	93.60		7.27	92.73		1.56	98.44		8.82	91.18	
>$100,000	553	(39.16)	4.52	95.48		6.87	93.13		1.08	98.92		4.70	95.30	
Don't Know	59	(4.18)	10.17	89.83		11.86	88.14		0.00	100.00		3.39	96.61	
Pre-Pregnancy BMI (Kg/m^2)														
Underweight (<18.5)	48	(3.40)	2.08	97.92	<0.01	10.42	89.58	0.64	2.08	97.92	0.62	2.08	97.92	<0.01
Normal (18.5-24.9)	869	(61.54)	5.18	94.82		7.48	92.52		1.27	98.73		4.49	95.51	
Overweight (25-29.9)	292	(20.68)	4.79	95.21		8.56	91.44		1.71	98.29		7.88	92.12	
Obese (≥30)	203	(14.38)	10.84	89.16		9.85	90.15		1.97	98.03		17.24	82.76	
Smoking Status														
Never	885	(62.68)	5.99	94.01	0.32	7.80	92.20	0.43	1.13	98.87	0.16	6.55	93.45	0.84
Current	77	(5.45)	3.90	96.10		11.69	88.31		2.60	97.40		6.49	93.51	
Quit during Pregnancy	80	(5.67)	10.00	90.00		5.00	95.00		0.00	100.00		8.75	91.25	
Former	370	(26.20)	4.86	95.14		8.92	91.08		2.43	97.57		7.57	92.43	
Parity														
0 live births	632	(44.76)	5.70	94.30	0.99	11.71	88.29	<0.01	2.06	97.94	0.047	9.02	90.98	0.02
1 live births	569	(40.30)	5.80	94.20		4.39	95.61		0.53	99.47		4.39	95.61	
2 live births	156	(11.05)	6.41	93.59		7.05	92.95		2.56	97.44		7.69	92.31	
3 or more live births	55	(3.90)	5.45	94.55		9.09	90.91		1.82	98.18		7.27	92.73	
Baby Sex														
Male	732	(51.84)	6.56	93.44	0.21	9.43	90.57	0.07	1.64	98.36	0.62	7.24	92.76	0.65
Female	680	(48.16)	5.00	95.00		6.76	93.24		1.32	98.68		6.62	93.38	
BPA (µg/L)														
< 0.37	384	(27.20)	4.17	95.83	0.46	8.59	91.41	0.28	1.82	98.18	0.85	5.99	94.01	0.70
0.37 – 0.82	358	(25.35)	6.42	93.58		10.06	89.94		1.68	98.32		6.42	93.58	
0.83 – 1.79	357	(25.28)	6.44	93.56		6.16	93.84		1.12	98.88		7.56	92.44	
> 1.80	313	(22.17)	6.39	93.61		7.67	92.33		1.28	98.72		7.99	92.01	
Average SBP (mmHg)														
Mean (SD)	1,412	108.28 (10.59)	111.50 (11.92)	108.10 (10.47)	<0.01	109.10 (11.34)	108.20 (10.52)	0.40	111.20 (11.99)	108.20 (10.56)	0.20	116.40 (9.86)	107.70 (10.39)	<0.01
Average DBP (mmHg)														
Mean (SD)	1,412	65.97 (7.71)	68.43 (8.91)	65.82 (7.60)	0.011	67.02 (8.15)	65.87 (7.66)	0.13	68.98 (8.57)	65.92 (7.69)	0.07	72.55 (7.89)	65.48 (7.47)	<0.01

Legend: p = p-value; SD = standard deviation; AS = Apgar score; BMI = body mass index; BPA = Bisphenol A; SBP = systolic blood pressure; DBP = diastolic blood pressure

[a]Row frequencies and percentages are presented unless otherwise specified

[b] Percentages reflect covariate distribution in the study sample (N=1412)

Table 3.2. Geometric means (SD) of SG-corrected phthalate concentrations (N=1,412).

Phthalates (µg/L)	LOD	N < LOD (%)[a]	Sample GM (SD)	Preterm Birth GM (SD)		Low AS at 1 minute GM (SD)		Low AS at 5 minutes GM (SD)		Gestational Hypertension GM (SD)	
				Case	Control	Case	Control	Case	Control	Case	Control
MBP	0.2	4 (0.24)	12.26 (2.31)	11.91 (2.40)	12.28 (2.31)	12.05 (2.60)	12.28 (2.29)	12.54 (2.03)	12.26 (2.32)	10.96 (2.47)	12.36 (2.30)
MBzP	0.2	8 (0.48)	5.61 (2.74)	5.79 (2.82)	5.60 (2.74)	4.53 (2.95)	5.72 (2.72)	4.65 (2.82)	5.62 (2.74)	7.63 (2.72)	5.48 (2.73)
MEP	0.5	2 (0.12)	34.32 (3.97)	41.34 (4.00)	33.93 (3.97)	34.72 (4.26)	34.28 (3.95)	36.47 (4.25)	34.29 (3.97)	48.37 (4.32)	33.45 (3.93)
MCPP	0.2	260 (15.53)	0.93 (3.01)	1.20 (3.42)	0.92 (2.98)	0.87 (3.07)	0.94 (3.01)	0.83 (4.02)	0.93 (3.00)	1.05 (3.55)	0.92 (2.97)
MEHP	0.2	26 (1.55)	2.42 (2.49)	2.42 (2.21)	2.42 (2.51)	2.16 (2.56)	2.45 (2.48)	2.24 (2.12)	2.43 (2.50)	2.29 (2.27)	2.43 (2.51)
MEHHP	0.4	11 (0.66)	9.88 (2.49)	10.31 (2.47)	9.85 (2.49)	8.44 (2.53)	10.02 (2.48)	9.34 (1.77)	9.89 (2.50)	9.77 (2.19)	9.89 (2.51)
MEOHP	0.2	5 (0.30)	6.90 (2.35)	7.07 (2.36)	6.89 (2.35)	6.13 (2.35)	6.97 (2.35)	6.41 (1.69)	6.90 (2.36)	6.61 (1.98)	6.92 (2.38)
DEHP	N/A	N/A	19.47 (2.39)	20.02 (2.36)	19.44 (2.39)	16.95 (2.42)	19.71 (2.38)	18.37 (1.72)	19.49 (2.40)	19.03 (2.06)	19.50 (2.41)

Legend: LOD = limit of detection; SG = specific gravity; GM = geometric means; SD = standard deviation; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; MEHP = Mono-(2-ethylhexyl) Phthalate; MEOHP = Mono-(2-ethyl-5-oxo-hexyl) Phthalate; MEHHP = Mono-(2-ethyl-5-hydroxy-hexyl) Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; AS = Apgar score

[a]Frequencies and percentages of observations below the LOD are based on the cohort of pregnant women with live singleton births and available exposure data

Table 3.3. Effect estimates from minimally-adjusted models for the association of first trimester phthalate levels with maternal and infant outcomes (N=1,412).

Phthalate Quartiles (µg/L)	Birth Weight (g) [a] β (95% CI)	Head Circumference (cm) [a] β (95% CI)	Gestational Age (weeks) [b] β (95% CI)	Preterm Birth [b] OR (95% CI)	Low AS at 1 minute [a] OR (95% CI)	Low AS at 5 minutes [d+] OR (95% CI)	Gestational Hypertension [c] OR (95% CI)
MBP							
<5.2	0	0	0	1.00	1.00	1.00	1.00
5.2-12.9	-4.99 (-68.80 – 58.82)	0.06 (-0.15 – 0.27)	**-0.30* (-0.56 – -0.04)**	1.81 (0.91 – 3.60)	0.98 (0.57 – 1.68)	2.00 (0.56 – 7.21)	0.78 (0.41 – 1.47)
13.0-25.0	6.61 (-69.26 – 82.47)	0.18 (-0.07 – 0.43)	-0.12 (-0.42 – 0.19)	1.22 (0.53 – 2.78)	0.78 (0.39 – 1.53)	1.58 (0.33 – 7.51)	0.61 (0.29 – 1.27)
>25.0	-69.15 (-155.85 – 17.54)	-0.06 (-0.34 – 0.23)	-0.13 (-0.48 – 0.21)	0.97 (0.38 – 2.53)	0.91 (0.42- 1.98)	0.82 (0.12 – 5.69)	**0.38 * (0.16 – 0.91)**
MBzP							
<2.4	0	0	0	1.00	1.00	1.00	1.00
2.4-5.1	48.54 (-15.76 – 112.85)	0.13 (-0.08 – 0.34)	-0.11 (-0.37 – 0.14)	0.82 (0.41 – 1.67)	0.61 (0.36 – 1.05)	1.19 (0.37 – 3.85)	1.03 (0.50 – 2.11)
5.2-12.0	**74.32* (4.32 – 144.32)**	0.16 (-0.07 – 0.39)	-0.17 (-0.45 – 0.11)	0.93 (0.45 – 1.96)	**0.45* (0.24 – 0.86)**	0.46 (0.09 – 2.22)	1.55 (0.75 – 3.18)
>12.0	60.00 (-16.80 – 136.80)	0.20 (-0.05 – 0.45)	-0.16 (-0.47 – 0.15)	1.08 (0.49 – 2.39)	**0.48 * (0.24 – 0.96)**	0.77 (0.17 – 3.58)	**2.36 * (1.11 – 5.01)**
MEP							
<12.0	0	0	0	1.00	1.00	1.00	1.00
12.0-28.9	-13.17 (-77.52 – 51.18)	0.07 (-0.14 – 0.28)	-0.23 (-0.49 – 0.03)	1.34 (0.67 – 2.65)	0.86 (0.50 – 1.50)	1.11 (0.30 – 4.12)	1.65 (0.84 – 3.25)
29.0-86.0	-22.24 (-87.94 – 43.46)	-0.17 (-0.39 – 0.05)	-0.03 (-0.29 – 0.24)	0.91 (0.43 – 1.95)	0.63 (0.34 – 1.16)	1.14 (0.30 – 4.37)	1.14 (0.55 – 2.39)
>86.0	-0.58 (-70.37 – 69.22)	-0.07 (-0.30 – 0.16)	-0.10 (-0.38 – 0.18)	1.42 (0.68 – 2.98)	1.00 (0.55 – 1.82)	1.49 (0.39 – 5.74)	2.01 (0.995 – 4.08)
MCPP							
<0.31	0	0	0	1.00	1.00	1.00	1.00
0.31-0.92	26.81 (-36.30 – 89.91)	0.05 (-0.16 – 0.25)	-0.22 (-0.47 – 0.04)	1.96 (0.95 – 4.04)	0.87 (0.50 – 1.51)	0.85 (0.25 – 2.94)	1.22 (0.64 – 2.31)
0.93-2.1	-52.47 (-123.35 – 18.40)	-0.12 (-0.36 – 0.11)	-0.08 (-0.36 – 0.21)	1.81 (0.81 – 4.03)	1.12 (0.60 – 2.10)	0.84 (0.21 – 3.44)	0.92 (0.44 – 1.93)
>2.1	**-82.40* (-162.00 – -2.81)**	-0.15 (-0.41 – 0.11)	-0.08 (-0.40 – 0.24)	2.19 (0.92 – 5.23)	1.21 (0.59 – 2.47)	0.90 (0.19 – 4.32)	1.12 (0.50 – 2.47)
∑DEHP							
<8.32	0	0	0	1.00	1.00	1.00	1.00
8.32-18.39	-25.51 (-90.32 – 39.30)	0.05 (-0.16 – 0.26)	-0.19 (-0.45 – 0.07)	1.59 (0.81 – 3.14)	0.67 (0.39 – 1.16)	**3.65* (1.03 – 12.94)**	1.04 (0.54 – 1.98)
18.40-37.9	-30.79 (-105.21 – 43.63)	0.04 (-0.21 – 0.28)	-0.04 (-0.34 – 0.26)	0.82 (0.35 – 1.93)	0.59 (0.30 – 1.14)	2.49 (0.49 – 12.54)	0.90 (0.42 – 1.91)
>37.9	-65.36 (-150.81 – 20.08)	-0.06 (-0.34 – 0.23)	-0.06 (-0.41 – 0.28)	1.13 (0.45 – 2.81)	0.57 (0.26 – 1.26)		0.83 (0.35 – 1.97)

Legend: OR = odds ratio; 95% CI = 95% Confidence Interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; AS = Apgar score

[+] In place of the concentrations presented, ∑DEHP was modelled as tertiles (T), with T1 as the reference, for AS at 5 minutes using the following cut-offs: <11.24µg/L (T1), 11.24-28.79µg/L (T2), and >28.80µg/L (T3)

[a] Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, and gestational age.

[b] Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, and specific gravity.

[c] Model adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, and baby sex.

[d] Model adjusted for maternal age, specific gravity, and gestational age.

* P<0.05; **P<0.01

Table 3.4. Effect estimates from minimally-adjusted models for the association of first trimester phthalate levels with infant outcomes among males (N=732).

Phthalate Quartiles (µg/L)	Birth Weight (g)[a] β (95% CI)	Head Circumference (cm)[a] β (95% CI)	Gestational Age (weeks)[b] β (95% CI)	Preterm Birth[b] OR (95% Cl)	Low AS at 1 minute[a] OR (95% CI)
MBP					
<5.2	0	0	0	1.00	1.00
5.2-12.9	-38.55 (-126.32 – 49.21)	-0.09 (-0.38 – 0.20)	**-0.45* (-0.83 – -0.07)**	1.65 (0.68 – 3.96)	0.82 (0.39 – 1.71)
13.0-25.0	-14.22 (-118.25 – 89.80)	-0.13 (-0.48 – 0.21)	-0.14 (-0.59 – 0.32)	1.12 (0.38 – 3.32)	0.92 (0.38 – 2.24)
>25.0	-103.20 (-222.11 – 15.72)	-0.30 (-0.70 – 0.10)	-0.42 (-0.94 – 0.10)	1.01 (0.29 – 3.55)	0.93 (0.34 – 2.58)
MBzP					
<2.4	0	0	0	1.00	1.00
2.4-5.1	36.94 (-50.92 – 124.81)	-0.02 (-0.32 – 0.27)	-0.05 (-0.44 -0.33)	1.05 (0.43- 2.56)	0.55 (0.26 – 1.14)
5.2-12.0	87.33 (-7.66 – 182.33)	0.08 (-0.24 – 0.40)	-0.18 (-0.59 – 0.24)	1.03 (0.39 – 2.71)	0.53 (0.23 – 1.20)
>12.0	84.77 (-18.72 – 188.26)	0.16 (-0.19 – 0.50)	-0.11 (-0.57 – 0.34)	1.14 (0.40 – 3.25)	0.52 (0.21 – 1.29)
MEP					
<12.0	0	0	0	1.00	1.00
12.0-28.9	-54.47 (-142.69 – 33.75)	-0.01 (-0.30 – 0.28)	0.004 (-0.38 – 0.39)	1.01 (0.43- 2.41)	1.02 (0.48 -2.17)
29.0-86.0	-40.06 (-130.51 – 50.39)	**-0.31* (-0.61 – -0.01)**	0.26 (-0.14 – 0.66)	0.60 (0.22 – 1.63)	0.94 (0.42- 2.08)
>86.0	11.58 (-83.10 – 106.27)	-0.19 (-0.51 – 0.12)	-0.01 (-0.42 – 0.41)	1.04 (0.40 – 2.71)	1.29 (0.58 – 2.85)
MCPP					
<0.31	0	0	0	1.00	1.00
0.31-0.92	25.48 (-61.61 – 112.57)	-0.17 (-0.46 – 0.12)	-0.36 (-0.74 -0.02)	2.06 (0.76 – 5.58)	0.59 (0.27 – 1.26)
0.93-2.1	-42.06 (-136.70 – 52.57)	**-0.34* (-0.66 – -0.03)**	-0.34 (-0.75 – 0.08)	**2.89* (1.03 – 8.12)**	0.90 (0.41 – 2.00)
>2.1	**-110.01* (-216.53 – -3.49)**	**-0.48** (-0.83 – -0.12)**	-0.27 (-0.74 – 0.19)	2.76 (0.86 – 8.85)	1.05 (0.42 – 2.62)
∑DEHP					
<8.32	0	0	0	1.00	1.00
8.32-18.39	-21.71 (-110.89 – 67.47)	-0.05 (-0.34 – 0.25)	-0.30 (-0.70 – 0.09)	1.45 (0.62 – 3.41)	0.62 (0.30- 1.26)
18.40-37.9	-35.62 (-136.18 – 64.95)	-0.19 (-0.53 – 0.14)	-0.11 (-0.55 – 0.33)	0.48 (0.15 – 1.55)	**0.31* (0.12 – 0.79)**
>37.9	-96.86 (-212.81 – 19.09)	-0.27 (-0.66 – 0.11)	-0.15 (-0.66 – 0.36)	0.89 (0.27 – 2.94)	0.50 (0.18 – 1.39)

Legend: OR = odds ratio; 95% CI = 95% Confidence Interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; AS = Apgar score

[a]Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, and gestational age.

[b] Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, and specific gravity.

* P<0.05; **P<0.01

Table 3.5. Effect estimates from minimally-adjusted models for the association of first trimester phthalate levels with infant outcomes among Females (N=680).

Phthalate Quartiles (µg/L)	Birth Weight (g)[a] β (95% CI)	Head Circumference (cm)[a] β (95% CI)	Gestational Age (weeks)[b] β (95% CI)	Low AS at 1 minute[a] OR (95% CI)
MBP				
<5.2	0	0	0	1.00
5.2-12.9	23.87 (-68.54 – 116.28)	0.18 (-0.11 – 0.47)	-0.15 (-0.49 – 0.20)	1.25 (0.56 – 2.83)
13.0-25.0	18.35 (-91.79 – 128.49)	**0.51** (0.17 – 0.85)**	-0.13 (-0.54 – 0.28)	0.62 (0.20 – 1.94)
>25.0	-42.59 (-168.70 – 83.53)	0.18 (-0.21 – 0.57)	0.11 (-0.36 – 0.58)	0.82 (0.23 – 2.99)
MBzP				
<2.4	0	0	0	1.00
2.4-5.1	67.22 (-26.72 – 161.17)	**0.32* (0.03 – 0.61)**	-0.18 (-0.53 – 0.17)	0.72 (0.32- 1.65)
5.2-12.0	54.61 (-48.20 – 157.43)	0.27 (-0.05 – 0.59)	-0.19 (-0.57 – 0.19)	0.35 (0.12 – 1.02)
>12.0	44.40 (-69.57 – 158.38)	0.31 (-0.04 – 0.67)	-0.26 (-0.68 – 0.17)	0.47 (0.15 – 1.50)
MEP				
<12.0	0	0	0	1.00
12.0-28.9	22.43 (-70.78 – 115.64)	0.11 (-0.18 – 0.40)	**-0.44* (-0.79 – -0.10)**	0.73 (0.32 – 1.71)
29.0-86.0	-8.54 (-103.91 – 86.83)	-0.04 (-0.34 – 0.26)	-0.33 (-0.68 – 0.02)	**0.32* (0.11 – 0.93)**
>86.0	-25.54 (-127.92 – 76.84)	0.01 (-0.31 – 0.33)	-0.17 (-0.55 – 0.21)	0.74 (0.29 – 1.93)
MCPP				
<0.31	0	0	0	1.00
0.31-0.92	31.01 (-59.94 – 121.96)	**0.28* (0.0003 – 0.57)**	-0.06 (-0.40 – 0.28)	1.45 (0.63 – 3.34)
0.93-2.1	-64.75 (-170.72 – 41.22)	0.11 (-0.22 – 0.44)	0.24 (-0.15 – 0.64)	1.60 (0.56 – 4.56)
>2.1	-47.21 (-166.01 – 71.59)	0.21 (-0.16 – 0.58)	0.19 (-0.26 – 0.63)	1.62 (0.50 – 5.25)
∑DEHP				
<8.32	0	0	0	1.00
8.32-18.39	-27.45 (-120.77 – 65.86)	0.18 (-0.11 – 0.47)	-0.08 (-0.43 – 0.27)	0.73 (0.30 – 1.80)
18.40-37.9	-24.87 (-134.91 – 85.17)	0.31 (-0.03 – 0.66)	0.01 (-0.39 – 0.42)	1.43 (0.53 – 3.90)
>37.9	-29.32 (-154.61 – 95.97)	0.23 (-0.16 – 0.62)	0.01 (-0.46 – 0.47)	0.73 (0.20 – 2.61)

Legend: OR = odds ratio; 95% CI = 95% Confidence Interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; AS = Apgar score

[a]Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, and gestational age.

[b] Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, and specific gravity.

* P<0.05; **P<0.01

Table 3.6. Effect estimates from maximally-adjusted models for the association of first trimester phthalate levels with maternal and infant outcomes (N=1,412).

Phthalate Quartiles (µg/L)	Birth Weight (g)[a] β (95% CI)	Head Circumference (cm)[a] β (95% CI)	Gestational Age (weeks)[b] β (95% CI)	Preterm Birth[b] OR (95% Cl)	Low AS at 1 minute[a] OR (95% CI)	Low AS at 5 minutes[d+] OR (95% CI)	Gestational Hypertension[c] OR (95% CI)
MBP							
<5.2	0	0	0	1.00	1.00	1.00	1.00
5.2-12.9	7.15 (-57.70 – 71.99)	0.07 (-0.14 – 0.29)	**-0.28* (-0.54 – -0.02)**	1.74 (0.86 – 3.51)	0.97 (0.56 – 1.67)	2.29 (0.62- 8.46)	0.78 (0.41 – 1.48)
13.0-25.0	20.70 (-57.26 – 98.67)	0.19 (-0.06 – 0.45)	-0.09 (-0.40 – 0.23)	1.07 (0.46 – 2.52)	0.77 (0.38 – 1.55)	1.88 (0.38 – 9.25)	0.60 (0.28 – 1.29)
>25.0	-55.50 (-143.88 – 32.88)	-0.06 (-0.35 – 0.23)	-0.13 (-0.48 – 0.23)	0.91 (0.34 – 2.41)	0.91 (0.41 – 2.00)	1.02 (0.14 – 7.35)	**0.38* (0.15 – 0.92)**
MBzP							
<2.4	0	0	0	1.00	1.00	1.00	1.00
2.4-5.1	53.27 (-11.52 – 118.05)	0.14 (-0.07 – 0.36)	-0.08 (-0.34 – 0.18)	0.76 (0.37 – 1.56)	0.60 (0.35 – 1.04)	1.19 (0.36 – 3.90)	1.04 (0.50 – 2.14)
5.2-12.0	**81.22* (10.32 – 152.12)**	0.17 (-0.07 – 0.40)	-0.14 (-0.43 – 0.14)	0.84 (0.40 – 1.77)	**0.44* (0.23 – 0.84)**	0.47 (0.10 – 2.31)	1.63 (0.78 – 3.38)
>12.0	66.82 (-10.93 – 144.58)	0.19 (-0.06 – 0.45)	-0.14 (-0.45 – 0.18)	0.97 (0.44 – 2.17)	**0.46* (0.23 – 0.94)**	0.78 (0.17 – 3.70)	**2.50* (1.17 – 5.38)**
MEP							
<12.0	0	0	0	1.00	1.00	1.00	1.00
12.0-28.9	-11.62 (-76.05 – 52.81)	0.07 (-0.14 – 0.29)	-0.22 (-0.47 – 0.04)	1.29 (0.64 – 2.56)	0.86 (0.50 – 1.50)	1.11 (0.30 – 4.12)	1.65 (0.84 – 3.25)
29.0-86.0	-22.72 (-88.59 – 43.16)	-0.17 (-0.39 – 0.04)	-0.01 (-0.28 – 0.25)	0.85 (0.40 – 1.83)	0.63 (0.34 – 1.17)	1.13 (0.30 – 4.31)	1.15 (0.55 – 2.41)
>86.0	-3.60 (-73.62 – 66.41)	-0.08 (-0.31 – 0.15)	-0.08 (-0.36 – 0.20)	1.32 (0.63 – 2.76)	0.99 (0.54 – 1.79)	1.36 (0.35 – 5.27)	**2.04* (1.01 – 4.14)**
MCPP							
<0.31	0	0	0	1.00	1.00	1.00	1.00
0.31-0.92	34.26 (-29.18 – 97.71)	0.05 (-0.16 – 0.26)	-0.21 (-0.47 – 0.04)	1.93 (0.92 – 4.02)	0.87 (0.50 – 1.52)	0.90 (0.26 – 3.15)	1.23 (0.64 – 2.34)
0.93-2.1	-43.53 (-115.03 – 27.97)	-0.12 (-0.36 – 0.11)	-0.07 (-0.36 – 0.22)	1.75 (0.78 – 3.95)	1.11 (0.59 – 2.09)	0.90 (0.21 – 3.76)	0.94 (0.45 – 1.97)
>2.1	-75.66 (-156.16 – 4.84)	-0.18 (-0.44 – 0.09)	-0.09 (-0.42 – 0.23)	2.16 (0.89 – 5.26)	1.20 (0.58 – 2.46)	0.98 (0.20 – 4.92)	1.16 (0.52 – 2.58)
∑DEHP							
<8.32	0	0	0	1.00	1.00	1.00	1.00
8.32-18.39	-13.20 (-79.84 – 53.44)	0.08 (-0.14 – 0.30)	-0.14 (-0.41 – 0.13)	1.46 (0.72 – 2.96)	0.63 (0.36 – 1.10)	**4.44* (1.21 – 16.24)**	1.07 (0.55 – 2.09)
18.40-37.9	-16.91 (-93.11 – 59.30)	0.05 (-0.20 – 0.30)	-0.01 (-0.32 – 0.29)	0.76 (0.32 – 1.82)	0.55 (0.28 – 1.09)	2.91 (0.57 – 14.74)	0.94 (0.43 – 2.04)
>37.9	-49.91 (-137.19 – 37.37)	-0.06 (-0.34 – 0.23)	-0.05 (-0.40 – 0.30)	1.06 (0.42 – 2.70)	0.53 (0.24 – 1.19)		0.88 (0.36 – 2.12)

Legend: OR = odds ratio; 95% CI = 95% Confidence Interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; AS = Apgar score

[+] In place of the concentrations presented, ∑DEHP was modelled as tertiles (T), with T1 as the reference, for AS at 5 minutes using the following cut-offs: <11.24µg/L (T1), 11.24-28.79µg/L (T2), and >28.80µg/L (T3)

[a] Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, gestational age, average SBP at V2, average DBP at V2, and BPA.

[b] Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, average SBP at V2, average DBP at V2, and BPA.

[c] Model adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, baby sex, and BPA.

[d] Model adjusted for maternal age, specific gravity, gestational age, average SBP at V2, average DBP at V2, and BPA.

* P<0.05; **P<0.01

Table 3.7. Effect estimates from maximally-adjusted models for the association of first trimester phthalate levels with infant outcomes among males (N=732).

Phthalate Quartiles (µg/L)	Birth Weight (g)[a] β (95% CI)	Head Circumference (cm)[a] β (95% CI)	Gestational Age (weeks)[b] β (95% CI)	Preterm Birth[b] OR (95% Cl)	Low Apgar score at 1 minute[a] OR (95% CI)
MBP					
<5.2	0	0	0	1.00	1.00
5.2-12.9	-21.11 (-111.16 – 68.94)	-0.06 (-0.36 – 0.24)	**-0.42* (-0.82 – -0.03)**	1.47 (0.59 – 3.67)	0.83 (0.39 – 1.77)
13.0-25.0	5.26 (-103.08 – 113.61)	-0.09 (-0.46 – 0.27)	-0.11 (-0.58 – 0.37)	0.88 (0.28 – 2.78)	1.04 (0.41 – 2.59)
>25.0	-85.40 (-208.14 – 37.33)	-0.28 (-0.69 – 0.13)	-0.44 (-0.97 – 0.10)	0.86 (0.23 – 3.17)	1.12 (0.39 – 3.20)
MBzP					
<2.4	0	0	0	1.00	1.00
2.4-5.1	48.26 (-40.67 – 137.20)	0.02 (-0.28 – 0.31)	-0.02 (-0.42 – 0.37)	0.97 (0.39 – 2.40)	0.54 (0.26 – 1.14)
5.2-12.0	**103.34* (5.89 – 200.78)**	0.12 (-0.20 – 0.45)	-0.15 (-0.58 – 0.28)	0.87 (0.32 – 2.37)	0.56 (0.24 – 1.31)
>12.0	101.58 (-4.65 – 207.81)	0.20 (-0.16 – 0.55)	-0.11 (-0.57 – 0.36)	0.99 (0.34 – 2.91)	0.57 (0.23 – 1.45)
MEP					
<12.0	0	0	0	1.00	1.00
12.0-28.9	-58.19 (-146.66 – 30.28)	-0.01 (-0.30 – 0.29)	0.02 (-0.37 – 0.41)	0.94 (0.39 – 2.27)	0.97 (0.46 – 2.08)
29.0-86.0	-39.20 (-130.18 – 51.77)	**-0.30* (-0.61 – -0.001)**	0.27 (-0.13 – 0.67)	0.56 (0.20 – 1.53)	0.95 (0.42 – 2.12)
>86.0	9.80 (-85.82 – 105.43)	-0.18 (-0.50 – 0.14)	0.02 (-0.40 – 0.43)	0.96 (0.37 – 2.49)	1.25 (0.56 – 2.78)
MCPP					
<0.31	0	0	0	1.00	1.00
0.31-0.92	31.83 (-56.00 – 119.67)	-0.16 (-0.45 – 0.13)	-0.33 (-0.72 – 0.05)	1.87 (0.68 – 5.13)	0.58 (0.27 – 1.26)
0.93-2.1	-28.22 (-124.62 – 68.18)	**-0.33* (-0.65 – -0.01)**	-0.31 (-0.73 – 0.12)	2.66 (0.92 – 7.71)	0.90 (0.40 – 2.03)
>2.1	-98.75 (-207.18 – 9.67)	**-0.48* (-0.84 – -0.11)**	-0.28 (-0.75 – 0.20)	2.59 (0.78 – 8.55)	1.16 (0.46 – 2.91)
∑DEHP					
<8.32	0	0	0	1.00	1.00
8.32-18.39	-1.72 (-94.45 – 91.00)	-0.004 (-0.31 – 0.31)	-0.26 (-0.66 – 0.15)	1.21 (0.49 – 3.03)	0.58 (0.27 – 1.22)
18.40-37.9	-14.68 (-119.41 – 90.05)	-0.16 (-0.51 – 0.19)	-0.10 (-0.55 – 0.36)	0.40 (0.12 – 1.36)	**0.31* (0.12 – 0.81)**
>37.9	-75.87 (-196.70 – 44.95)	-0.26 (-0.66 – 0.15)	-0.13 (-0.66 – 0.40)	0.70 (0.20 – 2.46)	0.51 (0.18 – 1.45)

Legend: OR = odds ratio; 95% CI = 95% Confidence Interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; AS = Apgar score

[a]Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, gestational age, average SBP at V2, average DBP at V2, and BPA.

[b]Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, average SBP at V2, average DBP at V2, and BPA.

* P<0.05; **P<0.01

Table 3.8. Effect estimates from maximally-adjusted models for the association of first trimester phthalate levels with infant outcomes among females (N=680).

Phthalate Quartiles (µg/L)	Birth Weight (g)[a] β (95% CI)	Head Circumference (cm)[a] β (95% CI)	Gestational Age (weeks)[b] β (95% CI)	Low AS at 1 minute[a] OR (95% CI)
MBP				
<5.2	0	0	0	1.00
5.2-12.9	34.65 (-58.87 – 128.17)	0.20 (-0.09 – 0.49)	-0.14 (-0.49 – 0.21)	1.17 (0.51 – 2.66)
13.0-25.0	34.74 (-77.93 – 147.41)	**0.53** (0.19 – 0.88)**	-0.11 (-0.53 – 0.31)	0.54 (0.17 – 1.74)
>25.0	-30.46 (-158.01 – 97.10)	0.20 (-0.20 – 0.60)	0.13 (-0.35 – 0.60)	0.75 (0.21- 2.75)
MBzP				
<2.4	0	0	0	1.00
2.4-5.1	69.49 (-24.93 – 163.91)	**0.33* (0.04 – 0.63)**	-0.15 (-0.50 – 0.20)	0.70 (0.30 – 1.63)
5.2-12.0	57.50 (-45.80 – 160.80)	0.25 (-0.07 – 0.57)	-0.17 (-0.55 – 0.22)	**0.31* (0.10 – 0.93)**
>12.0	50.05 (-64.49 – 164.58)	0.30 (-0.06 – 0.65)	-0.24 (-0.67 – 0.18)	0.44 (0.14 – 1.41)
MEP				
<12.0	0	0	0	1.00
12.0-28.9	26.10 (-67.79 – 120.00)	0.12 (-0.17 – 0.41)	**-0.42* (-0.77 – -0.08)**	0.69 (0.30 – 1.63)
29.0-86.0	-9.63 (-105.18 – 85.91)	-0.04 (-0.34 – 0.26)	-0.31 (-0.67 – 0.04)	**0.31* (0.11 – 0.94)**
>86.0	-27.57 (-130.00 – 74.85)	0.001 (-0.32 – 0.32)	-0.15 (-0.53 – 0.23)	0.72 (0.27 – 1.88)
MCPP				
<0.31	0	0	0	1.00
0.31-0.92	45.98 (-45.83 – 137.78)	**0.31* (0.02 – 0.60)**	-0.08 (-0.42 – 0.27)	1.37 (0.59 – 3.22)
0.93-2.1	-52.44 (-159.15 – 54.27)	0.11 (-0.23 – 0.44)	0.23 (-0.17 – 0.62)	1.44 (0.50 – 4.15)
>2.1	-33.70 (-154.26 – 86.86)	0.18 (-0.19 – 0.56)	0.15 (-0.30 – 0.60)	1.41 (0.43 – 4.65)
∑DEHP				
<8.32	0	0	0	1.00
8.32-18.39	-20.78 (-116.01 – 74.44)	0.20 (-0.10 – 0.50)	-0.04 (-0.39 – 0.32)	0.66 (0.27 – 1.66)
18.40-37.9	-7.81 (-119.66 – 104.03)	0.33 (-0.01 – 0.68)	0.02 (-0.40 – 0.44)	1.29 (0.47 – 3.58)
>37.9	-11.19 (-137.91 – 115.53)	0.25 (-0.15 – 0.64)	-0.003 (-0.48 – 0.47)	0.67 (0.18 – 2.47)

Legend: OR = odds ratio; 95% CI = 95% Confidence Interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; AS = Apgar score

[a]Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, gestational age, average SBP at V2, average DBP at V2, and BPA.

[b] Models adjusted for maternal age, pre-pregnancy BMI, parity, active smoking status, educational level, specific gravity, average SBP at V2, average DBP at V2, and BPA.

* P<0.05; **P<0.01

3.5. DISCUSSION

3.5.1. Summary of Study Findings

A significant ($p<0.01$) positive association was observed between first trimester concentrations of MBP in quartile 3 and head circumference among female infants. Although quartile 4 concentrations of MCPP were associated with a decrease in head circumference among male infants, this relationship only approached statistical significance ($p<0.05$) following the adjustment of additional covariates included in the maximally-adjusted model. Furthermore, associations of phthalate metabolites with birth weight, gestational age, preterm birth, Apgar scores, and gestational hypertension did not achieve statistical significance ($p>0.05$).

Several general trends among suggestive associations ($p<0.05$) may be noted. For instance, consistent increases among female infants, and decreases among male infants were observed for head circumference. This difference in the directionality of the stratum-specific effect estimates suggests the possibility of effect modification by infant sex. Furthermore, a consistent decrease in gestational age was observed in association with phthalate metabolites among male and female infants. Interestingly, quartile 3 concentrations of MCPP was also positively associated with the odds of preterm birth in male infants; however, this relationship was not observed following adjustment in the maximally-adjusted model. These study findings suggest that phthalates may potentially play a contributing role in decreasing the duration of pregnancy. Lastly, a consistent decrease in the odds of low Apgar scores at 1 minute were observed in association with select phthalate metabolites among male and female infants. Overall, a number of suggestive associations warranting investigation in other studies were observed.

3.5.2. The Association of Phthalates with Clinical Outcomes in the Mother and Infant

For consistency across studies, associations with a p-value of less than 0.05 were considered to be statistically significant in the discussion comparing the current research findings with results reported in the existing literature. The following specific issues are discussed below: (1) the effect of phthalates on birth weight, (2) the effect of phthalates on head circumference, (3) the effect of phthalates on gestational age, (4) the effect of phthalates on preterm birth, (5) the effect of phthalates on Apgar scores and gestational hypertension, and (6) potential explanations for inconsistencies in the literature.

The Effect of Phthalates on Birth Weight

A nested-case control study consisting of study participants with male infants, measured maternal urinary concentrations of phthalate metabolites between 6 and 30 weeks of gestation, and reported a 198g (95% Cl: -343 – -52) decrease in birth weight in association with MCPP concentrations in tertile 2, relative to tertile 1[36]. Based on the minimally-adjusted models developed for the current analysis, a similar inverse association was also observed between MCPP and birth weight among male infants. However, following the adjustment of additional covariates in the final model, the effect of MCPP was not apparent. This null finding was consistent with those reported by a cohort study conducted in New York City that assessed the exposure to phthalates using maternal urinary samples collected predominantly in the third trimester [41]. Su et al. (2014) conducted a cohort study consisting of participants from central Taiwan, and evaluated the exposure to phthalates using maternal urinary samples collected in the third trimester; the study authors reported an association of MBzP and birth weight that was not statistically significant [37]. Similar null associations were also reported in other studies

investigating the same relationship [36, 41]. In contrast, the present study found a positive association between MBzP and birth weight among male infants.

Three of the previously described studies also evaluated the effect of MEP on birth weight; consistently, all studies found associations that were not statistically significant [36, 37, 41]. Similar null effects of MEP on birth weight were also observed in the current study. The nested case-control study by Zhang et al. (2009) investigated the association of phthalates with low birth weight (LBW), defined as weight less than 2,500g [42]. A positive exposure-response relationship was observed between meconium concentrations of MBP and birth weight; in particular, elevated odds of LBW were observed in association with quartiles 2 (OR: 1.58; 95% Cl: 1.08 – 2.46), 3 (OR: 2.84; 95% Cl: 1.19 – 4.82), and 4 (OR: 4.68; 95% Cl: 2.14 – 6.85), compared to quartile 1 [42]. The study by Xie et al. (2015), measured meconium concentrations of mono-n-butyl phthalate (MnBP), and reported a 0.92g (95% Cl: -2.09 – -0.03) decrease in birth weight per unit increase in the exposure, as well as a 2.4 (95% Cl: 1.1 – 4.8) times greater odds of LBW, defined as weight less than 2,500g, among those exposed to concentrations above the median [11]. Contrary to the previous two studies, Huang et al. (2009), measured amniotic fluid concentrations of MBP, and observed greater birth weight among female infants with higher levels of the exposure; however, no significant effects were observed among male infants [49]. Also, previous research using maternal urine as the matrix for exposure assessment reported no significant associations of MBP or MnBP with birth weight [36, 37, 41, 43]. These findings are consistent with those observed in the current analysis.

Among studies examining ∑DEHP, a summary measure of DEHP metabolites, null effects on birth weight were reported [36, 37, 41, 43]. Similar non-significant associations were also observed in the present study. However, several other studies evaluating the effect of individual

DEHP metabolites have reported results that were of statistical significance [10, 11, 34, 42, 43]. Although most studies observed an inverse association with select DEHP metabolites, the study by De Cock et al. (2016), reported relative to tertile 1, a 504.3g (95% Cl: -974.83 – -33.84) decrease in birth weight in association with tertile 3 concentrations of mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), and a 436.4g (95% Cl: 65.22 – 807.67) increase in birth weight in association with tertile 2 concentrations of MEHHP among male infants [10, 11, 34, 42, 43].

The Effect of Phthalates on Head Circumference

A study of male newborns by Brucker-Davis et al. (2010) measured phthalate exposure using cord blood samples; in association with MBP, a moderate positive correlation of 0.43 ($p = 0.005$) was observed with head circumference [50]. Different study findings were observed in the present study where a similar relationship of MBP exposure with increased head circumference was found among females, but not among males. Furthermore, other studies assessing the effects of MBP or MnBP on head circumference have reported relationships that were not statistically significant [36, 37, 41]. Three studies investigating the association between MBzP and head circumference reported null findings [36, 37, 41]. In contrast, the current analysis identified a positive relationship between MBzP and head circumference among female infants. The cohort study by Wolff et al. (2008), reported an elevated head circumference per ln-unit increase in maternal urinary concentrations of MEP (0.12cm; 95% Cl: 0.01 – 0.23) [41]. This differed from the results identified in the present study, which observed an inverse association of MEP with head circumference among males. In addition, the studies conducted by Su et al (2014) and Philippat et al. (2012) reported null findings for the same relationship [36, 37].

Two studies assessing the association between MCPP and head circumference reported results that were not statistically significant [36, 41]. In contrast, the current analysis for the relationship between MCPP and head circumference demonstrated a decrease among males, and an increase among females. The study conducted by Su et al. (2014) reported a positive association between ∑DEHP and head circumference expressed as z-scores; in particular, a unit increase in ∑DEHP was associated with a 0.001cm (SE: 0.0004) increase in head circumference among males [37]. Other studies assessing the effect of ∑DEHP or its respective metabolites demonstrated associations that were not statistically significant [33, 36, 41]. These study findings were consistent with those identified in the current analysis.

The Effects of Phthalates on Gestational Age

The effects of MBP, MnBP, MEP, MBzP, or MCPP on gestational age were not statistically significant in several studies [37, 39, 41, 49]. In the present study, consistent null findings were identified for MBzP and MCPP; however, decreases in gestational age were also observed in association with MBP in males, and MEP in females. The study by Wolff et al. (2008), reported non-significant study findings for the relationship between ∑DEHP and gestational age [41]. Similar null results were also identified in the current analysis. However, conflicting results were observed in other studies [37, 40]. In particular, Su et al. (2014), reported a 0.0011 week (SE: 0.0005) increase in gestational age per unit increase in ∑DEHP among males [37]. In contrast, the study by Whyatt et al. (2009), reported a 0.18 week (-0.32 – -0.03) decrease in gestational age per log-unit increase in ∑DEHP [40]. Studies reporting on the association of gestational age with DEHP metabolites separately have also reported inconsistent results. In particular, among studies reporting on the relationship between select metabolites of DEHP and gestational age, 3 reported

positive associations, 3 identified inverse associations, and 2 did not detect any significant associations [30, 37, 39-41, 49, 51, 52].

The Effect of Phthalates on Preterm Birth

The cohort study by Adibi et al. (2009), measured maternal urinary concentrations of phthalates, and reported reduced odds of preterm birth per log-unit increase in MEHP (OR: 0.5; 95% Cl: 0.3 – 0.9), MEOHP (OR: 0.4; 95% Cl: 0.2 – 0.9), and MEHHP (OR: 0.5; 95% Cl: 0.3 – 0.9) [30]. In contrast, the nested case-control study by Ferguson et al. (2014a) estimated average maternal urinary concentrations of phthalates using samples collected a 3 different time points, and reported elevated odds of preterm birth for each ln-unit increase in MEHP (OR: 1.34; 95% Cl: 1.07 – 1.68), MECPP (OR: 1.40; 95% Cl: 1.13 – 1.74), and ∑DEHP (OR: 1.33; 95% Cl: 1.04 – 1.70) [23]. The study authors also conducted a subgroup analysis and reported increased odds of spontaneous preterm birth per ln-unit increase in MEHP (OR: 1.65; 95% Cl: 1.20 – 2.26), MEOHP (OR: 1.47; 95% Cl: 1.04 – 2.08), MECPP (OR: 1.56; 95% Cl: 1.15 – 2.13), ∑DEHP (OR: 1.63; 95% Cl: 1.15 – 2.31), MBzP (OR: 1.41; 95% Cl: 1.02 – 1.95), MnBP (OR: 1.49; 95% Cl: 1.08 – 2.06), and MCPP (OR: 1.36; 95% Cl: 1.02 – 1.81) [23].

The study by Ferguson et al. (2014b) separately evaluated maternal urinary concentrations of phthalates at 4 different time points, and reported elevated odds of preterm birth per ln-unit increase in MECPP (OR: 1.25; 95% Cl: 1.06 – 1.48) and MCPP (OR: 1.19; 95% Cl: 1.01 – 1.41) at visit 1, and MECPP at visit 3 (OR: 1.27; 95% Cl: 1.06 – 1.52). Although a reduced odds of preterm birth were observed per ln-unit increase in MEHHP at visit 4 (OR: 0.77; 95% Cl: 0.60 – 0.98), the study authors also reported a decrease in the number of cases at that time point as several had already delivered [31]. The study findings describing the odds of overall preterm birth were

acquired from the available supplementary data. After performing subgroup analyses, the study authors reported greater odds of placental preterm birth per ln-unit increase in MECPP at visit 1 (OR: 1.46; 95% Cl: 1.10 – 1.95), as well as elevated odds of spontaneous preterm birth per ln-unit increase in visit 3 concentrations of MECPP (OR: 1.33; 95% Cl: 1.04 – 1.70), ∑DEHP (OR: 1.33; 95% Cl: 1.02 – 1.73), MBzP (OR: 1.43; 95% Cl: 1.05 – 1.95), and MnBP (OR: 1.45; 95% Cl: 1.08 – 1.96) [31]. Similarly, the study by Meeker et al. (2009) measured maternal urinary concentrations of phthalates collected during the third trimester, and observed a positive relationship between preterm birth and MnBP concentrations above the median (OR: 4.5; 95% Cl: 1.2 – 16.6) [35]. Overall, most of the study findings discussed suggest a positive association between phthalates and preterm birth. The current analysis found MCPP to be associated with an increased odds of preterm birth among male infants. This association was not apparent following the adjustment of additional covariates in the maximally-adjusted model.

The Effect of Phthalates on the Apgar scores and Gestational Hypertension

The study by Latini et al. (2003) measured cord blood concentrations of DEHP and MEHP, and reported null associations for the Apgar score at 1 minute, and 5 minutes [51]. However, reduced odds of a low-Apgar score at 1 minute were observed in the present study in association with MBzP and MEP in females, as well as with ∑DEHP in males. Additionally, an elevated odds of a low-Apgar score at 5 minutes were observed in association with ∑DEHP among the sample consisting of both male and female infants. To our knowledge, there were no prior studies on the association between phthalates and gestational hypertension. Although research findings from the current analysis demonstrate a positive association of MBzP and MEP with gestational hypertension, an inverse relationship was also observed between MBP and this maternal clinical

endpoint. As a result of the scarcity of research assessing the association of phthalates with Apgar scores and gestational hypertension, validation of the current study findings with future research is essential.

Possible Explanations for Inconsistencies in the Literature

Although the current study shared some similar results with others reported in the existing literature, contradictory findings were also observed. Possible explanations include multiple potential sources of heterogeneity, such as the differences in the study population, methods of exposure assessment, and approaches to statistical analysis. Variability in the study populations and eligibility criteria for subject selection may lead to variation in the underlying characteristics of the study sample. Additionally, the recruitment of study participants from different areas or countries may contribute to varying magnitudes of exposure that can be attributable to distinct environmental levels of phthalates. The different exposure levels between study populations may produce research results that are not comparable, as evidence in the literature from human, animal, and in vitro exposure studies has demonstrated possible non-monotonic relationships of select phthalates or metabolites with several outcomes, including birth weight, birth length, aromatase activity, and pro-inflammatory cytokine stimulation [36, 46, 53].

Variability in the methods implemented to estimate the exposure to phthalates may consist of differences in the choice of biological specimen, the frequency of exposure measurement, and the time of exposure assessment. The biological matrix selected for exposure assessment may determine the likelihood of sample contamination, and the concentration of phthalates measured. For instance, urine samples present a lower risk of contamination when evaluating hydrolytic monoesters as this exposure matrix lacks the enzymes capable of breaking down external phthalate

contaminants following sample collection; as well, urine samples have been shown to yield higher average concentrations of phthalate metabolites when compared to other biological specimens, such as serum and breast milk [54-56]. Also, standards used in the phthalate analysis can cause errors due to differences between suppliers and batches, a factor that was corrected for in the MIREC Study [22]. As phthalates are quickly eliminated from the human body, and internal levels may vary by altered sources of exposure, there are concerns regarding temporal variability [7, 8, 57]. As a result, a more accurate estimate of gestational exposure to phthalates may be acquired via multiple measurements over time. As specific time points during pregnancy may be more vulnerable to the effects of phthalates, it is critical to consider the time of exposure assessment [31]. For this reason, studies using a single exposure measurement with differential timings across gestation may acquire attenuated measures of association.

Other sources of variability that should be considered includes the different approaches to statistical analysis, such as the handling of concentrations below the LOD, the covariates adjusted for as potential confounders, and the evaluation of exposures and outcomes as either continuous or categorical variables. In summary, to better understand the inconsistencies in the literature, it is imperative to consider the multiple factors that can contribute to the overall heterogeneity between studies, as each may have an influence on the reported research findings. A detailed discussion on heterogeneity between the studies described was previously reported in our systematic review of the association of gestational exposure to environmental phthalates with perinatal and pregnancy outcomes [20].

3.5.3. Study Strengths

The current study possesses a number of strengths that are essential to the validity of the research findings. The prospective cohort design is a major advantage, as temporality of the relationship between phthalates and clinical outcomes in the mother and infant is assured. Specifically, exposure measurements were conducted using maternal urinary samples collected during the first trimester of pregnancy, which precedes the assessment of the maternal and infant outcomes. Also previously mentioned, the accuracy of phthalate measurement methodology in this study is a strength as issues related to the standards used were adjusted for [22]. The recruitment of a relatively large number of study participants from multiple study sites in Canada contributes to a more representative study sample of pregnant women in the country, with enhanced generalizability of the study findings. The use of multiple questionnaires to collect information on participant characteristics is beneficial as an abundant amount of data on potential confounding factors were available. For this reason, residual confounding related to unmeasured covariates has likely been minimized. An additional strength of the study relates to the common time of exposure assessment. As exposure to phthalates were measured during the first trimester among all study participants, the study findings may contribute meaningful information to further research evaluating early pregnancy as a possible sensitive window of exposure.

3.5.4. Study Limitations

The study limitations include a sample comprised of women who were predominantly of white race (82.86%) and university educated (63.46%). Consequently, the generalizability of the study findings may be reduced, as they may be less applicable to women of other races or educational levels. Since phthalates have demonstrated to have relatively short half-lives, and

sources of exposure that may vary over time, the use of a single urine sample for the measurement of phthalates may not yield precise exposure estimates [7, 8, 57]. However, as misclassification of the exposure to phthalates is believed to be non-differential, the effect estimates are likely to be underestimated. As the adjustment of potential confounding factors were highly dependent on self-reported information collected through the use of questionnaires, there may be concerns associated with data accuracy. In particular, incorrect measures of covariates can consequently contribute to residual confounding. To fill in existing knowledge gaps in the literature, several statistical models were developed to examine the effects of phthalates in relation to maternal and infant clinical outcomes during the perinatal period. A limitation of performing multiple tests consists of the possibility of generating more false positives [58]. However, this issue was addressed to a certain extent by selecting a lower threshold for statistical significance ($p<0.01$).

3.6. CONCLUSION

In conclusion, the current study findings demonstrate a statistically significant association between MBP exposure and elevated head circumference among female infants. Although a significant decrease in head circumference among male infants was observed in association with MCPP exposure in the minimally-adjusted model, this effect was not demonstrated following the adjustment of additional covariates in the maximally-adjusted model. Several interesting trends were observed among associations approaching statistical significance ($p<0.05$). For instance, the analysis on the effect of phthalates on head circumference yielded consistent increases among females, and decreases among males. An inverse relationship between phthalates and gestational age were observed among both male and female stratums. As well, a consistent decrease in the odds of a low Apgar score at 1 minute was observed in males and females. While some similar

associations were demonstrated between results from the current study and those reported in the existing literature, inconsistencies were also observed. Possible explanations for the conflicting results reported in the literature may include heterogeneity in the study populations, methods employed for exposure assessment, and approaches implemented for statistical analysis. In order to more effectively evaluate the risks of phthalates among susceptible populations based on an examination of the currently available body of evidence as a whole, it is essential to identify, understand, and potentially minimize the potential sources of heterogeneity between studies being compared.

3.7. REFERENCES

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BRIDGE TO CHAPTER 4

In the previous manuscript, a secondary analysis was conducted using data from the MIREC Study to investigate the association of gestational exposure to environmental phthalates with clinical outcomes in the mother and infant. The results from this study demonstrated a significant ($p<0.01$) positive association between MBP exposure and head circumference in female infants. No significant relationship between exposure to phthalate metabolites in the first trimester were observed with birth weight, gestational age, preterm birth, Apgar scores, and gestational hypertension. Several interesting findings among those results that approached statistical significance ($0.01<p<0.05$) were noted. Consistent increases in head circumference in females and decreases in males were identified. As well, decreases in gestational age were observed among both male and female stratums. Some discrepancies with the literature were observed: possible explanations may include sources of heterogeneity and challenges with exposure assessment.

In the following manuscript, a secondary analysis was performed using data from the MIREC Study, to address the paucity of research evaluating phthalate-induced changes in maternal responses of inflammation as potential mediators of effect. Since inflammatory processes are tightly controlled during pregnancy, we were interested in identifying any perturbations on relevant inflammatory pathways due to phthalate exposures as such imbalances could potentially explain the adverse outcomes reported. Multinomial logistic regression models were constructed and employed to investigate the association of first trimester urinary phthalate metabolites with low ($\leq 10^{th}$ percentile) and high ($\geq 90^{th}$ percentile) levels of inflammatory biomarkers, in comparison to levels considered normal ($10^{th} – 90^{th}$ percentile).

PREFACE TO CHAPTER 4

The objective of the third manuscript was to address the paucity of research by investigating the association between maternal exposure to environmental phthalates and inflammatory responses among pregnant women sampled for the MIREC Study. Ethics approval for the secondary data-analysis performed was acquired from the Ottawa Health Science Network Research Ethics Board. A copy of the informed consent documents for the MIREC Study is provided in the appendix of this thesis (Appendix 1 and 2).

Dr. Premkumari Kumarathasan, Dr. Renaud Vincent, Dr. William Fraser, and Dr. Tye Arbuckle are main MIREC Study platform investigators that contributed to the establishment of the study. MIREC data handling for urinary phthalate levels was performed by Mandy Fisher. Erica Blais was involved with maternal blood biomarker analysis and biomarker data handling.

This study was a secondary data-analysis of the MIREC database to examine biomarkers related to inflammation from exposure to phthalates. Access to MIREC data, and content expertise on toxicology, inflammation biomarkers, and obstetrical outcomes was provided by Dr. Premkumari Kumarathasan. Expertise in statistics and SAS coding was provided by Dr. Sabit Cakmak. Guidance on development of this research and methodological guidance for statistical analysis was provided by Dr. Daniel Krewski, Dr. Premkumari Kumarathasan, and Dr. James Gomes.

CHAPTER 4: THE ASSOCIATION BETWEEN MATERNAL EXPOSURE TO ENVIRONMENTAL PHTHALATES AND INFLAMMATORY RESPONSES AMONG PREGNANT WOMEN SAMPLED FOR THE MIREC STUDY (MANUSCRIPT 3)

4.1. ABSTRACT

Background: Phthalates are present in products commonly used in trade and commerce, and have been detected as ubiquitous contaminants in the environment. Studies assessing the potential adverse health effects in relation to phthalate exposures during vulnerable periods, such as gestation, have demonstrated the role of these chemicals as possible reproductive and developmental toxicants. To investigate the effects of phthalates on maternal immunological changes, which may be a critical mediator resulting in adverse maternal and perinatal outcomes, the current study was conducted to evaluate the association between maternal exposure to environmental phthalates and inflammatory responses among pregnant women sampled for the MIREC Study.

Methods: A total of 2,001 pregnant women were recruited during the first trimester of pregnancy from multiple sites in Canada between 2008 and 2011. Eleven phthalate metabolite concentrations were estimated using maternal urinary samples collected during the first trimester. Plasma concentrations of 19 markers of inflammation were measured using blood samples collected during the third trimester. Participant characteristics were acquired using questionnaires, and details regarding the pregnancy and newborn were obtained from medical charts. In total, 1,286 study participants with live singleton births, and complete exposure, outcome, and confounder

information were included in the analysis. Multinomial logistic regression models were constructed to evaluate the association between quartiles of phthalates and low ($\leq 10^{th}$ percentile) and high ($\geq 90^{th}$ percentile) levels of inflammation, using moderate levels (10^{th}-90^{th} percentile) as the reference.

Results: The 2^{nd} (OR: 2.64; 95% Cl: 1.51-4.63), and 4^{th} (OR: 2.84; 95% Cl: 1.47-5.49) quartiles of MBzP were significantly associated with greater odds of high MMP-2 levels. Quartile 3 of MEP was inversely associated with low IL-12 levels (OR: 0.41; 95% Cl: 0.22-0.74). The 2^{nd} quartile of MBP was associated with reduced odds of low VCAM levels (OR: 0.37; 95% Cl: 0.21-0.68). Although ∑DEHP concentrations in quartile 2 (OR: 0.38; 95% Cl: 0.20-0.70), and 4 (OR: 0.27; 95% Cl: 0.12-0.62) exhibited an inverse association with low MMP-1 levels, greater odds of low VCAM levels were also observed in association with quartile 4 of ∑DEHP (OR: 2.81; 95% Cl: 1.41-5.62).

Conclusion: The study results demonstrate a significant positive association ($p<0.01$) of MBzP and ∑DEHP urinary concentrations with high MMP-2 and low VCAM plasma levels, respectively. In addition, suggestive associations ($p<0.05$) indicate possible roles of MCPP and MEP exposure in altering maternal inflammatory responses. Further research is needed to validate the current study findings, and to further evaluate the potential of phthalate-induced immunological changes as a plausible biological mechanism resulting in potential adverse outcomes during pregnancy.

4.2. INTRODUCTION

Phthalates are chemicals that have been incorporated into a number of products commonly used in trade and commerce. Potential sources of exposure to these chemicals include personal care products, toys, dietary supplements, and medications [1-3]. The widespread detection of phthalates in the environment demonstrates the ubiquitous nature of these chemicals. For instance, several studies have reported detecting these contaminants in air, water, dust, and soil [4-8]. As a consequence of the extensive use of phthalates and its vast distribution in the environment, humans are at risk of frequent exposure to these chemicals.

Human exposure to phthalates may occur through several possible pathways, including ingestion, inhalation, and dermal contact [9, 10]. In addition, biomonitoring research evaluating internal exposures to phthalates have reported identifying these chemicals in a number of biological fluids, including breast milk, urine, and blood [11]. Concerns regarding human exposure to phthalates exist, as these chemicals have exhibited endocrine disrupting properties, and may be linked to asthma, rhinitis and eczema in children, as well as reproductive outcomes, including male infertility, and endometriosis [12-15]. Epidemiological studies investigating the effects of these chemicals among vulnerable populations, such as pregnant women and the developing fetus, indicate a possible association of phthalates with clinical pregnancy loss, and reduced fetal growth [16, 17]. We previously conducted a systematic review on the effects of phthalates during pregnancy, and identified possible associations with decreased birth weight and gestational age, and increased head circumference and preterm births; however, contradictory results were also observed [18]. As exposure to these environmental chemicals may be hazardous to human health, especially during sensitive periods such as pregnancy, research into maternal biological changes

in relation to phthalate exposures is essential to a acquire a better understanding of the mechanisms underlying the effects of these chemicals.

Altered levels of inflammation during gestation may be a critical intermediate factor in the association between phthalate exposures and adverse clinical outcomes occurring during pregnancy. As the existing literature demonstrates the importance of both pro- and anti-inflammatory cytokines during gestation, disruptions to the tightly regulated inflammatory response may be contributing to the incidence of perinatal and pregnancy complications [19, 20]. Research findings reported by several observational studies indicate possible associations between markers of inflammation and outcomes, such as preterm premature rupture of membranes (pPROM), preterm births, severe pre-eclampsia, small for gestational age, and reduced birth weight [21-24]. The immunotoxic properties of phthalates have also been studied in a number of in-vitro experiments. For instance, a study exposing human macrophages to di-(2-ethyl-hexyl) phthalates (DEHP) reported elevated media levels of TNF-α, IL-1β, IL-8, and IL-6, and greater gene expressions of IL-8, MMP3, MMP10, and MMP14 [25]. Additionally, a study conducted on human epithelial cells reported observing initial potent stimulatory effects of mono-2-ethylhexyl phthalate (MEHP), mono-n-octyl phthalate (MnOP), mono-iso-nonyl phthalate (MINP), and mono-iso-decyl phthalate (MIDP) on IL-6 and IL-8, which was followed by suppressive effects at higher doses [26]. Overall, the evidence presented suggests that phthalate-induced inflammatory responses may be a plausible pathway leading to adverse pregnancy and perinatal outcomes; however, few epidemiological studies have been conducted to assess the relationship between phthalates and inflammation during pregnancy [27-29].

To address the paucity of research investigating phthalate-induced maternal immunological changes during gestation, which may be a critical building-block in identifying a

plausible biological mechanism for the relationship between phthalate exposure and adverse clinical outcomes during pregnancy, the current study was conducted to evaluate the association between maternal exposure to environmental phthalates and inflammatory responses among pregnant women sampled for the Maternal-Infant Research on Environmental Chemicals (MIREC) Study.

4.3. MATERIALS AND METHODS

4.3.1. Study Design

Details regarding the study design and eligibility criteria of the MIREC Study has been previously described [30]. In brief, MIREC Study recruited study participants from prenatal clinics located in 10 cities within Canada between 2008 and 2011. In total, 2,001 women in their first trimester of pregnancy who were 18 years of age or older, had agreed to take part in the current study. Information concerning participant characteristics, such as demographics, medical conditions, and lifestyle habits, were collected by trained individuals through the administration of questionnaires. Medical charts were used to obtain details regarding the pregnancy and newborn. Biological specimens were collected and analyzed for chemical compounds, including a number of environmental contaminants.

The study consisted of 1,983 pregnant women following the withdrawal of 18 study participants who requested to have their data and samples destroyed. Women were excluded from the analysis if they did not deliver a live singleton baby, or had incomplete information regarding urinary concentrations of phthalate metabolites, plasma levels of inflammatory biomarkers, and covariates identified as potential confounders; this resulted in a final sample size of 1,286 study participants. Ethics approval for the MIREC Study was granted by over 10 ethics committees

across Canada, including the Research Ethics Board at Health Canada. In addition, the current secondary data-analysis being conducted was approved by the Ottawa Health Science Network Research Ethics Board.

4.3.2. Phthalate Exposure

Maternal urine samples collected during the first trimester of pregnancy were used to measure the concentrations of 11 phthalate metabolites, including mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), mono-(2-ethylhexyl) phthalate (MEHP), mono-butyl phthalate (MBP), mono-ethyl phthalate (MEP), mono-benzyl phthalate (MBzP), mono-methyl phthalate (MMP), mono-cyclo-hexyl phthalate (MCHP), mono-isononyl phthalate (MiNP), mono-n-octyl phthalate (MnOP), and mono-(3-carboxypropyl) phthalate (MCPP). The analysis of these chemicals were conducted by the Centre de Toxicologie due Québec, Institut national de Santé Publique du Québec (INSPQ), which is accredited by the Standards Council of Canada. Phthalate metabolites were enzymatically deconjugated, extracted by solid phase extraction, and assessed by LC-MS/MS. The laboratory extraction and analysis methods have been previously reported in greater detail [31].

4.3.3. Inflammatory Responses

Plasma from maternal blood samples collected during the third trimester of pregnancy were analyzed for 19 biomarkers associated with inflammation, including matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-7 (MMP-7), matrix metalloproteinase-9 (MMP-9), matrix metalloproteinase-10 (MMP-10), interleukin-2 (IL-2), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), vascular

endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), human macrophage inflammatory protein 1-beta (MIP-1β), interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GMCSF), vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), and C-reactive protein (CRP). The analysis of these biomarkers were conducted by a laboratory at the Environmental and Radiation Health Science Directorate of Health Canada [30]. Plasma samples for the target biomarkers were analyzed by the methodology reported by Kumarathasan et al. (2014) [32]. Briefly, aliquots of stored plasma samples were analyzed for markers of inflammation by affinity-based multiplex protein array assays using Bio-Plex Pro Human panels (Biorad) and Milliplex Map kits (Millipore) with a Bioplex 100 instrument (Biorad).

4.3.4. Statistical Analysis

All statistical analyses were performed using SAS 9.4 (Statistical Analysis System). Observations that were visually identified as potential outliers were retained in the analysis if determined to be biologically plausible. A descriptive analysis was performed to describe the distribution of maternal characteristics according to the *a priori* selected categories of inflammatory biomarker concentrations: the descriptive statistics computed include means and standard deviations for continuous variables, and proportions and 95% confidence intervals for categorical variables. Associated p-values were generated using ANOVA tests for continuous covariates and Chi-square tests for categorical covariates; however, when the expected cell counts of categorical covariates were below 5, p-values were based on Fisher exact tests.

Phthalate concentrations below the limit of detection (LOD) were replaced by the LOD divided by 2. As a high frequency of concentrations below the LOD were observed for MCHP,

MMP, MiNP, and MnOP (85.4 to 98.5%), these metabolites were not considered in the current analysis. In addition, due to the high Spearman correlation coefficients (>0.90) between MEHP, MEHHP, and MEOHP, these three metabolites which originate from the same parent compound were summed into a single exposure variable (∑DEHP) [31]. These methods were implemented as reported by several other MIREC studies investigating the effects of phthalates [27, 33, 34]. To account for the skewed distribution of phthalate concentrations, geometric means were calculated and presented by categories of the inflammatory-related outcomes. To adjust for variability in urinary dilution, these concentrations were also corrected for using specific gravity (SG) measurements. SG adjusted concentrations were acquired using the following formula: Pc = Pi [(SGm – 1) / (SGi – 1)]. In the formula presented, 'Pc' represents the concentration adjusted for SG, 'Pi' represents the measured concentration, 'SGm' represents the median SG for the MIREC Study cohort, and 'SGi' represents the SG of the urine sample [35].

Multinomial logistic regression models were constructed to investigate the association between individual phthalate metabolites, with the exception of DEHP where sum of metabolites were considered, and biomarkers of inflammation. As in previous studies, phthalate concentrations were categorized into quartiles [33, 34, 36]. Given that these environmental contaminants are ubiquitous, the lowest quartile was selected as the reference group. As previously done, inflammatory biomarker levels were categorized into three groups: low ($\leq 10^{th}$ percentile), normal (10^{th} to 90^{th} percentile), and high ($\geq 90^{th}$ percentile); these cut-offs were selected based on the rationale that these biomarkers which are associated with inflammation have critical functions during pregnancy, and that irregular levels, such as lower or higher than normal concentrations, may consequently increase the risk of adverse pregnancy complications or perinatal outcomes [19,

20, 37]. For this reason, high and low levels of inflammation were modelled as the outcomes of interest, with normal levels of inflammation were selected as the reference category.

The minimally-adjusted models were developed using covariates that were identified as potential confounders by similar studies in the existing literature; these *a priori* selected covariates include maternal age, pre-pregnancy body mass index (BMI), and specific gravity [27-29]. As in several previous studies assessing the exposure to phthalates, specific gravity was directly adjusted for as a covariate in the regression model [36, 38]. Pre-pregnancy BMI was categorized according to cut-offs presented by the World Health Organization: underweight (BMI<18.5 Kg/m^2), normal weight (18.50-24.99 Kg/m^2), overweight (25.00-29.99 Kg/m^2), and obese (≥30.00 Kg/m^2) [39]. Maternal age (years) was modelled as a continuous variable.

The 10 percent change-in-estimate approach to variable selection was implemented in the construction of the maximally-adjusted models [40]. Specifically, the percent change in the effect of the exposure on the outcome was assessed following the introduction of a single covariate into the minimally-adjusted models. Covariates that altered the association of interest by 10% or more in a minimum of 4 models were identified as potential confounders, and included in the maximally-adjusted models. Covariates that were identified as potential confounders in 2 to 3 models were considered if their effects were considered biologically feasible. And lastly, covariates that were identified as a potential confounder in a single model were considered potentially spurious, and thus not retained. Additionally, to reduce the rate of false positives due to multiple comparisons, results were considered statistically significant at a p-value of less than 0.01. However, as the purpose of this study is exploratory, results with a p-value of less than 0.05 were also highlighted.

Covariates considered in the analysis of potential confounders were: highest education level (high school diploma or less/ college diploma or some college education/ university degree),

race (white/ black/ other), marital status (married/ same partner for ≥ 1 year/ widowed/ divorced/ separated/ single/ other), annual household income (≤ $50,000/ $50,001-100,000/ >100,000/ don't know), active smoking status at visit 1 and 3 (never/ current/ quit during pregnancy/ former), second-hand smoke exposure at home, work, public places, or private vehicles at visit 1 and 3 (no/ yes/ don't know), alcohol consumption at visit 1 and 3 (no/ yes/ refuse to answer), family history of maternal high blood pressure during pregnancy (no/ yes/ don't know), chronic illness: high blood pressure (no/ yes), chronic illness: diabetes (no/ yes), other chronic conditions (no/ yes), prenatal multivitamin preparation use (no/ yes), folic acid supplements use (no/ yes), other supplements use (no/ yes), weight measured at visit 1, visit 2, visit 3, and prior to delivery, height measured at visit 1, systolic and diastolic blood pressure measured at visit 1, visit 2, visit 3, and prior to or after delivery admission, spontaneous membrane rupture prior to labour onset (no/ yes), parity (0/ 1/ 2/ 3 or more), gestational diabetes (no/ yes), baby sex (male/ female), congenital anomalies (no/ yes), and bisphenol A (BPA) concentrations.

The categorization of the maternal education, and annual household income variables was done in a similar manner as in the study by Arbuckle et al. (2014) [31]. This study observed significantly higher concentrations of MnBP and MBzP among pregnant women with the lowest level of educational attainment, and greater concentrations of MEHP, MEOHP, and MEHHP in the highest education level. Additionally, while higher MnBP, MEP, and MBzP concentrations were reported among participants with an income of $50,000 or less per year, greater MEOHP and MEHHP concentrations were exhibited by those with an income of more than $100,000 per year. The questionnaire administered to study participants provided 'don't know' and 'refuse to answer' as possible answers in relation to annual household income: these responses were collapsed into one category. Maternal race/ethnicity was originally described as: White, Black, Latin American,

Japanese, Chinese, Filipino, Arab, Korean, South Asian, Southeast Asian, West Asian, Aboriginal, and other. Participants reporting more than one race/ethnicity were placed in the 'Other' category. This variable was further categorized as 'White', 'Black', and 'Other' according to the categories used by Ferguson et al. (2015) [41]. Using the 'White' category as the reference group, this study reported significantly higher MEHP and MBP concentrations among African Americans, whereas individuals of other races/ethnicities only demonstrated significantly higher MBP concentrations [41].

The single second-hand smoke exposure variable was derived from 4 different questions assessing exposure at home, in the workplace, in public places, and in vehicles. Participants indicating 'yes' to exposure at any one of four locations were considered as exposed. Participants indicating 'no' to exposure at all locations, with the exception of 'not applicable' in the workplace, were considered as unexposed. And individuals that responded 'don't know' or 'refuse to answer' were combined, and considered as having an unknown exposure to second-hand smoke. An average value for systolic and diastolic blood pressure was calculated per visit as the two measurements were taken a minute apart. Lastly, BPA concentrations were categorized into quartiles similar to those for phthalate concentrations; this was done for consistency, as both chemicals may share a common source as additives in plastics [42].

A sensitivity analysis applying more conservative criteria of selecting potential confounders was also performed. The conditions that a covariate needed to meet to satisfy these more stringent criteria were: (1) the covariate has a p-value of less than 0.05, and (2) the covariate changes the association of interest by 10% or more. Overall, with 5 phthalate exposures, and 19 biomarkers of inflammation, a total of 95 multivariable regression models were constructed as described.

4.4. RESULTS

Among the study participants included in the current analysis (N=1,286), most were women of white race (N=1,059), had a normal pre-pregnancy BMI (N=800), were university-educated (N=812), were not using folic acid supplements (N=884), had never smoked (N=807), and had an annual household income between $50,001 and $100,000 (N=530). Of the 19 markers of inflammation, a significant association ($p < 0.01$) with maternal age was noted for IL-8, maternal race with MMP-9, MCP-1, MIP-1β, and VCAM, annual household income with CRP, pre-pregnancy BMI with IL-6, TNF-α, ICAM, and CRP, average systolic blood pressure during the second trimester with VCAM, ICAM, and CRP, and average diastolic blood pressure during the second trimester with IL-6, and VCAM. The distribution of maternal characteristics by the *a priori* selected categories of markers of inflammation are displayed in Table 4.1. The LOD of the phthalate metabolites being studied ranged from 0.2 μg/L to 0.5 μg/L. Based on the cohort of pregnant women with live singleton births and available exposure data, the percentage of observations below the LOD ranged from 0.12% to 15.53%. The geometric means and standard deviations of specific gravity-corrected phthalate concentrations by low, moderate, and high inflammatory biomarker levels are presented in Table 4.2.

In total, 22 covariates were obtained from the analysis implemented to identify potential confounders that altered the odds ratios for the effect of the exposure on the outcome by 10% or more. The following covariates demonstrated a considerable influence in at least 4 models: maternal race, premature rupture of membranes (PROM), BPA levels, gestational diabetes status, and several repeated measures of weight and blood pressure. To avoid bias associated with over-adjustment, a single variable relating to each of maternal weight, systolic blood pressure, and diastolic blood pressure was retained. Considering that blood pressure may both influence, and be

effected by, inflammation, only measurements taken prior to the third trimester of pregnancy were considered; this ensures that measurements taken subsequent to the evaluation of inflammation are not adjusted for, as they may act as mediators in the causal pathway [43, 44]. The systolic and diastolic blood pressures measured during the second trimester were selected for inclusion, as this falls in the mid-point between the time of phthalate exposure assessment in the first trimester and inflammatory biomarker measurement in the third trimester. Pre-pregnancy BMI was identified *a priori* as a critical covariate to include in the minimally-adjusted models, as it was previously accounted for in similar studies evaluating the relationship between phthalates and inflammatory responses; for this reason, other weight-related measurements were not retained [28, 29]. As a result of the high number of missing observations (N=577), gestational diabetes status was also not included. In the literature, significantly greater IL-2, IFN-γ, and IL-12 placenta levels have been observed among those with PROM, and approximately 25% of preterm births may occur as a result of preterm PROM [45, 46]. For this reason, this variable was not adjusted for as it may lie in the causal pathway resulting in adverse birth outcomes in relation to phthalate exposures.

Folic acid supplement use demonstrated a change in the association of interest by 10% or more in 2 models. This variable was included as a potential confounder as recent studies have identified phthalates in several types of supplements, and demonstrated its attenuating effects on inflammation [3, 47]. Lastly, maternal educational level, and second-hand smoke exposure during the third trimester were only detected as influential in one model, respectively; these results were considered potentially spurious, and thus not retained. Overall, the following 5 covariates were additionally included in the maximally-adjusted models: maternal race, average systolic blood pressure at visit 2, average diastolic blood pressure at visit 2, BPA, and folic acid supplement use.

The odds ratios (OR) and 95% confidence intervals (95% Cl) generated from the minimally- and maximally-adjusted models, which were constructed to assess the relationship between phthalates and low (<10th percentile) and high (>90th percentile) levels of inflammation, are reported in Tables 4.3 and 4.4, respectively. The results acquired from the maximally-adjusted models that were statistically significant ($p<0.01$) were highly congruent with those obtained from the minimally-adjusted models, demonstrating the robustness of the model construction algorithm employed. The status of statistical significance and the directionality of the relationships did not change between the two sets of models, and the majority of the results demonstrated stronger associations following the adjustment of additional covariates. Due to the high degree of consistency between the two sets of models constructed in this analysis, only results from the maximally-adjusted models are presented. A significant positive association was observed between quartile 2 (OR: 2.64; 95% Cl: 1.51-4.63), and quartile 4 (OR: 2.84; 95% Cl: 1.47-5.49) of MBzP and high MMP-2 levels. MEP concentrations in quartile 3 were significantly associated with reduced odds of low IL-12 levels (OR: 0.41; 95% Cl: 0.22-0.74). A significant inverse association was observed between quartile 2 of MBP and low VCAM levels (OR: 0.37; 95% Cl: 0.21-0.68). Quartile 2 (OR: 0.38; 95% Cl: 0.20-0.70) and quartile 4 (OR: 0.27; 95% Cl: 0.12-0.62) of ∑DEHP were significantly associated with reduced odds of low MMP-1 levels. In contrast, ∑DEHP concentrations in the 4th quartile were significantly associated with elevated odds of low VCAM levels (OR: 2.81; 95% Cl: 1.41-5.62).

Other results highlighted in Table 4.4 include measures of associations with p-values below 0.05. Positive associations of ∑DEHP concentrations in quartile 4 with low MCP-1 (OR: 2.42; 95% Cl: 1.16-5.03) and ICAM (OR: 2.16; 95% Cl: 1.10-4.27) were observed. MBzP concentrations in quartile 3 (OR: 1.79; 95% Cl: 1.001-3.22) and 4 (OR: 1.96; 95% Cl: 1.04-3.70)

were associated with elevated odds of low CRP levels. Quartile 4 of MCPP was associated with greater odds of low GMCSF levels (OR: 2.18; 95% Cl: 1.13-4.22). An inverse association of MEP concentrations in quartile 3 with low GMCSF levels (OR: 0.55; 95% Cl: 0.31-0.97), and quartile 4 with low IL-8 levels (OR: 0.46; 95% Cl: 0.24-0.86) were observed. ∑DEHP concentrations in quartile 3 was associated with reduced odds of low MMP-1 levels (OR: 0.41; 95% Cl: 0.21-0.84). Quartile 2 of MCPP and MBP were associated with decreased odds of low IL-8 (OR: 0.54; 95% Cl: 0.31-0.94) and ICAM (OR: 0.57; 95% Cl: 0.33-0.99) levels, respectively. Positive associations of MEP concentrations in quartile 2 with high MMP-7 levels (OR: 1.74; 95% Cl: 1.01-3.00), and quartile 3 with high IL-2 levels (OR: 1.77; 95% Cl: 1.02-3.07) were observed. Quartile 4 levels of MBzP were associated with elevated odds of high ICAM levels (OR: 2.03; 95% Cl: 1.02-4.06). Inverse associations of ∑DEHP concentrations in quartile 3 were observed with high IL-8 (OR: 0.47; 95% Cl: 0.23-0.93) and TNF-α (OR: 0.44; 95% Cl: 0.21-0.93) levels. Quartiles 3 (OR: 0.50; 95% Cl: 0.27-0.93) and 4 (OR: 0.42; 95% Cl: 0.21-0.84) of MCPP were associated with reduced odds of high MMP-1 levels. Although these study findings were not considered statistically significant in the current analysis, these results are discussed as they may be informative to future research evaluating inflammatory-mediated toxicity pathways in relation to phthalate exposures.

Table 4.1a. Maternal characteristics by categories (≤10th percentile, 10th-90th percentile*, ≥90th percentile) of inflammatory biomarkers (N=1,286).[a]

Characteristics	N	MMP-1 (%)				MMP-2 (%)				MMP-7 (%)				MMP-9 (%)				MMP-10 (%)			
		≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	P	≤10	10-90	≥90	p	≤10	10-90	≥90	p
Maternal Age																					
Mean (SD)	1,286	32.47 (4.65)	33.17 (5.01)	33.98 (4.92)	0.06	32.82 (5.13)	33.24 (4.94)	33.15 (5.12)	0.65	32.96 (4.97)	33.24 (4.93)	32.96 (5.39)	0.72	33.59 (5.32)	33.20 (4.90)	32.59 (5.26)	0.29	32.80 (4.85)	33.21 (5.04)	33.39 (4.61)	0.62
Maternal Race																					
White	1,059	9.07	81.30	9.63	0.67	11.24	78.00	10.76	0.47	10.39	79.70	9.92	0.80	9.16	82.44	8.40	<0.01	9.82	80.55	9.63	0.47
Black	33	12.12	81.82	6.06		9.09	81.82	9.09		3.03	87.88	9.09		27.27	63.64	9.09		3.03	84.85	12.12	
Other	194	8.25	79.38	12.37		7.73	84.02	8.25		10.31	79.90	9.79		15.98	75.77	8.25		9.28	84.02	6.70	
Maternal Education																					
High School or Less	106	9.43	82.08	8.49	0.95	14.15	77.36	8.49	0.31	11.32	76.42	12.26	0.42	13.21	78.30	8.49	0.56	6.60	83.02	10.38	0.23
College	366	9.84	80.05	10.11		12.57	77.87	9.56		12.30	78.69	9.02		8.47	83.06	8.47		7.10	83.33	9.56	
University	812	8.62	81.28	10.10		9.36	79.68	10.96		9.11	80.91	9.98		11.33	80.42	8.25		10.96	80.05	8.99	
Missing	2																				
Household Income																					
≤ $50,000	201	11.94	80.10	7.96	0.03	9.95	78.11	11.94	0.40	9.95	75.12	14.93	0.15	13.43	78.11	8.46	0.38	10.45	81.59	7.96	0.42
$50,001-$100,000	530	9.43	78.68	11.89		10.57	79.43	10.00		10.94	79.25	9.81		9.25	81.13	9.62		8.11	83.40	8.49	
>$100,000	500	8.20	82.20	9.60		10.20	79.00	10.80		9.20	82.40	8.40		11.40	81.80	6.80		10.60	78.40	11.00	
Don't Know	55	1.82	96.36	1.82		18.18	78.18	3.64		12.73	81.82	5.45		7.27	81.82	10.91		10.91	83.64	5.45	
Pre-Pregnancy BMI																					
Underweight (<18.5)	43	6.98	86.05	6.98	0.33	6.98	79.07	13.95	0.44	18.60	67.44	13.95	0.01	16.28	72.09	11.63	0.27	9.30	81.40	9.30	0.18
Normal (18.5-24.9)	800	8.63	81.25	10.13		10.13	78.50	11.38		9.38	80.25	10.38		11.50	80.50	8.00		8.50	81.00	10.50	
Overweight (25-29.9)	265	7.55	81.13	11.32		10.94	80.38	8.68		9.43	78.49	12.08		8.30	81.13	10.57		10.19	81.51	8.30	
Obese (≥30)	178	13.48	78.65	7.87		13.48	79.21	7.30		12.92	83.71	3.37		8.99	84.83	6.18		13.48	81.46	5.06	
Smoking Status																					
Never	807	8.67	81.66	9.67	0.80	9.29	79.80	10.90	0.26	9.54	80.92	9.54	0.45	9.91	82.03	8.05	0.01	10.41	81.16	8.43	0.72
Current	64	7.81	82.81	9.38		18.75	73.44	7.81		10.94	71.88	17.19		7.81	75.00	17.19		6.25	82.81	10.94	
Quit during Pregnancy	78	14.10	75.64	10.26		12.82	79.49	7.69		8.97	80.77	10.26		17.95	69.23	12.82		7.69	80.77	11.54	
Former	337	8.90	80.42	10.68		11.87	78.04	10.09		11.87	78.93	9.20		11.28	82.20	6.53		8.61	81.01	10.39	
Folic Acid Use																					
Yes	402	6.22	82.34	11.44	0.04	12.69	78.11	9.20	0.22	7.96	81.34	10.70	0.18	10.45	82.09	7.46	0.70	8.46	83.33	8.21	0.41
No	884	10.29	80.43	9.28		9.73	79.41	10.86		11.20	79.30	9.50		10.75	80.43	8.82		10.07	80.20	9.73	
BPA (µg/L)																					
< 0.37	339	9.44	82.30	8.26	0.22	13.27	75.81	10.91	0.17	11.80	77.58	10.62	0.59	10.91	84.66	4.42	0.02	8.85	82.01	9.14	0.81
0.37 – 0.82	340	6.18	84.41	9.41		7.94	81.76	10.29		9.71	80.88	9.41		11.47	81.18	7.35		9.12	79.71	11.18	
0.83 – 1.79	323	11.15	77.09	11.76		12.69	76.47	10.84		11.46	78.64	9.91		8.98	80.50	10.53		10.84	80.80	8.36	
> 1.80	284	9.51	79.93	10.56		8.45	82.39	9.15		7.39	83.10	9.51		11.27	76.76	11.97		9.51	82.39	8.10	
Average SBP (mmHg)																					
Mean (SD)	1,286	108.30 (8.79)	108.47 (10.63)	108.77 (11.20)	0.94	108.32 (10.46)	108.50 (10.59)	108.57 (10.22)	0.98	108.97 (9.79)	108.45 (10.74)	108.29 (9.53)	0.85	108.21 (9.61)	108.51 (10.66)	108.60 (10.50)	0.95	109.96 (11.26)	108.20 (10.44)	109.51 (10.47)	0.12
Average DBP (mmHg)																					
Mean (SD)	1,286	67.60 (7.18)	65.81 (7.43)	66.45 (8.43)	0.04	66.40 (7.48)	65.93 (7.50)	66.47 (7.77)	0.61	67.37 (8.81)	65.92 (7.36)	65.57 (7.28)	0.09	66.00 (8.09)	66.01 (7.43)	66.29 (7.71)	0.93	66.97 (7.50)	65.85 (7.45)	66.63 (8.17)	0.20

Legend: p = p-value; SD = standard deviation; MMP-1 = Matrix Metalloproteinase-1; MMP-2 = Matrix Metalloproteinase-2; MMP-7 = Matrix Metalloproteinase-7; MMP-9 = Matrix Metalloproteinase-9; MMP-10 = Matrix Metalloproteinase-10; BMI = body mass index; BPA = Bisphenol A; SBP = systolic blood pressure; DBP = diastolic blood pressure

[a] Row frequencies and percentages are presented unless otherwise specified

*The middle category includes values greater than the 10th percentile and smaller than the 90th percentile

Table 4.1b. Maternal characteristics by categories (≤10th percentile, 10th-90th percentile*, ≥90th percentile) of inflammatory biomarkers (N=1,286).[a]

Characteristics	N	IL-2 (%)				IL-6 (%)				IL-8 (%)				IL-10 (%)				IL-12 (%)			
		≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	p
Maternal Age																					
Mean (SD)	1,286	33.06 (4.55)	33.20 (4.96)	33.20 (5.48)	0.96	33.45 (4.82)	33.13 (4.99)	33.35 (5.11)	0.71	31.73 (4.57)	33.40 (5.00)	32.91 (5.03)	<0.01	33.41 (4.98)	33.21 (4.93)	32.83 (5.40)	0.62	32.58 (5.14)	33.19 (4.91)	33.80 (5.30)	0.14
Maternal Race																					
White	1,059	8.69	80.83	10.48	0.41	10.58	79.51	9.92	0.77	9.92	81.21	8.88	0.02	9.25	80.08	10.67	0.09	9.35	80.83	9.82	0.33
Black	33	12.12	78.79	9.09		6.06	81.82	12.12		15.15	60.61	24.24		6.06	72.73	21.21		12.12	75.76	12.12	
Other	194	12.89	77.32	9.79		12.89	77.84	9.28		10.31	76.80	12.89		11.86	81.44	6.70		13.40	75.26	11.34	
Maternal Education																					
High School or Less	106	8.49	82.08	9.43	0.75	9.43	72.64	17.92	0.07	11.32	75.47	13.21	0.66	7.55	80.19	12.26	0.52	12.26	75.47	12.26	0.82
College	366	8.74	82.24	9.02		10.66	79.51	9.84		10.11	79.23	10.66		7.65	82.24	10.11		9.56	80.87	9.56	
University	812	9.85	79.06	11.08		11.08	80.05	8.87		9.98	80.91	9.11		10.47	79.31	10.22		9.85	80.05	10.10	
Missing	2																				
Household Income																					
≤ $50,000	201	10.95	78.61	10.45	0.41	8.96	75.62	15.42	0.01	9.95	77.61	12.44	0.66	7.46	78.61	13.93	0.43	7.96	78.11	13.93	0.50
$50,001-$100,000	530	11.13	78.68	10.19		11.89	77.55	10.57		10.94	78.68	10.38		9.62	80.57	9.81		10.19	81.13	8.68	
>$100,000	500	7.40	82.40	10.20		10.60	83.00	6.40		9.60	82.00	8.40		10.40	80.60	9.00		10.60	79.40	10.00	
Don't Know	55	5.45	81.82	12.73		9.09	76.36	14.55		7.27	83.64	9.09		9.09	76.36	14.55		10.91	78.18	10.91	
Pre-Pregnancy BMI																					
Underweight (<18.5)	43	11.63	79.07	9.30	0.92	13.95	74.42	11.63	<0.01	16.28	72.09	11.63	0.07	9.30	81.40	9.30	0.49	16.28	79.07	4.65	0.60
Normal (18.5-24.9)	800	8.88	80.50	10.63		12.25	79.63	8.13		11.00	80.75	8.25		10.63	80.00	9.38		9.25	80.13	10.63	
Overweight (25-29.9)	265	9.43	79.62	10.94		10.94	77.74	11.32		9.06	78.87	12.08		7.92	80.75	11.32		11.70	78.87	9.43	
Obese (≥30)	178	11.24	80.34	8.43		3.37	81.46	15.17		6.18	80.34	13.48		7.30	79.21	13.48		9.55	80.34	10.11	
Smoking Status																					
Never	807	9.42	80.05	10.53	0.80	10.53	80.05	9.42	0.42	11.03	78.56	10.41	0.64	9.54	79.31	11.15	0.58	9.79	79.93	10.29	0.53
Current	64	7.81	81.25	10.94		15.63	76.56	7.81		9.38	81.25	9.38		14.06	79.69	6.25		9.38	79.69	10.94	
Quit during Pregnancy	78	6.41	79.49	14.10		10.26	73.08	16.67		10.26	78.21	11.54		6.41	82.05	11.54		5.13	80.77	14.10	
Former	337	10.39	80.71	8.90		10.68	79.53	9.79		8.01	83.68	8.31		9.50	81.60	8.90		11.87	79.53	8.61	
Folic Acid Use																					
Yes	402	7.71	81.59	10.70	0.37	12.19	79.85	7.96	0.20	11.19	79.10	9.70	0.68	9.70	82.09	8.21	0.24	9.20	81.34	9.45	0.67
No	884	10.18	79.64	10.18		10.18	79.07	10.75		9.62	80.43	9.95		9.50	79.19	11.31		10.41	79.19	10.41	
BPA (µg/L)																					
< 0.37	339	8.55	78.76	12.68	0.35	11.21	79.94	8.85	0.85	11.21	77.88	10.91	0.21	10.32	79.65	10.03	0.58	12.09	80.24	7.67	0.18
0.37 – 0.82	340	8.24	83.53	8.24		9.41	79.41	11.18		9.71	81.47	8.82		7.06	82.94	10.00		9.71	78.24	12.06	
0.83 – 1.79	323	9.60	79.26	11.15		11.46	79.88	8.67		8.98	83.90	7.12		9.60	78.95	11.46		8.05	83.28	8.67	
> 1.80	284	11.62	79.23	9.15		11.27	77.82	10.92		10.56	76.41	13.03		11.62	78.52	9.86		10.21	77.46	12.32	
Average SBP (mmHg)																					
Mean (SD)	1,286	109.48 (9.93)	108.43 (10.65)	108.06 (10.13)	0.52	105.97 (9.13)	108.81 (10.59)	108.69 (11.20)	0.01	107.55 (9.02)	108.34 (10.57)	110.65 (11.39)	0.04	108.40 (9.56)	108.29 (10.59)	110.10 (10.84)	0.17	107.77 (9.75)	108.68 (10.71)	107.71 (9.81)	0.44
Average DBP (mmHg)																					
Mean (SD)	1,286	67.07 (7.95)	66.02 (7.43)	65.15 (7.78)	0.13	64.31 (5.97)	66.14 (7.58)	67.07 (8.28)	<0.01	65.61 (6.09)	65.89 (7.53)	67.66 (8.60)	0.03	65.85 (6.93)	65.88 (7.53)	67.38 (7.92)	0.09	65.43 (7.45)	66.18 (7.57)	65.45 (7.23)	0.37

Legend: p = p-value; SD = standard deviation; IL-2 = Interleukin 2; IL-6 = Interleukin 6; IL-8 = Interleukin 8; IL-10 = Interleukin 10; IL-12 = Interleukin 12; BMI = body mass index; BPA = Bisphenol A; SBP = systolic blood pressure; DBP = diastolic blood pressure

[a] Row frequencies and percentages are presented unless otherwise specified

*The middle category includes values greater than the 10th percentile and smaller than the 90th percentile

Table 4.1c. Maternal characteristics by categories (≤10th percentile, 10th-90th percentile*, ≥90th percentile) of inflammatory biomarkers (N=1,286).[a]

Characteristics	N	VEGF (%)				MCP-1 (%)				MIP-1β (%)				IFN-γ (%)				TNF-α (%)			
		≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	p
Maternal Age																					
Mean (SD)	1,286	33.41 (5.51)	33.18 (4.91)	32.98 (5.04)	0.80	34.05 (5.22)	33.16 (4.95)	32.56 (4.91)	0.06	32.74 (5.44)	33.31 (4.92)	32.68 (4.92)	0.23	32.34 (5.15)	33.25 (4.92)	33.55 (5.23)	0.09	32.50 (4.98)	33.20 (4.97)	33.78 (5.01)	0.13
Maternal Race																					
White	1,059	9.07	81.40	9.54	0.04	8.50	81.49	10.01	<0.01	10.29	80.26	9.44	<0.01	10.39	79.98	9.63	0.20	9.16	81.87	8.97	0.11
Black	33	6.06	75.76	18.18		39.39	51.52	9.09		3.03	63.64	33.33		3.03	75.76	21.21		9.09	72.73	18.18	
Other	194	14.95	77.84	7.22		9.79	78.87	11.34		9.28	80.93	9.79		11.34	77.84	10.82		13.92	77.84	8.25	
Maternal Education																					
High School or Less	106	7.55	83.96	8.49	0.03	9.43	76.42	14.15	0.57	12.26	72.64	15.09	0.33	16.04	71.70	12.26	0.08	12.26	81.13	6.60	0.76
College	366	8.20	78.69	13.11		8.47	80.87	10.66		10.11	79.51	10.38		12.30	78.14	9.56		9.84	80.05	10.11	
University	812	10.96	81.16	7.88		9.98	80.54	9.48		9.61	81.03	9.36		8.74	81.16	10.10		9.61	81.40	8.99	
Missing	2																				
Household Income																					
≤ $50,000	201	12.44	77.11	10.45	0.13	9.95	80.60	9.45	0.73	9.95	76.62	13.43	0.73	13.43	74.63	11.94	0.52	10.45	80.10	9.45	0.99
$50,001-$100,000	530	7.74	83.58	8.68		8.30	82.45	9.25		9.62	81.13	9.25		10.38	79.25	10.38		10.19	80.57	9.25	
>$100,000	500	11.80	78.60	9.60		10.40	78.00	11.60		10.00	80.20	9.80		9.40	81.20	9.40		9.60	81.80	8.60	
Don't Know	55	3.64	85.45	10.91		10.91	80.00	9.09		12.73	78.18	9.09		7.27	85.45	7.27		7.27	81.82	10.91	
Pre-Pregnancy BMI																					
Underweight (<18.5)	43	2.33	83.72	13.95	0.23	2.33	88.37	9.30	0.06	13.95	81.40	4.65	0.39	13.95	74.42	11.63	0.55	11.63	72.09	16.28	<0.01
Normal (18.5-24.9)	800	10.63	81.25	8.13		10.75	80.75	8.50		10.75	79.38	9.88		9.38	79.50	11.13		11.25	81.63	7.13	
Overweight (25-29.9)	265	8.30	80.75	10.94		8.30	78.11	13.58		8.68	78.87	12.45		11.70	80.38	7.92		9.43	80.38	10.19	
Obese (≥30)	178	10.67	77.53	11.80		7.30	79.78	12.92		7.30	83.71	8.99		11.80	79.78	8.43		3.93	81.46	14.61	
Smoking Status																					
Never	807	10.29	81.16	8.55	0.04	9.91	79.31	10.78	0.21	9.42	80.17	10.41	0.48	11.15	78.69	10.16	0.16	10.53	80.17	9.29	0.76
Current	64	3.13	78.13	18.75		6.25	85.94	7.81		17.19	70.31	12.50		12.50	73.44	14.06		7.81	82.81	9.38	
Quit during Pregnancy	78	15.38	74.36	10.26		15.38	71.79	12.82		11.54	79.49	8.97		15.38	75.64	8.97		7.69	79.49	12.82	
Former	337	8.90	81.60	9.50		7.72	83.68	8.61		9.50	81.31	9.20		6.82	83.68	9.50		9.20	83.09	7.72	
Folic Acid Use																					
Yes	402	9.95	80.60	9.45	1.00	10.95	77.61	11.44	0.25	9.20	81.34	9.45	0.70	10.95	77.86	11.19	0.58	9.95	81.84	8.21	0.76
No	884	9.84	80.77	9.39		8.82	81.56	9.62		10.29	79.30	10.41		10.07	80.32	9.62		9.84	80.66	9.50	
BPA (μg/L)																					
< 0.37	339	9.73	82.30	7.96	0.76	10.62	80.24	9.14	0.90	12.98	78.76	8.26	0.04	10.03	81.12	8.85	0.36	12.09	78.76	9.14	0.63
0.37 – 0.82	340	11.76	78.53	9.71		7.94	81.47	10.59		7.65	80.59	11.76		7.94	82.65	9.41		10.00	82.35	7.65	
0.83 – 1.79	323	8.98	81.42	9.60		9.91	78.95	11.15		10.22	82.35	7.43		11.15	76.78	12.07		8.36	81.41	10.22	
> 1.80	284	8.80	80.63	10.56		9.51	80.63	9.86		8.80	77.82	13.38		12.68	77.11	10.21		8.80	81.69	9.51	
Average SBP (mmHg)																					
Mean (SD)	1,286	109.49 (10.81)	108.25 (10.43)	109.48 (11.06)	0.25	107.77 (10.91)	108.47 (10.50)	109.29 (10.40)	0.52	108.93 (10.06)	108.25 (10.65)	109.92 (9.97)	0.21	109.66 (10.89)	108.40 (10.52)	108.00 (10.21)	0.37	107.16 (10.26)	108.62 (10.29)	108.77 (12.73)	0.32
Average DBP (mmHg)																					
Mean (SD)	1,286	66.20 (7.00)	65.94 (7.55)	66.63 (7.82)	0.61	65.48 (7.73)	66.09 (7.41)	66.07 (8.21)	0.69	65.47 (6.41)	65.97 (7.58)	67.06 (8.05)	0.20	66.33 (8.13)	66.08 (7.45)	65.38 (7.47)	0.54	65.61 (7.41)	65.97 (7.47)	67.06 (8.07)	0.26

Legend: p = p-value; SD = standard deviation; VEGF = Vascular endothelial growth factor; MCP-1 = Monocyte chemoattractant protein-1; MIP-1β = Human macrophage inflammatory protein 1-beta; IFN-γ = Interferon gamma; TNF-α = Tumor necrosis factor alpha; BMI = body mass index; BPA = Bisphenol A; SBP = systolic blood pressure; DBP = diastolic blood pressure

[a] Row frequencies and percentages are presented unless otherwise specified

*The middle category includes values greater than the 10th percentile and smaller than the 90th percentile

Table 4.1d. Maternal characteristics by categories (≤10th percentile, 10th-90th percentile*, ≥90th percentile) of inflammatory biomarkers (N=1,286).[a]

Characteristics	N	GMCSF (%)				VCAM (%)				ICAM (%)				CRP (%)			
		≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	p	≤10	10-90	≥90	p
Maternal Age																	
Mean (SD)	1,286	32.47 (4.32)	33.20 (5.03)	33.78 (5.13)	0.10	32.62 (5.03)	33.21 (4.98)	33.63 (4.95)	0.26	32.40 (4.87)	33.35 (4.99)	32.65 (4.90)	0.052	33.90 (4.86)	33.11 (5.00)	33.03 (4.92)	0.21
Maternal Race																	
White	1,059	10.10	79.70	10.20	0.56	9.44	80.08	10.48	<0.01	10.67	79.98	9.35	0.50	9.92	81.30	8.78	0.24
Black	33	15.15	69.70	15.15		24.24	75.76	0.00		9.09	72.73	18.18		6.06	81.82	12.12	
Other	194	9.79	81.44	8.76		12.37	81.44	6.19		9.79	81.96	8.25		13.40	74.74	11.86	
Maternal Education																	
High School or Less	106	7.55	83.96	8.49	0.51	10.38	79.25	10.38	0.85	10.38	76.42	13.21	0.09	8.49	78.30	13.21	0.047
College	366	12.02	78.69	9.29		11.48	80.05	8.47		11.20	76.78	12.02		10.38	77.32	12.30	
University	812	9.61	79.68	10.71		9.73	80.42	9.85		10.22	82.02	7.76		10.59	82.02	7.39	
Missing	2																
Household Income																	
≤ $50,000	201	8.46	82.09	9.45	0.74	9.95	80.10	9.95	0.25	9.95	78.61	11.44	0.29	11.94	76.62	11.44	<0.01
$50,001-$100,000	530	10.00	79.62	10.38		11.51	81.32	7.17		10.94	80.19	8.87		6.79	82.45	10.75	
>$100,000	500	11.60	78.20	10.20		9.00	79.40	11.60		10.60	81.20	8.20		13.20	79.20	7.60	
Don't Know	55	5.45	85.45	9.09		10.91	76.36	12.73		7.27	74.55	18.18		12.73	83.64	3.64	
Pre-Pregnancy BMI																	
Underweight (<18.5)	43	13.95	72.09	13.95	0.91	13.95	79.07	6.98	0.01	13.95	72.09	13.95	<0.01	20.93	76.74	2.33	<0.01
Normal (18.5-24.9)	800	10.00	79.75	10.25		10.75	81.00	8.25		11.00	81.50	7.50		12.13	80.63	7.25	
Overweight (25-29.9)	265	10.19	79.62	10.19		11.70	78.87	9.43		10.57	82.64	6.79		6.79	81.51	11.70	
Obese (≥30)	178	10.11	81.46	8.43		5.06	78.65	16.29		7.30	71.91	20.79		5.06	78.09	16.85	
Smoking Status																	
Never	807	9.91	78.93	11.15	0.63	10.53	80.05	9.42	0.80	10.41	80.30	9.29	0.99	10.66	79.93	9.42	0.39
Current	64	12.50	78.13	9.38		14.06	79.69	6.25		7.81	81.25	10.94		3.13	89.06	7.81	
Quit during Pregnancy	78	8.97	79.49	11.54		11.54	78.21	10.26		10.26	79.49	10.26		7.69	79.49	12.82	
Former	337	10.68	81.90	7.42		8.61	81.01	10.39		11.28	79.53	9.20		11.57	79.82	8.61	
Folic Acid Use																	
Yes	402	9.95	78.61	11.44	0.56	11.19	77.86	10.95	0.36	11.69	78.36	9.95	0.55	9.70	80.85	9.45	0.88
No	884	10.29	80.20	9.50		9.84	81.22	8.94		9.95	80.88	9.16		10.63	80.09	9.28	
BPA (µg/L)																	
< 0.37	339	12.68	76.70	10.62	0.12	11.80	80.24	7.96	0.54	11.50	78.76	9.73	0.89	14.45	76.99	8.55	0.06
0.37 – 0.82	340	10.59	79.41	10.00		7.94	82.65	9.41		8.82	82.65	8.53		10.88	80.29	8.82	
0.83 – 1.79	323	5.88	83.28	10.84		11.46	78.02	10.53		11.15	79.57	9.29		8.98	80.80	10.22	
> 1.80	284	11.62	79.58	8.80		9.86	79.58	10.56		10.56	79.23	10.21		6.34	83.80	9.86	
Average SBP (mmHg)																	
Mean (SD)	1,286	107.56 (10.18)	108.79 (10.51)	107.03 (10.89)	0.11	106.25 (9.68)	108.38 (10.42)	111.82 (11.57)	<0.01	107.43 (9.57)	108.28 (10.48)	111.44 (11.51)	<0.01	106.89 (10.00)	108.41 (10.41)	110.96 (11.76)	<0.01
Average DBP (mmHg)																	
Mean (SD)	1,286	66.03 (7.62)	66.27 (7.41)	64.15 (8.11)	0.01	65.01 (7.34)	65.94 (7.57)	67.91 (7.07)	<0.01	66.08 (7.16)	65.86 (7.54)	67.48 (7.68)	0.08	64.77 (6.72)	66.05 (7.56)	67.32 (7.84)	0.03

Legend: p = p-value; SD = standard deviation; GMCSF = Granulocyte-macrophage colony-stimulating factor; VCAM = Vascular cell adhesion molecule; ICAM = Intercellular adhesion molecule; CRP = C-reactive protein; BMI = body mass index; BPA = Bisphenol A; SBP = systolic blood pressure; DBP = diastolic blood pressure

[a] Row frequencies and percentages are presented unless otherwise specified

*The middle category includes values greater than the 10th percentile and smaller than the 90th percentile

Table 4.2a. Geometric means (SD) of SG-corrected phthalate concentrations by categories (≤10th percentile, 10th-90th percentile*, ≥90th percentile) of inflammatory biomarkers (N=1,286).

Phthalates (µg/L)	LOD	N < LOD (%)[a]	MMP-1 GM (SD)			MMP-2 GM (SD)			MMP-7 GM (SD)			MMP-9 GM (SD)			MMP-10 GM (SD)		
			≤10	**10-90**	**≥90**	**≤10**	**10-90**	**≥90**	**≤10**	**10-90**	**≥90**	**≤10**	**10-90**	**≥90**	**≤10**	**10-90**	**≥90**
MBP	0.2	4 (0.24)	12.85 (2.38)	12.49 (2.34)	12.84 (2.15)	11.93 (2.32)	12.60 (2.33)	12.84 (2.26)	12.84 (2.16)	12.63 (2.33)	11.71 (2.40)	12.32 (2.19)	12.39 (2.35)	14.64 (2.15)	12.74 (2.11)	12.57 (2.36)	12.21 (2.24)
MBzP	0.2	8 (0.48)	5.95 (2.81)	5.77 (2.81)	5.12 (2.79)	5.24 (2.81)	5.67 (2.82)	6.68 (2.72)	5.95 (2.48)	5.69 (2.82)	5.74 (3.05)	5.14 (2.73)	5.77 (2.83)	6.04 (2.67)	6.88 (2.92)	5.57 (2.74)	5.92 (3.25)
MEP	0.5	2 (0.12)	38.00 (3.99)	34.67 (3.91)	31.83 (3.78)	34.95 (3.63)	34.80 (3.97)	33.30 (3.68)	34.69 (3.76)	34.64 (3.90)	34.80 (4.08)	31.53 (3.37)	35.23 (3.97)	33.43 (3.95)	33.66 (3.27)	34.87 (4.02)	33.93 (3.59)
MCPP	0.2	260 (15.53)	0.94 (2.62)	0.98 (3.02)	0.79 (3.02)	0.97 (3.03)	0.96 (2.94)	0.87 (3.30)	0.92 (2.77)	0.96 (2.95)	0.97 (3.52)	1.00 (2.94)	0.95 (3.04)	0.96 (2.61)	1.02 (2.96)	0.94 (2.97)	1.00 (3.22)
MEHP	0.2	26 (1.55)	2.30 (2.69)	2.52 (2.50)	2.40 (2.47)	2.70 (2.73)	2.46 (2.51)	2.53 (2.37)	2.42 (2.46)	2.55 (2.54)	2.14 (2.33)	2.60 (2.43)	2.46 (2.50)	2.63 (2.75)	2.21 (2.27)	2.55 (2.51)	2.27 (2.78)
MEHHP	0.4	11 (0.66)	9.40 (2.52)	10.20 (2.49)	10.19 (2.28)	10.62 (2.81)	10.04 (2.45)	10.25 (2.32)	9.39 (2.57)	10.36 (2.48)	9.10 (2.29)	10.86 (2.46)	9.96 (2.49)	10.84 (2.26)	9.79 (2.21)	10.25 (2.48)	9.41 (2.65)
MEOHP	0.2	5 (0.30)	6.74 (2.36)	7.12 (2.35)	7.01 (2.09)	7.48 (2.61)	7.02 (2.30)	7.04 (2.23)	6.75 (2.43)	7.23 (2.33)	6.19 (2.17)	7.38 (2.35)	6.99 (2.33)	7.50 (2.19)	6.71 (2.12)	7.17 (2.33)	6.61 (2.50)
∑DEHP	N/A	N/A	18.71 (2.44)	20.10 (2.39)	19.92 (2.18)	20.84 (2.70)	19.83 (2.34)	20.04 (2.26)	18.84 (2.46)	20.41 (2.38)	17.64 (2.20)	21.17 (2.37)	19.67 (2.38)	21.28 (2.26)	18.96 (2.14)	20.23 (2.38)	18.63 (2.53)

Legend: LOD = limit of detection; SG = specific gravity; GM = geometric mean; SD = standard deviation; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; MEHP = Mono-(2-ethylhexyl) Phthalate; MEHHP = Mono-(2-ethyl-5-hydroxy-hexyl) Phthalate; MEOHP = Mono-(2-ethyl-5-oxo-hexyl) Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; MMP-1 = Matrix Metalloproteinase-1; MMP-2 = Matrix Metalloproteinase-2; MMP-7 = Matrix Metalloproteinase-7; MMP-9 = Matrix Metalloproteinase-9; MMP-10 = Matrix Metalloproteinase-10

[a] Frequencies and percentages of observations below the LOD are based on the cohort of pregnant women with live singleton births and available exposure data

*The middle category includes values greater than the 10th percentile and smaller than the 90th percentile

Table 4.2b. Geometric means (SD) of SG-corrected phthalate concentrations by categories (≤10th percentile, 10th-90th percentile*, ≥90th percentile) of inflammatory biomarkers (N=1,286).

Phthalates (µg/L)	LOD	N < LOD (%)[a]	IL-2 GM (SD)			IL-6 GM (SD)			IL-8 GM (SD)			IL-10 GM (SD)			IL-12 GM (SD)		
			≤10	10-90	≥90	≤10	10-90	≥90	≤10	10-90	≥90	≤10	10-90	≥90	≤10	10-90	≥90
MBP	0.2	4 (0.24)	12.58 (2.18)	12.56 (2.30)	12.50 (2.58)	11.27 (2.26)	12.69 (2.31)	12.93 (2.49)	12.10 (2.22)	12.72 (2.35)	11.70 (2.17)	12.57 (2.45)	12.44 (2.30)	13.45 (2.38)	12.18 (2.34)	12.60 (2.33)	12.53 (2.24)
MBzP	0.2	8 (0.48)	5.85 (2.94)	5.76 (2.83)	5.29 (2.56)	4.87 (2.61)	5.84 (2.85)	5.74 (2.70)	5.63 (2.44)	5.73 (2.89)	5.75 (2.58)	5.66 (2.92)	5.67 (2.81)	6.18 (2.71)	5.45 (3.04)	5.63 (2.79)	6.79 (2.72)
MEP	0.5	2 (0.12)	41.75 (4.53)	33.23 (3.84)	40.59 (3.79)	33.44 (3.51)	34.77 (3.99)	35.15 (3.69)	28.83 (3.98)	35.05 (3.86)	38.26 (4.17)	37.74 (4.06)	34.29 (3.91)	34.84 (3.71)	29.04 (4.08)	35.43 (3.87)	34.77 (3.93)
MCPP	0.2	260 (15.53)	1.04 (2.96)	0.95 (2.97)	0.89 (3.14)	0.94 (2.98)	0.96 (2.98)	0.94 (3.08)	0.92 (3.17)	0.97 (2.99)	0.89 (2.81)	1.02 (3.26)	0.94 (2.96)	1.04 (2.94)	0.98 (2.91)	0.93 (2.97)	1.15 (3.20)
MEHP	0.2	26 (1.55)	2.32 (2.19)	2.52 (2.61)	2.40 (2.11)	2.65 (2.53)	2.49 (2.53)	2.32 (2.37)	2.88 (2.42)	2.49 (2.54)	2.15 (2.35)	2.72 (2.47)	2.44 (2.51)	2.66 (2.61)	2.43 (2.49)	2.48 (2.54)	2.67 (2.34)
MEHHP	0.4	11 (0.66)	9.03 (2.13)	10.33 (2.53)	9.61 (2.26)	10.48 (2.55)	10.10 (2.46)	9.92 (2.51)	10.53 (2.37)	10.24 (2.49)	8.83 (2.39)	10.06 (2.32)	10.04 (2.46)	10.88 (2.67)	9.21 (2.42)	10.13 (2.49)	11.03 (2.39)
MEOHP	0.2	5 (0.30)	6.35 (1.97)	7.17 (2.40)	6.94 (2.07)	7.24 (2.40)	7.06 (2.31)	6.93 (2.34)	7.30 (2.28)	7.15 (2.34)	6.24 (2.21)	7.11 (2.16)	7.00 (2.32)	7.55 (2.51)	6.58 (2.25)	7.07 (2.35)	7.62 (2.21)
∑DEHP	N/A	N/A	17.89 (2.04)	20.30 (2.44)	19.29 (2.11)	20.54 (2.47)	19.93 (2.36)	19.55 (2.36)	20.97 (2.31)	20.15 (2.39)	17.52 (2.26)	20.20 (2.24)	19.74 (2.37)	21.41 (2.55)	18.44 (2.33)	19.95 (2.39)	21.65 (2.27)

Legend: LOD = limit of detection; SG = specific gravity; GM = geometric mean; SD = standard deviation; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; MEHP = Mono-(2-ethylhexyl) Phthalate; MEHHP = Mono-(2-ethyl-5-hydroxy-hexyl) Phthalate; MEOHP = Mono-(2-ethyl-5-oxo-hexyl) Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; IL-2 = Interleukin 2; IL-6 = Interleukin 6; IL-8 = Interleukin 8; IL-10 = Interleukin 10; IL-12 = Interleukin 12

[a] Frequencies and percentages of observations below the LOD are based on the cohort of pregnant women with live singleton births and available exposure data

*The middle category includes values greater than the 10th percentile and smaller than the 90th percentile

Table 4.2c. Geometric means (SD) of SG-corrected phthalate concentrations by categories (≤10th percentile, 10th-90th percentile*, ≥90th percentile) of inflammatory biomarkers (N=1,286).

Phthalates (µg/L)	LOD	N < LOD (%)[a]	VEGF GM (SD)			MCP-1 GM (SD)			MIP-1β GM (SD)			IFN-γ GM (SD)			TNF-α GM (SD)		
			≤10	10-90	≥90	≤10	10-90	≥90	≤10	10-90	≥90	≤10	10-90	≥90	≤10	10-90	≥90
MBP	0.2	4 (0.24)	11.23 (2.19)	12.62 (2.31)	13.50 (2.53)	12.96 (2.74)	12.48 (2.28)	12.77 (2.29)	11.69 (2.23)	12.46 (2.33)	14.28 (2.28)	11.88 (2.36)	12.58 (2.32)	13.09 (2.26)	11.13 (2.14)	12.70 (2.36)	12.88 (2.11)
MBzP	0.2	8 (0.48)	5.29 (2.80)	5.74 (2.82)	6.04 (2.78)	5.88 (2.96)	5.71 (2.77)	5.68 (2.99)	5.51 (2.82)	5.69 (2.82)	6.15 (2.74)	6.02 (2.72)	5.75 (2.84)	5.18 (2.64)	5.14 (2.81)	5.76 (2.83)	5.99 (2.58)
MEP	0.5	2 (0.12)	34.02 (4.30)	34.37 (3.84)	37.98 (4.06)	36.20 (3.19)	34.35 (3.98)	35.76 (4.01)	30.49 (3.42)	35.14 (3.94)	35.31 (4.13)	39.02 (3.76)	34.51 (3.95)	31.78 (3.71)	32.67 (4.12)	35.15 (3.89)	32.62 (3.81)
MCPP	0.2	260 (15.53)	0.80 (2.64)	0.96 (3.03)	1.07 (2.95)	0.97 (2.88)	0.95 (2.97)	1.01 (3.22)	0.99 (2.76)	0.94 (3.00)	1.01 (3.15)	1.09 (2.70)	0.94 (3.04)	0.95 (2.87)	1.05 (3.38)	0.95 (2.97)	0.92 (2.73)
MEHP	0.2	26 (1.55)	2.06 (2.23)	2.53 (2.56)	2.62 (2.38)	3.42 (2.84)	2.40 (2.46)	2.47 (2.53)	2.59 (2.44)	2.48 (2.54)	2.48 (2.45)	2.56 (2.91)	2.49 (2.50)	2.42 (2.29)	2.33 (2.42)	2.53 (2.52)	2.32 (2.63)
MEHHP	0.4	11 (0.66)	8.67 (2.29)	10.25 (2.50)	10.71 (2.41)	13.36 (2.70)	9.88 (2.40)	9.48 (2.68)	10.08 (2.53)	10.13 (2.47)	10.08 (2.40)	10.10 (2.70)	10.17 (2.47)	9.77 (2.24)	9.14 (2.34)	10.30 (2.48)	9.68 (2.49)
MEOHP	0.2	5 (0.30)	5.95 (2.15)	7.17 (2.35)	7.53 (2.23)	9.14 (2.54)	6.89 (2.27)	6.80 (2.47)	7.11 (2.35)	7.08 (2.32)	6.94 (2.32)	6.99 (2.58)	7.10 (2.32)	6.92 (2.10)	6.36 (2.24)	7.20 (2.33)	6.73 (2.32)
∑DEHP	N/A	N/A	16.88 (2.19)	20.23 (2.40)	21.16 (2.28)	26.33 (2.62)	19.43 (2.31)	19.04 (2.52)	20.08 (2.40)	20.00 (2.37)	19.47 (2.35)	20.00 (2.63)	20.02 (2.37)	19.43 (2.14)	18.13 (2.28)	20.29 (2.38)	19.07 (2.38)

Legend: LOD = limit of detection; SG = specific gravity; GM = geometric mean; SD = standard deviation; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; MEHP = Mono-(2-ethylhexyl) Phthalate; MEHHP = Mono-(2-ethyl-5-hydroxy-hexyl) Phthalate; MEOHP = Mono-(2-ethyl-5-oxo-hexyl) Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; VEGF = Vascular endothelial growth factor; MCP-1 = Monocyte chemoattractant protein-1; MIP-1β = Human macrophage inflammatory protein 1-beta; IFN-γ = Interferon gamma; TNF-α = Tumor necrosis factor alpha

[a] Frequencies and percentages of observations below the LOD are based on the cohort of pregnant women with live singleton births and available exposure data

*The middle category includes values greater than the 10th percentile and smaller than the 90th percentile

Table 4.2d. Geometric means (SD) of SG-corrected phthalate concentrations by categories (≤10th percentile, 10th-90th percentile*, ≥90th percentile) of inflammatory biomarkers (N=1,286).

Phthalates (µg/L)	LOD	N < LOD (%)[a]	GMCSF GM (SD)			VCAM GM (SD)			ICAM GM (SD)			CRP GM (SD)		
			≤10	10-90	≥90	≤10	10-90	≥90	≤10	10-90	≥90	≤10	10-90	≥90
MBP	0.2	4 (0.24)	12.04 (2.27)	12.63 (2.32)	12.47 (2.35)	12.13 (2.49)	12.68 (2.34)	11.97 (1.97)	11.36 (2.69)	12.56 (2.26)	13.94 (2.40)	11.90 (2.42)	12.56 (2.31)	13.29 (2.28)
MBzP	0.2	8 (0.48)	5.41 (2.81)	5.79 (2.84)	5.50 (2.56)	5.01 (2.65)	5.80 (2.85)	5.85 (2.65)	5.43 (2.93)	5.64 (2.76)	6.79 (3.06)	6.01 (2.73)	5.55 (2.83)	6.96 (2.70)
MEP	0.5	2 (0.12)	30.85 (4.10)	35.17 (3.84)	34.73 (4.20)	40.24 (3.90)	34.29 (3.93)	32.35 (3.71)	41.72 (4.15)	33.33 (3.84)	39.29 (4.10)	38.64 (4.34)	33.56 (3.81)	40.55 (4.25)
MCPP	0.2	260 (15.53)	1.05 (3.17)	0.94 (2.95)	1.00 (3.08)	1.06 (3.16)	0.94 (2.95)	0.98 (3.17)	1.02 (3.11)	0.94 (3.00)	0.98 (2.78)	0.87 (2.96)	0.96 (3.02)	1.02 (2.71)
MEHP	0.2	26 (1.55)	2.33 (2.34)	2.48 (2.54)	2.71 (2.47)	2.99 (2.85)	2.47 (2.49)	2.17 (2.35)	2.93 (2.70)	2.47 (2.49)	2.25 (2.52)	2.56 (2.50)	2.54 (2.55)	2.04 (2.15)
MEHHP	0.4	11 (0.66)	9.47 (2.10)	10.16 (2.52)	10.56 (2.43)	11.69 (2.69)	10.04 (2.47)	9.29 (2.22)	11.85 (2.64)	9.99 (2.46)	9.51 (2.34)	10.26 (2.40)	10.19 (2.50)	9.42 (2.26)
MEOHP	0.2	5 (0.30)	6.48 (2.06)	7.09 (2.37)	7.52 (2.26)	8.18 (2.48)	7.02 (2.31)	6.38 (2.23)	8.28 (2.46)	6.99 (2.30)	6.51 (2.37)	7.08 (2.26)	7.14 (2.35)	6.46 (2.16)
∑DEHP	N/A	N/A	18.52 (2.08)	19.99 (2.42)	21.18 (2.31)	23.31 (2.58)	19.79 (2.36)	18.09 (2.17)	23.40 (2.54)	19.70 (2.36)	18.60 (2.29)	20.19 (2.32)	20.14 (2.40)	18.20 (2.15)

Legend: LOD = limit of detection; SG = specific gravity; GM = geometric mean; SD = standard deviation; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; MEHP = Mono-(2-ethylhexyl) Phthalate; MEHHP = Mono-(2-ethyl-5-hydroxy-hexyl) Phthalate; MEOHP = Mono-(2-ethyl-5-oxo-hexyl) Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; GMCSF = Granulocyte-macrophage colony-stimulating factor; VCAM = Vascular cell adhesion molecule; ICAM = Intercellular adhesion molecule; CRP = C-reactive protein

[a] Frequencies and percentages of observations below the LOD are based on the cohort of pregnant women with live singleton births and available exposure data

*The middle category includes values greater than the 10th percentile and smaller than the 90th percentile

Table 4.3a. Minimally-adjusted models: Odds ratios for the association of phthalate exposure with low (≤10th percentile) and high (≥90th percentile) levels of inflammatory biomarkers (N=1,286). [a] [b]

Phthalates (µg/L)	MMP-1		MMP-2		MMP-7		MMP-9		MMP-10	
	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)
MBP										
<5.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.2-12.0	0.58 (0.32-1.05)	0.89 (0.50-1.58)	0.71 (0.42-1.21)	0.94 (0.55-1.59)	0.80 (0.47-1.37)	1.32 (0.78-2.22)	0.96 (0.56-1.64)	0.94 (0.50-1.78)	1.33 (0.77-2.31)	1.01 (0.59-1.74)
13.0-25.0	0.87 (0.45-1.69)	1.58 (0.84-2.97)	0.82 (0.44-1.50)	1.17 (0.63-2.19)	1.02 (0.55-1.90)	0.85 (0.43-1.66)	1.50 (0.81-2.75)	1.44 (0.71-2.90)	0.99 (0.51-1.93)	0.90 (0.46-1.77)
>25.0	0.93 (0.44-1.94)	0.99 (0.47-2.08)	0.64 (0.31-1.30)	1.06 (0.52-2.16)	0.92 (0.45-1.89)	0.93 (0.44-1.95)	1.11 (0.55-2.27)	1.59 (0.74-3.44)	1.22 (0.58-2.55)	1.30 (0.63-2.69)
MBzP										
<2.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.4-5.1	0.73 (0.41-1.32)	0.68 (0.39-1.18)	0.59 (0.35-1.00)	**2.58** (1.48-4.48)**	0.81 (0.48-1.38)	0.69 (0.39-1.22)	0.83 (0.50-1.38)	0.76 (0.40-1.45)	1.18 (0.66-2.11)	0.72 (0.41-1.26)
5.2-12.0	1.07 (0.58-1.96)	0.68 (0.37-1.24)	**0.55* (0.31-0.99)**	1.60 (0.84-3.06)	0.93 (0.52-1.67)	1.08 (0.60-1.95)	0.70 (0.39-1.26)	1.35 (0.71-2.55)	1.46 (0.79-2.71)	0.85 (0.46-1.58)
>12.0	0.91 (0.47-1.77)	0.71 (0.37-1.34)	0.67 (0.36-1.24)	**2.79** (1.46-5.34)**	1.10 (0.59-2.05)	1.47 (0.79-2.71)	0.75 (0.40-1.40)	1.06 (0.53-2.14)	1.84 (0.96-3.51)	1.26 (0.67-2.37)
MEP										
<12.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.0-28.0	0.75 (0.42-1.36)	1.30 (0.76-2.21)	1.29 (0.75-2.21)	1.14 (0.67-1.93)	1.04 (0.60-1.80)	**1.74* (1.01-2.97)**	0.98 (0.58-1.67)	0.79 (0.43-1.45)	1.11 (0.63-1.96)	1.59 (0.93-2.74)
29.0-86.0	0.87 (0.48-1.57)	0.81 (0.45-1.46)	1.55 (0.90-2.67)	1.17 (0.68-2.01)	1.50 (0.88-2.54)	1.15 (0.64-2.07)	1.12 (0.65-1.91)	0.87 (0.47-1.60)	1.36 (0.77-2.38)	1.11 (0.61-2.00)
>86.0	1.05 (0.58-1.91)	0.82 (0.44-1.51)	1.07 (0.59-1.94)	0.94 (0.52-1.70)	0.88 (0.48-1.61)	1.25 (0.68-2.30)	0.89 (0.50-1.60)	0.83 (0.44-1.58)	1.01 (0.55-1.87)	1.12 (0.60-2.09)
MCPP										
<0.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.31-0.92	1.32 (0.75-2.32)	0.77 (0.46-1.31)	1.42 (0.84-2.40)	0.65 (0.39-1.09)	1.10 (0.65-1.85)	1.13 (0.66-1.91)	0.95 (0.56-1.62)	0.97 (0.54-1.76)	1.16 (0.66-2.02)	0.88 (0.51-1.51)
0.93-2.1	1.04 (0.54-2.00)	0.54 (0.29-1.00)	1.16 (0.64-2.12)	0.57 (0.31-1.04)	1.29 (0.71-2.33)	0.78 (0.41-1.49)	1.21 (0.68-2.17)	0.85 (0.43-1.66)	1.27 (0.68-2.36)	0.91 (0.48-1.70)
>2.1	1.24 (0.61-2.54)	**0.46* (0.24-0.91)**	0.92 (0.46-1.82)	0.74 (0.39-1.41)	1.17 (0.60-2.31)	1.49 (0.77-2.87)	1.01 (0.52-1.95)	0.82 (0.39-1.71)	1.27 (0.64-2.54)	1.25 (0.64-2.46)
∑DEHP										
<8.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.32-18.30	**0.40** (0.22-0.73)**	1.00 (0.57-1.77)	0.81 (0.47-1.39)	1.10 (0.65-1.86)	1.05 (0.63-1.77)	1.13 (0.66-1.93)	1.17 (0.68-2.03)	1.12 (0.60-2.09)	1.06 (0.60-1.87)	0.93 (0.54-1.60)
18.40-37.9	**0.48* (0.25-0.92)**	1.17 (0.62-2.21)	0.76 (0.41-1.41)	0.87 (0.46-1.64)	0.78 (0.42-1.47)	0.82 (0.44-1.55)	1.44 (0.79-2.66)	0.93 (0.46-1.91)	1.15 (0.61-2.19)	0.80 (0.42-1.52)
>37.9	**0.34** (0.16-0.74)**	0.80 (0.38-1.66)	0.93 (0.47-1.85)	1.24 (0.62-2.48)	0.82 (0.40-1.68)	0.51 (0.24-1.10)	1.84 (0.93-3.63)	1.73 (0.81-3.70)	1.10 (0.53-2.27)	0.72 (0.34-1.53)

Legend: OR = odds ratio; 95% CI = 95% confidence interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; MMP-1 = Matrix Metalloproteinase -1; MMP-2 = Matrix Metalloproteinase-2; MMP-7 = Matrix Metalloproteinase-7; MMP-9; Matrix Metalloproteinase-9; MMP-10 = Matrix Metalloproteinase-10

[a] Inflammatory biomarker levels between the 10th and 90th percentiles were used as the reference group.

[b] Models are adjusted for specific gravity, maternal age, and pre-pregnancy BMI.

*P<0.05; **P<0.01

Table 4.3b. Minimally-adjusted models: Odds ratios for the association of phthalate exposure with low (≤10th percentile) and high (≥90th percentile) levels of inflammatory biomarkers (N=1,286). [a] [b]

Phthalates (µg/L)	IL-2		IL-6		IL-8		IL-10		IL-12	
	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)
MBP										
<5.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.2-12.0	1.22 (0.69-2.13)	0.85 (0.50-1.45)	0.89 (0.53-1.48)	0.89 (0.52-1.54)	0.93 (0.55-1.58)	0.71 (0.41-1.24)	1.10 (0.64-1.89)	1.04 (0.61-1.78)	0.77 (0.45-1.32)	0.82 (0.47-1.41)
13.0-25.0	1.25 (0.64-2.44)	1.23 (0.67-2.27)	0.61 (0.32-1.16)	0.89 (0.47-1.68)	0.81 (0.43-1.54)	0.70 (0.37-1.33)	0.98 (0.51-1.88)	0.91 (0.48-1.73)	0.80 (0.42-1.52)	1.02 (0.55-1.92)
>25.0	1.55 (0.75-3.23)	1.07 (0.53-2.18)	0.52 (0.25-1.06)	0.98 (0.48-2.02)	0.59 (0.28-1.24)	0.48 (0.23-1.02)	0.84 (0.40-1.79)	1.41 (0.71-2.81)	0.89 (0.44-1.81)	0.83 (0.40-1.71)
MBzP										
<2.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.4-5.1	0.77 (0.43-1.37)	0.87 (0.51-1.47)	0.95 (0.57-1.57)	1.20 (0.71-2.03)	1.03 (0.61-1.74)	1.11 (0.64-1.94)	0.75 (0.43-1.32)	1.20 (0.71-2.06)	1.02 (0.60-1.73)	1.29 (0.73-2.27)
5.2-12.0	1.30 (0.72-2.36)	1.16 (0.66-2.05)	0.64 (0.36-1.16)	0.94 (0.51-1.73)	0.80 (0.44-1.47)	1.11 (0.60-2.03)	0.93 (0.51-1.69)	1.14 (0.62-2.07)	0.94 (0.52-1.70)	1.58 (0.86-2.89)
>12.0	1.06 (0.55-2.04)	0.96 (0.51-1.81)	**0.51*** **(0.27-0.98)**	0.92 (0.48-1.77)	0.81 (0.42-1.54)	0.99 (0.51-1.91)	0.94 (0.49-1.78)	1.28 (0.68-2.40)	0.81 (0.42-1.56)	1.75 (0.93-3.31)
MEP										
<12.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.0-28.0	0.89 (0.50-1.60)	1.45 (0.83-2.51)	0.74 (0.42-1.32)	0.75 (0.43-1.29)	0.79 (0.47-1.35)	0.94 (0.54-1.64)	0.59 (0.33-1.06)	1.07 (0.62-1.82)	0.65 (0.38-1.11)	0.88 (0.51-1.53)
29.0-86.0	1.00 (0.55-1.80)	**1.74*** **(1.004-3.01)**	1.57 (0.94-2.64)	0.69 (0.39-1.24)	0.75 (0.44-1.30)	0.94 (0.53-1.66)	0.80 (0.45-1.41)	1.06 (0.61-1.83)	**0.40**** **(0.22-0.74)**	0.69 (0.39-1.24)
>86.0	1.59 (0.89-2.83)	1.65 (0.92-2.99)	0.90 (0.49-1.62)	0.99 (0.56-1.74)	**0.49*** **(0.27-0.92)**	0.98 (0.54-1.76)	1.09 (0.62-1.93)	1.06 (0.59-1.90)	0.81 (0.46-1.42)	1.15 (0.65-2.03)
MCPP										
<0.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.31-0.92	1.27 (0.74-2.20)	0.61 (0.36-1.03)	0.78 (0.45-1.35)	0.66 (0.39-1.13)	**0.54*** **(0.31-0.94)**	0.59 (0.34-1.03)	0.90 (0.52-1.58)	1.13 (0.66-1.94)	1.06 (0.62-1.82)	1.09 (0.63-1.90)
0.93-2.1	0.98 (0.51-1.87)	0.81 (0.46-1.43)	1.20 (0.67-2.12)	0.83 (0.45-1.50)	0.81 (0.45-1.47)	0.70 (0.38-1.27)	1.06 (0.57-1.98)	1.37 (0.75-2.48)	1.21 (0.66-2.23)	1.23 (0.66-2.27)
>2.1	1.24 (0.62-2.47)	0.63 (0.32-1.22)	0.88 (0.46-1.69)	0.74 (0.38-1.46)	0.76 (0.39-1.48)	0.60 (0.31-1.18)	1.48 (0.76-2.88)	1.28 (0.65-2.49)	1.34 (0.69-2.62)	1.70 (0.88-3.27)
∑DEHP										
<8.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.32-18.30	1.21 (0.70-2.07)	1.23 (0.73-2.08)	1.31 (0.76-2.26)	0.99 (0.58-1.70)	0.76 (0.44-1.33)	0.67 (0.38-1.15)	1.17 (0.68-2.01)	1.46 (0.84-2.54)	0.85 (0.49-1.47)	1.40 (0.79-2.48)
18.40-37.9	0.65 (0.33-1.28)	1.02 (0.55-1.89)	0.93 (0.49-1.75)	0.73 (0.38-1.40)	0.75 (0.40-1.40)	**0.45*** **(0.23-0.87)**	0.80 (0.42-1.56)	1.46 (0.78-2.74)	0.88 (0.47-1.65)	1.55 (0.82-2.94)
>37.9	0.67 (0.32-1.43)	0.95 (0.47-1.93)	1.42 (0.71-2.82)	1.23 (0.61-2.48)	1.08 (0.54-2.17)	0.51 (0.25-1.06)	1.03 (0.50-2.13)	1.76 (0.88-3.55)	1.01 (0.50-2.04)	1.63 (0.80-3.31)

Legend: OR = odds ratio; 95% CI = 95% confidence interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; IL-2 = Interleukin 2; IL-6 = Interleukin 6; IL-8 = Interleukin 8; IL-10 = Interleukin 10; IL-12 = Interleukin 12

[a] Inflammatory biomarker levels between the 10th and 90th percentiles were used as the reference group.

[b] Models are adjusted for specific gravity, maternal age, and pre-pregnancy BMI.

*P<0.05; **P<0.01

Table 4.3c. Minimally-adjusted models: Odds ratios for the association of phthalate exposure with low (≤10th percentile) and high (≥90th percentile) levels of inflammatory biomarkers (N=1,286). [a b]

Phthalates (µg/L)	VEGF		MCP-1		MIP-1β		IFN-γ		TNF-α	
	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)
MBP										
<5.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.2-12.0	0.82 (0.48-1.39)	0.55 (0.30-1.03)	0.63 (0.36-1.12)	1.16 (0.67-2.01)	0.77 (0.45-1.30)	1.48 (0.84-2.61)	0.76 (0.44-1.31)	1.06 (0.61-1.84)	1.00 (0.60-1.67)	1.14 (0.63-2.06)
13.0-25.0	0.65 (0.33-1.25)	0.89 (0.46-1.71)	0.62 (0.32-1.21)	0.93 (0.49-1.78)	0.79 (0.42-1.50)	1.69 (0.89-3.24)	0.66 (0.34-1.27)	1.38 (0.73-2.60)	0.76 (0.39-1.46)	1.50 (0.77-2.91)
>25.0	0.76 (0.37-1.58)	0.92 (0.45-1.92)	0.97 (0.47-1.98)	0.89 (0.43-1.86)	0.71 (0.34-1.47)	1.30 (0.62-2.74)	1.08 (0.54-2.16)	1.26 (0.61-2.59)	0.60 (0.28-1.27)	0.98 (0.45-2.13)
MBzP										
<2.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.4-5.1	1.60 (0.94-2.73)	0.88 (0.48-1.59)	0.80 (0.46-1.38)	0.80 (0.46-1.39)	0.59 (0.33-1.03)	1.33 (0.77-2.30)	0.70 (0.40-1.24)	0.95 (0.55-1.63)	0.84 (0.49-1.42)	1.09 (0.60-1.98)
5.2-12.0	0.85 (0.45-1.63)	1.10 (0.59-2.06)	0.67 (0.36-1.25)	0.64 (0.34-1.19)	0.99 (0.56-1.73)	1.04 (0.55-1.95)	0.85 (0.47-1.56)	1.28 (0.72-2.28)	0.88 (0.49-1.59)	1.69 (0.91-3.15)
>12.0	1.34 (0.70-2.56)	1.09 (0.56-2.11)	0.94 (0.50-1.79)	1.11 (0.60-2.07)	0.82 (0.44-1.55)	1.43 (0.75-2.71)	1.15 (0.63-2.13)	0.90 (0.47-1.73)	0.79 (0.42-1.51)	1.15 (0.57-2.29)
MEP										
<12.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.0-28.0	1.31 (0.78-2.22)	0.88 (0.48-1.60)	1.19 (0.67-2.13)	0.98 (0.56-1.71)	0.75 (0.44-1.28)	0.82 (0.48-1.42)	0.99 (0.57-1.74)	0.91 (0.53-1.56)	0.85 (0.50-1.45)	0.99 (0.56-1.75)
29.0-86.0	0.65 (0.35-1.20)	0.84 (0.46-1.54)	1.38 (0.77-2.46)	0.94 (0.53-1.66)	0.86 (0.50-1.47)	0.60 (0.33-1.08)	1.02 (0.58-1.79)	0.87 (0.50-1.52)	0.84 (0.49-1.46)	0.83 (0.46-1.50)
>86.0	1.13 (0.63-2.03)	1.37 (0.76-2.48)	1.44 (0.79-2.65)	1.13 (0.63-2.03)	0.61 (0.33-1.11)	1.01 (0.57-1.77)	1.28 (0.72-2.28)	0.87 (0.48-1.57)	0.73 (0.40-1.33)	0.81 (0.43-1.51)
MCPP										
<0.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.31-0.92	0.98 (0.58-1.65)	1.16 (0.64-2.08)	1.16 (0.67-2.01)	0.77 (0.44-1.36)	0.85 (0.50-1.44)	1.02 (0.59-1.77)	1.03 (0.58-1.83)	1.21 (0.71-2.05)	0.57 (0.32-1.01)	0.94 (0.54-1.66)
0.93-2.1	0.70 (0.38-1.30)	1.07 (0.55-2.06)	1.22 (0.66-2.26)	1.10 (0.61-2.00)	1.16 (0.64-2.10)	1.25 (0.69-2.29)	1.41 (0.77-2.59)	1.00 (0.54-1.86)	1.00 (0.55-1.82)	1.00 (0.53-1.88)
>2.1	0.76 (0.39-1.50)	1.47 (0.74-2.94)	0.78 (0.38-1.59)	0.87 (0.45-1.70)	1.01 (0.52-1.99)	1.02 (0.52-2.00)	1.83 (0.95-3.53)	1.23 (0.63-2.41)	1.46 (0.77-2.78)	0.84 (0.41-1.72)
∑DEHP										
<8.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.32-18.30	1.17 (0.69-1.99)	1.16 (0.64-2.13)	1.40 (0.78-2.51)	1.16 (0.65-2.04)	1.31 (0.77-2.22)	1.44 (0.82-2.51)	1.12 (0.65-1.92)	1.26 (0.72-2.22)	0.67 (0.38-1.16)	1.12 (0.64-1.95)
18.40-37.9	0.72 (0.38-1.39)	1.27 (0.65-2.46)	1.39 (0.71-2.72)	1.39 (0.74-2.60)	1.09 (0.59-2.05)	1.13 (0.59-2.16)	0.72 (0.38-1.38)	1.32 (0.70-2.49)	0.70 (0.38-1.30)	**0.45* (0.22-0.94)**
>37.9	0.50 (0.23-1.07)	1.11 (0.52-2.34)	**2.39* (1.18-4.84)**	0.99 (0.47-2.07)	0.93 (0.45-1.93)	1.08 (0.52-2.23)	0.90 (0.44-1.84)	1.70 (0.84-3.41)	0.82 (0.40-1.65)	0.74 (0.35-1.59)

Legend: OR = odds ratio; 95% CI = 95% confidence interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; VEGF = Vascular endothelial growth factor; MCP-1 = Monocyte chemoattractant protein-1; MIP-1β = Human macrophage inflammatory protein 1-beta; IFN-γ = Interferon gamma; TNF-α = Tumor necrosis factor alpha

[a] Inflammatory biomarker levels between the 10th and 90th percentiles were used as the reference group.

[b] Models are adjusted for specific gravity, maternal age, and pre-pregnancy BMI.

*P<0.05; **P<0.01

Table 4.3d. Minimally-adjusted models: Odds ratios for the association of phthalate exposure with low (≤10th percentile) and high (≥90th percentile) levels of inflammatory biomarkers (N=1,286). [a] [b]

Phthalates (µg/L)	GMCSF		VCAM		ICAM		CRP	
	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)
MBP								
<5.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.2-12.0	0.87 (0.51-1.48)	0.65 (0.37-1.14)	**0.36** (0.20-0.64)**	0.76 (0.42-1.35)	**0.58* (0.34-0.99)**	0.66 (0.35-1.23)	1.02 (0.61-1.71)	1.37 (0.76-2.45)
13.0-25.0	0.79 (0.41-1.51)	0.97 (0.52-1.80)	0.65 (0.35-1.21)	1.09 (0.57-2.07)	1.00 (0.55-1.83)	1.37 (0.72-2.63)	1.13 (0.60-2.12)	0.74 (0.36-1.52)
>25.0	1.18 (0.59-2.38)	1.00 (0.49-2.02)	0.56 (0.27-1.13)	0.74 (0.35-1.59)	0.62 (0.30-1.27)	1.23 (0.59-2.59)	1.08 (0.53-2.21)	1.22 (0.57-2.59)
MBzP								
<2.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.4-5.1	0.98 (0.58-1.66)	0.76 (0.44-1.31)	0.58 (0.34-1.01)	1.15 (0.64-2.06)	0.93 (0.56-1.56)	1.50 (0.82-2.73)	1.14 (0.67-1.95)	1.00 (0.55-1.84)
5.2-12.0	0.91 (0.50-1.65)	0.98 (0.55-1.76)	0.84 (0.48-1.48)	1.46 (0.78-2.71)	0.78 (0.43-1.40)	1.26 (0.65-2.47)	1.60 (0.90-2.83)	0.99 (0.51-1.94)
>12.0	0.84 (0.44-1.60)	0.87 (0.46-1.63)	**0.48* (0.25-0.92)**	1.09 (0.55-2.14)	0.89 (0.47-1.65)	1.92 (0.98-3.77)	1.69 (0.90-3.15)	1.79 (0.93-3.45)
MEP								
<12.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.0-28.0	0.64 (0.37-1.08)	1.12 (0.66-1.90)	0.71 (0.39-1.31)	0.91 (0.53-1.59)	0.89 (0.51-1.55)	0.82 (0.46-1.48)	1.45 (0.85-2.46)	1.22 (0.68-2.20)
29.0-86.0	**0.52* (0.30-0.92)**	0.79 (0.45-1.39)	1.65 (0.97-2.81)	0.72 (0.40-1.32)	1.14 (0.66-1.96)	0.80 (0.43-1.47)	1.26 (0.73-2.19)	0.96 (0.51-1.79)
>86.0	0.63 (0.35-1.11)	0.87 (0.48-1.57)	1.38 (0.77-2.46)	0.87 (0.48-1.58)	1.44 (0.82-2.52)	1.16 (0.64-2.11)	1.29 (0.72-2.32)	1.39 (0.76-2.57)
MCPP								
<0.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.31-0.92	0.92 (0.52-1.61)	1.07 (0.63-1.81)	1.12 (0.65-1.95)	0.93 (0.54-1.61)	0.73 (0.41-1.27)	0.95 (0.54-1.68)	1.09 (0.65-1.82)	1.35 (0.75-2.41)
0.93-2.1	1.39 (0.76-2.53)	1.09 (0.59-2.00)	1.38 (0.75-2.52)	0.76 (0.40-1.43)	1.69 (0.96-2.97)	0.99 (0.52-1.89)	1.17 (0.65-2.12)	0.97 (0.50-1.88)
>2.1	**2.03* (1.06-3.88)**	1.31 (0.68-2.54)	1.45 (0.75-2.83)	0.78 (0.39-1.56)	1.31 (0.68-2.52)	0.98 (0.48-2.00)	1.10 (0.56-2.15)	1.09 (0.54-2.22)
∑DEHP								
<8.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.32-18.30	1.20 (0.72-2.02)	1.45 (0.83-2.52)	1.03 (0.58-1.83)	0.89 (0.51-1.56)	0.93 (0.53-1.62)	1.07 (0.60-1.92)	1.50 (0.89-2.52)	1.04 (0.59-1.85)
18.40-37.9	0.54 (0.28-1.06)	1.43 (0.76-2.67)	1.15 (0.61-2.16)	0.59 (0.30-1.17)	1.25 (0.68-2.29)	1.17 (0.60-2.28)	1.13 (0.61-2.11)	0.49 (0.24-1.00)
>37.9	0.84 (0.41-1.72)	1.52 (0.75-3.08)	**2.66** (1.36-5.20)**	0.78 (0.37-1.63)	**2.12* (1.09-4.11)**	1.07 (0.50-2.30)	1.14 (0.56-2.32)	0.61 (0.28-1.31)

Legend: OR = odds ratio; 95% CI = 95% confidence interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; GMCSF = Granulocyte-macrophage colony-stimulating factor; VCAM = Vascular cell adhesion molecule; ICAM = Intercellular adhesion molecule; CRP = C-reactive protein

[a] Inflammatory biomarker levels between the 10th and 90th percentiles were used as the reference group.

[b] Models are adjusted for specific gravity, maternal age, and pre-pregnancy BMI.

*P<0.05; **P<0.01

Table 4.4a. Maximally-adjusted models: Odds ratios for the association of phthalate exposure with low (≤10th percentile) and high (≥90th percentile) levels of inflammatory biomarkers (N=1,286). [a] [b]

Phthalates (µg/L)	MMP-1		MMP-2		MMP-7		MMP-9		MMP-10	
	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)
MBP										
<5.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.2-12.0	0.59 (0.32-1.09)	0.85 (0.47-1.52)	0.77 (0.45-1.34)	0.95 (0.55-1.64)	0.81 (0.47-1.41)	1.34 (0.78-2.29)	0.99 (0.57-1.73)	0.78 (0.40-1.50)	1.28 (0.73-2.25)	0.99 (0.57-1.73)
13.0-25.0	0.79 (0.39-1.59)	1.41 (0.73-2.72)	0.93 (0.49-1.77)	1.17 (0.61-2.23)	0.97 (0.51-1.85)	0.86 (0.43-1.71)	1.50 (0.79-2.84)	1.07 (0.52-2.23)	0.89 (0.45-1.79)	0.85 (0.43-1.71)
>25.0	0.82 (0.37-1.79)	0.89 (0.41-1.92)	0.74 (0.35-1.55)	1.07 (0.52-2.23)	0.94 (0.45-1.97)	0.94 (0.44-2.01)	0.99 (0.47-2.08)	1.17 (0.53-2.59)	1.16 (0.54-2.49)	1.27 (0.60-2.68)
MBzP										
<2.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.4-5.1	0.76 (0.42-1.39)	0.64 (0.37-1.12)	0.61 (0.35-1.05)	**2.64** (1.51-4.63)**	0.82 (0.48-1.40)	0.68 (0.38-1.22)	0.83 (0.49-1.39)	0.69 (0.35-1.32)	1.15 (0.64-2.06)	0.68 (0.38-1.20)
5.2-12.0	1.01 (0.54-1.90)	0.61 (0.33-1.13)	0.60 (0.33-1.10)	1.59 (0.82-3.06)	0.90 (0.49-1.63)	1.10 (0.60-2.00)	0.67 (0.37-1.24)	1.07 (0.55-2.08)	1.37 (0.73-2.57)	0.78 (0.42-1.46)
>12.0	0.86 (0.43-1.71)	0.63 (0.33-1.21)	0.74 (0.39-1.40)	**2.84** (1.47-5.49)**	1.09 (0.58-2.07)	1.48 (0.79-2.78)	0.72 (0.38-1.36)	0.83 (0.40-1.71)	1.73 (0.89-3.35)	1.18 (0.62-2.25)
MEP										
<12.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.0-28.0	0.74 (0.40-1.34)	1.25 (0.73-2.13)	1.31 (0.76-2.26)	1.14 (0.67-1.95)	1.01 (0.58-1.77)	**1.74* (1.01-3.00)**	0.99 (0.58-1.70)	0.75 (0.40-1.39)	1.08 (0.61-1.92)	1.63 (0.95-2.82)
29.0-86.0	0.84 (0.46-1.52)	0.77 (0.43-1.38)	1.61 (0.93-2.78)	1.16 (0.67-2.01)	1.47 (0.86-2.50)	1.15 (0.64-2.07)	1.08 (0.63-1.86)	0.81 (0.44-1.50)	1.33 (0.75-2.34)	1.10 (0.61-2.00)
>86.0	1.04 (0.56-1.91)	0.77 (0.41-1.44)	1.13 (0.62-2.07)	0.95 (0.52-1.72)	0.91 (0.49-1.67)	1.23 (0.66-2.27)	0.74 (0.40-1.35)	0.76 (0.39-1.46)	1.02 (0.55-1.90)	1.07 (0.57-2.02)
MCPP										
<0.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.31-0.92	1.30 (0.73-2.30)	0.74 (0.43-1.25)	1.43 (0.84-2.43)	0.63 (0.37-1.07)	1.10 (0.65-1.88)	1.13 (0.66-1.93)	0.95 (0.56-1.62)	0.86 (0.47-1.58)	1.16 (0.66-2.03)	0.87 (0.50-1.52)
0.93-2.1	0.98 (0.49-1.95)	**0.50* (0.27-0.93)**	1.26 (0.68-2.32)	0.56 (0.30-1.04)	1.30 (0.71-2.38)	0.79 (0.41-1.52)	1.20 (0.66-2.17)	0.70 (0.35-1.39)	1.25 (0.66-2.36)	0.91 (0.48-1.73)
>2.1	1.19 (0.56-2.50)	**0.42* (0.21-0.84)**	0.99 (0.50-1.99)	0.73 (0.38-1.41)	1.21 (0.61-2.43)	1.50 (0.77-2.90)	0.98 (0.50-1.92)	0.66 (0.31-1.40)	1.24 (0.61-2.50)	1.25 (0.63-2.48)
∑DEHP										
<8.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.32-18.30	**0.38** (0.20-0.70)**	0.95 (0.53-1.72)	0.91 (0.53-1.58)	1.12 (0.65-1.92)	1.10 (0.64-1.88)	1.16 (0.67-2.00)	1.12 (0.63-1.97)	0.93 (0.49-1.78)	1.05 (0.59-1.89)	0.88 (0.50-1.53)
18.40-37.9	**0.41* (0.21-0.84)**	1.11 (0.58-2.12)	0.88 (0.47-1.65)	0.88 (0.46-1.67)	0.79 (0.42-1.52)	0.84 (0.44-1.60)	1.41 (0.75-2.64)	0.73 (0.35-1.54)	1.13 (0.59-2.19)	0.75 (0.39-1.46)
>37.9	**0.27** (0.12-0.62)**	0.74 (0.35-1.57)	1.09 (0.54-2.20)	1.27 (0.63-2.57)	0.86 (0.41-1.78)	0.52 (0.24-1.14)	1.65 (0.82-3.32)	1.35 (0.61-2.97)	1.11 (0.53-2.35)	0.69 (0.32-1.47)

Legend: OR = odds ratio; 95% CI = 95% confidence interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; MMP-1 = Matrix Metalloproteinase -1; MMP-2 = Matrix Metalloproteinase-2; MMP-7 = Matrix Metalloproteinase-7; MMP-9; Matrix Metalloproteinase-9; MMP-10 = Matrix Metalloproteinase-10

[a] Inflammatory biomarker levels between the 10th and 90th percentiles were used as the reference group.

[b] Models are adjusted for specific gravity, maternal age, pre-pregnancy BMI, maternal race/ethnicity, average systolic blood pressure during 2nd trimester, average diastolic blood pressure during 2nd trimester, BPA and folic acid supplement use.

*P<0.05; **P<0.01

Table 4.4b. Maximally-adjusted models: Odds ratios for the association of phthalate exposure with low (≤10th percentile) and high (≥90th percentile) levels of inflammatory biomarkers (N=1,286). [a] [b]

Phthalates (µg/L)	IL-2		IL-6		IL-8		IL-10		IL-12	
	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)
MBP										
<5.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.2-12.0	1.20 (0.67-2.14)	0.90 (0.52-1.54)	0.87 (0.51-1.47)	0.83 (0.48-1.45)	0.98 (0.57-1.68)	0.79 (0.45-1.40)	1.11 (0.64-1.94)	1.08 (0.62-1.87)	0.80 (0.46-1.40)	0.73 (0.41-1.28)
13.0-25.0	1.12 (0.56-2.24)	1.32 (0.70-2.50)	0.60 (0.31-1.17)	0.79 (0.40-1.53)	0.88 (0.45-1.71)	0.78 (0.40-1.52)	0.95 (0.48-1.89)	0.89 (0.45-1.75)	0.87 (0.45-1.69)	0.90 (0.47-1.72)
>25.0	1.33 (0.62-2.87)	1.11 (0.53-2.30)	0.49 (0.23-1.04)	0.86 (0.41-1.81)	0.59 (0.28-1.28)	0.46 (0.21-1.02)	0.79 (0.36-1.73)	1.35 (0.66-2.77)	0.93 (0.45-1.95)	0.70 (0.33-1.49)
MBzP										
<2.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.4-5.1	0.76 (0.42-1.38)	0.89 (0.52-1.52)	0.98 (0.58-1.63)	1.21 (0.70-2.06)	1.06 (0.62-1.80)	1.20 (0.68-2.11)	0.77 (0.43-1.35)	1.23 (0.71-2.11)	1.08 (0.64-1.84)	1.26 (0.71-2.23)
5.2-12.0	1.18 (0.64-2.18)	1.21 (0.67-2.16)	0.67 (0.36-1.22)	0.88 (0.47-1.64)	0.85 (0.46-1.57)	1.16 (0.62-2.17)	0.90 (0.49-1.67)	1.08 (0.58-1.99)	1.01 (0.55-1.85)	1.51 (0.82-2.79)
>12.0	0.94 (0.48-1.84)	1.00 (0.53-1.90)	0.52 (0.27-1.01)	0.86 (0.44-1.67)	0.83 (0.43-1.60)	1.01 (0.51-1.99)	0.87 (0.45-1.69)	1.26 (0.66-2.39)	0.86 (0.44-1.68)	1.65 (0.86-3.15)
MEP										
<12.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.0-28.0	0.88 (0.48-1.58)	1.47 (0.84-2.55)	0.74 (0.41-1.31)	0.74 (0.43-1.28)	0.82 (0.48-1.39)	1.00 (0.57-1.76)	0.59 (0.32-1.07)	1.09 (0.63-1.86)	0.67 (0.39-1.14)	0.87 (0.50-1.52)
29.0-86.0	0.95 (0.52-1.72)	**1.77* (1.02-3.07)**	1.60 (0.95-2.70)	0.69 (0.38-1.23)	0.75 (0.43-1.30)	0.92 (0.52-1.64)	0.77 (0.44-1.37)	1.04 (0.60-1.82)	**0.41** (0.22-0.74)**	0.69 (0.38-1.24)
>86.0	1.44 (0.80-2.61)	1.68 (0.92-3.06)	0.96 (0.53-1.75)	1.00 (0.56-1.78)	**0.46* (0.24-0.86)**	0.81 (0.44-1.50)	1.03 (0.58-1.85)	1.00 (0.55-1.82)	0.79 (0.45-1.40)	1.16 (0.65-2.08)
MCPP										
<0.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.31-0.92	1.28 (0.73-2.23)	0.61 (0.36-1.05)	0.79 (0.46-1.37)	0.63 (0.36-1.08)	**0.54* (0.31-0.94)**	0.62 (0.36-1.09)	0.91 (0.52-1.61)	1.12 (0.65-1.93)	1.12 (0.65-1.93)	1.06 (0.61-1.86)
0.93-2.1	0.92 (0.47-1.78)	0.82 (0.46-1.47)	1.18 (0.66-2.12)	0.75 (0.41-1.37)	0.84 (0.46-1.53)	0.74 (0.40-1.38)	1.04 (0.55-1.97)	1.40 (0.76-2.57)	1.26 (0.68-2.33)	1.12 (0.60-2.09)
>2.1	1.13 (0.55-2.31)	0.64 (0.33-1.24)	0.88 (0.46-1.71)	0.68 (0.34-1.35)	0.77 (0.39-1.52)	0.59 (0.29-1.18)	1.40 (0.71-2.77)	1.29 (0.65-2.56)	1.41 (0.72-2.78)	1.58 (0.81-3.06)
∑DEHP										
<8.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.32-18.30	1.15 (0.65-2.01)	1.33 (0.78-2.27)	1.35 (0.77-2.36)	0.90 (0.51-1.56)	0.77 (0.44-1.36)	0.68 (0.39-1.20)	1.21 (0.69-2.13)	1.49 (0.85-2.62)	0.88 (0.50-1.54)	1.25 (0.70-2.24)
18.40-37.9	0.59 (0.29-1.19)	1.07 (0.57-2.01)	0.94 (0.49-1.80)	0.64 (0.32-1.25)	0.78 (0.41-1.48)	**0.47* (0.23-0.93)**	0.80 (0.41-1.59)	1.48 (0.78-2.82)	0.91 (0.48-1.74)	1.36 (0.70-2.61)
>37.9	0.57 (0.26-1.26)	1.02 (0.49-2.10)	1.43 (0.71-2.92)	1.04 (0.50-2.13)	1.07 (0.53-2.19)	0.47 (0.22-1.02)	1.00 (0.47-2.12)	1.77 (0.87-3.62)	1.01 (0.49-2.08)	1.39 (0.67-2.88)

Legend: OR = odds ratio; 95% CI = 95% confidence interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; IL-2 = Interleukin 2; IL-6 = Interleukin 6; IL-8 = Interleukin 8; IL-10 = Interleukin 10; IL-12 = Interleukin 12

[a] Inflammatory biomarker levels between the 10th and 90th percentiles were used as the reference group.

[b] Models are adjusted for specific gravity, maternal age, pre-pregnancy BMI, maternal race/ethnicity, average systolic blood pressure during 2nd trimester, average diastolic blood pressure during 2nd trimester, BPA, and folic acid supplement use.

*P<0.05; **P<0.01

Table 4.4c. Maximally-adjusted models: Odds ratios for the association of phthalate exposure with low (≤10th percentile) and high (≥90th percentile) levels of inflammatory biomarkers (N=1,286). [a] [b]

Phthalates (µg/L)	VEGF		MCP-1		MIP-1β		IFN-γ		TNF-α	
	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)
MBP										
<5.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.2-12.0	0.79 (0.45-1.36)	0.55 (0.29-1.04)	0.70 (0.39-1.26)	1.14 (0.65-2.00)	0.82 (0.48-1.41)	1.58 (0.88-2.83)	0.75 (0.42-1.31)	1.03 (0.58-1.81)	1.03 (0.61-1.75)	1.23 (0.67-2.27)
13.0-25.0	0.61 (0.31-1.19)	0.89 (0.45-1.76)	0.69 (0.34-1.39)	0.91 (0.47-1.79)	0.85 (0.44-1.65)	1.79 (0.91-3.53)	0.63 (0.32-1.25)	1.28 (0.66-2.47)	0.78 (0.40-1.54)	1.59 (0.79-3.21)
>25.0	0.75 (0.35-1.58)	0.92 (0.43-1.96)	0.90 (0.42-1.92)	0.87 (0.41-1.85)	0.75 (0.35-1.60)	1.20 (0.55-2.61)	1.05 (0.51-2.15)	1.06 (0.50-2.24)	0.61 (0.28-1.32)	0.96 (0.43-2.16)
MBzP										
<2.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.4-5.1	1.53 (0.89-2.64)	0.87 (0.47-1.58)	0.85 (0.48-1.50)	0.77 (0.44-1.35)	0.59 (0.34-1.04)	1.33 (0.76-2.33)	0.69 (0.39-1.23)	0.92 (0.53-1.61)	0.86 (0.51-1.47)	1.16 (0.63-2.13)
5.2-12.0	0.83 (0.43-1.60)	1.10 (0.59-2.08)	0.71 (0.37-1.36)	0.64 (0.34-1.21)	1.02 (0.57-1.81)	0.99 (0.52-1.89)	0.83 (0.45-1.54)	1.22 (0.67-2.20)	0.94 (0.51-1.71)	1.74 (0.92-3.29)
>12.0	1.29 (0.67-2.50)	1.10 (0.56-2.17)	1.06 (0.55-2.07)	1.13 (0.60-2.13)	0.84 (0.44-1.61)	1.36 (0.70-2.63)	1.08 (0.58-2.04)	0.85 (0.44-1.66)	0.83 (0.43-1.60)	1.20 (0.59-2.43)
MEP										
<12.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.0-28.0	1.28 (0.75-2.18)	0.89 (0.49-1.62)	1.28 (0.71-2.29)	0.96 (0.55-1.68)	0.76 (0.44-1.31)	0.86 (0.50-1.50)	0.99 (0.56-1.74)	0.89 (0.52-1.53)	0.85 (0.50-1.46)	1.01 (0.57-1.78)
29.0-86.0	0.62 (0.34-1.16)	0.85 (0.46-1.56)	1.40 (0.77-2.53)	0.92 (0.52-1.64)	0.86 (0.50-1.48)	0.58 (0.32-1.05)	1.00 (0.56-1.77)	0.83 (0.47-1.46)	0.83 (0.48-1.44)	0.82 (0.45-1.51)
>86.0	1.06 (0.58-1.93)	1.36 (0.74-2.48)	1.22 (0.65-2.31)	1.10 (0.61-1.99)	0.60 (0.32-1.11)	0.86 (0.48-1.54)	1.26 (0.71-2.26)	0.77 (0.42-1.41)	0.70 (0.38-1.28)	0.76 (0.40-1.45)
MCPP										
<0.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.31-0.92	1.01 (0.59-1.71)	1.14 (0.63-2.07)	1.13 (0.65-1.98)	0.77 (0.44-1.36)	0.89 (0.52-1.53)	1.01 (0.58-1.78)	1.06 (0.60-1.90)	1.14 (0.67-1.95)	0.58 (0.32-1.03)	0.92 (0.52-1.64)
0.93-2.1	0.71 (0.38-1.32)	1.09 (0.56-2.11)	1.31 (0.69-2.47)	1.12 (0.62-2.04)	1.24 (0.68-2.27)	1.27 (0.68-2.35)	1.46 (0.78-2.70)	0.95 (0.50-1.78)	1.03 (0.56-1.89)	0.98 (0.51-1.89)
>2.1	0.78 (0.39-1.54)	1.50 (0.74-3.02)	0.83 (0.40-1.72)	0.90 (0.46-1.75)	1.05 (0.53-2.08)	0.98 (0.49-1.96)	1.80 (0.92-3.52)	1.17 (0.60-2.31)	1.53 (0.80-2.94)	0.84 (0.41-1.75)
∑DEHP										
<8.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.32-18.30	1.14 (0.66-1.97)	1.15 (0.62-2.13)	1.46 (0.80-2.67)	1.15 (0.64-2.06)	1.49 (0.87-2.58)	1.35 (0.76-2.41)	1.17 (0.67-2.04)	1.18 (0.66-2.11)	0.67 (0.38-1.19)	1.13 (0.63-2.00)
18.40-37.9	0.73 (0.37-1.42)	1.27 (0.65-2.51)	1.49 (0.74-2.97)	1.42 (0.75-2.70)	1.21 (0.64-2.30)	1.07 (0.55-2.09)	0.74 (0.38-1.44)	1.23 (0.64-2.36)	0.73 (0.39-1.37)	**0.44* (0.21-0.93)**
>37.9	0.51 (0.23-1.10)	1.11 (0.51-2.38)	**2.42* (1.16-5.03)**	1.03 (0.48-2.18)	1.06 (0.50-2.25)	0.93 (0.44-1.97)	0.92 (0.44-1.93)	1.57 (0.77-3.21)	0.82 (0.40-1.68)	0.69 (0.32-1.51)

Legend: OR = odds ratio; 95% CI = 95% confidence interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; VEGF = Vascular endothelial growth factor; MCP-1 = Monocyte chemoattractant protein-1; MIP-1β = Human macrophage inflammatory protein 1-beta; IFN-γ = Interferon gamma; TNF-α = Tumor necrosis factor alpha

[a] Inflammatory biomarker levels between the 10th and 90th percentiles were used as the reference group.

[b] Models are adjusted for specific gravity, maternal age, pre-pregnancy BMI, maternal race/ethnicity, average systolic blood pressure during 2nd trimester, average diastolic blood pressure during 2nd trimester, BPA, and folic acid supplement use.

*P<0.05; **P<0.01

Table 4.4d. Maximally-adjusted models: Odds ratios for the association of phthalate exposure with low (≤10th percentile) and high (≥90th percentile) levels of inflammatory biomarkers (N=1,286). [a] [b]

Phthalates (µg/L)	GMCSF		VCAM		ICAM		CRP	
	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)	Low OR (95% CI)	High OR (95% CI)
MBP								
<5.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.2-12.0	0.95 (0.55-1.63)	0.65 (0.37-1.14)	**0.37** (0.21-0.68)**	0.72 (0.40-1.31)	**0.57* (0.33-0.99)**	0.72 (0.38-1.36)	1.10 (0.65-1.88)	1.43 (0.79-2.60)
13.0-25.0	0.96 (0.49-1.88)	1.00 (0.52-1.92)	0.68 (0.35-1.30)	0.99 (0.51-1.95)	0.99 (0.53-1.86)	1.56 (0.79-3.09)	1.27 (0.66-2.41)	0.76 (0.36-1.60)
>25.0	1.40 (0.68-2.88)	0.97 (0.47-2.02)	0.52 (0.25-1.10)	0.73 (0.34-1.60)	0.60 (0.28-1.27)	1.34 (0.62-2.91)	1.23 (0.59-2.57)	1.26 (0.57-2.77)
MBzP								
<2.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.4-5.1	1.07 (0.63-1.81)	0.77 (0.44-1.33)	0.62 (0.35-1.07)	1.07 (0.59-1.93)	0.94 (0.56-1.59)	1.54 (0.84-2.81)	1.18 (0.69-2.03)	1.01 (0.55-1.88)
5.2-12.0	1.05 (0.57-1.92)	1.04 (0.58-1.89)	0.90 (0.50-1.61)	1.35 (0.71-2.54)	0.78 (0.43-1.42)	1.33 (0.67-2.63)	**1.79* (1.001-3.22)**	1.01 (0.51-2.00)
>12.0	0.95 (0.49-1.82)	0.93 (0.49-1.77)	0.51 (0.26-1.01)	0.97 (0.48-1.95)	0.87 (0.46-1.66)	**2.03* (1.02-4.06)**	**1.96* (1.04-3.70)**	1.86 (0.95-3.65)
MEP								
<12.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.0-28.0	0.68 (0.40-1.16)	1.13 (0.67-1.92)	0.72 (0.39-1.34)	0.90 (0.52-1.57)	0.88 (0.50-1.54)	0.85 (0.47-1.52)	1.48 (0.87-2.52)	1.21 (0.67-2.19)
29.0-86.0	**0.55* (0.31-0.97)**	0.81 (0.45-1.43)	1.67 (0.97-2.87)	0.71 (0.38-1.29)	1.12 (0.65-1.94)	0.80 (0.43-1.47)	1.30 (0.75-2.27)	0.93 (0.50-1.75)
>86.0	0.64 (0.36-1.15)	0.88 (0.48-1.61)	1.35 (0.74-2.44)	0.88 (0.48-1.63)	1.45 (0.82-2.54)	1.09 (0.59-2.02)	1.34 (0.73-2.43)	1.31 (0.70-2.46)
MCPP								
<0.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.31-0.92	0.96 (0.54-1.69)	1.05 (0.62-1.80)	1.13 (0.64-1.97)	0.91 (0.52-1.59)	0.71 (0.40-1.24)	0.98 (0.55-1.74)	1.14 (0.68-1.92)	1.41 (0.78-2.55)
0.93-2.1	1.49 (0.81-2.75)	1.09 (0.59-2.02)	1.45 (0.78-2.68)	0.77 (0.41-1.47)	1.70 (0.95-3.02)	1.07 (0.55-2.05)	1.26 (0.69-2.30)	1.02 (0.52-2.00)
>2.1	**2.18* (1.13-4.22)**	1.36 (0.70-2.64)	1.55 (0.79-3.05)	0.72 (0.36-1.46)	1.29 (0.67-2.51)	1.02 (0.49-2.10)	1.22 (0.62-2.39)	1.15 (0.56-2.38)
∑DEHP								
<8.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.32-18.30	1.27 (0.75-2.17)	1.45 (0.82-2.56)	1.10 (0.61-1.99)	0.87 (0.49-1.55)	0.96 (0.54-1.69)	1.12 (0.61-2.04)	1.64 (0.96-2.81)	1.07 (0.60-1.94)
18.40-37.9	0.58 (0.29-1.15)	1.42 (0.75-2.70)	1.25 (0.65-2.41)	0.59 (0.30-1.18)	1.29 (0.69-2.41)	1.29 (0.65-2.55)	1.25 (0.66-2.35)	0.52 (0.25-1.07)
>37.9	0.84 (0.40-1.76)	1.55 (0.75-3.19)	**2.81** (1.41-5.62)**	0.79 (0.37-1.70)	**2.16* (1.10-4.27)**	1.14 (0.52-2.50)	1.31 (0.63-2.70)	0.64 (0.29-1.41)

Legend: OR = odds ratio; 95% CI = 95% confidence interval; MBP = Monobutyl Phthalate; MBzP = Monobenzyl Phthalate; MEP = Monoethyl Phthalate; MCPP = Mono-3-carboxypropyl Phthalate; DEHP = Di-(2-ethylhexyl) Phthalate; GMCSF = Granulocyte-macrophage colony-stimulating factor; VCAM = Vascular cell adhesion molecule; ICAM = Intercellular adhesion molecule; CRP = C-reactive protein

[a] Inflammatory biomarker levels between the 10th and 90th percentiles were used as the reference group.

[b] Models are adjusted for specific gravity, maternal age, pre-pregnancy BMI, maternal race/ethnicity, average systolic blood pressure during 2nd trimester, average diastolic blood pressure during 2nd trimester, BPA, and folic acid supplement use.

*P<0.05; **P<0.01

4.5.DISCUSSION

4.5.1. Summary of Study Findings

Study findings from the maximally-adjusted models demonstrate significantly greater odds of high MMP-2 levels among pregnant women exposed to quartiles 2 and 4 concentrations of MBzP, and low VCAM levels among those exposed to quartile 4 concentrations of ∑DEHP. In addition, reduced odds of low MMP-1, IL-12, and VCAM levels were significantly associated with quartiles 2 and 4 of ∑DEHP, quartile 3 of MEP, and quartile 2 of MBP, respectively. These results were consistent with those generated by the minimally-adjusted models in terms of the significance and directionality of the reported measures of associations.

The sensitivity analysis conducted to evaluate the effect of implementing a more conservative approach to model building on the selection of potential confounders did not identify a substantially different selection of covariates. In particular, a total of 18 variables met the more stringent criteria of having a p-value of less than 0.05, and altering the association of interest by 10% or more. The covariate representing folic acid supplement use, which was accounted for in the maximally-adjusted models constructed for the primary analysis, was not retained using this approach as it was only identified as being influential in one model. Overall, the covariates identified using the model construction methods applied for the primary and sensitivity analyses differed by one variable. The following 4 variables were identified in the sensitivity analysis: maternal race, average systolic blood pressure at visit 2, average diastolic blood pressure at visit 2, and BPA.

4.5.2. Non-Monotonic Exposure-Response

Although a monotonic biological gradient among phthalate quartiles was not consistently observed in this study, research in the existing literature have reported more complex non-monotonic exposure-response relationships in relation to the exposure to phthalates. An animal study was conducted to examine the effects of DEHP on aromatase activity, an enzyme involved with transforming testosterone to estradiol, by exposing rat dams to five high and low doses and evaluating the enzyme activity in newborn rats [48]. On postnatal day 1, the study results show statistically significant reductions in aromatase activity at lower doses (0.135 and 0.405mg DEHP/kg body weight/day), and elevations in activity at higher doses (15, 45, and 405mg DEHP/kg body weight/day) among males but not females [48]. However, on postnatal day 22, statistically significant elevations in aromatase activity were reported for several high and low doses in females, whereas only a single low dose was significantly associated with greater enzyme activity among males [48]. These previous study findings indicate that exposure concentration, time of outcome assessment, and sex of study subjects may all be critical factors influencing the response to phthalate exposure.

An experimental study investigating the effects of monophthalates on pro-inflammatory cytokines exposed human epithelial cells to various doses, and measured the supernatant concentrations of IL-6 and IL-8 as the response [26]. Initial lower concentrations of MEHP, MnOP, MINP, and MIDP were potent stimulators of IL-6 and IL8, whereas higher concentrations exhibited a suppressive effect; in contrast, MnBP and MBzP were non-significant or weak stimulators of these cytokines, as well as less potent suppressors at higher doses [26]. Overall, greater cytokine stimulating effects were observed among monophthalates with alkyl side chains consisting of 8 or more carbons [26]. These study findings demonstrate that the stimulating or

suppressive effects of monophthalates on cytokines may be dependent on the exposure concentration, and that the chemical structure may potentially be an important determining potency.

An experimental study was conducted to evaluate the impact of short-term exposure to house dust consisting of high (2.09mg/g) or low (0.41mg/g) concentrations of DEHP, on immune responses in the nasal mucosa of 32 individuals that were either healthy or allergic to house dust mites [49]. Although cytokine levels were not significantly altered among healthy individuals, allergic study participants exposed to low concentrations of DEHP exhibited greater levels of granulocyte-colony-stimulating factor (G-CSF), interleukin-5 (IL-5), and IL-6 compared to controls [49]. In contrast, the allergic study participants who were exposed to high concentrations of DEHP yielded reduced levels of G-CSF and IL-6 compared to the allergic study participants exposed to low concentrations of DEHP [49]. These findings demonstrate that among individuals who are allergic to house dust mites, short-term exposure to DEHP may have cytokine stimulatory effects at low doses, as well as suppressive effects at high doses.

The research results observed in these experimental, animal, and epidemiological studies discussed demonstrate that the effects of phthalate may be influenced by a number of factors, including exposure concentration, chemical structure, time of outcome assessment, and sex of the study subjects. Collectively, these results provide evidence supporting non-monotonic exposure-response curves for phthalates and associated adverse health outcomes. .

4.5.3. Phthalates and Markers of Inflammation

Studies assessing the effects of phthalates on biomarkers of inflammation may be essential to identifying a plausible biological mechanism of action, leading to a better understanding of the

detrimental effects that may occur following human exposure. However, few epidemiological studies have been conducted to evaluate the potential immunotoxic properties of phthalates during vulnerable periods such as gestation, which may be critical factors mediating adverse perinatal and pregnancy outcomes. A prospective cohort study consisting of pregnant women from Northern Puerto Rico, assessed the relationship of urine concentrations of MEHP, MEHHP, MEOHP, mono-2-ethyl-5-carboxypentyl phthalate (MECPP), MEP, MCPP, monocarboxyisooctyl phthalate (MCOP), monocarboxyisononyl phthalate (MCNP), MBzP, MBP, and monoiso-butyl phthalate (MiBP), with plasma concentrations of CRP, interleukin-1β (IL-1β), IL-6, IL-10, and TNF-α [28]. While most study findings yielded null results regarding the association between phthalates and markers of inflammation, statistically significant increases in IL-6 and IL-10 were observed in relation to increases in exposure (corresponding to the interquartile range) to MCNP and MECPP, respectively [28]. However, as these two metabolites were not evaluated in the current analysis, the effects of these chemicals could not be compared for consistency.

The study conducted by Ferguson et al. (2015), which included pregnant women participating in a nested-case control study in Boston, investigated the relationship of urine concentrations of MEHP, MEHHP, MEOHP, MECPP, MBzP, MBP, MiBP, MEP, and MCPP with plasma concentrations of CRP, IL-1β, IL-6, IL-10, and TNF-α [29]. As in the previous study discussed, most of the study findings were not statistically significant. However, statistically significant elevations in IL-6 levels were associated with an interquartile range increase in MCPP concentrations [29]. Similar results were not observed in the current analysis conducted, as null findings were identified for the association between MCPP and IL-6. The study by Ashley-Martin et al. (2015) included pregnant women from the MIREC Study, and evaluated the association of urine concentrations of MEP, MBP, MBzP, MCPP, and ∑DEHP with elevated umbilical cord

plasma levels of immunoglobulin E (IgE), and a composite variable of thymic stromal lymphopoietin (TSLP) and interleukin-33 (IL-33) [27]. The study authors identified an inverse non-linear relationship of MCPP concentrations with increased IL-33/TSLP levels (>80%) [27]. Similar effects of MCPP on inflammation were not observed in the current analysis (all associations evaluated with MCPP in the present study were not significant).

In summary, inconsistent results were observed between those reported in the current analysis and studies in the existing literature. However, differences in the biomarkers of inflammation investigated, and in the study methods implemented may have contributed to these inconsistent findings.

4.5.4. Inflammation and Adverse Outcomes during Pregnancy

During pregnancy, both pro- and anti-inflammatory responses are necessary yet tightly regulated to enable critical processes to occur at various stages of gestation [50]. However, stimulants of inflammation can result in a cascade of events, including an increase pro-inflammatory cytokines, such as TNF-α, IL-8, and IL6, which can subsequently increase matrix metalloproteinases, and thus have an effect on functions, such as the ripening of the cervix, and the rupture of membranes [51]. Evidence in the existing literature have linked altered immune responses during pregnancy to a number of adverse complications. A case-control study compared MMP-8 concentrations measured in amniotic fluid among women with preterm PROM and term pregnancies [21]. Although no significant differences were observed among median MMP-8 concentrations between the two groups, participants with MMP-8 concentrations above the 90th percentile had a significantly greater odds of preterm PROM (OR: 3.4; 95% Cl: 1.2-9.9) [21]. Whitcomb et al. (2009) compared serum concentrations of G-CSF among participants with normal

pregnancies to those with preterm births, and reported a positive association (OR: 1.52; 95% Cl: 1.07, 2.16) between G-CSF and preterm birth [22].

A cross-sectional study was conducted to compare the inflammatory responses between women with healthy pregnancies, mild pre-eclampsia, and severe pre-eclampsia [23]. No significant differences among plasma concentrations of IL-4, IL-12, and IFN-γ were observed between the different groups considered in this study. Compared to women with healthy pregnancies, significantly greater IL-8 and CRP concentrations were reported among those with severe pre-eclampsia [23]. Although not statistically significant, women with mild pre-eclampsia exhibited IL-8 and CRP concentrations between levels measured among participants with healthy pregnancies and severe pre-eclampsia [23]. The study by Ernst et al. (2011) measured plasma concentrations of CRP, and reported a reduction in birth weight (-128g; 95% Cl: -195, -60) and an increased odds of small for gestational age newborns (OR: 2.94; 95% Cl: 1.61-5.36) among pregnant women with concentrations of 25mg/L or more, compared to those with levels less than 5mg/L [24]. Overall, evidence in the existing literature demonstrates the importance of inflammation during pregnancy, and suggest that disruptions to the tightly regulated immune response may potentially result in the manifestation of number of adverse perinatal and pregnancy complications.

4.5.5. Study Strengths

The MIREC Study encompasses a number of strengths that add to both the internal and external validity of the study findings. The current study recruited participants from multiple sites across the country, and is based on a relatively large sample size, thereby contributing to a representative sample of pregnant women living in Canada. The prospective design of the study

is an additional strong point as a clear temporal relationship between the exposures and outcomes can be established; this provides greater confidence that the observed changes to maternal inflammatory responses are due to the exposure to phthalates which were measured at an earlier time point during pregnancy. To the best of our knowledge, the current study has evaluated the largest selection of inflammatory biomarkers in response to phthalate exposures during pregnancy to date. As the purpose of conducting this study is exploratory, the extensive list of measured biomarkers may be imperative for filling in current gaps in the literature, and informing future studies in this area of research. The administration of multiple comprehensive questionnaires for data collection has yielded a wealth of information, enabling thorough investigations into potential confounders, minimizing to a certain extent the potential for residual confounding. Finally, a major strength of the current study is the common time of exposure assessment during the first trimester of pregnancy, thereby contributing critical information for the evaluation of sensitive windows of exposure during vulnerable periods such as gestation.

4.5.6. Study Limitations

There are several limitations that should be considered when interpreting the study findings. The study participants included in the current analysis were predominantly white pregnant women with a university education; for this reason, the generalizability of the study findings may be reduced as they may be more applicable to populations with these cohort characteristics. As phthalates are quickly excreted from the human body following exposure, and have yielded low to moderate temporal reproducibility, the use of a single urine sample to estimate phthalate exposure may increase the risk of exposure misclassification [52-54]. However, as

exposure misclassification is expected to be non-differential, the study results are more likely to be biased towards the null, resulting in an underestimation of the measures of associations.

Specific biomarkers related to inflammation were selected for measurement in MIREC Study; however, high content biomarker analysis may provide a more superior approach as greater biomarker information would have been acquired, enabling the effects of phthalates to be better validated mechanistically. As the purpose of this study is exploratory, multiple multivariate regression models were constructed to evaluate the effects of phthalates on maternal inflammatory responses. The implementation of multiple tests can consequently result in an increase the detection of false positives [55]; however, this was partially addressed by reducing the threshold for statistical significance to a p-value of less than 0.01. A considerable amount of data used in this study relied on self-reported information that were collected through questionnaires; as self-reported information may yield inaccuracies in the data collected, there is potential for residual confounding due to erroneous measurements of covariates. And finally, although the current analysis implemented statistical methods that have been applied in similar studies, the categorization of both the exposure and outcome is a less sensitive approach to evaluating the association of interest compared to modelling the exposure and outcome as continuous variables. Since information is lost as phthalates and inflammatory biomarker concentrations are grouped, the power to detect statistically significant relationships may have been reduced.

4.6. CONCLUSION

Overall, the study results demonstrate statistically significant ($p<0.01$) associations of urinary MBzP and ∑DEHP concentrations with elevated odds of high MMP-2 levels and low VCAM levels, respectively. Other results that may be of interest for exploratory purposes,

although not considered to be statistically significant ($p<0.05$), include a positive association of ∑DEHP with low MCP-1 and ICAM levels, MCPP with low GMCSF levels, MBzP with low CRP and high ICAM levels, and MEP with high MMP-7 and IL-2 levels. In general, a monotonic biological gradient among exposure quartiles was not observed; however, studies in the existing literature provide evidence supporting non-monotonic exposure-responses in relation to phthalates. As the current literature has linked altered markers of inflammation with various adverse outcomes during pregnancy, the results reported in this study suggest that phthalates may potentially be a risk factor for adverse perinatal and pregnancy complications. However, more research is needed to validate the current study findings, and to further evaluate the potential of phthalate-induced maternal immunological changes as a plausible biological mechanism.

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CHAPTER 5: DISCUSSION

There is increasing interest on the impact of phthalates during pregnancy due to previous reports on possible links between environmental contaminants and detrimental health outcomes, such as decreased birth weight and preterm birth [1]. In this respect, potential toxicities of phthalates resulting in adverse birth outcomes and possible etiological pathways are of particular importance, as they have important implications for public and population health, health policy development, and future research. With the aim to provide new insights into the effects of phthalates on maternal and perinatal health, the current master's thesis evaluated the present state of evidence by performing a systematic review (manuscript 1), and by filling some existing knowledge gaps in the literature through original research (manuscripts 2 and 3).

The objective of the first manuscript was to summarize the existing literature, and evaluate the possible risks of phthalates for pregnant women and their developing offspring, by performing a systematic review of observational studies investigating the effects of gestational exposure to environmental phthalates on several *a priori* selected outcomes. Following a comprehensive search of MEDLINE, EMBASE, PubMed, CINAHL, and POPLINE, supplemented by citation tracking and hand-searching of reference lists, a total of 24 articles were eligible for inclusion. While birth weight (BW), gestational age (GA), head circumference (HC), and preterm birth (PB) were the most frequently reported outcomes, studies investigating intrauterine growth restriction (IUGR), pre-eclampsia (PE), pregnancy-induced hypertension (PIH), gestational diabetes mellitus (GDM), and Apgar scores (AS) were either scarce or not detected (n = 0 to 1).

Although contradicting results were observed between studies, most of the statistically significant results supported an association of phthalates with a decrease in BW and GA, and an increase in HC and PB. After exploring possible sources of heterogeneity as a likely explanation for the discrepancies in the literature, it was observed that a major challenge in conducting research on phthalates consisted of issues related to exposure assessment. Specific issues that warrant consideration in this regard include the following: the influence of contamination risk by the biological matrix of study, the impact of participant characteristics on adjustment methods for urinary dilution, the effect of temporal variability of phthalates on the risk of exposure misclassification, and the identification of critical time windows of exposure. This demonstrates that the accuracy and precision of measured phthalates, and biomarkers of exposure may be dependent on a number of factors. In reviewing the literature, inconsistencies between studies were observed, and a scarcity of research reporting on select phthalates and outcomes were identified. Therefore, as current evidence on the risks of phthalates during pregnancy are largely inconclusive, more research is warranted. (Chapter 2; Manuscript 1)

In the second manuscript, secondary data-analysis was conducted to address some of the inconsistencies in the literature by investigating the association of gestational exposure to environmental phthalates with clinical outcomes among maternal-infant pairs participating in the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. The exposure to phthalates was determined using maternal urinary samples collected during the first trimester of pregnancy, and assessed as quartiles, with the lowest group selected as the reference. Multiple linear and logistic regression models were employed for statistical analysis, with results were considered statistically significant at $p<0.01$ and suggestive at $p<0.05$. To account for the possibility of effect modification by infant sex, a stratified analysis was performed. However, as

the identification of potential effect modifiers was not the primary objective of the current study, an interaction analysis was not conducted. Based on this analysis, a significant positive association was identified between 3rd quartile of maternal exposure to monobutyl phthalate (MBP) and HC among female infants. Relationships observed for PIH, BW, GA, PB, and AS with phthalate levels did not reach statistical significance at the $p<0.01$ level.

For greater comparability across studies in the literature, and to accentuate general trends that may be informative to future studies, results approaching statistical significance ($p<0.05$) were also noted. In particular, HC showed consistent increases in females and decreases in males. The identification of effect measures in opposite directions indicate that effect modification by baby sex is a possibility, and thus should be further explored. Moreover, an inverse association with GA among both male and female stratums was also identified. This decrease in pregnancy duration reinforces the general observation made in the first manuscript. In sum, the current analysis presents little evidence supporting the effects of phthalates on pregnancy and birth outcomes. Interesting trends were observed among suggestive associations; however, these study findings should be interpreted with caution as validation through further research is needed. (Chapter 3; Manuscript 2)

Since previous research has demonstrated a possible relationship of inflammatory biomarkers with phthalate exposures, and unfavourable maternal and infant outcomes, maternal inflammatory responses may play a critical role as mediators of effect in the mechanistic pathway [2-5]. Despite this, there is a lack of human research investigating the potential immunotoxic properties of phthalates during pregnancy [6-8]. In the third manuscript, the paucity of research was addressed by performing secondary data-analysis on the association between maternal exposure to environmental phthalates and inflammatory responses among pregnant women in the

MIREC Study. The phthalate exposures examined were analogous to those in the second manuscript. In addition, biomarkers of inflammation were measured in plasma from samples of maternal blood collected in the third trimester of pregnancy. Multinomial logistic regression models were employed to investigate the odds of low ($\leq 10^{th}$ percentile) and high ($\geq 90^{th}$ percentile) levels of inflammation, in comparison to levels considered normal (10^{th} to 90^{th} percentile).

Significant ($p<0.01$) positive associations of exposure quartiles 2 and 4 of monobenzyl phthalate (MBzP) with high matrix metalloproteinase-2 (MMP-2) levels, and of quartile 4 of ∑di-(2-ethylhexyl) phthalate (∑DEHP) with low vascular cell adhesion molecule (VCAM) levels were identified. These results suggest that select phthalates may be capable of interfering with normal maternal responses of inflammation. Additionally, a significant inverse association of quartile 2 and 4 of ∑DEHP with low matrix metalloproteinase-1 (MMP-1) levels, quartile 3 of monoethyl phthalate (MEP) with low interleukin-12 (IL-12) levels, and quartile 2 of MBP with low VCAM levels were observed. Although the study findings presented do not show an increased odds of abnormal biomarker levels of inflammation at the lower end of the spectrum, the results collectively reinforce the inflammatory potential of phthalates as pregnant women exposed to concentrations greater than the first quartile were less likely to experience low levels of MMP-1, IL-12, and VCAM.

As the nature of this study is exploratory, results approaching statistical significance ($p<0.05$) were also highlighted. In particular, positive associations of ∑DEHP with low monocyte chemoattractant protein-1 (MCP-1) and intracellular adhesion molecule (ICAM) levels, MBzP with low C-reactive protein (CRP) and high ICAM levels, mono-(3-carboxypropyl) phthalate (MCPP) with low granulocyte-macrophage colony stimulating factor (GMCSF) levels, and MEP with high matrix metalloproteinase-7 (MMP-7) and interleukin-2 (IL-2) levels were observed. In

agreement with previous research reporting on the effects of phthalates on inflammatory cytokines, (non-monotonic) exposure-response relationships were also observed among a number of measures of associations reported [2]. This is a key issue that should be addressed with further research, as such information would be vital to understanding and accurately interpreting results in the literature. Overall, the study findings identified demonstrate that MBzP, ∑DEHP, MCPP and MEP may impact on the inflammatory process during pregnancy. However, in spite of this study, evidence on this topic is limited. Therefore, more research is fundamental to elucidating a potential etiological mechanism associated with the incidence of adverse outcomes during the gestational period. (Chapter 4; Manuscript 3)

In the secondary-data analyses performed for the MIREC Study, biomarker indicators of exposure were used, as these may be more direct exposure metrics than external measures of exposure. However, studies in the literature have reported relatively short half-lives for several phthalates, and this suggest that the phthalate metabolites measured may not accurately reflect long-term exposure [9, 10]. Additionally, multiple statistical tests were conducted to investigate the association of phthalates with inflammatory responses and clinical outcomes. One way to reduce the number of comparisons is to use potency weighted combined measures of phthalate exposure. However, this was not possible in the current study as the relative potency of all phthalates being analyzed were not found in the literature. To be consistent with previous analyses, phthalate exposures and most outcomes were evaluated as categorical variables. BW, HC, and GA were the only outcomes modelled as continuous variables, as this was done in past studies. Although categorical analyses does result in some information loss, it is more robust against outlying values.

A primary strength of this thesis consists of the exhaustive search and inclusive eligibility criteria implemented in the first manuscript. In particular, the multiple search methods employed reduces the likelihood of missing articles pertinent to the systematic review, and the lenient eligibility criteria captures studies reporting on phthalates, irrespective of the parent compound, which contributes to a more comprehensive review. Although discrepancies were identified among included studies, a critical discussion on heterogeneity yielded a number of factors to be considered for the improvement of exposure assessment, and should be followed-up on with further research. A major strong point of the second and third manuscripts consist of the different methods implemented for covariate selection. In regards to the construction of the minimally-adjusted models, the identification of *a priori* covariates among similar studies in the literature ensures the adjustment of common potential confounders even if they may not have been identified as confounders in the MIREC Study when considered alone. Furthermore, covariates that altered the association of interest by 10% or more were considered for inclusion in the maximally-adjusted models; this step was essential to highlight additional covariates that were relevant but not detected in other study populations.

The research conducted for this thesis is also subjected to a number of limitations. Since the systematic review consisted solely of published articles, there is potential for publication bias. In particular, research shows that the likelihood of publication is greater among studies reporting findings that are statistically significant [11]. Therefore, there is a possibility that more studies with null results were not accounted for in the systematic review, which could be problematic as the impact of phthalates may appear greater than it truly is. Also, as the systematic review was limited to humans, animal studies where important toxicological insights may have been obtained may have been overlooked. The restriction to articles in English is another limitation of the

systematic review which may have resulted in the exclusion of relevant studies published in other languages. However, as English-language restrictions are not uncommon in systematic reviews, the decision to only consider studies published in English as part of the eligibility criteria was made for reasons of practicality. With regards to the analyses conducted for the MIREC Study, a weakness that stands out includes the selection of cut-offs for the categorization of exposure and outcome variables, based on the distribution of phthalates and inflammatory biomarkers in the study sample. This presents a possible issue that could reduce the generalizability of the study findings, as concentrations of both phthalates and biomarkers of inflammation may not be comparable across different populations. Consequently, caution should be applied when interpreting the research findings.

The investigations into the effects of phthalates on maternal and perinatal health conducted to date, as well as the maternal-biological changes that may occur as a result of the exposure to these environmental chemicals, have provided important findings that require clarification and expansion through further research. The results of future studies may influence public health initiatives aimed at reducing exposures among susceptible subgroups of the general population. Additional studies will also inform public and population health policy development, if regulations of phthalates employed in commonly used products are deemed necessary. At this point in time, it is clear that more work is required prior to fully understanding the impact of these chemical compounds on vulnerable populations. And for this reason, the current master's thesis is an important contribution in this relatively early stage of research, by (1) summarizing the existing evidence, (2) providing new knowledge through exploratory research, (3) highlighting the challenges in evaluating phthalates, and (4) proposing recommendations for future studies.

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biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. Environ Sci Technol, 2014. **48**(12): p. 7018-25.

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APPENDIX

Appendix 1: Informed Consent for the MIREC Study

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants

Université de Montréal

INFORMED CONSENT

MIREC
Maternal-Infant Research on Environmental Chemicals

Maternal-Infant Research on Environmental Chemicals (The MIREC Study): A National Profile of *In Utero* and Lactational Exposure to Environmental Contaminants

Investigators:

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Local Site Investigator: Dr. William D. Fraser, M.D., M.Sc., FRCSC

Funding agencies and research partners:
Health Canada
Ontario Ministry of the Environment
Canadian Institutes of Health Research

We are asking for your participation in a study on the effects of exposure to metals such as lead and mercury on pregnancy outcomes. The study will also measure the levels of various environmental chemicals in your blood, urine, hair and in your infant's cord blood and feces. We will also conduct a survey of nutrients, immune characteristics and environmental chemicals in breast milk. The information on breast milk together with the information during pregnancy could help improve the health of mothers and babies. We invite you to read this consent form to learn more about the study. Please feel free to ask the study personnel (local site investigator or research nurse) any questions which you may have.

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1. Why is the MIREC study being done?
Some recent reports have raised concerns about the number of chemicals in our bodies and what, if any, health effects may be associated with the levels measured. For some chemicals such as lead, government policies banning lead in gasoline and paint have helped to reduce blood lead levels in children. Lead may still persist in soil, dust and water. Work continues on assessing the health risks of low lead exposure in children.

Smoking in pregnancy has long been linked with a higher risk of low birth weight, and other harmful effects on the baby. Currently, there is little information about exposure to tobacco smoke among pregnant women in Canada. In particular, there is a lack of information on whether smoking behavior changes during pregnancy. Governments and public health workers could use this information to develop policies and programs that help pregnant women quit smoking and avoid exposure to second-hand tobacco smoke.

Most often breast feeding is the best method of feeding your infant. It gives good nutrition, immune protection and emotional benefits to your infant. There are also health benefits to you

when you breastfeed. This study will collect information on nutrients and environmental chemicals found in breast milk as well as, the immune protection that it provides to the baby. This study will be the largest ever conducted in Canada and will provide valuable information. Many other studies conducted on pregnant women in Canada have been very small or limited to certain locations. Information from this research may also assist in the development of nutrition programs and policy for breast-feeding women.

2. What is the purpose of this project?

There are three main purposes for this research study:

(1) to measure the extent to which pregnant women and their babies are exposed to environmental chemicals including; lead, mercury, cadmium, arsenic and manganese (heavy metals); persistent organic pollutants (POPs); pesticides; flame retardants; plastic softeners; surface coatings; food packaging and processing related chemicals and smoking by-products. Exposure will be measured in mother's blood, urine, hair, breast milk as well as cord blood and meconium (baby's first stool).

(2) to measure some of the beneficial components in human breast milk (nutrients and immune factors); and

(3) to assess what health risks, if any, are associated with the levels of heavy metals measured.

We know that not everyone who experiences the same risks will develop a certain health problem. Therefore, we will also test characteristics of women (genomic testing: to identify genes that could affect reactions to certain chemicals or diseases; nutritional status; and other markers), which might make mothers or their babies more or less likely to experience harmful effects from exposure to environmental chemicals.

3. Number of participants in this study

We aim to recruit 2,000 women in their first trimester of pregnancy from 8 to 10 sites across Canada.

4. What will be the process of this study?

If you agree to participate we will arrange our meetings with you during your regularly scheduled prenatal visits (6 – 13 weeks, 16 – 21 weeks, and 32 – 34 weeks) and at delivery, as well as a home visit 3 to 8 weeks after the birth of your child. During these visits you will be asked to:

a) Complete questionnaires.

The first questionnaire takes close to 45 minutes to complete. It will collect information on you and the baby's father including general health, any previous pregnancies, occupation, education, lifestyle, and potential sources of exposure to chemicals. At each visit during your pregnancy and at delivery we will ask you to complete some short follow-up questionnaires (about 10-20 *minutes*). Some of the questions will help us determine your nutritional status based on the foods you eat and your use of dietary supplements. The study nurse will

administer these questionnaires and help you to understand the questions if needed. You may refuse to answer any questions that make you uncomfortable.

b) Allow us access to your medical records to record information on the health of your pregnancy and your baby.
The information to be collected from your medical records includes any health problems you experienced during pregnancy (e.g., elevated blood pressure) and the health of your baby (e.g., birth weight).

c) Provide us with samples of your blood and urine.
At each visit (once in each trimester and at delivery) you will be asked to provide about 120 *ml* of urine (about ½ cup) and an extra 38 ml of blood (2.5 tablespoons).

d) Allow us to collect cord blood and meconium (baby's first stools).
The study will need to collect 100 *ml* (approximately 7 tablespoons) of cord blood and 20 grams (0.8 oz.) of meconium. Meconium is the first stools that your baby excretes over the 2 days following birth. The collections of these samples are non-invasive and safe to you and your baby.

e) When your baby is between 3 and 8 weeks of age a research nurse will call you to arrange a home visit. At this home visit you will be asked to:

- complete a 20 minute questionnaire about your diet, lifestyle and baby's feeding
- provide about 200 ml (about . of cup) of your breast milk expressed by hand or with a breast pump. We will provide you with a kit for collecting the milk and step-by-step instructions. We will also give you contact information for the nurse and the site investigator if you have any questions.
- provide a small hair sample. The hair strands will be collected from the lower back of your head where it would not be noticeable. The nurses are trained to perform this task. (NOTE: If you do not wish to participate in the breast milk collection, we will request a hair sample when we collect your baby's meconium.)

5. What advantages and benefits can I expect?

In regards to communication of individual results, if you have a result that is higher than health-based guidelines, and there are preventive measures or treatments, you will be informed through your doctor. These individual results will be added to your medical chart.

We will also provide you with some material to help you understand these chemicals. You should also know that it could be several months to years after your pregnancy before all the chemical results will be available, given the time and costs for these analyses.

For the first time in Canada we will have some measures of levels of important environmental chemicals in pregnant women and their babies that can be used as a baseline and to direct further research. This research may also help to develop useful recommendations for pregnant women to reduce the risk of poor pregnancy outcomes associated with pollutants in their environment and identify potential sources of exposure.

This study will produce new knowledge on smoking behaviour, use of methods to quit smoking, which are being encouraged for use during pregnancy, and smoking restrictions in the home.

6. What are the possible risks for me or my baby?

There are minimal risks to you or your baby. There are no additional medications or treatments for this study. We will ask you to donate an extra 38 ml (2.5 tablespoons) of blood at first visit, and at each subsequent prenatal visit and at delivery. You might experience slight bleeding, pain/discomfort at the site of the needle insertion. Very few may develop a bruise, discomfort, dizziness or infection. Cord blood collection will be done using a standard procedure and only if there are no risks for you or your baby.

For some people, hand expressing of breast milk can be difficult. The inconvenience you may experience is small. You will be given a pamphlet to explain how to hand express; also you can contact the nurse who gave you the kit for extra help. If you are having difficulty with the hand expression technique, you may use your breast pump to collect the requested amount of milk.

Some of the questions in the questionnaires are of a personal nature and may cause you some discomfort or anxiety. You can discuss this with the research nurse. You may choose not to answer any questions that make you feel uncomfortable.

The lab results for heavy metals and environmental chemicals may cause you some anxiety and we strongly recommend that you discuss them with your health care provider or local site investigator. There is also a small potential risk that a life or health insurance company may consider the lab results in your medical chart when determining your insurance premium.

7. Who will have access to my records and know that I am in a study?

Only the study team will have access to your records. Confidentiality will be respected and no information that contains your name or identity will be released or published without consent unless required by law. This legal obligation includes a number of circumstances, such as suspected child abuse, infectious disease, or expression of suicidal ideas where researchers are obliged to report to the appropriate authorities. As a partner or founder of this research, a member of Health Canada, the hospital research ethics committee, the Ontario Ministry of Environment and the Canadian Institutes of Health Research, will have the right to access the research data file and the medical chart for audit and quality control purposes only.

The lab results described above will be used for research purposes only to meet the purposes of this study. No names of individuals will appear on the questionnaires or on any of the samples collected, only a unique code. This unique code will be linked back to the information collected during the research study but only the local site investigator and study nurse team will have access to the key to link this code with your name and contact information. The consent forms and an electronic key file with your name and address will be kept separately in a secure location at the local study site.

Other coded information will be located in the study coordinating center at Ste. Justine's Hospital in Montreal, and temporarily at Génome Québec (for genome studies, these results

will not be shared with you but will be kept confidential and will not be documented in your medical chart), at Institut National de Santé Publique du Québec (for analysis of environmental chemicals) and at Health Canada laboratories (for analysis of milk and hair as well as nutritional and other factors in blood and urine that might make you more or less likely to experience harmful effects of environmental chemicals).

We will notify your doctor that you are participating in this study if you have any results higher than health-based guidelines that are scientifically proven to be significant for your health and there are preventive measures or treatments.

The study samples will be kept until all chemical analyses are complete, and the study data will be kept until all MIREC related research papers have been published. The general results of this study will be presented during scientific conferences and published in scientific journals, but no information that would allow individuals to be identified will be presented.

8. Commercialization

The knowledge gained from your data and biological samples could contribute to the creation of commercial products or to the broader commercialization of existing products. However, you will not be entitled to share the potential economic benefits.

9. Who can participate in this study?

Pregnant women in each city selected for the study can participate if they are enrolled during weeks 6 – 13 of pregnancy, are 18 years or older, are able to consent, can speak and understand English or French and are generally healthy.

10. Who can I contact if I have further questions?

If you have further questions, you can call the local site investigator, Dr. William D. Fraser, at (514) 345-4931, extension 4948, or the research nurse, Susanne Andersen, extension 4334. For all information regarding your rights as a research project participant you can contact the CHU Sainte-Justine local quality service and complaints commissioner at (514) 345-4749.

11. Will I get paid for my participation in the study?

No payment will be provided for participating in this study.

12. Can I refuse to be in the study and can I be asked to leave the study?

Your participation in this study is strictly voluntary. You can choose not to take part in the study, or, if you choose to participate, you can quit at any time. Your decision not to participate or decision to withdraw from the study will in no way adversely affect the quality of care that you will receive during your pregnancy, or during and after delivery. In addition, you will not be prevented from participating in future studies. You may be asked to leave the study by the local site investigator without your consent if you do not follow the study plan, if you have a study-related injury or for any other reason. There are two levels of study withdrawals: you can withdraw partially from the study follow-up (e.g. study visits, questionnaires), but authorize the keeping of the data already collected as well as the data collection from your clinical chart. You

can also fully withdraw. In this case, we will destroy all your data and specimens collected. You will be asked to specify what level of withdrawal you choose.

13. Investigator's responsibility

In the unlikely event of problems resulting from a procedure during this study, you will receive all the care needed by your health status and covered by your hospitalization and medical insurance.

By signing this consent form, you are not, in any way, giving up your legal rights. Moreover you are not freeing the investigators or the sponsors from their legal and professional responsibilities.

14. Participant Follow-up

If funds become available at a later date, the researchers are interested in following you and your baby as he or she grows and develops to assess the health risks, if any, from exposure to these environmental chemicals during pregnancy. At this time, we are only asking if we can contact you again to see if you are interested in participating in these follow-up studies.
You will receive a signed copy of this consent form.

CONSENT:
By signing this form, I agree that:

	Yes	No	Initials
• The MIREC study has been explained to me.	☐	☐	_____
• All of my questions were answered.	☐	☐	_____
• The possible risks discomforts and benefits of this study have been explained to me.	☐	☐	_____
• I understand that I have the right not to participate and the right to withdraw at any time.	☐	☐	_____
• I understand that I may refuse to participate without consequence to my continuing medical care.	☐	☐	_____
• I am free now, and in the future to ask any questions about the study.	☐	☐	_____
• I understand that my personal information will be kept confidential unless required by law to be disclosed.	☐	☐	_____
• I understand that no information that would identify me will be released or printed without asking me first.	☐	☐	_____
• I understand that I will receive a signed copy of this consent form.	☐	☐	_____
• I agree to be contacted after the birth of my infant to see if I am interested in further research on the health of my baby.	☐	☐	_____
• The research staff may access my medical charts and those of my baby to record information on the health of my pregnancy and my baby.	☐	☐	_____

In case I have moved during that time, you may contact this person, who should know my new address:

Name: _____

Address: _____

Phone number: _____

I was informed of the nature and the content of the research project. I have read the informed consent, and a copy has been given to me. I received answers to the questions that I asked. After thinking about it, I agree to participate with my child in this research project. I authorize the research team to access my and my child's medical records to collect the information pertinent to the project. I hereby consent to the participation of both myself and my child in this study.

Name of participant:	Age
Participant signature	Date

I consent that my spouse can answer questions related to my fertility, my work and my activities that could expose my family to environmental chemicals.

Name of the spouse of the participant	Age
Spouse signature	Date

I have explained to the participant all the significant aspects of the research and have answered the questions she asked me. I have explained that participation in this research project is free and voluntary and it can be stopped at any time.

Name of person who obtained consent (block letters)	Signature	Date

The research project as well as the terms of participation must be described to the participant. A member of the research team must answer her questions and must explain her that participation in the research project is free and voluntary. The research team agrees to respect what has been agreed to in the consent form.

Name of the responsible researcher	Signature	Date

Appendix 2: Informed Consent for the MIREC Study (Future Research on Stored Biological Samples)

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants

Université de Montréal

INFORMED CONSENT

MIREC
Maternal-Infant Research on Environmental Chemicals

Maternal-Infant Research on Environmental Chemicals (MIREC): FUTURE RESEARCH ON STORED BIOLOGICAL SAMPLES (blood, urine, hair, breast milk, meconium)

Co- principal investigators:
Dr William D. Fraser — Obstetrician, CHU Ste-Justine
Tye Arbuckle — Epidemiologist, Health Canada

Local Site Investigator: Dr. William D. Fraser, M.D., M.Sc., FRCSC

1. Why is it important to store your biological samples for future research as part of the MIREC study?

Little is known about the potential long-term health effects, if any, of prenatal or early life exposure to the levels of environmental chemicals seen today. Studies have shown that some of these chemicals may affect the child's growth and development, including effects on behavior and ability to learn. However, this research remains preliminary and needs to be tested in larger populations such as the MIREC study. The environmental chemicals that will be measured in you and your baby were selected based on the current science and limited by the budget available. In future years, based on the latest scientific information (along with the funds to do the work), we would like to measure additional chemicals, as well as markers that might help us understand how a particular chemical causes harm and why some people are more likely to be exposed or suffer health effects. We may also want to do research on fetal growth, pregnancy and health of mothers and their babies. If we did not have your stored samples to test these new research questions, we would have to start a whole new study which would be very costly and result in long delays to obtain the results.

2. What is the purpose of this project?

The MIREC study is being conducted to fulfill three main purposes:
(1) To measure the extent to which pregnant women and their babies are exposed to environmental chemicals. Exposure will be measured in maternal blood, urine, hair, breast milk as well as cord blood and meconium (infant's first stools);
(2) To measure some of the beneficial components in human breast milk (i.e. nutrients and immune factors); and

(3) To assess what health risks, if any, are associated with the levels of heavy metals measured.

Another purpose of the study is to create a data and biological sample bank. Your coded data and samples (unused blood, urine, hair, cord blood, meconium and breastmilk) would be stored in this "bank" and used for future research on fetal growth, pregnancy, health of mothers and their children, and to measure new environmental chemicals.

3. Number of participants in this study

For the MIREC study, we aim to recruit approximately 2,000 women in their first trimester of pregnancy from 8 to 10 sites across Canada.

4. What will be the process of this study?

Future Research on the Data and Biological Sample Bank

To access the information and/or samples in the "bank", any future research would first have to be approved by the MIREC biological sample bank management committee to make sure that the research met the purposes of the MIREC study. The research plan would also have to be approved by the research ethics committees at Health Canada and Ste. Justine's Hospital (the coordinating center).

The type of data and samples that will be stored in the data and biological sample bank includes:

- Maternal blood, urine, breast milk and hair
- Newborn cord blood, and meconium
- Questionnaire and medical chart information from each visit:
    - Visit 1: between 6 and 13 weeks
    - Visit 2: between 16 and 21 weeks
    - Visit 3: between 32 and 34 weeks
    - Visit 4: Delivery
    - Visit 5: About 2 days after delivery
    - Visit 6: Home visit, between 2 to 8 weeks after delivery.

The study data will be stored in secure servers at CHU Sainte-Justine. All of the samples will be coded with a unique bar code so that they can be linked with the questionnaire and medical chart information collected. Only the local site investigator and study nurse will have the key to link the unique code to the names and contact information for the mothers and babies. The samples will be stored at CHU Ste-Justine's clinical research unit and at Health Canada in Ottawa in freezers at -20□C or -80□C (depending on what is required for proper storage of the sample). The data and any remaining biological samples will be destroyed after 30 years of storage, following strict procedures. If a participant wishes to withdraw from the study, she must contact her local site investigator or research nurse and request that her data and specimens be destroyed.

5. What advantages and benefits can I expect?

There is no direct benefit for you personally. The future research will be targeted at children's health and the environment. With new and emerging manufactured chemicals being measured

in the environment each year, these future studies may give new knowledge into the health risks, if any, that these chemicals may pose.

6. What are the possible risks for me or my baby?
There are no known risks to you and your baby. The biological samples and data will already have been collected for the MIREC study.

7. Who will have access to my stored records and samples for future research and know that I am in a study?
Researchers who will submit a proposal to the bank management committee will have access to your coded records and samples, if and only after their request has been approved by the bank management committee and the research ethics committees. Confidentiality will be respected and no information that discloses your name or identity will be released or published without consent unless required by law. This legal obligation includes a number of circumstances, such as suspected child abuse, infectious disease, or expression of suicidal ideas where research documents are ordered to be produced by a court of law and where researchers are obliged to report to the appropriate authorities. As a partner in this research, a member of Health Canada, the CHU Sainte-Justine and Health Canada ethics committees, the Ministry of Environment of Ontario and the Canadian Institutes of Health Research, will have the right to accessthe research data file and the medical chart for audit and quality control purposes only.

No names of individuals will appear on the questionnaires, in the data bank or on any of the samples collected, only a unique code. This unique code will be linked back to the information collected during the research study but only the local site investigator and his research team will have access to your name and contact information. The consent forms and an electronic file with your name and address will be kept separately in a secure location at the local study site.

8. Commercialization
The knowledge gained from your data and biological samples could contribute to the creation of commercial products or to the broader commercialization of existing products. However, you will not be entitled to share the potential economic benefits.

9. Who can participate in this study?
Anyone who has agreed to participate in the MIREC study can have their data and samples stored for future research on fetal growth, pregnancy, health of mothers and their babies and environmental chemicals.

10. Who can I contact if I have further questions?
If you have further questions, you can call the local site investigator; Dr. William D. Fraser at (514) 345-4931 extension 4948, or the research nurse, Susanne Andersen, at the extension 4334. For all information regarding your rights as a research project participant you can contact the CHU Ste-Justine local quality service and complaints commissioner at (514) 345-4749.

11. Will I get paid for my participation in the study?
No payment will be made for participating in the long-term storage of your data and samples for future research.

12. Can I refuse to have my data and samples stored for a maximum of 30 years for future research?
Your participation in the long-term storage of your data and samples for future research as part of the MIREC study is strictly voluntary. You can choose to participate in MIREC but not agree to the long-term storage of your data and samples for future research. You may also withdraw your consent for the long-term storage at any time by contacting the local research nurse or site investigator. Your decision not to participate or decision to withdraw from the study will in no way adversely affect the quality of care that you will receive during your pregnancy, or during and after delivery. In addition, you will not be prevented from participating in future studies.

13. Investigator's responsibility
By signing this consent form, you are not, in any way, giving up your legal rights. Moreover you are not freeing the investigators or the sponsors from their legal and professional responsibilities.

14. Results
Any biological samples used in future research will first require approval from the research ethics board.

Only group level results of any future research will be presented during scientific conferences and published in scientific journals. No information that would allow individuals to be identified will be presented.

You will receive a signed copy of this consent form.

CONSENT:

I understand that any future research that uses my stored data and samples will only be used if that research has been approved by the MIREC biological sample bank management committee and by the research ethics committees at Health Canada and Ste. Justine's Hospital.

I understand that I may withdraw my consent for the long-term storage of my data and samples for future research at any time by contacting the local site investigator or research nurse.

Mother's Specimens: I agree that my blood, urine, breast milk and hair may be stored and used for future research on fetal growth, pregnancy, and health of mothers and their children.

Baby's Specimens: I agree that my baby's cord blood and meconium may be used for future research on fetal growth, pregnancy, and health of mothers and their children.

Name of participant	Age
Participant signature	Date

Spouse of the participant:
I consent to use my personal information collected in the questionnaires for the purpose of future research.

Name of the spouse of the participant	Age
Spouse signature	Date

I have explained to the participant all the significant aspects of the research and have answered the questions she asked me. I have explained that participation in this research project is free and voluntary and it can be stopped at any time.

Name of person who obtained consent (block letters)	Signature	Date

The research project as well as the terms of participation must be described to the participant. A member of the research team must answer her questions and must explain her that participation in the research project is free and voluntary. The research team agrees to respect what has been agreed to in the consent form.

Name of the responsible researcher	Signature	Date