

THE IMPACT OF ENVIRONMENTAL HEAVY METAL EXPOSURES ON PREGNANCY AND BIRTH OUTCOMES

THESIS

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ABSTRACT

Background: There is still a paucity of information on maternal biological mechanisms specific to adverse birth outcomes despite maternal environmental exposure and health status being known to influence neonatal morbidity and mortality. The purpose of this study was to explore maternal biomarkers pertinent to infant development in utero, specifically matrix metalloproteinases (MMPs), and determine their relationships to environmental heavy metals such as arsenic, cadmium, lead, mercury, and manganese as well as their relationships to outcomes such as preterm birth, low birth weight and small for gestational age infant outcomes. **Methods:** A secondary data analysis on 1533 mother-infant pairs from the Maternal and Infant Research on Environmental Chemicals (MIREC) cohort was conducted to statistically test relationships between metals and biomarkers, as well as biomarkers and outcome. A systematic literature review and meta-analysis was also conducted to identify the interdependencies between maternal blood biomarkers relating to adverse pregnancy outcomes. **Results:** Multivariate regression models were used to estimate odds ratios (OR) for the association between metal concentrations in quartiles and both high (90%) and low (10%) maternal MMP levels. Significant metal-related effects were observed with different MMP responses. A total of 54 studies (35 for meta-analysis), including 43,702 women and evaluating 50 biomarkers, met the inclusion criteria and all subgroups of biomarkers showed significant associations with birth outcomes with no apparent publication bias. **Conclusions:** Maternal plasma markers may serve as potentially valuable tools in the investigation of maternal molecular mechanisms, especially select toxicity pathways underlying metal-mediated adverse infant outcomes. Further research is still needed to evaluate biomarkers such as proteomic and genetic profiles in other various maternal biological samples.

RÉSUMÉ

Contexte: Il y a encore un manque d'information sur les mécanismes biologiques maternels, spécifiques à des résultats défavorables de la grossesse, malgré l'exposition environnementale maternelle et l'état de santé étant connus pour influencer la morbidité et la mortalité néonatale. Le but de cette étude était d'explorer les biomarqueurs maternels, en particulier les métalloprotéinases matricielles (MMP), pertinents au développement du nourrisson *in utero* et de déterminer leurs relations avec les métaux lourds environnementales tels que l'arsenic, le cadmium, le plomb, le mercure et le manganèse ainsi que leurs relations des résultats tels que la naissance prématurée, poids sous la norme et plutôt petit pour l'âge gestationnel. **Méthodes:** Une analyse des données secondaires sur 1533 couples mère-enfant dans l'étude mère-enfant sur les composés chimiques de l'environnement (MIREC) cohorte a été menée pour vérifier les relations statistique d'essai entre les métaux et les biomarqueurs ainsi que les biomarqueurs et les aboutissement de la grossesse. Une revue systématique de la littérature et méta-analyse a également été réalisée pour identifier les interdépendances entre les biomarqueurs sanguins maternels liés à des résultats défavorables de la grossesse. **Résultats:** Les modèles de régression multivariés ont été utilisés pour estimer les rapports de cotes (RC) pour l'association entre les concentrations de métaux dans les quartiles et les niveaux élevé (90%) et faible (10%) des MMP maternels. Les effets liés à des métaux importants ont été observés avec des réponses MMP différentes. Un total de 54 études (35 méta-analyse), y compris 43,702 femmes et l'évaluation des 50 biomarqueurs, ont rencontré les critères d'inclusion et tous les sous-groupes de biomarqueurs ont montré des associations significatives avec les résultats de naissance avec sans biais de publication apparente. **Conclusions:** Les marqueurs plasmatiques maternels peuvent servir d'outils potentiellement utiles dans l'étude des mécanismes moléculaires maternels, et sélectionner particulièrement les voies de toxicité selon les résultats infantiles défavorables médiate-métal. D'autres recherches sont encore nécessaires afin d'évaluer les biomarqueurs tels que les profils protéomiques et génétiques incluant divers échantillons biologiques maternels supplémentaires.

STATEMENT OF CONTRIBUTIONS

As the study coordinator, I completed this study in partial fulfillment of the requirements for the Master of Science in Interdisciplinary Health Sciences Program at the University of Ottawa. In congruence with my role as the study coordinator, I conceptualized the study with the help of my TAC committee, designed the study and analyzed the quantitative data and led the development of the manuscript.

This project also involved other integral members of the study team. My supervisor, Dr. James Gomes, alongside Dr. Premkumari Kumarathasan, worked with me to design a feasible project and guided me throughout the research process. Together, they helped me create the proposal, connect me with the statistical team members from Health Canada and provided me feedback and guidance on the study software and tools. They oversaw the project in its entirety including study design, implementation, analysis and dissemination.

As the study coordinator of the project, I led the writing of the manuscripts. We determined authorship and authorship order for each article based on discussions as a team. All listed authors for each article meet the criteria for authorship as outlined by the individual journal. The listed co-authors of the manuscript reviewed early drafts and provided substantive and editorial feedback. All co-authors have approved the manuscript included in this thesis.

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LIST OF ABBREVIATIONS

As	Arsenic
Cd	Cadmium
EDC	Endocrine Disrupting Chemicals
Hg	Mercury
IUGR	Intrauterine Growth Restriction
LBW	Low Birth Weight
LOD	Level Of Detection
LOAEL	Low Observed Adverse Effect Level
MIREC	Maternal Infant Research on Environmental Chemicals
MMP	Matrix Metalloproteinase
Mn	Manganese
NOAEL	No Observed Adverse Effect Level
Pb	Lead
PTB	Preterm Birth
Se	Selenium
SGA	Small-for-Gestational-Age

CHAPTER 1: INTRODUCTION TO THE STUDY

1.1 The Problem

Ecology, often associated with biology, is defined as the “study of relationships between organisms and their environment” (“Ecology”, 2014). Some of the most pressing problems in human affairs, air pollutants and their adverse outcomes, for example, are largely ecological. As a result, it is of great importance that a more comprehensive understanding is achieved, so the problem may be properly addressed to move to potentially affecting public health policies. According to Shoeters et al., “mothers share their chemical burden with the fetus and their infant,” so sampling maternal biological samples during pregnancy would allow for an evaluation of the relative exposure of the fetus at different periods of development (2011). Subsequently, the study of maternal biomarkers has become a growing interest in improving the diagnosis of adverse birth outcome cases.

Scientific research has continued to provide evidence in support of the concern regarding environmental pollutants contributing to early origins of disease in childhood and adult life. It has long been known that maternal chemical burdens are shared with the fetus and infant. Thus, the sampling of maternal blood or urine samples during pregnancy would allow for an evaluation of the relative exposure to the fetus at several different critical periods of development (Shoeters et al., 2011). Subsequently, the study of maternal biomarkers should become a main focus in order to understand underlying mechanisms and pathways that lead to adverse birth outcomes.

The Maternal-Infant Research on Environmental Chemical (MIREC) Study was developed by researchers from Health Canada to investigate the relationships between environmental chemicals and their effects on maternal biomarkers and adverse health outcomes, both in the

mother and the infant. Pregnancy is a very sensitive period with respect to human development and, although not yet conclusive, numerous studies have begun to identify several harmful factors associated with negatively affecting pregnancy outcomes. Lifestyle factors like smoking and alcohol-consumption, and nutritional or dietary factors such as insufficient folic acid intake during pregnancy, are just a few examples of the many risk factors that have demonstrated strong correlations to several birth complications. These complications include, but are not limited to, preeclampsia, premature rupture of membranes (PROM), preterm delivery/birth, gestational diabetes, low birth weight (LBW), intrauterine growth restriction (IUGR), and small for gestational age infants (SGA) (Health Canada, 2014).

The concern that maternal chemical exposures to environmental pollutants such as heavy metals is genuine for it has been reported, by an increasing number of researchers, that environmental exposures contribute to early onset of adulthood diseases (Ferguson et al., 2015; Kumarathasan et al., 2014). Epidemiological evidence has repeatedly supported early exposures to environmental chemicals as potential causes for disease and disability in infants, children, and across the entire lifespan. Therefore, it is very important that a more comprehensive understanding of factors that affect the etiology of particular adverse health outcomes is achieved. Understanding chemical exposures to heavy metals before and during pregnancy to assess the effects on the regulation of maternal biological pathways, as well as pregnancy outcomes, is critical for risk assessment. New knowledge on maternal and fetal toxicity of heavy metals may aid in the identification of potential protective factors or nutritional factors that could be translated to reducing possible immediate and long-term sequelae of the aforementioned environmental hazards. Current research on the mechanistic basis of adverse outcome has been mostly limited to animal models and the dose-response curves relating to the reproductive

toxicity in the Canadian population have yet to be further explored. As such, this thesis hopes to instigate an investigation regarding the knowledge gap between exposure to environmental metals, biomarkers during pregnancy and adverse health outcomes in the infant.

1.2 Purpose Statement

This thesis project will include the analysis and interpretation of data collected from a large prospective cohort study conducted in Canada that had a primary objective of examining prenatal and maternal exposure to environmental chemicals and the potential effects on maternal and infant health. It will also include the analysis and interpretation of literature data collected through a review of literature and meta-analysis to explore the relationship between maternal blood biomarkers and adverse infant outcomes. The purpose of this thesis project is to gain insight into the association between maternal blood levels of environmental metals and markers of health in pregnant mothers, while controlling for potential confounding variables.

Research Questions:

- 1) What are the biomarkers of exposure to heavy metals in pregnant women and how do they affect the pregnancy outcome?
- 2) What are the characteristics of environmental exposures to heavy metals (As, Cd, Mn, Pb, Hg), MMPs, and their subsequent impact on pregnancy and birth outcomes?
- 3) What is the nature of relationships among exposure to heavy metals, expression of MMPs as biomarkers of effect, and pregnancy outcomes affecting the mother and the infant?

1.3 Specific Objectives

The objectives of this thesis project are:

Objective 1: To describe/explore maternal heavy metal exposures, maternal biomarkers and pregnancy outcomes.

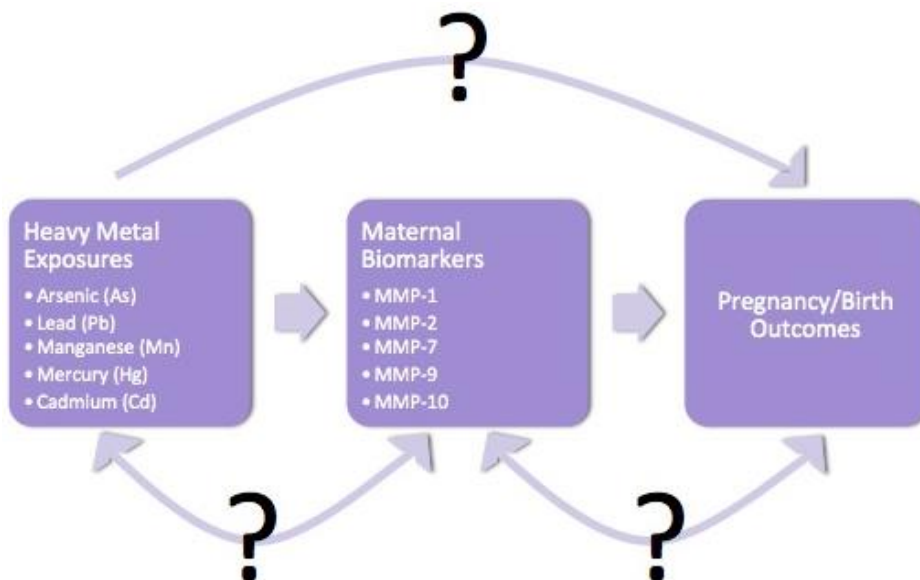
Objective 2: To explore statistical associations among heavy metal exposures, MMPs and pregnancy outcomes (preterm birth, low birth-weight and small-for-gestational-age).

Objective 3: To investigate the nature of relationships among heavy metal levels in maternal blood, maternal plasma MMPs, and adverse birth outcomes.

1.4 Hypotheses

Maternal heavy metal exposures to arsenic, cadmium, manganese, lead, and mercury before and during pregnancy may be positively associated with the regulation of matrix metalloproteinases, which may, in turn, lead to adverse pregnancy outcomes.

FIGURE 1: VISUAL REPRESENTATION OF OBJECTIVES.



CHAPTER 2: REVIEW OF LITERATURE

This literature review provides a brief introduction to heavy metal toxicity during pregnancy and its impact on the mother and the infant. The review also examines endocrine disruption, including the endocrine disrupting activity of heavy metals such as arsenic, cadmium, mercury, manganese, and lead. It also presents evidence surrounding possible reproductive toxic effects of these chemicals, specifically low birth weight and preterm birth. Furthermore, a brief summary of biomonitoring is also presented.

2.1 Endocrine Disrupting Chemicals

Gilbert and Epel (2009) defined “ecological developmental biology” as an approach to embryonic development that studies interactions between a developing organism and its environment. Standard embryology has often focused on the internal dynamics, though recent discoveries have revealed that cell-cell communication is key to fetal development. Endocrine disrupting chemicals (EDCs), toxicants attributed to the hindrance of normal endocrine signalling processes in the body, have been suggested to be potentially responsible for the adverse effects on cellular functions leading to undesirable health outcomes (Health Canada, 2014). Health Canada (2014) has identified EDCs to include, though not restricted to, heavy metals such as lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), manganese (Mn), and selenium (Se), as well as organics like polychlorinated biphenyls (PCBs), dioxins, phthalates, and bisphenol-A (BPA). Human teratogens including drugs and environmental chemicals, such as heavy metals, have been associated with teratogenic effects and observed birth defects. According to Health Canada (2014), current knowledge on the exposure of EDCs to pregnant women and infants, two of the most vulnerable members of the population, is limited and little is

known about the potential health effects of EDCs following maternal exposure.

Heavy metals are considered to be highly toxic since they have the capability to cross the blood-placenta barrier accumulating in the amniotic fluid or fetal tissues and human exposure to heavy metals occurs primarily through environmental contamination by industrial wastes (Vahter, Berglund, Akeeson, and Liden, 2002; Georgescu, Georgescu, Daraban, Bouary, & Pascalau, 2011). The mother's chemical burden will be shared with her fetus, which may expose the neonate, in some instances, to a larger dose relative to body weight and lead to a wide range of functional deficits that manifest after birth and have lifelong health outcomes (Shoeters et al., 2011). In utero exposures to heavy metals have been correlated with developmental, neurological and endocrine disorders and certain metals like lead have a higher chemical burden as a result from mobilization from stores in the mother due to pregnancy-related metabolic changes (Caserta, Graziano, Lo Monte, & Moscarini, 2013; Bellinger, 2005). Although lead and mercury have been extensively studied, there is a paucity in research on the human reproductive effects of low-level exposures in women to other heavy metals such as cadmium, manganese, selenium and arsenic, with studies indicating adverse effects occur at lower exposure levels than previously anticipated (Au et al., 2016; Rahman et al., 2016). Heavy metals also exhibit metal-related toxicity independent from the endocrine disrupting capability and these effects were examined for biological effects in the mother, though not from a clinical toxicity point of view but from a reproductive toxicity point of view.

2.2 Adverse Birth Outcomes

The quality of an infant's development is typically assessed by two measures, gestational age at delivery and infant birth weight. Preterm birth (PTB), or live birth before 37-weeks of gestation, is a growing concern in developed countries. Buhimschi, Schatz, Krikun, Buhimschi

and Lockwood report that \$26 billion is spent annually to treat the 540 000 premature infants that are delivered each year (2010). Likewise, low infant birth weight, defined as birth weight lower than 2500g, has been associated with several chronic health consequences in adulthood such as hypertension, diabetes mellitus and obesity (Goldenberg, Goepfert & Ramsey, 2005). Since a shortened gestational age and decreased birth weight may lead to increased disease burden and growth restriction, preterm birth has become one of the leading causes of perinatal mortality and morbidity (Conde-Agudelo, Papageorgiou, Kennedy, & Villar, 2011).

Normal term pregnancy is expected to last between 37 to 41 completed weeks, while PTB is defined as live birth before 37 weeks of gestation (Ferguson et al., 2014; Bhat, Williams, Saade & Menon, 2014). Low infant birth weight can be the result of either preterm birth or intrauterine growth restriction (IUGR), or a combination of the two. The most common measurement for IUGR is small-for-gestational-age (SGA), which is generally defined as birth weight below the 10th percentile for gestational age given a reference population (Brou et al., 2012; Coussons-Read et al., 2012).

Elevated blood pressure (BP) in pregnant women has been related to IUGR resulting in low infant birth weights, while oxidative stress has also been reported to cause maternal and fetal morbidity and is implicated in preeclampsia leading to PTB (Curry et al., 2007; Goldenberg et al., 2005; Ernst et al., 2011). Previous research on environmental pollutants have suggested air contaminants as triggers, increasing markers of oxidative stress and, subsequently, have been linked to adverse pregnancy outcomes (Fransson et al., 2012). Despite significant research being conducted and compelling evidence to suggest several underlying pathogenic pathways and factors, the precise etiology of preterm birth still remains largely elusive. Nevertheless, although each purported pathway has unique genetic and environmental predispositions, most have been

identified with immunologically mediated or inflammatory components (Buhimschi et al., 2010).

Prediction of spontaneous preterm birth is important because it could allow for the identification of women at highest risk, in whom, appropriate risk-specific interventions could be implemented (Goldenberg et al., 2005). The ability to predict cases may also help acquire important insights into the mechanisms or pathways that lead to preterm birth. According to Shoeters et al. (2011), mothers share their chemical burden with the fetus and their infant, so sampling maternal samples during pregnancy would allow for an evaluation of the relative exposure of the fetus at different periods of development. Consequently, the study of maternal biomarkers has become a growing interest in improving the diagnosis of preterm birth cases and allowing for a better understanding of the events leading to outcome manifestation.

2.3 Toxicology of Heavy Metals

Ubiquitous in the environment, exposure to metals can occur through contaminated food, water, soil, dust, or air, as well as direct contact with consumer products (Arbuckle et al., 2016). During pregnancy, physiological changes can alter the toxicokinetics and toxicodynamics of metal contaminants in the mother's body resulting in an endogenous source of prenatal exposure (Thomas et al., 2015). According to Health Canada (2015), metal concentrations in the Canadian population have shown variability and comparing cycles of the Canadian Health Measures Survey between 2007 and 2013, no significant difference in blood Cd or Hg was observed, however, Pb concentrations declined and Mn increased. According to Arbuckle et al. (2016), despite a decline in metal levels over the past few decades, Canadians can still be exposed to lead in food, drinking water, air, and manufactured products, to cadmium from cigarette smoke and diet, to mercury from certain fish species and dental fillings, and to manganese from a variety of foods and nutritional supplements on top of environmental exposure.

Rahman et al. (2016) reported epidemiological associations between maternal exposures to: lead with LBW, PTB, stillbirths, spontaneous abortions and hypertension; cadmium with LBW, neurological dysfunction and low Apgar score; and mercury with spontaneous abortions and neurotoxic effects. Correspondingly, lead toxicity also appeared to increase in the presence of higher levels of cadmium, mercury or manganese (Claus Henn, Coull & Wright, 2014).

Manganese, a required trace element and an important antioxidant nutrient during pregnancy, was reported by several studies to exhibit associations between elevated concentration of blood manganese during pregnancy with gestational hypertension and infant birth weight (Mistry and Williams, 2011; Vigeh et al., 2013; Zota et al., 2009). Toxic metals present in the blood stream may alter placental function and impair nutrient function have been hypothesized as potential risk factors in the induction of growth restriction via oxidative stress mediated pathways (Thomas et al., 2015).

Health Canada (2016) reports the Permitted Daily Exposure (PDE) for oral, parenteral and inhalation exposures to arsenic, cadmium, mercury and lead. Arsenic was reported to have PDEs of 15µg/day for oral and parenteral exposures and 1.9 for inhalation exposures, 5.0µg/day for cadmium oral exposures and 1.7µg/day for parenteral and inhalation exposures to cadmium, 5.0µg/day for all exposures to lead, and 30µg/day, 3.0µg/day, and 1.2µg/day for oral, parenteral and inhalation mercury exposures, respectively (Health Canada, 2016). According to the Ontario Drinking Water Quality Standards (2016), the maximum allowable concentration (MAC) of arsenic is 0.025mg/L, cadmium is 0.005mg/L, lead is 0.010mg/L, and mercury is 0.001mg/L, while manganese has an aesthetic objective of 0.05mg/L (Safe Drinking Water Act, 2016). Since manganese is an essential nutrient, it has been given a tolerable upper intake level of 11mg/day (Agency for Toxic Substances and Disease Registry [ASTDR], 2011d).

Normal blood levels are reported for arsenic, cadmium, mercury, manganese and lead to be $<1\mu\text{gAs/L}$, $0.315\mu\text{gCd/L}$, $<10\mu\text{gHg/L}$, $4\text{--}15\mu\text{gMn/L}$, and $1.9\mu\text{gPb/dL}$ respectively (ASTDR, 2007a, 2007b, 2012a, 2012b; Mayo Medical Laboratories, 2016). **Table 1** reports a summary of body burden implications for arsenic, cadmium, mercury, manganese and lead, according the Agency for Toxic Substances and Disease Registry (ASTDR) using LOAELs (low observed adverse effect level) and NOAELs (no observed adverse effect level). Arsenic demonstrated no observable neurological effects at 0.003mg/kg/day but effects were observed at 0.004mg/kg/day (ATSDR, 2011a). Similarly, no cardiovascular effects were observable for cadmium levels at 0.53mg/kg/day , however low observed effects were seen at 1.71mg/kg/day (ATSDR, 2011b). **Table 2** highlights the regulations and guidelines summarized by the ASTDR and reports values given by several agencies including the American Conference of Governmental Industrial Hygienists (ACGIH), the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), the Occupational Safety and Health Administration (OSHA), and the World Health Organization (WHO).

TABLE 1. BODY BURDEN OF SELECT METALS ACCORDING TO AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY (ASTDR).

Metal	Route of Exposure	LOAEL	NOAEL
Arsenic	Oral	0.05 mg/kg/day	0.0008mg/kg/day
	Dermal	0.0008mg/kg/day	0.014mg/kg/day
	Inhalation		0.067µg/m ³
Cadmium	Oral		0.0021mg/kg/day
	Inhalation	0.088mg/m ³	
	Parenteral	0.6mg/kg	
Mercury	Oral		0.93mg/kg/day
Manganese	Oral		0.11mg/kg/day
	Inhalation	0.15mg/m ³	
Lead	Oral	0.005mg/kg/day	
	Inhalation	3.2µg/m ³	400µg/m ³

TABLE 2. REGULATIONS AND GUIDELINES APPLICABLE TO METALS ACCORDING TO THE AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY (ASTDR).

Metal	Exposure	Agency	Description	Information
Arsenic (As)	Air	WHO ACGIH	Inhalation unit risk Threshold limit value	1.5x10 ⁻³ /µg/m ³ 0.01mg/m ³
	Water	WHO EPA	Drinking water quality guidelines Maximum contaminant level	0.01mg/L 0.01mg/L
	Food	FDA	Bottled drinking water	0.01mg/L
Cadmium (Cd)	Air	WHO ACGIH	Air quality guidelines Biological exposure indices in blood Threshold limit value	5ng/m ³ 5µg/L 0.01mg/m ³
	Water	WHO EPA	Drinking water quality guidelines Drinking water standards lifetime	0.003mg/L 0.005mg/L
	Food	FDA	Bottled drinking water	0.005mg/L
	Other	EPA	Inhalation unit risk	1.8x10 ⁻³ /µg/m ³
Mercury (Hg)	Air	WHO OSHA ACGIH	Drinking-water guideline values Permissible tolerable weekly intake Permissible exposure limit Short-term exposure limit	0.001mg/L 5µg/kg total 0.1mg/m ³ 0.3mg/m ³
	Water	EPA	Maximum contaminant level	0.002mg/L
	Food	FDA	Bottled drinking water Action level for poisonous or deleterious substances in fish/seafood	0.002µg/L 1ppm
	Other	FDA	Eye area cosmetics	<65ppm
Manganese (Mn)	Air	WHO ACGIH	Air quality guidelines Threshold limit value	0.15 µg/m ³ 0.2mg/m ³
	Water	WHO EPA	Drinking water quality guidelines Lifetime advisory	0.4mg/L 0.3mg/L
	Food	FDA	Bottled drinking water	0.05mg/L
Lead (Pb)	Air	WHO ACGIH	Air quality guidelines Threshold limit value	0.5 µg/m ³ 0.05mg/m ³
	Water	WHO	Drinking water quality guidelines	0.01mg/L
	Food	FDA	Bottled drinking water	0.005mg/L

ACGIH = American Conference of Governmental Industrial Hygienists

EPA = Environmental Protection Agency

FDA = Food and Drug Administration

OSHA = Occupational Safety and Health Administration

WHO = World Health Organization

2.4 Heavy Metals and Adverse Birth Outcomes

Lead is an extensively documented toxic heavy metal known to produce a number of adverse effects at higher levels of exposure and can be found in food, water, air, as well as numerous consumer products including paints and ceramics used for storage and food preparation (Georgescu et al., 2011; Vahter et al., 2002). Lead has the ability to pass the blood-placenta barrier, has been found in maternal blood, and is also able to deposit into bone tissue, where it decomposes slowly and is gradually released at a half-life of ten years (Vahter et al., 2002). Maternal exposures to lead have been associated with PROM related preterm delivery, LBW, preterm birth, and IUGR (Andrews, Savitz & Hertz-Picciotto, 1994; Angell & Lavery, 1982; Falcon, Vinas & Luna, 2003; Srivastava et al., 2001).

Similar to lead, which can easily cross the placental barrier, mercury is also a well-studied neurotoxic metal that can also pass from mother to child in utero and can produce long-lasting harm to the child's neurological development (O'Reilly, McCarty, Steckling, & Lettmeier, 2010). Mercury is found naturally in the environment as methylmercury, mercuric sulfide and mercuric chloride and it is microbial biotransformation that is responsible for virtually all the methylmercury found in nature (Wigle, 2003). This neurotoxic heavy metal can also be found in fungicides, industrial aerosols, dental amalgams, herbal drugs, and women's beauty products (Georgescu et al., 2011). Methylmercury exposure is most commonly attributed to the consumption of seafood in contaminated waters and interferes with steroidogenesis, disrupts DNA replication, impairs protein synthesis, and inhibits normal cell division and cell migration (Georgescu et al., 2011; Wigle, 2003).

Arsenic, another toxic heavy metal capable of crossing the placental barrier, is often used in industrial processes and can be found as a component in a large number of pesticides used in

agriculture (Vahter et al., 2002). As a result, human exposure to arsenic is most commonly due to contaminated groundwater and well water. Arsenic exposure has been suggested to play a role in increasing the risk of low birth weight (McDermott et al., 2014; Yang et al., 2003; Hopenhayn et al., 2003) and contributes as a factor in the development of hypertension (Lee et al., 2003).

Unlike lead, mercury and arsenic, cadmium is a heavy metal that does not easily cross the placental barrier, but may affect placental function and subsequently fetal growth and development (Iyengar & Rapp, 2001; Yang, Julian, Rubio, Sharma & Guan, 2006). The main source for cadmium exposure is through dietary intake of fiber-rich foods such as cereals, vegetables and potatoes (Vahter et al., 2002). Some researchers have associated cadmium absorption into the blood as being synchronized with iron absorption, so an increased absorption of iron during pregnancy may also result in the accumulation of cadmium in the blood. Since elevated cadmium levels, whose transport is mitigated by selenium, in cord blood or maternal blood have been shown to demonstrate negative effects on neonatal birth height, cadmium may be responsible for SGA infants (Y. Zhang et al., 2004; Y.L. Zhang et al., 2004). Cadmium has also been hypothesized to play a role in the etiology of eclampsia and preeclampsia with studies reporting higher blood cadmium concentrations in pregnant women with hypertension (Semczuk & Semczuk-Sikora, 2001; Dawson, Evans & Nosovitch, 1999; Chisolm & Handorf, 1996; Eisenmann & Miller, 1995; Kosanovic, Jokanovic, Jevremovic, Dobric & Bokanjic, 2002).

Finally, manganese is an essential trace element for humans, but both deficiency and excess intake can result in adverse developmental outcomes such as a lower birth weight (Eum et al., 2014). Several other studies have also associated manganese levels with preeclampsia and IUGR, gestational diabetes and lowered head circumference, preterm birth, and reduced gestational age (Marriott et al., 2007; McDermott et al., 2014; Mora et al., 2014; Sarwar et al., 2013; Wood,

2009). Although manganese might not be considered a heavy metal like nickel, which has also been documented to have an association with fetal death, IUGR, and pulmonary emphysema, it shares similarities with zinc, copper and selenium. Also, elemental exposures to zinc, copper and selenium have revealed inconsistent results with respect to pregnancy outcomes (Dagouassat et al., 2012; Vahter et al., 2002).

2.5 Biomarkers

Biomarkers, described by Vogel, Thorsen, Curry, Sandager and Uldbjerg (2005), are parameters that can be measured in a biological sample and provide information on an exposure, or the actual potential effects of that exposure in an individual. Some controversy has risen around predictive biomarkers since biomarkers, by definition, reflect the current status of the biological sample at the time of analysis. Benford et al. (2000) suggested that the status of *biomarkers of exposure* could either reflect recent or long-term exposure, so it may only serve as a predictor of changes in risk of diseases, but cannot predict a toxic effect. Conversely, a *biomarker of effect* might reflect an earlier stage of a disease, which may be predictive of eventual disease, though such biomarkers are dependent upon the understanding of the aetiology of the disease in question (Benford et al., 2000).

Although the precise aetiologies of pregnancy complications remain poorly understood, it has been implicated that pathological pathways and important risk factors may stem from maternal immune and inflammatory responses (Dibble et al., 2014). Low-grade inflammation has been associated with endothelial dysfunction and subsequent vascular dysfunction leading to suboptimal placental development (Ernst et al., 2011). Maternal systemic inflammation due to elevated pro-inflammatory markers and decreased anti-inflammatory markers have been linked with shortened gestational age, preterm birth and resulting small-for-gestational-age infants

(Koucky et al., 2010). Given the substantial involvement of inflammation and the immune system during pregnancy, pregnancy complications have been attributed to the disturbances in cytokine networks and inflammatory cytokine are logical candidates for predictive biomarkers.

Among the biomarkers included, this present study focuses on matrix-metalloproteinases due to their highly affinitive metal domain as well as their implication in tissue remodelling and membrane breakdown. MMPs and their relationship with other maternal biomarkers (i.e. interleukins, TNF, IFN, CRP, etc.) are also investigated to better understand their true role during pregnancy and their direct, as well as indirect, roles in mediating adverse birth outcomes.

2.5.1 MMPs as Biomarkers of Outcome

Among the biomarkers that have been identified, many of the inflammation-related biomarkers including matrix metalloproteinases (MMPs), have been shown to have a strong association with increased risk for preterm birth and may play a predictive role in low birth weight (Tu et al., 1998; Yoon et al., 2001; Biggio et al., 2005; Poon et al., 2009). Matrix metalloproteinases are a family of zinc-dependent proteinases that are involved in the degradation of extracellular matrix during normal and pathological tissue remodelling. During human development, MMPs play a critical role in embryo implantation, placentation, cervical dilation and fetal-maternal membrane lysis, indicating their importance during pregnancy (Tu et al., 1998). Various studies have reported findings that support the hypothesis that the up-regulation or down-regulation of matrix metalloproteinases may be directly correlated with spontaneous preterm delivery and lower infant birth weights (Myers et al., 2005; Nissi et al., 2013).

The roles of MMPs on cell behaviour in culture has not been well studied, however, they have been shown to affect cell proliferation, apoptosis, differentiation and organization (Vu and

Werb, 2016). Likewise, MMPs also regulate the bioavailability of growth factors by cleaving the proteins that bind to them. For example, cleavage of insulin-like growth factor binding protein (IGFBP) by MMP-1/-2 or MMP-3/-8 cleavage of angiotensin-I to generate angiotensin-II or IL-1 β degradation resulting in loss of its biological activity (Rajah, 1995; Fowlkes et al., 1994a, b, 1995; Schönbeck et al., 1998; Ito et al., 1996; Diekmann & Tscesche, 1994).

Evaluation of the levels of multiple biomarkers in maternal plasma has been reported in several studies to support the premise implicating the predictive nature of matrix metalloproteinase concentrations for low infant birth weight and have also begun to demonstrate promising potential for application to the analyses of adverse outcome pathways (Tu et al., 1998; Yoon et al., 2001; Biggio et al., 2005; Poon et al., 2009). A few studies have implicated MMPs as players associated with preterm birth and low birth weight infants in relation to prenatal exposure to heavy metals, more specifically lead and arsenic (Gonzalez-Puebla et al., 2012; Remy et al., 2014).

2.5.2 MMPs as Biomarkers of Exposure

MMPs are only a small sample of the large group of inflammation-related biomarkers that are linked to adverse birth outcomes. Consequently, to establish a more complete picture of the mechanisms and pathways affecting fetal development, research must be conducted to investigate the relationship between exposures and outcome as well as exposures to intermediary biomarkers. Of the maternal samples collected, with data on MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10, several MMPs have demonstrated to be affected by exposures to certain metals. MMP-1 has only been linked to arsenic exposure and selenium, while both MMP-2 and MMP-9 have been associated to arsenic, manganese, cadmium, mercury and lead. MMP-2 has also displayed associations with selenium and MMP-9 with zinc.

CHAPTER 3: METHODS

3.1 Study Design

This thesis project involved a secondary analysis of data acquired from the Health Canada MIREC database to statistically test the plausibility of interdependent relationships between metal exposure to biomarkers as well as biomarker concentrations and infant birth outcomes. This thesis project also included a systematic literature review and meta-analysis to synthesize all current knowledge and evaluate the relationships between maternal biomarkers and adverse infant birth outcomes in order to identify any potential pathways recognized to link biomarker levels to outcome manifestation. Statistical analysis on data acquired from the MIREC database will provide further support for any probable causative relationships isolated during the systematic review.

3.2 Secondary Data Analysis

3.2.1 Study Population

The MIREC study recruited pregnant women from 10 cities across Canada between 2008-2011 during their first trimester of pregnancy (6-14 weeks): Vancouver, Edmonton, Winnipeg, Toronto, Hamilton, Sudbury, Kingston, Ottawa, Montreal and Halifax.

Of the 8,716 women who were initially approached for participation in the MIREC study, 5,108 were eligible and 2,001 consented. After 18 women withdrew, the 1,983 remaining subjects gave birth to 1,959 infants. Of this subset, 426 women were excluded for multiple births, missing metal biomonitoring data and anthropometric measurements, or unknown sex resulting in a final sample size of 1533 mother-infant pairs. As the study progressed, some participants partially withdrew and some were lost to follow-up, which yielded a total of 1,727

women who completed the final delivery assessment of MIREC.

This thesis project involved the analysis of data collected from the MIREC and MIREC-ID studies, which included administered questionnaires, endocrine sensitive endpoint measures and maternal blood specimen results from the Institut National de Santé Publique du Québec (INSPQ) lab (Ashley-Martin & Dodds et al., 2015; Ashley-Martin & Levy et al., 2015). All MIREC and MIREC biobank datasets were imported from DACIMA – an online tool used to manage large datasets (Dacima Software, Montréal, Québec) – into Statistical Package for the Social Sciences (SPSS) v.21 (IBM Corporation, Armonk, NY, USA) and Statistical Analysis Software (SAS) EG v4.2 (SAS Institute, Inc., Cary, NC, USA).

3.2.2 Participant Recruitment

As the MIREC and MIREC-ID were multi-centered studies, the research staff from each site participated in standardized training. This included patient screening, recruitment, consenting, specimen and data collection and processing, as well as biospecimen handling and shipping. In addition, the examination techniques of the research staff were monitored and checked regularly (Arbuckle et al., 2013).

The eligibility criteria included being pregnant between 6^{0/7} and 12^{6/7} completed weeks, 18 years of age or older, with the ability to consent and communicate in English or French and planning to deliver at a participating study hospital. In order to ensure a healthy obstetric population, women with known fetal abnormalities or chromosomal anomalies in their current pregnancy were excluded from the study along with women with a history of multiple medical conditions. This includes: renal disease with altered renal function, any collagen vascular disease such as lupus erythematosus or scleroderma, active hepatitis, epilepsy, heart disease, serious pulmonary disease, cancer, hematologic disorder, threatened abortion or illicit drug use.

3.2.3 Ethics and Informed Consent

The MIREC study received ethics approval from human subjects' research ethics committees for all protocols, questionnaires, consent forms and recruitment posters/pamphlets. These included the Research Ethics Board at Health Canada, the research ethics committee at the coordinating center at Ste-Justine's Hospital in Montréal, as well as the academic and hospital ethics committees at each research site across Canada. Prior to beginning the study, potential participants were provided with the study objectives along with the study design and asked to sign consent forms. Consent forms agreeing to participate in the MIREC study as well as for the use of the stored biological specimens for follow-up studies and MIREC-ID study are included in **Appendix 1, Appendix 2 and Appendix 3**, respectively. At any point, participants had the option of partially withdrawing (all data and biospecimens retained) or completely withdrawing (all data and biospecimens destroyed) from the study.

3.2.4 Data/Biospecimen Collection

During each trimester, at delivery and in the early postnatal period (up to 10 weeks), biospecimens were collected from participants and stored for laboratory analyses (i.e. maternal blood, umbilical cord blood, urine, infant meconium, human milk and postnatal maternal hair) (Arbuckle et al., 2013; Fisher et al., 2016). In addition to the collection of biospecimens, prenatal questionnaires were administered during each visit by trained research staff. During the 1st visit (between 6^{0/7} and 13^{6/7} weeks), information on sociodemographic characteristics, medical history (obstetrical and non-obstetrical), family history, employment status, environmental exposures, smoking history (active and passive), alcohol consumption, place of residence, daily activities, and nutrition /diet and sunlight exposure were collected. During the 2nd visit (between 16^{0/7} and 21^{6/7} weeks), information on gestational age and current medication use was obtained and any

clinical laboratory tests the mother had completed were obtained from medical chart reviews. During the 3rd visit (between 32^{0/7} and 34^{6/7} weeks), similar questions on lifestyle as collected during the 1st trimester visit were asked. During all three pre-delivery visits and at delivery, sampling characteristics of the biospecimens collected were also recorded, in addition to blood pressure and anthropometric measurements of the mother. The detailed status of the pregnancy was recorded during all visits as well. For pregnancies greater than 20 weeks, a chart review questionnaire was completed after delivery. This questionnaire assessed: glucose tolerance during pregnancy, corticosteroid use during pregnancy, blood pressure during pregnancy prior to admission for delivery, blood pressure after admission for delivery, anthropometric measurements prior to delivery, maternal conditions after admission and maternal outcomes after delivery. Lastly, information on the labour and delivery of the baby and neonatal characteristics were recorded. In cases where there were multiple pregnancies, neonatal information for all babies was obtained.

3.2.5 Definition of Potential Risk Factors (Confounding Variables)

The independent variables or exposures of interest for this thesis project are the maternal plasma concentrations of As, Cd, Hg, Mn, and Pb ($\mu\text{g/L}$) in pregnant women, measured in blood samples taken at first and third trimester of pregnancy.

The covariates that were considered included several demographic variables, anthropometric measurement variables and postnatal measurements. Maternal active and passive smoke exposure was also included as smoking during pregnancy and exposure to environmental tobacco smoke (ETS) has been known to be associated with many adverse events, including preterm birth, low birth weight and decreased length at birth (Kharrazi et al., 2004; Hegaard et al., 2006). Prenatal exposure to nicotine (the active component in cigarettes) significantly reduced birth

weight and length at birth (Wickström, 2007; Kirchengast and Hartmann, 2003).

The following variables determined during visit 1 of the MIREC study were derived:

- **Maternal age**, determined from the following questions:

1. What is today's visit date? (MIREC participant visit 1 date)
2. What is your year of birth?

For the analysis, maternal age was kept as a continuous variable. For the descriptive analysis, maternal age was categorized as follows: 1= $\leq$ 19 years, 2=20-24 years, 3=25-29 years, 4=30-34 years, 5= $\geq$ 35 years.

- **Maternal education**, determined from the following question:

- Highest education level achieved? (Grade 8 or less/some high school/high school diploma/some college classes/college diploma/trade school diploma/undergraduate university degree/graduate university degree)

For the analysis, maternal education level categories were collapsed as follows: 1= $\leq$ High School Diploma, 2=Some College, 3=College or Trade Diploma, 4=University Degree.

- **Annual household income**, determined from the following question:

- From all sources from Jan-Dec last year, what was your annual household income before taxes? (Including other sources of income, help from family or friends) (Less than \$10,000/\$10,001-\$20,000/\$20,001-\$30,000/\$30,001-\$40,000/\$40,001-\$50,000/\$50,001-\$60,000/\$60,001-\$70,000/\$70,001-\$80,000/\$80,001-\$100,000/More than \$100,000/don't know/refuse to answer)

Annual household income categories were collapsed as follows: 1= $\leq$ \$20,000, 2=\$20,001-\$40,000, 3=\$40,001-\$60,000, 4=\$60,001-\$80,000, 5=\$80,001-\$100,000, 6= $\geq$ \$100,000.

- **Active smoke exposure**, determined from the following questions:
 - Have you ever smoked at least 100 cigarettes over your lifetime (about 4 packs)?
(yes/no)
 - At the present time, do you smoke cigarettes daily, occasionally or not at all?
(daily/occasionally/not at all)

For the analysis, active smoke exposure was categorized as follows: 1=Prior to Pregnancy, 2=Occasionally during Pregnancy, 3=Daily during Pregnancy, 4=Never. If the participant answered 'yes' to the first question and 'not at all' to the second question, they were considered past smokers and were classified in the 'prior to pregnancy' group.

- **Maternal pre-pregnancy body mass index (BMI)**, determined from the following questions:
 - Pre-pregnancy weight? (Kg/Pounds, don't know)
 - Weight measured at this visit (Kg/Pounds)
 - Height measured at this visit (cm)

For the analysis, BMI was kept as a continuous variable. For the descriptive analysis, maternal BMI was categorized into the following categories: 1= ≤ 18.5 kg/m², 2=18.5-24.9 kg/m², 3=25.0-29.9 kg/m², 4= ≥ 30 kg/m²

- **PROM**, determined from the following question:
 - In a previous pregnancy, have you suffered from gestational hypertension?
- **Parity**, determined from the following question:
 - Have you ever been pregnant before this pregnancy?
 - If yes, how many pregnancies including the current pregnancy?

Furthermore, infant birthweight, length at birth, cephalic perimeter, placental weight, measured at delivery, were included. Additionally, gestational age at delivery, baby sex and APGAR score at 1, 5, and 10 minutes were also included. For the analysis, gender of baby was categorized as follows: 1=Male, 2=Female, 3=Unknown. All questions were taken from the case-report forms (CRFs).

3.2.6 Data Cleaning

This thesis project involved the analysis of secondary datasets obtained from the MIREC research platform. Of the datasets included for this thesis, one contained the biomonitoring data for all biospecimens included in the MIREC biological specimens bank (MIREC biobank). The dataset included the laboratory measurement of each environmental metabolite for each participant, measured as a concentration and identified by unique participant identification numbers. The datasets from various sources were linked and cross-checked to ensure the final dataset was complete and accurate using a program in Microsoft Excel. Cleaning of this dataset included the assessment of the laboratory results, which included identifying missing data (which may have been due to damaged specimens, insufficient quantity of specimen for testing, other reasons) and the identification of values below the LOD (application of LOD/2). Metal concentrations from the 1st and 3rd trimester were averaged to create an estimate of gestational exposure and in the case where the value for one time point was missing the other values were used.

In biomonitoring studies, when the concentration of a chemical is less than the laboratory's detection or reporting limit for that specific chemical, the value is referred to as below the 'limit of detection' or 'censored data'. There are multiple ways to account for values below the LOD in a statistical analysis including, but not limited to, substituting zero for the value, the LOD itself

or dividing the LOD by 2. There is evidence that these substitution methods may be biased (known to artificially reduce the standard error), with the recommendation that a censoring method be used (Helsel, 2012), where the characteristics of the distribution of the values above the LOD are used to estimate the values below the LOD. In our analysis, all contaminants for which greater than 50% of the samples were below the LOD were excluded from further analysis. However, as the overall percentage of samples below the LOD was small for the included contaminants (<10%), the LOD/2 was substituted for values below the LOD. Other studies conducted in this field of research have used the same approach to deal with values below the LOD (Ashley-Martin et al., 2015).

A final dataset containing only laboratory data pertaining to the environmental chemicals of interest for this thesis project was obtained. SPSS was then used for the descriptive characterization via frequency distributions of heavy metal concentrations and MMP levels in maternal blood samples. Although SAS was used to determine confounding variables, SPSS was also used to conduct the majority of statistical tests such as t-tests, logistic regressions, univariate and multivariate analyses to assess the statistical relationships between variables.

3.2.7 Descriptive Statistics

The study population in the birth cohort consisted of 1533 mother-infant pairs. The total sample size decreased from the initial sample size of 2034 due to exclusion of participants with missing contaminant data and multiple births. **Table 3** describes this population by various maternal demographic categories and blood collection parameters. Among the 1533 mothers (sample size used for descriptive statistics purposes), 541 (35.3%) were between the ages of 30 and 34 years, 896 (58.4%) had a pre-pregnancy body mass index between 18.5-24.9 kg/m², 361 (23.5%) had a college/trade school diploma while 962 (62.8%) had a university degree, 590

(38.5%) had a household income greater than \$100,000. In regards to exposure to environmental tobacco smoke, 507 (33.1%) of women had actively been exposed to tobacco smoke prior to pregnancy, while 948 (61.8%) of women had never smoked. There were 78 (5.1%) women who smoked during pregnancy (either occasionally or daily).

There was a relatively even distribution of female and male babies in this cohort, 47.3% and 52.7% respectively. **Table 4** summarizes the infant demographic categories and birth outcome measurements. On average, babies weighed 3.47-kg, had a birth length of 51.16-cm and had cephalic perimeters of 34.80-cm. The average gestational age was determined to be 276.06 days or 39.44 weeks. At 1 minute, 83 (5.4%) of babies had an APGAR score between 1 and 5 and 1447 (94.4%) had a score between 6 and 10, while at 5 minutes, 11 (0.8%) of babies had an APGAR score between 1 and 5 and 1518 (99.0%) of babies had a score between 6 and 10.

Table 5 reports the overall summary statistics (sample size, minimum, maximum, geometric mean with 95% lower and upper confidence intervals, sample median and sample 25th, 50th and 75th percentiles) for each contaminant, detected from blood samples in mothers from the birth cohort. The first entry in the table represents the first trimester statistics (e.g. As), while the second entry represents the third trimester statistics (e.g. As_third), and the last entry represents the average between first and third (e.g. As_avg). The column labeled 'LOD' reports the Level of Detection for each contaminant, while the %<LOD reports the proportion of observations below this value. All values below the LOD were substituted using the ½ LOD substitution methods and reported.

TABLE 3. MATERNAL DEMOGRAPHIC COVARIATES OF A SAMPLE OF MIREC PARTICIPANTS (BIRTH COHORT) PROVIDING 1ST AND 3RD TRIMESTER BLOOD SAMPLES FOR ANALYSIS OF MMPs (N=1533).

	Frequency	Percentage (%)
Maternal Age (Years)		
<20	10	0.7
20-24	89	5.8
25-29	375	24.5
30-34	541	35.3
≥35	518	33.8
Maternal Education		
≤High School	127	8.3
Some College	81	5.3
College/Trade School Diploma	361	23.5
University Degree	962	62.8
Missing	2	0.1
Household Income (\$)		
≤20,000	59	3.8
20,001 – 40,000	115	7.5
40,001 – 60,000	151	9.8
60,001 – 80,000	252	16.4
80,001 – 100,000	297	19.4
>100,000	590	38.5
Missing	69	4.5
Active Smoke Exposure		
Prior To Pregnancy	507	33.1
Daily During Pregnancy	58	3.8
Occasionally During Pregnancy	20	1.3
Never	948	61.8
Pre-Pregnancy Body Mass Index (kg/m²)		
≤18.50	84	5.5
18.50-24.99	896	58.4
25.00-29.99	333	21.7
>30	220	14.4
Parity		
0	682	44.5
1	618	40.3
2	175	11.4
>3	58	3.8
PROM		
Yes	382	24.9
No	1095	71.4
Missing	56	3.7
Maternal High BP		
Yes	20	1.3
No	1513	98.7
Maternal Diabetes?		
Yes	18	1.2
No	1515	98.8
Hypertension Prior to Admission (after 20w)		
Yes	74	4.8
No	1442	94.1
Missing	17	1.1
Hypertension After Admission		
Yes	70	4.6
No	1449	94.5
Missing	14	0.9

TABLE 4. INFANT DEMOGRAPHIC COVARIATES OF A SAMPLE OF MIREC PARTICIPANTS (BIRTH COHORT) PROVIDING 1ST AND 3RD TRIMESTER BLOOD SAMPLES FOR ANALYSIS OF MMPs (N=1533)

	Frequency	Percentage (%)
Sex of Baby		
Male	809	52.7
Female	724	47.3
APGAR 1 min		
1	6	0.4
2	15	1
3	19	1.2
4	18	1.2
5	25	1.6
6	36	2.3
7	83	5.4
8	317	20.7
9	1007	65.7
10	4	0.3
Missing	3	0.2
APGAR 5 min		
2	1	0.1
3	2	0.1
4	1	0.1
5	7	0.5
6	10	0.7
7	19	1.2
8	63	4.1
9	1345	87.7
10	81	5.3
Missing	4	0.3
Gestational Age		
Preterm (<265 days)	181	11.8
Term (266-280 days)	790	51.5
Post-term (>280 days)	562	36.7
Birth Weight		
LBW (<2499g)	37	2.4
NBW (2500g-4499g)	1467	95.7
HBW (>4500g)	29	1.9
Size for Gestational Age		
SGA	156	10.2
AGA	1227	80.0
LGA	150	9.8
Mean Birth Weight (kg) [Std. Dev.]	3.470 [0.491]	
Mean Gestational Age (days) [Std. Dev.]	276.060 [10.192]	

TABLE 5. SUMMARY OF METAL EXPOSURES IN MATERNAL BLOOD SAMPLES FROM MIREC COHORT.

Contaminant (nmol/L)	N	LOD (µg/L)	%<LOD	Min	Max	Geometric Mean	95% Confidence Interval		Sample Median	Sample 25 th Percentile	Sample 50 th Percentile	Sample 75 th Percentile
							Lower	Upper				
As	1533	0.22	7.2	1.47	460.00	9.86	9.47	10.25	11.00	7.00	11.00	16.00
As_third	1533	0.22	7.2	1.47	440.00	8.52	8.14	8.93	9.20	5.50	9.20	15.00
As_avg	1533	0.22	7.2	1.47	440.00	10.09	9.74	10.46	10.25	6.75	10.25	15.50
Cd	1533	0.04	2.6	0.18	45.00	1.86	1.78	1.94	1.80	1.20	1.80	2.70
Cd_third	1533	0.04	2.6	0.18	38.00	1.73	1.67	1.81	1.70	1.20	1.70	2.60
Cd_avg	1533	0.04	2.6	0.18	41.50	1.90	1.84	1.97	1.78	1.20	1.78	2.65
Hg	1533	0.12	9.7	0.30	50.00	3.05	2.88	3.22	3.50	1.70	3.50	6.70
Hg_third	1533	0.12	9.7	0.30	62.00	2.41	2.29	2.54	2.80	1.30	2.80	5.05
Hg_avg	1533	0.12	9.7	0.30	34.00	2.85	2.71	3.01	3.25	1.65	3.25	5.90
Mn	1533	0.05	0	37.00	530.00	160.68	158.09	163.34	160.00	130.00	160.00	200.00
Mn_third	1533	0.05	0	5.00	610.00	222.40	218.57	226.26	230.00	180.00	230.00	280.00
Mn_avg	1533	0.05	0	66.50	550.00	193.61	190.77	196.47	195.00	160.00	195.00	235.00
Pb	1533	10	0	7.50	200.00	29.98	29.26	30.70	30.00	21.00	30.00	41.00
Pb_third	1533	10	0.3	2.41	200.00	27.53	26.82	28.26	27.00	20.00	27.00	38.50
Pb_avg	1533	10	0	8.00	195.00	29.22	28.56	29.91	28.50	21.00	28.50	39.50

TABLE 6. SUMMARY OF BIOMARKERS FOUND IN MATERNAL BLOOD SAMPLES FROM MIREC COHORT.

Marker (pg/mL)	N	LOD (pg/mL)	%<LOD	Min.	Max.	Geometric Mean	95% Confidence Interval		Sample Median	Sample 25 th Percentile	Sample 50 th Percentile	Sample 75 th Percentile
							Lower	Upper				
MMP-1	1533	3	0	89.07	16435.52	722.14	700.68	760.69	702.86	445.26	702.86	1107.86
MMP-2	1533	200	0	6096.81	273765.57	65734.42	60172.77	62492.80	66882.04	53376.51	66882.04	81543.54
MMP-7	1533	97	0	395.19	64731.56	5878.38	5775.11	6202.97	6192.10	4013.69	6192.10	9032.12
MMP-9	1533	2	0	4376.08	316744.28	26941.15	25914.33	28311.96	25034.33	15461.38	25034.33	43666.80
MMP-10	1533	5	0	21.40	3281.06	263.61	248.78	262.88	258.89	194.72	258.89	350.59
IL-2	1192	0.26	10.8	0.13	1596.01	1.38	1.26	1.47	1.32	0.63	1.32	3.15
IL-6	1193	0.2	0.7	0.10	70031.17	1.87	1.77	1.98	1.71	1.04	1.71	3.02
IL-8	1193	0.05	0	0.18	58.15	1.99	1.90	2.04	1.84	1.37	1.84	2.60
IL-10	1193	0.48	0.2	0.24	1470.65	21.05	20.01	22.14	20.31	13.40	20.31	31.66
IL-12	1193	0.34	6.1	0.17	10429.17	3.02	2.76	3.28	2.75	1.30	2.75	6.74
CRP*	1395	0.004	0	233.10	1602600.00	17197.00	15951.44	18251.56	17532.84	8381.16	17532.84	36826.07
TNF-a	1193	0.07	0	0.28	1062.93	4.63	4.49	4.76	4.57	3.43	4.57	6.06
IFN-g	1193	0.18	0.4	0.09	554.47	5.07	4.74	5.40	5.01	2.71	5.01	9.86
GMCSF	1193	0.15	2.4	0.08	1484.93	1.42	1.32	1.51	1.44	0.67	1.44	2.75
MCP-1	1392	1.1	0	6.49	3755.09	41.64	38.82	41.50	39.72	29.18	39.72	56.80
MIP-1B	1401	2.4	0.1	1.20	2112.75	59.99	57.86	61.63	58.58	46.12	58.58	76.10
VCAM	1405	0.6	0	1.16	711947.90	243895.89	228349.47	243220.40	260000.71	187496.81	260000.71	330457.56
ICAM	1401	2.4	0	163.60	577275.65	132013.48	117327.55	128854.62	160637.80	98632.17	160637.80	204204.50
VEGF	1196	3.1	49.7	1.55	115.20	3.09	2.97	3.25	3.14	1.55	3.14	5.54

* Measured in ng/mL

TABLE 7. SUMMARY OF DESCRIPTIVE STATISTICS OF OUTCOMES.

	N	Minimum	Maximum	Mean	Std. Dev.
Gestational Age in Days (Weeks)	1533	185 (26.4)	297 (42.4)	276.06 (39.44)	10.19 (1.46)
Birth Weight (g)	1533	780.00	5070.00	3470.27	490.89
Length at Birth (cm)	1339	34.00	59.70	51.16	2.68
Cephalic Perimeter (cm)	1444	23.00	39.50	34.80	1.47

3.3 Systematic Review and Meta-Analysis

Systematic review of literature was conducted in an attempt to investigate the relationship between maternal blood biomarkers and adverse birth outcomes. Due to the broad scope of this topic, the outcome measurements were limited to infant birth outcomes such as preterm birth, low birth weight, and small for gestational age infants. The search strategy was designed to identify observational studies that described association between biomarkers found in maternal blood (whole blood, plasma, or serum) and adverse birth outcomes in the infant. The search strategy is discussed in further detail in **Chapter 5**.

CHAPTER 4: BLOOD METAL LEVELS AND THIRD TRIMESTER MATERNAL PLASMA MATRIX METALLOPROTEINASES (MMPS)

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Running Title: Metals and MMPs in pregnancy

4.1 Abstract

While it is known that in utero exposure to environmental toxicants, namely heavy metals, can adversely affect the neonate, there remains a significant paucity of information on maternal biological changes specific to metal exposures during pregnancy. This study aims at identifying associations between maternal metal exposures and matrix metalloproteinases (MMPs) that are known to be engaged in pregnancy process. Third trimester maternal plasma (n=1533) from a pregnancy cohort (Maternal-Infant Research on Environmental Chemicals Study, MIREC) were analyzed for MMP-1,-2,-7,-9 and -10 by affinity-based multiplex protein array analyses.

Maternal metal concentrations (mercury, cadmium, lead, arsenic and manganese) in 1st and 3rd trimesters exhibited strong correlations ($p < 0.05$). Multivariate regression models were used to estimate odds ratio (OR) for the association between metal concentrations in quartiles and high (90%) and low (10%) maternal MMP levels. Significant ($p < 0.05$) metal exposure-related effects were observed with the different MMP isoform responses. MMP profiles were specific to the trimester at which the maternal blood metals were analyzed. Our findings suggest that the profiles of these MMP isoforms vary with the type of metal exposure, blood metal concentrations and the trimester at which metal levels were determined. These new findings on maternal metal-MMP relationships can guide future explorations on toxicity mechanisms relevant to metal exposure-mediated adverse birth outcomes.

Keywords: Maternal biomarkers, matrix metalloproteinases, metals, birth cohort.

4.2 Introduction

Maternal chemical burdens are shown to influence maternal and infant health (Stieb et al., 2012; Stillerman et al., 2008; Perera et al., 2007). Previous studies have revealed associations between factors such as maternal life style and adverse pregnancy outcomes (Wigle et al., 2008; Matthews et al., 1999; Jolly et al., 2000; Gelson & Johnson, 2010; Alsuwaida et al., 2011; Abu-Saad & Fraser, 2010; Dechanet et al., 2011). Adverse birth outcomes include preeclampsia, premature rupture of membranes (PROM), gestational diabetes, intrauterine growth restriction (IUGR), preterm birth (PTB), low birth weight (LBW), and small for gestational age infants (Godfrey et al., 1996; King, 2003; Hackshaw et al., 2011; Howe et al., 2012).

Early life exposures to environmental metals can cause disease and disability in infants and across the entire lifespan (Shoeters et al., 2011; Wigle et al., 2007). Metals namely lead (Pb), mercury (Hg), cadmium (Cd), manganese (Mn) and arsenic (As) may act as endocrine disruptors

(Caserta et al., 2013; Bellinger, 2005). Lead, mercury, and arsenic can cross the placental barrier and accumulate in amniotic fluid or fetal tissues (Vahter et al., 2002). Lead is associated with premature rupture of membrane (PROM)-related preterm delivery, LBW, PTB and IUGR (Andrews et al., 1994; Angell and Lavery, 1982; Falcon et al., 2003; Srivastava et al., 2001). Mercury is reported to alter neurological development and disrupt DNA replication among other effects (O'Reilly et al., 2010; Georgescu et al., 2011; Wigle, 2003). Arsenic has been suggested to play a role in the risk of having LBW infants (Vahter et al., 2002; McDermott et al., 2014; Yang et al., 2003; Hopenhayn et al., 2003) and in maternal hypertension (Lee et al., 2003) and gestational diabetes (Ettinger et al., 2009; Saldana et al., 2007; Shapiro et al., 2015). Cadmium does not easily cross the placental barrier, but may affect placental function and thus fetal development (Iyengar and Rapp, 2001; Zhang et al., 2004a; b; Nishijo et al., 2004; Yang et al., 2006), and is implicated in preeclampsia (Semczuk & Semczuk-Sikora, 2001; Dawson et al., 1999; Eisenmann & Miller, 1995; Kosanovic et al., 2002). Manganese is an essential element, yet deficiency and excess intake may result in adverse pregnancy outcomes such as IUGR, LBW and other effects (Eum et al., 2014; Marriott et al., 2007; McDermott et al., 2014; Mora et al., 2014; Sarwar et al., 2013; Wood, 2009). Some studies have indicated adverse birth effects at lower exposure levels than previously anticipated (Wigle et al., 2007; Vahter et al., 2002).

Heavy metal exposures are associated with oxidative stress and inflammatory pathways (Dagouassat et al., 2012; Sivanesan et al., 2007; Valko et al., 2005), which may be relevant to PTB and LBW (Buhimschi et al., 2010; Conde-Agudelo et al., 2011; Ferguson et al., 2015). A family of zinc-dependent proteinases involved in the degradation of extracellular matrix during normal and pathological tissue remodelling that are known as matrix metalloproteinases (MMPs) play a critical role in embryo implantation, placentation, cervical dilation and fetal-maternal

membrane lysis (Tu et al., 1998), and are associated with inflammatory conditions (Zhou et al., 2000; Dagouassat et al., 2012; Conde-Agudelo et al., 2011). Both arsenic and mercury, at high levels, have been shown to affect MMPactivity (Jacob-Ferreira et al., 2009; Liang et al., 2012). There is a paucity of information on the mechanistic basis for environmental metal exposure-mediated adverse pregnancy outcomes (Dagouassat et al., 2012; Kumarathasan et al., 2014). An understanding of how pre- and peri-natal metal exposures affect maternal biological pathways and pregnancy outcomes including long term effects is critical for risk estimation.

In this study, the objective was to investigate the relationships between maternal prenatal (first and third trimester) blood metal concentrations and maternal third trimester circulating MMPs, key enzymes in the process of pregnancy, to gain information towards the understanding of environmental chemical exposure-mediated adverse pregnancy outcomes. For this purpose, we have employed participants from the Maternal-Infant Research on Environmental Chemicals (MIREC) study.

4.3 Materials and Methods

4.3.1 Study Design

Details on the MIREC study have been reported by Arbuckle et al. (2013). To summarize, 2001 women were recruited from 10 Canadian sites from 2008-2011 during their first trimester of pregnancy. From the 2001 women, 18 withdrew and the 1983 remaining subjects gave birth to 1959 infants. Of this subset, 426 women were excluded for multiple births, missing metal biomonitoring data and anthropometric measurements, or unknown sex of the baby, resulting in a final sample size of 1533 mother-infant pairs.

4.3.2 Ethics

The research protocol, questionnaires, consent forms and recruitment posters and pamphlets

were reviewed and approved by human studies research ethics committees, including the Research Ethics Board at Health Canada, the ethics committee at the coordinating center at St-Justine's Hospital in Montreal and more than ten academic and hospital ethics committees across Canada. All participants signed informed consent forms.

4.3.3 Metal Exposure

Whole blood samples collected during the first and third trimesters were analysed for As, Cd, Hg, Mn and Pb using inductively coupled plasma mass spectrometry (PerkinElmer ELAN ICP-MS DRC II). These analyses were performed by the Laboratoire de Toxicologie, Institut National de Santé Publique du Québec (INSPQ) (Québec, QC, Canada), accredited by the Standards Council of Canada.

4.3.4 Plasma Sample Preparation and MMP Biomarker Analyses

Plasma samples were from the 3rd trimester maternal blood samples (n=1533), which were stabilized with preservatives EDTA and PMSF following a procedure described by Kumarathan et al. (2014). These plasma samples were treated with DETPA, BHT and an antiprotease (HaltTM protease inhibitor) cocktail, (Thermo Fisher Scientific Canada), vortexed, and frozen at -80°C for storage (Kumarathan et al., 2014).

Aliquots of plasma samples were analyzed for MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10 by affinity (antibody)-based multiplex liquid suspension (fluorescence-coded magnetic beads) protein array analyses using Milliplex Map kits (Millipore, Canada). Briefly, plasma was treated with the corresponding MMP capture antibody-coated magnetic beads and was incubated for two hours. Beads were then washed and reacted with biotinylated-detection antibodies followed by incubation with streptavidin-phycoerythrin, re-washed, re-suspended in sheath fluid (Biorad, Canada) and analysed by Bioplex 100 using Bioplex Manager (version 6.0) software

(Biorad, Canada).

4.3.5 Statistical Analysis

Data were extracted from questionnaires and hospital charts to test whether maternal characteristics and infant sex influenced maternal plasma MMPs. Initially, descriptive statistics was conducted using frequency distributions and chi-square tests of significance for the difference between the low ($\leq 10^{\text{th}}$ percentile), moderate (>10 – $<90^{\text{th}}$ percentiles), and elevated ($\geq 90^{\text{th}}$ percentile) MMP categories by maternal and birth characteristics [maternal age at delivery, maternal education, household income, smoking status prior to pregnancy, pre-pregnancy body mass index (BMI) according to WHO guidelines (World Health Organization, 2006), premature rupture of membranes, baby sex and parity)]. In addition, the geometric mean (GM) and standard deviation (SD) of the metal concentrations were determined according to MMP categories. All samples with metal concentrations below the level of detection (LOD) were imputed as one half the LOD. The LOD values for plasma MMP-1, -2, -7, -9 and -10, were 3, 200, 97, 2 and 5 pg/mL, respectively.

Since both metals and maternal physiological measurements may influence circulating MMP levels, multivariate regression analyses, chi-square tests and one-way ANOVAs were conducted to select any maternal and infant physiological traits that were significantly associated with each individual MMP to identify potential confounders. The maternal parameters tested include: hypertension status, diabetic status, diagnosis of PROM, education, smoking history, age, pre-pregnancy BMI, pre-pregnancy weight, 1st trimester measurements (weight, height, systolic blood pressure BP, diastolic BP), 2nd trimester measurements (weight, systolic BP, diastolic BP), 3rd trimester measurements (weight, systolic BP and diastolic BP), pre-delivery measurements (weight, height, systolic BP and diastolic BP), post-partum BP measurements (systolic and

diastolic), and parity. Those parameters that were significant at the $p < 0.05$ level were selected as covariates, and further multivariate analyses were carried out to identify relationships between blood metal concentrations and circulating MMPs.

Although the first trimester was deemed as the more critical period during pregnancy of the two trimesters, metal exposure at both 1st and 3rd trimesters were considered in the analyses of unadjusted and adjusted odds ratios for MMPs. In addition, metal concentrations from the two time-points were averaged to create an estimate of gestational exposure. In cases where the value for one of the time-points was missing, the other value was used. Pearson correlation coefficient analyses were done with both 1st and 3rd trimester blood metal levels. The maternal blood metal levels (Hg, Pb, Cd, As and Mn) were categorized into quartiles since the concentration range was wide and we intended to resolve meaningfully any non-monotonous effects. Here, Q_1 was considered as the lowest and Q_4 was set as the highest metal concentration quartiles. Since MMPs play a crucial role in pregnancy, low and high plasma levels of these enzymes can potentially be associated with adverse effects. Maternal blood levels of MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10 were thus categorized into the $\leq 10^{\text{th}}$ (low), $10^{\text{th}}-90^{\text{th}}$ (normal) and $\geq 90^{\text{th}}$ (high) percentiles, with $10^{\text{th}}-90^{\text{th}}$ percentile being the reference group (Shapiro et al., 2015; Ashley-Martin et al., 2015).

Multinomial logistic regression was initially employed to examine the unadjusted relationships between the quartiles of maternal heavy metal concentrations for both first trimester and third trimester maternal blood metal levels and the odds of observing low ($\leq 10^{\text{th}}$ percentile) and high ($\geq 90^{\text{th}}$ percentile) MMPs, relative to the corresponding reference $10^{\text{th}}-90^{\text{th}}$ percentiles groups. Multinomial logistic regression was then performed including the aforementioned maternal characteristics that were identified as influencing each MMP as covariates to determine

the adjusted odds ratio for low ($\leq 10^{\text{th}}$ percentile) and high ($\geq 90^{\text{th}}$ percentile) MMPs, relative to 10th-90th percentiles, for the different metal concentration quartiles. The first quartile was used as the baseline when odds ratios were calculated for As, Cd, Hg and Pb based on the assumption that very low levels led to no observed effects on MMPs. However, for manganese, the first quartile concentration was not chosen as the reference level because it is a nutrient for humans and a deficiency can also lead to adverse health outcomes. Instead, Q₂ was selected as the reference level based on values reported for healthy controls (ca. 130 nmol/L). All statistical analysis was conducted using either SPSS v.21 (IBM Corporation, Armonk, NY, USA), or SAS EG v4.2 (SAS Institute, Inc., Cary, NC, USA).

4.4 Results

The majority of MIREC study participants were over 30 years of age at the time of pregnancy, never smoked, were of normal BMI, were university educated, and had a household income greater than \$100,000 (**Table 8**). Maternal plasma MMP-2 and MMP-7 concentrations were significantly ($p < 0.05$) associated with the diagnosis of hypertension after admission (data not shown), while PROM was related to MMP-1 ($p < 0.1$) and MMP-2 ($p < 0.05$) levels, and maternal diabetic status only was associated with MMP-2 ($p < 0.05$) and parity with MMP-2 ($p < 0.1$) and MMP-7 ($p < 0.05$). Maternal education and smoking were significantly associated with MMP-7 ($p < 0.1$) and MMP-2 ($p < 0.1$) respectively (**Table 8**). Since there were no differences in the levels of MMPs between genders of the infants, categorization of MMPs for further analysis was not done based on sex-specific cut-offs.

The geometric mean and standard deviation values (SD) of the average of first and third trimester maternal blood metal levels were as follows: arsenic=0.22 (16.81) nmol/L, cadmium=1.91 (3.29) nmol/L, mercury=2.90 (4.20) nmol/L, manganese=193.61 (60.86) nmol/L,

and lead=29.22 (17.54) nmol/L. The first and third trimester blood metal concentrations were all significantly correlated ($p<0.05$): arsenic $r=0.45$, cadmium $r=0.80$, mercury $r=0.63$, manganese $r=0.64$, and lead $r=0.74$. Geometric means and SD for first trimester metals according to low and high levels of MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10 are presented in **Table 9**. About 97% of the samples from this cohort exhibited geometric mean blood concentrations $>LOD$ for all metals with the exceptions of arsenic (92.8%) and mercury (90.3%) (**Table 9**).

Multivariate regression models revealed that the MMP-1 level was associated with 2nd trimester weight (kg) and systolic and diastolic BP, while MMP-2 level was linked with 2nd trimester weight (kg) and maternal diabetic status. Both MMP-7 and MMP-9 levels were associated with 2nd trimester weight (kg), whereas MMP-10 level was related to 1st trimester weight (kg) and pre-pregnancy BMI (data not shown).

Unadjusted and adjusted multinomial logistic regression analysis results for associations between the first trimester blood metal concentrations and plasma MMPs are given in **Tables 10 and 11**, respectively. Although, Q₂ & Q₃ levels of maternal blood arsenic were associated ($p<0.05$) with increased risk for low ($\geq 10\%$) MMP-2 without adjustment for covariates, with adjustment, only Q₂ remained to be significantly ($p<0.05$) associated with increased odds for low MMP-2 levels (**Table 10 and 11**). Mercury at Q₂ level exhibited significant increased risk for high MMP-9, after adjustment for covariates (**Table 11**). Increased ($p<0.05$) risk for low ($\leq 10\%$) MMP-2 and high ($\geq 90\%$) MMP-9 were observed at high concentrations of lead levels Q₄ and Q₂-Q₃, respectively (**Table 11**). Manganese at Q₄ appeared to decrease ($p < 0.05$) the risk for high ($\geq 90\%$) MMP-10, and this relationship disappeared with adjustment for covariates. Decreased ($p<0.05$) risk for high ($\geq 90\%$) MMP-7 and high ($\geq 90\%$) MMP-10 were observed for lead (Q₂) and arsenic (Q₃), respectively after adjustment for covariates (**Table 11**).

Similar associations were seen with MMPs and third trimester blood metal levels (**Tables 12 and 13**). The associations seen between metal concentrations and MMPs with the unadjusted multinomial logistic regression analyses (**Table 12**) were modified by inclusion of covariates into the models (**Table 13**). Q₂ of cadmium was associated ($p<0.05$) with increased risk for low ($\leq 10\%$) MMP-2 levels, whereas, Q₃ and Q₄ were associated ($p<0.05$) with decreased risk for low ($\leq 10\%$) MMP-10 and MMP-7, respectively. Q₂-Q₄ mercury levels were associated with high ($\geq 90\%$) MMP-7. Q₄ level of blood lead was associated ($p<0.05$) with decreased risk for high ($\geq 90\%$) MMP-9, meanwhile Q₃ level of manganese was associated with increased risk for low ($\leq 10\%$) MMP-9.

4.5 Discussion

Prenatal exposure to metals is associated with developmental, neurological and endocrine disorders. In the case of Pb, pregnancy-related metabolic changes can result in higher exposure from mobilization of metals from stores in the mother (Caserta et al., 2013; Bellinger, 2005).

In this study, we have investigated the relationship between prenatal exposures to various metals (Hg, Pb, Cd, As and Mn) as expressed by maternal blood levels and the circulating levels of a class of zinc-dependent metalloenzymes MMPs, in the MIREC study mothers. The MMPs play a crucial role in remodelling of the extracellular matrix (ECM) in pregnancy and parturition. Their activities are regulated by tissue inhibitors of metalloproteinases (TIMPs). Aberrant degradation of ECM or imbalance in MMPs and TIMPs has been linked to preterm labor. MMPs are involved in multiple cellular functions in different stages of pregnancy and both low and high levels can affect the process of pregnancy (Myers et al., 2005; Nissi et al., 2013).

Initial descriptive statistics revealed that among all the maternal parameters tested in this study, PROM was associated with maternal plasma MMP-2 levels whereas, parity was

significantly related to plasma MMP-7 (**Table 8**). In addition, multinomial regression analyses results also indicated that the different MMP isoforms were associated with different sets of maternal characteristics. During the tests for relationships between maternal metal burdens and plasma MMP levels, these factors were considered as a rich set of covariates.

The distribution of the different MMP isoforms with respect to the mean metal concentrations of As, Cd, Hg, Mn and Pb (**Table 9**) exhibited metal-specific changes in plasma MMP profiles. Unadjusted and adjusted odds ratio results revealed that the metals explored in this work were either associated with up- or down-regulation of the different MMP isoforms, in a monotonic or nonmonotonic manner (**Tables 10-12**). It was interesting to note that the first and third trimester blood metal concentrations while exhibiting some similarities in terms of the plasma MMP levels, revealed clear trimester-specific differences in MMP responses. For instance, the trends in association between Q₂-Q₄ arsenic and increased risk for low plasma MMP-2 levels were similar for the first and third trimester blood metal concentrations (**Tables 11 and 13**). However, first trimester blood mercury and lead at Q₂-Q₄ levels were associated with increased risk for high ($\geq 90\%$) MMP- 9, whereas, the third trimester blood levels of these heavy metals were observed to be associated with decreased risk for high ($\geq 90\%$) MMP-9 levels (**Tables 11 and 13**). In addition, any significant association between manganese or cadmium and MMPs were noticed only with the third trimester exposures, not with the first trimester levels. These results suggested that environmental metal exposure period may be important in triggering or altering MMP-related biochemical events, notably inflammatory cascades. There are reports on alteration in MMP profiles and inflammation during pregnancy, for instance during gingival inflammation (Gursoy et al., 2014) and in case of intraamniotic infection (Olgun & Reznik, 2010).

The MMP isoforms 1, -2, -7, -9 and -10 are known to play crucial roles in reproductive

function (Hulboy et al., 1997). Especially in women with normal pregnancy, basal elevated levels of MMP-9 are associated with cervical ripening before labor, and MMP-9 is also associated with events during preterm birth (Sawicki et al., 2000; Olgun & Reznik, 2010). In addition, altered MMP levels are linked with spontaneous preterm delivery and low birth weight (Tu et al., 1998; Yoon et al., 2001; Biggio et al., 2005; Poon et al., 2009). Prenatal exposures to metals can modify these MMP responses in pregnancy process.

The high maternal third trimester plasma MMP-9 (mean concentration for the $\geq 90\%$ group – 121.1 ng/mL) associated ($p < 0.05$) with first trimester mercury and lead (**Table 11**) are in the range of that reported for preterm birth (Romero et al., 2002). The mean concentration of the low ($\leq 10\%$) maternal plasma MMP-2 group associated with the first trimester arsenic and lead exposures (**Table 11**) and third trimester cadmium exposure (**Table 13**) is 33.5 ng/mL. Lower maternal serum MMP-2 levels are reported in women with preterm labor compared to normal pregnancy (Koucký et al., 2010). Mean plasma concentration for the high MMP-7 group associated with third trimester mercury exposure is 17.3 ng/mL. There are no reports so far on maternal third trimester plasma MMP-7 concentrations, to our knowledge. Yet, MMP-7 is reported to be found in human villous trophoblasts (Sawicki et al., 2000). Also, MMP-7 is shown to cause vasoconstriction in rat mesenteric arteries (Hao et al., 2004, 2006). Plasma MMPs appear to perturb maternal vasoregulatory process during pregnancy. We have previously shown associations between third trimester plasma MMP-2 and MMP-9 levels and infant birth weight and gestational age (Kumarathan et al., 2014, 2016).

Despite the strengths of this study, one of the limitations associated with the MIREC study population stems from the fact that the study subjects, on average, were from a higher socioeconomic background, exhibited relatively lower BMI and were less likely to smoke than

the general population. Thus caution is needed before generalizing these findings to other populations.

Our findings on the association of maternal blood metal levels with MMP changes in this MIREC cohort are in line with the limited reports so far on prenatal exposures to Hg, Pb and As (Jacob-Ferreira et al., 2009; Gonzalez-Puebla et al., 2012; Liang et al., 2012; Remy et al., 2014). Thus inference could be made that metal exposures may mediate the adverse pregnancy outcomes via MMP-related pathways. Nevertheless, further assessment of these metal exposure-mediated MMP enzyme activities and related mechanistic information can strengthen the notion of metals acting through MMP-related pathways to precipitate adverse birth outcomes.

4.6 Conclusions

Our data suggest that prenatal exposures to metals such as Hg, Cd, As, Pb and Mn can influence third trimester maternal plasma MMP levels and may potentially influence birth outcomes. Furthermore, although first and third trimester blood metal levels were correlated, they exhibited different patterns of third trimester plasma MMP responses implying time of exposure-related effects on this class of enzymes. These findings need to be validated by future studies on different pregnancy cohorts. The new information on associations between maternal blood metal levels and third trimester plasma MMPs from this study can assist future research on identification of metal exposure-related MMP-mediated toxicity pathways relevant to adverse pregnancy outcomes.

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TABLE 8. EFFECT OF MATERNAL/INFANT CHARACTERISTICS ON FREQUENCIES OF PERCENTILE PLASMA MMP CONCENTRATIONS (PG/ML).

Characteristic	N	MMP-1			p-value	MMP-2			p-value	MMP-7			p-value	MMP-9			p-value	MMP-10			p-value
		Percentiles				Percentiles				Percentiles				Percentiles				Percentiles			
		≤10	10-90	≥90		≤10	10-90	≥90		≤10	10-90	≥90		≤10	10-90	≥90		≤10	10-90	≥90	
Maternal Age					0.249				0.134				0.414				0.203				0.411
<20	10	10.0	90.0	0.0		10.0	70.0	20.0		20.0	50.0	30.0		0.0	90.0	10.0		10.0	80.0	10.0	
20-24	89	10.1	83.1	6.7		19.1	73.0	7.9		9.0	77.5	13.5		12.4	74.2	13.5		16.9	76.4	6.7	
25-29	375	10.7	78.9	10.4		8.0	80.5	11.5		10.7	79.2	10.1		7.7	79.7	12.5		9.1	79.5	11.5	
30-34	541	12.2	78.2	9.6		10.4	79.9	9.8		10.0	81.0	9.1		10.2	80.0	9.8		10.7	80.2	9.1	
35+	518	7.1	82.0	10.8		9.5	81.3	9.3		9.7	80.5	9.8		11.2	81.1	7.7		8.7	80.9	10.4	
Maternal Education					0.962				0.239				0.057**				0.137				0.720
Grade 8 or Less	2	0.0	100.0	0.0		0.0	100.0	0.0		0.0	100.0	0.0		50.0	50.0	0.0		50.0	50.0	0.0	
Some High School	32	9.4	84.4	6.3		18.8	68.8	12.5		18.8	78.1	3.1		6.3	90.6	3.1		9.4	78.1	12.5	
HS Diploma	93	9.7	80.6	9.7		12.9	79.6	7.5		7.5	75.3	17.2		9.7	76.3	14.0		9.7	78.5	11.8	
Some College	81	11.1	82.7	6.2		6.2	82.7	11.1		12.3	74.1	13.6		3.7	88.9	7.4		12.3	79.0	8.6	
College Diploma	327	12.2	76.5	11.3		13.8	75.5	10.7		14.1	78.3	7.6		8.6	80.4	11.0		6.7	82.3	11.0	
Trade School	34	8.8	79.4	11.8		11.8	85.3	2.9		5.9	88.2	5.9		8.8	85.3	5.9		8.8	82.4	8.8	
Undergraduate	566	9.5	80.2	10.2		8.8	80.6	10.6		9.2	80.6	10.2		11.7	77.0	11.3		11.5	79.2	9.4	
Graduate	396	8.8	81.6	9.6		7.8	82.8	9.3		7.8	82.1	10.1		10.4	82.1	7.6		9.8	80.3	9.8	
Missing	2																				
Household Income					0.532				0.779				0.242				0.114				0.656
<\$10 000	23	13.0	73.9	13.0		26.1	65.2	8.7		0.0	95.7	4.3		13.0	87.0	0.0		8.7	82.6	8.7	
\$10 001-20 000	36	16.7	75.0	8.3		8.3	77.8	13.9		8.3	72.2	19.4		11.1	77.8	11.1		11.1	75.0	13.9	
\$21 001-30 000	45	17.8	71.1	11.1		11.1	75.6	13.3		6.7	75.6	17.8		2.2	77.8	20.0		11.1	82.2	6.7	
\$30 001-40 000	70	5.7	87.1	7.1		10.0	78.6	11.4		10.0	74.3	15.7		11.4	85.7	2.9		14.3	78.6	7.1	
\$40 001-50 000	73	13.7	80.8	5.5		8.2	78.1	13.7		15.1	72.6	12.3		12.3	71.2	16.4		9.6	80.8	9.6	
\$50 001-60 000	78	10.3	80.8	9.0		10.3	79.5	10.3		9.0	85.9	5.1		7.7	87.2	5.1		10.3	76.9	12.8	
\$60 001-70 000	112	8.9	79.5	11.6		13.4	76.8	9.8		12.5	78.6	8.9		7.1	82.1	10.7		10.7	81.3	8.0	
\$70 001-80 000	140	12.1	73.6	14.3		8.6	82.9	8.6		12.1	75.7	12.1		11.4	80.0	8.6		5.7	82.9	11.4	
\$80 000-100 000	297	9.4	78.1	12.5		8.8	80.1	11.1		8.8	81.5	9.8		9.1	79.1	11.8		6.7	84.8	8.4	
>\$100 000	590	9.5	81.4	9.2		9.2	81.5	9.3		9.8	81.2	9.0		11.2	79.8	9.0		11.5	77.3	11.2	
Missing	69																				
Ever Smoked?					0.900				0.081**				0.220				0.619				0.457
Yes	585	9.9	79.7	10.4		12.1	78.5	9.4		11.5	77.8	10.8		10.4	78.8	10.8		9.4	79.5	11.1	
No	948	10.0	80.3	9.7		8.6	81.0	10.3		9.2	81.3	9.5		9.7	80.8	9.5		10.3	80.4	9.3	
Pre-pregnancy BMI					0.365				0.858				0.635				0.855				0.422
Underweight (<18.5)	84	4.8	81	14.3		10.7	82.1	7.1		6	88.1	6		8.3	83.3	8.3		6	83.3	10.7	
Normal (18.5-24.9)	896	10.7	78.9	10.4		9.4	80.1	10.5		10.3	79.7	10		9.9	80.1	9.9		9.2	80.9	9.9	
Overweight (25-29.9)	333	9	82.9	8.1		11.4	78.4	10.2		9.6	80.2	10.2		11.7	78.4	9.9		11.4	77.5	11.1	
Obese (≥30)	220	10.5	80	9.5		10	81.4	8.6		11.4	77.7	10.9		8.2	80.9	10.9		12.7	79.1	8.2	
PROM					0.066**				0.046*				0.673				0.211				0.224
Yes	382	8.9	78.3	12.8		12.3	80.4	7.3		10.7	78.5	10.7		8.1	80.1	11.8		12.3	78.5	9.2	
No	1095	10.5	80.6	8.9		9	80.4	10.6		9.6	80.6	9.8		10.4	80.2	9.4		9.2	81.1	9.7	
Missing	56																				
Parity					0.557				0.055**				0.020*				0.412				0.361
0	682	11.1	79.5	9.4		8.4	79.9	11.7		8.5	78.6	12.9		10.3	78.7	11.0		10.4	81.4	8.2	
1	618	9.9	80.1	10.0		11.5	81.2	7.3		10.8	81.9	7.3		10.4	79.8	9.9		9.7	79.8	10.5	
2	175	6.9	80.6	12.6		10.3	76.6	13.1		12.0	78.9	9.1		8.0	86.3	5.7		9.1	76.6	14.3	
3+	58	6.9	84.5	8.6		12.1	79.3	8.6		13.8	79.3	6.9		8.6	79.3	12.1		10.3	77.6	12.1	
Baby Sex					0.534				0.925				0.977				0.164				0.449
Male	809	10.8	79.1	10.1		10.0	79.7	10.3		9.9	80.1	10.0		8.7	81.6	9.8		10.8	78.9	10.4	
Female	724	9.1	81.1	9.8		9.9	80.4	9.7		10.2	79.8	9.9		11.5	78.3	10.2		9.1	81.4	9.5	

* p<0.05

** p< 0.1

TABLE 9. FIRST TRIMESTER GEOMETRIC MEAN BLOOD CONCENTRATION OF METALS (NMOL/L) ACCORDING TO CATEGORIES OF THIRD TRIMESTER PLASMA MMPs (PG/ML) (MIREC STUDY, 2008–2011) (N=1533).

Metals (nmol/L)	%>LOD ^a	MMP-1			MMP-2		
		≤10%	10-90%	≥90%	≤10%	10-90%	≥90%
Arsenic	92.8	9.595 (8.253)	9.887 (19.796)	10.027 (37.214)	11.020 (24.928)	9.801 (22.053)	9.365 (8.289)
Cadmium	97.4	1.639 (2.050)	1.891 (3.937)	1.880 (4.029)	1.851 (3.981)	1.879 (3.961)	1.751 (1.736)
Mercury	90.3	2.645 (4.079)	3.058 (5.034)	3.392 (6.700)	2.921 (3.997)	3.048 (5.325)	3.152 (4.735)
Manganese	100	157.580 (61.391)	160.561 (56.538)	164.812 (61.158)	163.426 (52.947)	161.302 (58.094)	153.159 (56.835)
Lead	100	26.805 (14.232)	30.385 (19.039)	30.056 (16.237)	31.312 (21.849)	29.759 (17.386)	30.408 (22.094)
		MMP-7			MMP-9		
		≤10%	10-90%	≥90%	≤10%	10-90%	≥90%
Arsenic	92.8	9.372 (7.854)	10.038 (20.181)	9.093 (35.642)	9.840 (9.304)	9.921 (22.997)	9.505 (16.251)
Cadmium	97.4	1.785 (4.152)	1.860 (3.751)	1.966 (3.859)	1.935 (2.406)	1.841 (3.879)	1.968 (4.291)
Mercury	90.3	2.898 (5.044)	3.097 (5.293)	2.797 (3.948)	3.383 (6.406)	3.054 (4.868)	2.678 (5.885)
Manganese	100	158.110 (50.576)	161.731 (58.342)	154.996 (57.057)	164.014 (58.983)	161.093 (57.931)	154.207 (52.036)
Lead	100	28.703 (20.491)	30.150 (18.219)	29.886 (17.632)	30.573 (18.139)	30.003 (18.902)	29.169 (13.961)
		MMP-10					
		≤10%	10-90%	≥90%			
Arsenic	92.8	10.428 (10.845)	10.007 (23.387)	8.374 (9.017)			
Cadmium	97.4	1.879 (3.822)	1.844 (3.779)	2.006 (3.986)			
Mercury	90.3	2.835 (5.774)	3.073 (5.074)	3.041 (5.125)			
Manganese	100	167.506 (61.437)	160.968 (58.197)	151.931 (45.277)			
Lead	100	28.801 (15.416)	29.981 (18.715)	31.145 (18.484)			

^a Percent greater than level of detection (LOD) based on first trimester measurements (arsenic=0.22µg/L, cadmium=0.04µg/L, mercury=0.12µg/L, manganese=0.05µg/L, lead=1.0µg/dL).

TABLE 10. UNADJUSTED ODDS RATIOS FOR HIGH ($\geq 90\%$) AND LOW ($\leq 10\%$) THIRD TRIMESTER PLASMA MMPs (pg/mL) AND FIRST TRIMESTER MATERNAL BLOOD METAL CONCENTRATIONS IN QUANTILES WITH 95% CONFIDENCE INTERVALS (MIREC STUDY, 2008-2011) (N=1533).⁺

Metals (nmol/L)	Low MMP-1 OR (95% CI)	High MMP-1 OR (95% CI)	Low MMP-2 OR (95% CI)	High MMP-2 OR (95% CI)	Low MMP-7 OR (95% CI)	High MMP-7 OR (95% CI)	Low MMP-9 OR (95% CI)	High MMP-9 OR (95% CI)	Low MMP-10 OR (95% CI)	High MMP-10 OR (95% CI)
<i>Arsenic</i>										
<7.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
7.0-11.0	0.92 (0.59-1.46)	1.16 (0.73-1.87)	2.33 (1.39-3.90)*	1.29 (0.83-1.99)	1.24 (0.80-1.93)	1.05 (0.67-1.65)	0.85 (0.54-1.35)	0.99 (0.63-1.55)	1.01 (0.64-1.60)	0.78 (0.51-1.20)
11.0-16.0	0.97 (0.60-1.48)	1.18 (0.71-1.95)	1.95 (1.13-3.38)*	0.68 (0.40-1.16)	0.61 (0.35-1.05)	0.83 (0.51-1.37)	0.92 (0.56-1.51)	1.05 (0.65-1.70)	1.04 (0.64-1.70)	0.58 (0.35-0.95)*
>16.0	0.99 (0.59-1.69)	1.13 (0.66-1.94)	1.78 (0.98-3.23)	0.83 (0.49-1.43)	1.04 (0.62-1.76)	0.83 (0.48-1.43)	0.92 (0.54-1.55)	0.72 (0.41-1.27)	0.91 (0.53-1.57)	0.52 (0.30-0.90)*
<i>Cadmium</i>										
<1.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.2-1.8	0.82 (0.52-1.29)	1.29 (0.81-2.05)	0.77 (0.49-1.23)	0.73 (0.45-1.19)	0.76 (0.48-1.21)	0.90 (0.56-1.45)	1.13 (0.70-1.83)	1.04 (0.65-1.67)	1.22 (0.77-1.94)	0.74 (0.45-1.23)
1.8-2.7	0.75 (0.46-1.22)	1.01 (0.61-1.68)	0.73 (0.45-1.20)	1.02 (0.64-1.63)	0.93 (0.58-1.49)	0.88 (0.53-1.47)	1.23 (0.75-2.02)	1.05 (0.64-1.72)	1.13 (0.69-1.85)	1.28 (0.80-2.05)
>2.7	0.80 (0.49-1.29)	1.08 (0.66-1.77)	0.81 (0.50-1.30)	0.90 (0.55-1.45)	0.79 (0.49-1.29)	1.32 (0.83-2.09)	1.33 (0.82-2.16)	1.23 (0.77-1.99)	1.06 (0.64-1.73)	1.05 (0.65-1.70)
<i>Mercury</i>										
<1.7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.7-3.5	1.22 (0.77-1.95)	0.84 (0.51-1.41)	1.26 (0.78-2.04)	1.43 (0.89-2.31)	0.77 (0.47-1.24)	0.84 (0.52-1.37)	1.03 (0.62-1.70)	1.54 (0.98-2.44)	1.07 (0.67-1.71)	1.17 (0.72-1.91)
3.5-6.7	1.16 (0.72-1.89)	1.10 (0.68-1.79)	0.95 (0.57-1.58)	1.08 (0.65-1.81)	0.85 (0.52-1.38)	1.00 (0.62-1.62)	1.04 (0.63-1.71)	1.00 (0.61-1.66)	0.85 (0.52-1.40)	1.13 (0.69-1.86)
>6.7	0.80 (0.46-1.38)	1.06 (0.64-1.77)	0.92 (0.54-1.56)	1.32 (0.79-2.22)	0.87 (0.53-1.44)	0.90 (0.54-1.51)	1.21 (0.73-2.02)	0.89 (0.52-1.53)	0.87 (0.52-1.47)	1.31 (0.78-2.18)
<i>Lead</i>										
<21	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
21-30	0.81 (0.52-1.27)	1.04 (0.64-1.70)	1.11 (0.69-1.79)	0.87 (0.54-1.39)	0.94 (0.58-1.50)	0.73 (0.45-1.18)	1.09 (0.68-1.75)	1.69 (1.05-2.73)*	0.88 (0.55-1.40)	0.94 (0.58-1.54)
30-41	0.87 (0.55-1.40)	1.31 (0.79-2.14)	0.77 (0.45-1.31)	0.79 (0.48-1.30)	1.23 (0.76-1.98)	0.93 (0.57-1.52)	1.03 (0.62-1.70)	1.52 (0.91-2.53)	1.03 (0.64-1.66)	1.11 (0.67-1.84)
>41	0.47 (0.27-0.83)*	0.99 (0.58-1.67)	1.29 (0.78-2.14)	0.98 (0.60-1.63)	0.78 (0.45-1.33)	0.87 (0.53-1.44)	0.89 (0.53-1.50)	0.99 (0.56-1.74)	0.74 (0.43-1.25)	1.29 (0.78-2.13)
<i>Manganese</i>										
<130	1.02 (0.64-1.62)	0.91 (0.55-1.49)	0.77 (0.46-1.28)	1.14 (0.72-1.80)	0.97 (0.60-1.57)	0.98 (0.62-1.54)	0.89 (0.54-1.45)	1.18 (0.74-1.87)	0.59 (0.35-1.01)	1.07 (0.69-1.68)
130-160**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
160-200	0.83 (0.53-1.31)	0.88 (0.56-1.38)	1.31 (0.85-1.99)	1.13 (0.74-1.75)	0.95 (0.61-1.46)	0.66 (0.42-1.04)	0.92 (0.59-1.43)	1.00 (0.64-1.55)	0.80 (0.51-1.24)	0.77 (0.50-1.21)
>200	1.03 (0.64-1.66)	1.18 (0.75-1.86)	0.86 (0.53-1.40)	0.65 (0.38-1.10)	0.80 (0.49-1.31)	0.71 (0.44-1.15)	0.96 (0.61-1.53)	0.80 (0.48-1.32)	1.11 (0.71-1.72)	0.58 (0.34-0.98)*

⁺ Odd ratios are relative to the group including percentiles from 10th to 90th of MMPs

* p<0.05

** The second quartile was used as the reference base since manganese is a nutrient and a priori knowledge that adverse effects of manganese can be observed at both low and high levels.

TABLE 11. ADJUSTED ODDS RATIOS OF HIGH ($\geq 90\%$) AND LOW ($\leq 10\%$) THIRD TRIMESTER PLASMA MMPs (pg/mL) AND FIRST TRIMESTER MATERNAL BLOOD METAL CONCENTRATIONS IN QUANTILES WITH 95% CONFIDENCE INTERVALS (MIREC STUDY, 2008-2011) (N=1533).⁺

Metal (nmol/L)	Low MMP-1^a OR (95% CI)	High MMP-1^a OR (95% CI)	Low MMP-2^b OR (95% CI)	High MMP-2^b OR (95% CI)	Low MMP-7^c OR (95% CI)	High MMP-7^c OR (95% CI)	Low MMP-9^c OR (95% CI)	High MMP-9^c OR (95% CI)	Low MMP-10^d OR (95% CI)	High MMP-10^d OR (95% CI)
<i>Arsenic</i>										
<7.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
7.0-11.0	0.85 (0.53-1.36)	1.18 (0.72-1.93)	2.20 (1.30-3.73)*	1.32 (0.84-2.07)	1.26 (0.80-1.98)	1.14 (0.71-1.82)	0.92 (0.57-1.48)	1.02 (0.64-1.62)	1.07 (0.66-1.72)	0.76 (0.49-1.18)
11.0-16.0	0.92 (0.55-1.54)	1.22 (0.72-2.09)	1.68 (0.95-2.98)	0.67 (0.38-1.16)	0.58 (0.33-1.03)	0.90 (0.53-1.51)	0.91 (0.54-1.52)	1.00 (0.60-1.65)	1.17 (0.70-1.97)	0.59 (0.35-0.99)*
>16.0	1.12 (0.63-1.99)	1.21 (0.67-2.18)	1.66 (0.88-3.13)	0.92 (0.51-1.65)	1.13 (0.63-2.01)	0.93 (0.51-1.68)	0.91 (0.51-1.61)	0.70 (0.38-1.30)	1.29 (0.72-2.33)	0.61 (0.34-1.07)
<i>Cadmium</i>										
<1.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.2-1.8	1.00 (0.62-1.62)	1.26 (0.76-2.08)	0.78 (0.48-1.28)	0.70 (0.42-1.17)	0.90 (0.55-1.47)	0.88 (0.52-1.47)	1.15 (0.69-1.91)	1.02 (0.62-1.67)	1.42 (0.87-2.32)	0.85 (0.50-1.44)
1.8-2.7	1.03 (0.58-1.81)	0.94 (0.52-1.70)	0.81 (0.46-1.44)	0.88 (0.50-1.55)	1.25 (0.72-2.18)	0.90 (0.49-1.65)	1.22 (0.68-2.17)	1.02 (0.58-1.81)	1.44 (0.81-2.56)	1.63 (0.95-2.81)
>2.7	0.93 (0.49-1.78)	1.19 (0.63-2.26)	0.75 (0.40-1.42)	0.86 (0.46-1.62)	1.39 (0.74-2.62)	1.32 (0.70-2.49)	1.31 (0.69-2.46)	1.21 (0.65-2.27)	1.03 (0.53-2.01)	1.59 (0.85-2.97)
<i>Mercury</i>										
<1.7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.7-3.5	1.39 (0.82-2.38)	0.92 (0.51-1.64)	1.14 (0.65-1.99)	1.31 (0.76-2.26)	0.69 (0.40-1.20)	0.60 (0.34-1.05)	1.03 (0.59-1.81)	1.92 (1.14-3.23)*	1.32 (0.77-2.27)	1.02 (0.59-1.77)
3.5-6.7	1.26 (0.69-2.29)	1.19 (0.65-2.19)	0.84 (0.45-1.55)	0.95 (0.51-1.75)	0.76 (0.42-1.37)	0.70 (0.39-1.25)	1.01 (0.55-1.86)	1.31 (0.71-2.40)	1.04 (0.56-1.92)	0.96 (0.53-1.74)
>6.7	0.81 (0.39-1.68)	1.22 (0.60-2.45)	0.75 (0.37-1.51)	1.07 (0.54-2.12)	0.67 (0.34-1.32)	0.54 (0.28-1.06)	1.00 (0.50-1.99)	1.37 (0.67-2.78)	1.02 (0.50-2.07)	1.15 (0.60-2.23)
<i>Lead</i>										
<21	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
21-30	0.83 (0.50-1.38)	1.09 (0.62-1.90)	1.17 (0.69-1.99)	0.81 (0.48-1.37)	0.96 (0.57-1.63)	0.57 (0.34-0.97)*	1.10 (0.65-1.86)	2.02 (1.19-3.44)*	0.92 (0.55-1.56)	0.86 (0.51-1.47)
30-41	0.90 (0.50-1.64)	1.48 (0.79-2.79)	1.08 (0.57-2.06)	0.82 (0.45-1.53)	1.48 (0.82-2.69)	0.60 (0.32-1.10)	0.93 (0.49-1.77)	2.20 (1.17-4.12)*	1.28 (0.70-2.34)	1.01 (0.55-1.87)
>41	0.54 (0.25-1.16)	1.00 (0.47-2.12)	2.12 (1.03-4.37)*	1.04 (0.49-2.19)	1.35 (0.64-2.84)	0.51 (0.24-1.07)	0.85 (0.40-1.78)	1.99 (0.93-4.26)	1.32 (0.63-2.76)	1.09 (0.53-2.26)
<i>Manganese</i>										
<130	1.05 (0.63-1.76)	0.94 (0.54-1.64)	0.74 (0.42-1.32)	0.98 (0.58-1.65)	1.06 (0.62-1.80)	0.82 (0.49-1.38)	0.93 (0.53-1.62)	1.31 (0.78-2.21)	0.60 (0.33-1.07)	1.10 (0.67-1.80)
130-160**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
160-200	0.99 (0.61-1.62)	0.93 (0.57-1.52)	1.18 (0.75-1.86)	1.23 (0.77-1.97)	0.86 (0.54-1.38)	0.68 (0.41-1.13)	0.80 (0.49-1.28)	1.06 (0.66-1.71)	0.76 (0.47-1.23)	0.80 (0.50-1.27)
>200	1.17 (0.65-2.12)	1.19 (0.68-2.10)	0.69 (0.39-1.23)	0.80 (0.43-1.50)	0.58 (0.32-1.04)	0.94 (0.52-1.69)	0.93 (0.40-1.62)	0.86 (0.47-1.56)	0.77 (0.45-1.32)	0.60 (0.32-1.12)

⁺ Odd ratios are relative to the group including percentiles from 10th to 90th of MMPs

^a Adjusted for 2nd trimester weight (kg), 2nd trimester systolic blood pressure and 2nd trimester diastolic blood pressure.

^b Adjusted for 2nd trimester weight (kg) and maternal diabetic status.

^c Adjusted for 2nd trimester weight (kg).

^d Adjusted for 1st trimester weight (kg) and pre-pregnancy BMI.

* p<0.05

** The second quartile was used as the reference base since manganese is a nutrient and a priori knowledge that adverse effects of manganese can be observed at both low and high levels.

TABLE 12. UNADJUSTED ODDS RATIOS OF HIGH ($\geq 90\%$) AND LOW ($\leq 10\%$) THIRD TRIMESTER PLASMA MMPs (pg/mL) AND THIRD TRIMESTER MATERNAL BLOOD METAL CONCENTRATIONS IN QUANTILES WITH 95% CONFIDENCE INTERVALS (MIREC STUDY, 2008-2011) (N=1533).⁺

Metals (nmol/L)	Low MMP-1 OR (95% CI)	High MMP-1 OR (95% CI)	Low MMP-2 OR (95% CI)	High MMP-2 OR (95% CI)	Low MMP-7 OR (95% CI)	High MMP-7 OR (95% CI)	Low MMP-9 OR (95% CI)	High MMP-9 OR (95% CI)	Low MMP-10 OR (95% CI)	High MMP-10 OR (95% CI)
<i>Arsenic</i>										
<5.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
5.5-9.2	1.34 (0.85-2.14)	1.33 (0.82-2.14)	1.23 (0.73-2.06)	0.96 (0.61-1.52)	0.80 (0.50-1.28)	0.97 (0.62-1.52)	0.90 (0.56-1.45)	1.54 (0.95-2.49)	1.19 (0.76-1.85)	0.83 (0.52-1.32)
9.2-15.0	1.26 (0.79-2.03)	1.22 (0.75-1.98)	1.62 (0.98-2.67)	0.89 (0.56-1.43)	0.79 (0.49-1.26)	0.70 (0.43-1.12)	0.63 (0.38-1.04)	1.46 (0.89-2.38)	0.65 (0.39-1.07)	0.95 (0.61-1.50)
>15.0	0.73 (0.42-1.28)	0.93 (0.54-1.59)	1.56 (0.92-2.66)	0.69 (0.40-1.17)	0.72 (0.43-1.19)	0.53 (0.31-0.91)*	1.08 (0.66-1.76)	1.36 (0.79-2.36)	0.66 (0.39-1.11)	0.51 (0.29-0.88)*
<i>Cadmium</i>										
<1.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1.2-1.7	1.09 (0.70-1.71)	1.07 (0.66-1.72)	1.74 (1.09-2.77)*	1.47 (0.91-2.38)	1.00 (0.64-1.56)	1.06 (0.64-1.76)	0.75 (0.45-1.24)	1.09 (0.68-1.74)	0.78 (0.49-1.25)	0.90 (0.56-1.44)
1.7-2.6	0.57 (0.34-0.94)*	0.94 (0.59-1.51)	0.83 (0.49-1.39)	1.20 (0.74-1.94)	0.78 (0.49-1.23)	1.16 (0.71-1.88)	0.97 (0.61-1.55)	0.96 (0.59-1.55)	0.56 (0.35-0.92)*	0.76 (0.47-1.23)
>2.6	0.88 (0.54-1.43)	0.89 (0.54-1.47)	1.30 (0.78-2.16)	1.31 (0.79-2.17)	0.54 (0.32-0.93)*	1.43 (0.88-2.34)	1.08 (0.67-1.77)	1.12 (0.72-1.96)	0.86 (0.53-1.40)	0.91 (0.56-1.48)
<i>Mercury</i>										
<1.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1.3-2.8	0.96 (0.61-1.53)	0.89 (0.55-1.45)	1.12 (0.64-1.81)	1.53 (0.96-2.45)	0.99 (0.62-1.59)	1.36 (0.84-2.22)	0.80 (0.49-1.31)	0.98 (0.63-1.53)	0.91 (0.57-1.45)	1.25 (0.77-2.01)
2.8-5.05	0.95 (0.57-1.58)	1.18 (0.72-1.91)	0.91 (0.54-1.52)	1.13 (0.66-1.91)	1.00 (0.60-1.65)	1.46 (0.87-2.44)	1.11 (0.67-1.82)	0.83 (0.51-1.37)	0.89 (0.54-1.47)	1.32 (0.80-2.18)
>5.05	1.16 (0.70-1.93)	0.99 (0.59-1.66)	1.01 (0.64-1.79)	1.38 (0.81-2.34)	1.30 (0.79-2.16)	1.54 (0.91-2.62)	1.06 (0.64-1.76)	0.64 (0.37-1.10)	1.09 (0.66-1.79)	1.09 (0.64-1.86)
<i>Lead</i>										
<20.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
20.0-27.0	0.75 (0.48-1.18)	0.86 (0.53-1.40)	1.20 (0.75-1.91)	1.19 (0.75-1.90)	1.34 (0.85-2.12)	1.24 (0.77-2.01)	0.88 (0.54-1.44)	1.02 (0.65-1.60)	1.22 (0.78-1.93)	1.61 (0.99-2.61)
27.0-38.5	0.86 (0.54-1.39)	0.96 (0.57-1.60)	0.77 (0.45-1.31)	0.87 (0.52-1.46)	1.17 (0.71-1.93)	1.14 (0.68-1.92)	1.12 (0.68-1.84)	1.15 (0.72-1.85)	0.97 (0.59-1.61)	1.02 (0.58-1.77)
>38.5	0.53 (0.31-0.91)*	1.09 (0.66-1.80)	0.90 (0.53-1.52)	0.97 (0.57-1.62)	0.70 (0.40-1.24)	1.04 (0.61-1.76)	0.89 (0.53-1.50)	0.49 (0.27-0.87)*	0.68 (0.39-1.18)	1.64 (0.97-2.77)
<i>Manganese</i>										
<180	0.94 (0.60-1.47)	1.00 (0.62-1.61)	1.06 (0.65-1.74)	1.10 (0.70-1.71)	1.04 (0.65-1.67)	0.95 (0.61-1.48)	1.09 (0.66-1.79)	0.79 (0.79-1.94)	0.78 (0.48-1.29)	1.01 (0.65-1.57)
180-230**	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
230-280	0.68 (0.42-1.11)	0.87 (0.54-1.40)	1.44 (0.92-2.26)	0.93 (0.59-1.47)	0.98 (0.61-1.57)	0.61 (0.38-0.99)*	1.71 (1.09-2.67)*	0.76 (0.76-1.89)	0.76 (0.46-1.25)	0.75 (0.47-1.20)
>280	0.87 (0.54-1.40)	1.19 (0.76-1.87)	1.08 (0.67-1.75)	0.64 (0.39-1.06)	1.18 (0.74-1.87)	0.65 (0.40-1.04)	1.12 (0.69-1.81)	0.46 (0.46-1.29)	1.55 (1.00-2.41)*♦	0.70 (0.43-1.15)

⁺ Odd ratios are relative to the group including percentiles from 10th to 90th of MMPs

* p< 0.05

** The second quartile was used as the reference base since manganese is a nutrient and a priori knowledge that adverse effects of manganese can be observed at both low and high levels.

♦ Lower limit of confidence interval is 1.004

TABLE 13. ADJUSTED ODDS RATIOS OF HIGH ($\geq 90\%$) AND LOW ($\leq 10\%$) THIRD TRIMESTER PLASMA MMPs (pg/mL) AND THIRD TRIMESTER MATERNAL BLOOD METAL CONCENTRATIONS IN QUANTILES WITH 95% CONFIDENCE INTERVALS (MIREC STUDY, 2008-2011) (N=1533).⁺

Metal (nmol/L)	Low MMP-1 ^a OR (95% CI)	High MMP-1 ^a OR (95% CI)	Low MMP-2 ^b OR (95% CI)	High MMP-2 ^b OR (95% CI)	Low MMP-7 ^c OR (95% CI)	High MMP-7 ^c OR (95% CI)	Low MMP-9 ^c OR (95% CI)	High MMP-9 ^c OR (95% CI)	Low MMP-10 ^d OR (95% CI)	High MMP-10 ^d OR (95% CI)
<i>Arsenic</i>										
<5.5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.5-9.2	1.28 (0.79-2.07)	1.27 (0.77-2.09)	1.32 (0.77-2.25)	1.07 (0.67-1.72)	0.93 (0.57-1.51)	1.00 (0.62-1.60)	0.95 (0.58-1.54)	1.54 (0.94-2.53)	1.13 (0.71-1.78)	0.90 (0.55-1.45)
9.2-15.0	1.29 (0.78-2.14)	1.13 (0.68-1.89)	1.56 (0.92-2.63)	0.94 (0.57-1.53)	0.92 (0.56-1.53)	0.73 (0.44-1.21)	0.62 (0.36-1.05)	1.38 (0.83-2.30)	0.63 (0.37-1.06)	1.04 (0.65-1.66)
>15.0	0.75 (0.41-1.37)	0.87 (0.48-1.56)	1.64 (0.92-2.91)	0.74 (0.41-1.31)	0.92 (0.53-1.60)	0.53 (0.29-0.96)*	1.19 (0.70-2.01)	1.51 (0.85-2.70)	0.64 (0.36-1.13)	0.59 (0.33-1.05)
<i>Cadmium</i>										
<1.2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.2-1.7	1.21 (0.76-1.95)	1.08 (0.65-1.81)	1.99 (1.22-3.24)*	1.44 (0.87-2.40)	1.06 (0.66-1.70)	1.02 (0.60-1.74)	0.69 (0.41-1.18)	1.22 (0.75-1.99)	0.76 (0.46-1.25)	0.84 (0.51-1.38)
1.7-2.6	0.63 (0.35-1.13)	1.00 (0.57-1.75)	1.05 (0.58-1.91)	1.18 (0.67-2.08)	0.69 (0.40-1.20)	1.03 (0.58-1.83)	0.94 (0.55-1.60)	1.04 (0.59-1.82)	0.53 (0.30-0.92)*	0.59 (0.34-1.02)
>2.6	1.04 (0.54-1.99)	0.86 (0.44-1.67)	1.60 (0.82-3.11)	1.29 (0.68-2.48)	0.44 (0.22-0.87)*	1.16 (0.61-2.21)	0.90 (0.48-1.70)	1.05 (0.55-2.02)	0.92 (0.49-1.74)	0.57 (0.30-1.08)
<i>Mercury</i>										
<1.3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.3-2.8	0.80 (0.47-1.37)	0.75 (0.42-1.33)	1.10 (0.63-1.91)	1.54 (0.89-2.67)	1.28 (0.74-2.20)	1.83 (1.04-3.21)*	0.78 (0.44-1.37)	0.85 (0.51-1.41)	0.87 (0.51-1.48)	1.39 (0.81-2.39)
2.8-5.05	0.85 (0.46-1.58)	0.97 (0.52-1.80)	1.06 (0.56-2.02)	1.17 (0.61-2.21)	1.33 (0.70-2.50)	1.93 (1.02-3.66)*	1.07 (0.58-1.97)	0.74 (0.41-1.36)	0.88 (0.47-1.64)	1.40 (0.76-2.58)
>5.05	1.26 (0.65-2.47)	0.61 (0.30-1.26)	1.36 (0.68-2.71)	1.39 (0.69-2.79)	1.88 (0.95-3.73)	2.33 (1.16-4.71)*	0.95 (0.47-1.89)	0.55 (0.27-1.11)	1.18 (0.60-2.33)	1.15 (0.58-2.27)
<i>Lead</i>										
<20.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
20.0-27.0	0.81 (0.49-1.34)	0.75 (0.43-1.29)	1.23 (0.73-2.06)	1.29 (0.77-2.15)	1.32 (0.79-2.20)	1.58 (0.92-2.70)	0.83 (0.48-1.42)	0.76 (0.46-1.26)	1.29 (0.76-2.16)	1.63 (0.96-2.76)
27.0-38.5	1.08 (0.60-1.97)	0.79 (0.41-1.51)	0.66 (0.34-1.25)	0.88 (0.46-1.68)	0.95 (0.51-1.77)	1.53 (0.79-2.93)	1.16 (0.63-2.17)	0.78 (0.44-1.41)	0.83 (0.44-1.57)	1.00 (0.51-1.95)
>38.5	0.78 (0.37-1.67)	1.08 (0.52-2.23)	0.54 (0.26-1.14)	0.95 (0.45-2.03)	0.53 (0.25-1.13)	1.76 (0.83-3.74)	0.99 (0.48-2.06)	0.33 (0.15-0.71)*	0.54 (0.25-1.16)	1.47 (0.70-3.08)
<i>Manganese</i>										
<180	0.93 (0.57-1.54)	1.12 (0.66-1.93)	1.14 (0.66-1.97)	1.29 (0.78-2.13)	0.95 (0.56-1.59)	1.16 (0.70-1.93)	1.14 (0.65-1.99)	1.02 (0.61-1.71)	0.82 (0.48-1.42)	0.90 (0.55-1.46)
180-230**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
230-280	0.63 (0.37-1.07)	0.90 (0.54-1.51)	1.47 (0.91-2.39)	1.00 (0.61-1.64)	1.20 (0.72-1.99)	0.74 (0.44-1.25)	1.86 (1.15-3.01)*	1.22 (0.75-1.99)	0.72 (0.42-1.22)	0.85 (0.52-1.41)
>280	0.73 (0.40-1.32)	1.09 (0.61-1.92)	1.25 (0.71-2.20)	0.79 (0.43-1.44)	1.52 (0.86-2.67)	0.74 (0.41-1.34)	1.14 (0.62-2.00)	0.80 (0.43-1.48)	1.54 (0.91-2.62)	0.87 (0.49-1.57)

⁺ Odd ratios are relative to the group including percentiles from 10th to 90th of MMPs

^a Adjusted for 2nd trimester weight (kg), 2nd trimester systolic blood pressure and 2nd trimester diastolic blood pressure.

^b Adjusted for 2nd trimester weight (kg) and maternal diabetic status.

^c Adjusted for 2nd trimester weight (kg).

^d Adjusted for 1st trimester weight (kg) and pre-pregnancy BMI.

* p< 0.05

** The second quartile was used as the reference base since manganese is a nutrient and a priori knowledge that adverse effects of manganese can be observed at both low and high levels.

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CHAPTER 5: MATERNAL BLOOD BIOMARKERS AND ADVERSE PREGNANCY OUTCOMES – A SYSTEMATIC LITERATURE REVIEW AND META-ANALYSIS

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Running Title: Maternal Blood Biomarkers and Pregnancy

5.1 Abstract

Background: Pregnancy is a vulnerable period and environmental chemical exposures in utero can influence neonatal morbidity and mortality. There is a momentum towards understanding and exploring the current maternal biological mechanisms specific to in utero effects, to improve birth outcomes. Currently, this study aims at exploring the current status of understanding on biomarker changes that may be associated with pregnancy term, infant birth weights and infant development in utero. **Methods:** Electronic searches were conducted through PubMed, Embase, OvidMD, and Scopus databases to retrieve only articles with full text articles in English. Studies were selected if they evaluated maternal blood plasma/serum biomarkers proposed to influence adverse birth outcomes in the neonate. Data was extracted on characteristics, quality and odds

ratios from each study and synthesized with meta-analysis. **Results:** A total of 54 studies (35 for meta-analysis), including 43,702 women and 50 biomarkers, were included in the present study. The random effect point estimates were, 1.61(95%CI:1.39-1.85, $p<0.0001$) for inflammatory-related biomarkers, 1.65(95%CI:1.22-2.25, $p=0.0013$) for growth factor-related and hormone-related biomarkers. All subgroups of biomarkers showed significant associations with adverse birth outcomes with no apparent publication bias. **Conclusions:** The two subsets of plasma markers identified in this study (inflammatory-related and growth factor/hormone-related) may serve as potentially valuable tools in the investigation of maternal molecular mechanisms, especially select pathways underlying inflammatory and immunological mediation in terms of modulating adverse infant outcomes. Further large, prospective cohort studies are needed to evaluate promising biomarkers in plasma and other maternal biological samples such as cervico-vaginal fluid and urine. Generation of high content proteomic, metabolic, epigenomic information can be useful in conducting systems biology analyses.

Keywords: Maternal blood biomarkers, preterm birth, infant birth weight, small for gestational age, pregnancy

5.2 Introduction

Perinatal health outcomes are considered important markers of both child and adult health.¹⁻³ Although not yet conclusive, a number of studies have begun to identify several harmful factors associated with negatively affecting pregnancy outcomes as pregnancy is a very sensitive period with respect to human development. Alcohol-consumption and smoking during pregnancy are just a few of the many risk factors that have demonstrated strong correlations to several birth complications such as preterm birth (PTB), low birth weight (LBW), small for gestational age

(SGA) infants, intrauterine growth restriction (IUGR), and preeclampsia.⁴⁻¹²

Defined by Benford et al. (2000), biomarkers are parameters that can be measured in a biological sample and provide information on the level of exposure, or the exposure effects in an individual.¹³ The status of *biomarkers of effect* might reflect an earlier stage of a disease, which may be predictive of eventual disease, though such biomarkers are dependent upon the understanding of the aetiology of the disease in question.¹³ Maternal markers of oxidative stress and inflammation that lead to an increased risk for preterm birth and low infant birth weight outcomes are continuously being reported.^{14,15} Notwithstanding, there still is a gap in information on the mechanistic basis of maternal blood biomarkers and subsequent pregnancy outcomes.^{16,17}

The aetiology of birth, along with other adverse birth outcomes, still remains largely elusive, despite emerging evidence suggesting multiple underlying pathogenic pathways and factors affecting pregnancy outcomes. Albeit each pathway given for adverse birth outcome has unique genetic and environmental predispositions, many have been identified to be immunologically mediated or consist of inflammatory components.¹⁴ Predicting potentially negative birth outcomes is critical because it could allow for the identification of women at high risk, in whom, risk-specific interventions could be employed.¹⁸ The ability to predict cases may also help gain important insights into the maternal mechanistic drivers or biochemical pathways involved in affecting birth outcomes pertinent for the child's future health. Consequently, the study of maternal biomarkers has become a growing interest in improving the diagnosis and preventive interventions during pregnancy. The objective of this work, therefore, was to explore the nature of relationships between maternal blood biomarker levels to understand maternal biochemical changes and if, or how, they can the fetal development in utero.

Assessment of the quality of an infant's development typically considers two measures:

infant birth weight and gestational age at delivery. Low infant birth weight, defined as birth weight less than 2500g, has been associated with several chronic health consequences in adulthood such as obesity, diabetes and.¹⁹ Preterm birth (PTB) is defined as live birth before 37 weeks of gestation, compared to a normal term pregnancy, which is expected to last between 37-41 completed weeks.^{20,21} Low infant birth weight can result from either preterm birth or intra uterine growth restriction (IUGR), or a combination of the two. Small-for-gestational-age (SGA) is the most common surrogate measurement for IUGR and is defined generally as birth weight below the 10th percentile for gestational age in comparison with a reference population.^{22,23} Elevated circulating levels of endothelin-1, a vasoactive peptide, and high blood pressure (BP) in pregnant women have been linked to IUGR resulting in low infant birth weights.²⁴ Similarly, oxidative stress has also been reported to cause maternal and fetal morbidity and is implicated in preeclampsia leading to PTB.^{19,25} Previous work on environmental pollutants have suggested air contaminants as triggers of increased markers of oxidative stress and, as a result, environmental toxicants have also been associated to adverse pregnancy outcomes.²⁶

Prior to this study, there was no comprehensive synthesis of research on all maternal blood biomarkers and adverse infant birth outcomes. Through comprehensive synthesis and meta-analysis of relevant literature, this study aims to identify and confirm the associations between biomarkers in maternal blood and adverse infant birth outcomes such as low birth weight, preterm birth and small-for-gestational-age infants. Research on the relationship between maternal blood biomarkers and adverse infant health outcomes have continued to produce inconsistent results. In order to collectively analyze existing research on this topic and yield more reliable results, a quality systematic review and meta-analysis was conducted.

5.3 Methodology

This systematic review was conducted following a prospectively prepared protocol, and is reported using widely recommended guidelines for systematic reviews and meta-analyses.

5.3.1 Literature Search

The search strategy was devised with the aim of isolating observational and experimental studies that described the association between maternal blood biomarkers and the risk of adverse birth outcomes. An initial search was performed in PubMed using a combination of keywords and text words related to biomarkers ('cytokine', 'chemokine', 'inflammatory markers', 'interferon (IFN)', 'vascular endothelial growth factor (VEGF)', 'vascular cell adhesion molecule (VCAM)', 'intercellular adhesion molecule (ICAM)', 'tissue necrosis factor (TNF)', 'granulocyte macrophage colony stimulating factor (GM-CSF)', 'C-reactive protein (CRP)', 'matrix metalloproteinase (MMP)', 'monocyte chemoattractant protein (MCP-1)', 'macrophage inhibitory protein (MIP-1)', 'endothelin(ET)', 'interleukin(IL)') and birth outcomes ('preterm', 'birth weight', 'preterm birth', 'small for gestational age', 'SGA', 'gestational age'). These biomarkers were selected as previous research has linked PTB, LBW and SGA with inflammatory, hormonal and angiogenesis-related pathways.^{15,16}

Search terms, as well as inclusion and exclusion criteria, were used to isolate the most relevant studies (see **Table 14**). The inclusion criteria were selected to explore a lesser-known sample since research on maternal predictors of adverse birth outcome has previously been primarily focused on amniotic fluid and cervico-vaginal fluid. The exclusion criterion was chosen in a similar manner as our study aims to study the relationships between biomarkers and outcome rather than interventions or treatments. Studies were included if they identified biomarkers in maternal blood (whole blood, plasma or serum) and excluded if they assessed

biomarkers in other biological samples such as urine, cervico-vaginal fluid, saliva or if the study was focused on a therapy, treatment or therapeutics.

In the initial search, we chose those biomarkers identified in maternal blood (whole blood, plasma or serum) for the prediction of adverse birth outcomes such as preterm birth, low or high birth weight, and small or large for gestational age. Language restrictions were applied to include only English full text. Limitations were applied in the search strategy to include only human data, although animal data was drawn upon to formulate conceptual pathways linking biomarkers and outcome. The initial search criterion was developed using PubMed and when the criterion was operational, it was applied to other databases. A comprehensive search was then conducted using SCOPUS, ToxLine and OvidMD databases using keywords and text words for each of the biomarkers identified in the initial search and keywords and text words for adverse infant birth outcomes described previously.

The process used to identify and select studies is summarized in **Figure 2**. The searches produced 6159 citations, of which, 391 were considered relevant. After filtering out studies that lacked human data or did not have English full text available, we identified a total of 267 empirical studies of all study types (both observational and review studies). Upon reviewing the abstracts of the 267 selected articles, we further excluded 213 studies because there were no comparisons of biomarker levels and outcomes or the biomarker analysis was not conducted in maternal blood (whole blood, plasma, or serum) yielding a final sample size of 54 for systematic review (53 observational studies and 1 review article).

Selected articles were collected in RefWorks and categorized according to the type of study. The categories include observational studies (case-control and cohort studies) and reviews. Furthermore, studies that were referenced in the articles being reviewed were hand-searched for

potentially relevant studies that could be included to yield six additional studies. References for excluded studies can be obtained from the authors.

5.3.2 Study Selection

Titles and abstracts of all citations acquired from the electronic search were reviewed by one reviewer (FA) and full-text articles were then assessed by the same reviewer for inclusion and data extraction. A second independent reviewer (JG) then examined a 10% sample of the papers and any disagreements were resolved by discussion and consensus. In the case there were multiple or duplicate publications of the same data, we included only the most recent or complete study.

Studies were included if: (i) they were cohort, cross-sectional, case-control, or review studies that evaluated the biomarkers in relation to intrauterine growth restriction (IUGR), preterm birth, low birth weight or small-for-gestational age (SGA) infants in women at any level of risk (ii) the biological samples were collected from maternal blood (whole blood, serum or plasma); for the meta-analysis: (iii) if they allowed for the assessment of cases with higher biomarker levels and adverse birth outcomes. Studies were excluded if: (i) they were case series or reports, editorials, or comments; and for the meta-analyses, if (ii) biomarker data were reported only as mean or median values or (iii) they reported insufficient data on cases or control groups to compute pooled odds ratios. The biomarkers that were included in this study were selected based on previous evidence suggesting that inflammation and immunological biomarkers are key regulators of labour as well as infant growth and development.^{14-17,20,23,32-35}

A total of 54 studies were included for the systematic review (30 case-control, 22 cohort, 1 systematic review, 1 cross-sectional) and from the 54 studies, 35 studies (18 case-control and 17

cohort), evaluating a total of 50 biomarkers were identified for the meta-analysis to exclude 19 studies due to insufficient information pertaining to elevated biomarker levels and corresponding outcomes, or the lack of odds ratios or confidence intervals. The biomarkers related to the adverse birth outcomes in question for this review are listed in **Figure 3**.

5.3.3 Study Quality Assessment

The methodological quality of the included studies were assessed by at least one reviewer using a 15-point checklist (see supplemental material – **Table 19**) adapted from Downs and Black.²⁸ The quality of each study was determined based on external and internal validity, methodology, confounders and population demographics. Two authors (FA and JG) reviewed the articles and assigned a numerical value representative of its quality (**Table 15**). The scores were categorized into 4 groups. Studies with scores between 0 and 3 were classified as “poor quality” studies, 4-7 were deemed “low quality”, 8-11 were “medium quality”, and 12-15 were “good quality” studies. 43 studies were deemed medium quality and the remaining 11 were categorized as good quality studies and there were no studies in the poor quality. There were no major discrepancies between good and poor quality studies. Study quality was conducted in order to exclude poor and low quality studies, however, there were no studies that fell into those categories.

5.3.4 Data Extraction and Data Cleaning

Potentially relevant articles were obtained, and data was extracted in duplicates from all reports and recorded on a form designed independently by the reviewers. There was no blinding of authorship. The following information was extracted from each article: study characteristics (design and prospective or retrospective data collection); participants (sample size, country

where research was conducted and date of publication); description of the biomarkers (gestational age at sampling, and biological sample); and reference standard used (preterm definition, SGA definition, and LBW definition). Both data extraction and quality assessment were conducted individually and any differences of opinions or disagreements were discussed and resolved among authors

This study entails the analysis of data collected from multiple databases. All of the data that was extracted was imported from Microsoft Excel v.14.5.7 (Microsoft Office, Redmond, WA) into Comprehensive Meta-Analysis Software (CMA) v.2 (Biostat, Eaglewood, NJ).^{28,29}

5.3.5 Statistical Analysis

Extracted data from each study were arranged in CMA and odds ratios were analysed using Comprehensive Meta-Analysis Software v3 (Biostat, Inc., Englewood, NJ).²⁹ Meta-analyses were performed using subgroups of studies with similar biomarker families to minimize clinical heterogeneity. Pooled estimates with 95% confidence intervals were calculated using a multivariate, random-effects meta-regression model.

5.4 Results

Table 16 summarizes the main findings of this present study and highlights the studies included for meta-analysis as well as for the systematic review. Of the studies included for meta-analysis, eighteen studies were performed in European countries, thirteen in North America and the four remaining studies were conducted in other countries worldwide. The sample size in cohort studies ranged from 62 to 9,450. The number of case participants enrolled in case-control studies ranged from 29 to 3,539 and the corresponding number of controls ranged from 21 to 46,262.

Biomarkers in the following biological samples were included in the meta-analysis: maternal serum (24 studies) and maternal plasma (11 studies). Nineteen studies provided data on preterm birth before 37 weeks, 4 provided data on preterm birth before 34 weeks, 2 provided data on preterm birth before 33 weeks of gestation, and 4 provided data on preterm birth prior to 32 weeks. 5 studies provided data on small for gestational age infants, 1 study reported findings on low birth weight and 1 study reported findings on IUGR. Both groups of biomarkers exhibited high heterogeneity with all pooled I^2 values demonstrating high heterogeneity $I^2=91.941$ for inflammation-related biomarkers and $I^2=74.110$ for growth factor/hormone-related biomarkers. Subsequently, random effect point estimates were noted and individual odds ratios for biomarkers included in meta-analyses are reported in **Tables 17 and 18**.

5.4.1 Inflammation-Related Biomarkers

The findings of the inflammatory biomarkers and their odds ratios with 95% confidence intervals are reported in **Table 17**. A total of six studies evaluated IL-1 β ^{20,22,34,51,61,63}, five studies addressed IL-2^{22,24,32,51,64}, four addressed IL-12^{34,49,61,65}, three addressed IL-18^{33,49,61}, while the remaining IL-7 and IL-8 were addressed by six studies^{21,22,26,34,51,65}. Forty-one articles evaluated other markers of inflammation such as interferon-gamma^{24,26,32,34,49,56,61,65}, tumour necrosis factor-alpha^{20,22,23,24,32,37,45,49,51,61,63,66,67}, intercellular adhesion molecule-1^{27,39}, vascular cell adhesion molecule-1³⁹, C-reactive protein^{20,25,27,31,40,42,45,46,50,53,58,65,67,68,69,70,71,72}, granulocyte macrophage colony-stimulating factor^{24,32,42,48,52,73}, ferritin^{27,43,44}, and matrix metalloproteinase-8^{70,77}. Other inflammation-related biomarkers are also addressed by 9 studies evaluating biomarkers including, IL-1RA^{22,34,47}, transforming growth factor- α/β ^{49,61}, TNFR1^{21,22,49,62}, and matrix metalloproteinase-3/-7^{22,48} (**Table 17**). Nineteen studies addressed IL-6^{20,23,24,26,27,32,45,47,49,50,51,58,61,63,64,66,67,70,74}, ten addressed IL-10^{20,27,45,47,49,56,61,63,66,70}, and the

remaining five inflammatory related biomarkers were evaluated by eight studies^{21,22,34,49,51,56,64,65}. Chemokines, such as eotaxin were addressed by two studies^{34,39}, macrophage inflammatory protein-1 were evaluated by four studies^{21,22,39,49}, and monocyte chemotactic proteins were addressed by three studies^{21,34,49}. Tissue inhibiting metalloproteinases were addressed by three studies^{22,48,77}, while macrophage migration inhibiting factor was also addressed by three studies^{45,49,57}.

IL-1 β and IL-2 were reported by one study as a marker for PTB before 37 weeks with an odds ratio of 3.74 (95%CI:1.31-10.7) and 4.02 (95%CI:1.53-10.52) respectively.²² Three other studies found statistically non-significant associations regarding increased IL-2 and PTB with OR=1.13, 1.02, and 2.37^{24,32,51}. IL-12 was positively associated with SGA infants by one study with an odds ratio of 3.62 (95%CI:1.74-7.54)³⁴ while another study found no statistically significant association with PTB (OR=0.60, 95%CI:0.20-1.65)³³. Two studies found associations between elevated IFN- γ levels and PTB as well as SGA OR=1.36 (95%CI:1.03-1.81)²⁴ and OR=3.72 (95%CI:1.54-9.03)³⁴, respectively.

All three studies that addressed IL-1RA found statistically significant associations with elevated concentrations and PTB <37 weeks (OR=3.08, 95%CI:1.16-8.2; OR=2.55, 95%CI:1.24-5.24)^{22,47} as well as SGA (OR=5.32, 95%CI:2.40-11.79)³⁴. TNF-R1 was also significantly associated with PTB <37w with OR=6.42 (95%CI:2.24-18.41)²¹ and OR=3.70 (95%CI:1.60-8.50)⁴⁹. IL-6 was reported statistically significant to PTB by three studies with OR=1.29 (95%CI:1.07-1.56)²⁰, OR=1.27 (95%CI:1.02-1.59)⁵⁰, and OR=30.8 (95%CI:3.93-241.20)⁵¹. The six studies that addressed IL-6 found an association between elevated IL-6 in maternal plasma with increased risk of PTB, with ORs ranging from 1.15 to 30.80 although not all were statistically significant, except for the samples collected at delivery^{24,27,32,47,50}. Similarly, IL-10

was found to be statistically significant with respect to PTB <37w reporting ORs of 2.30 to 4.60. Two studies found elevated IFN- γ levels to be associated with PTB and SGA OR=1.36 (95%CI:1.03-1.81)²⁴ and OR=3.72 (95%CI:1.54-9.03)³⁴, respectively.

According to three studies, MMP-9 was significantly associated with PTB yielding OR=33.75 (95%CI:5.84-194.80)³⁸, OR=2.21 (95%CI:1.59-3.07)⁴⁸, and OR=6.00 (95%CI:2.90-12.70)⁴⁹. MMPs are particularly important during pregnancy and parturition, as they are key players in remodelling the extracellular matrix⁴⁸. During human development, MMPs play a critical role in during pregnancy^{4,5,24,41}. MMP-9 at higher levels was determined to be significantly associated with higher risk of all preterm births^{38,48,49,60,62,70,77}, but not with SGA or LBW^{58,76}.

The I² value for the inflammatory biomarkers was 91.941 demonstrates high heterogeneity. The random effect point estimate for pooled odds ratios was chosen 1.606 (95% CI: 1.391-1.853, p<0.0001). The results obtained from the meta-analysis confirm a significant association between inflammatory markers and adverse birth outcomes.

5.4.2 Growth Factor/Hormone-Related Biomarkers

The second group of biomarkers consist of a combination of cytokines, growth factors and hormones affecting pregnancy and parturition. Decreased PAPP-A concentrations were reported significant in all studies addressing this biomarker with respect to SGA and PTB^{41,55,53,54}. Similarly, SGA was found to be in strong association with lower levels of β -human chorionic gonadatropin^{41,53,54,55,75} reporting ORs 1.31 and 1.91. High maternal blood levels of CRH were found to be positively associated with PTB^{27,45}, but only one study found statistical significance OR=3.2 (95%CI:1.7-6.2)⁴⁵. VEGF and ANGPT2 were associated strongly with PTB before 37w,

given ORs 3.83 and 0.32, respectively. With an I^2 value of 74.110, the random point estimate for growth factor/hormone-related biomarkers was OR=1.654 (95% CI:1.216-2.251, $p=0.0013$).

5.5 Discussion

One of the most well established causes of preterm birth is attributed to inflammation at the maternal-fetal interface.²⁰ Pro-inflammatory cytokines are the most well studied pathway although reduced levels of anti-inflammatory cytokines may also be involved in mechanisms as markers of premature birth.²⁰ Increased odds of preterm birth (significantly different from control group), for example, have been associated with increased IL-6 concentrations, while PTB due to infection has been associated with lower MMP-2 levels.^{27,51,70,77} Preterm birth was significantly associated to Substantially higher levels of IL-2²², while some studies also observed non-statistically significant increase in expression of IL-2.^{24,32,51,64} Similar to IL-1 β and TNF- α , some found a significant association^{22,27,32,45,49}, while others found non-significant associations.^{20,24,51} Three studies found IL-1Ra to be significantly increased in PTB cases.^{22,34,47} IL-18, a cytokine capable of inducing Th1 and Th2 immune responses, was proposed to interact synergistically with IL-12 to induce an immunological response leading to the premature rupture of membranes and preterm birth.³³ An increase in IL-12 has also been suggested to down regulate IFN- γ ¹⁸, while elevated levels of CRP are inversely related to birth weight and high levels in the mother increase the odds of SGA infants, PTB as well as LBW.^{21,25,34}

Matrix metalloproteinases, a novel family of proteinases that play a role in the degradation of extracellular matrix during tissue remodelling, have been identified as important players during embryo implantation, placentation, cervical dilation and imbalance in MMPs have been correlated with PTB and lower birth weights.⁵⁸⁻⁶⁰ According to Lyon et al., IL-1 β increases the

production of MMPs and TNF- α induces MMP activity and IL-8 aids in cervical remodelling membrane lysis.⁶¹ Poon et al., also observed an association between MMP-9 and TNFR1, suggesting the important role of inflammation on PTB.⁶² Conversely, low levels of IL-10 combined with high levels of IL1-Ra greatly increased the probability of PTB.⁴⁷ MMP-8 and IL-10 were positively correlated with CRP levels and increase in these markers correlated negatively with gestational age, however, MMP-8 did not exhibit significant increases when comparing cases to controls.^{70,77}

Consequently, inflammatory biomarkers are key regulators during pregnancy for normal infant growth and development. Pro-inflammatory biomarkers are associated with being especially important during the onset of labour and the pathways leading to labour. Often linked with hypertension and preeclampsia, inflammation, oxidative stress and vascular dysfunction are crucial to the analysis of preterm birth, which may result in SGA infants along with other birth complications¹⁴. A better understanding of pro-inflammatory markers, and the anti-inflammatory markers that regulate them, may help us achieve a more comprehensive biochemical pathways leading to the onset of adverse health outcomes at birth and in adulthood.

In utero, a number of hormones are responsible for the normal growth and development of a fetus, and any imbalance in maternal hormones, may negatively impact the infant birth outcome. Therefore, understanding the hormonal biomarkers associated with pregnancy is critical, since the lack, or abundance, of any factor affecting fetal growth may potentially result in IUGR, PTB, or SGA infants. Elevated G-CSF levels were found to be associated with PTB and LBW, while high estriol levels predicted higher risk of PTB.^{47,52} On the contrary, several studies found low PAPP-A to be associated SGA infants as well as PTB and Poon et al. identified decreased levels of PAPP-A to be linked with increased MMP-9 and risk for PTB.⁶² Correspondingly, extensive

research has been conducted on β -hCG however, only one study reported a significant association between low β -hCG levels and SGA.⁵³ PAPP-A and β -hCG are both produced in the placenta and present upon implantation and increase as the pregnancy progresses. As such, dramatic fluctuations in either biomarker may critically impact pregnancy term and infant development.

Since the mother's biochemical make-up and her physiology change constantly during pregnancy, the evaluation of biomarkers must be conducted at several stages during pregnancy. This review of literature strengthens pre-existing hypotheses suggesting a link between common biomarkers, such as interleukins and countless inflammation-related biomarkers, and the pathways leading to preterm birth or IUGR. Although the results are still far from conclusive, further research on novel longitudinal high-content biomarkers is needed along with the investigation of causal pathways, which may aid in the identification of risk factors and the development of preventative screening methods.

This systematic review demonstrates that there is still no conclusive pathway, presently, linking maternal biomarkers and adverse infant outcomes. However, our findings confirm that molecular mechanisms in the mother can play a role by affecting vascular remodelling and function as well as inflammatory and immunological responses, which subsequently, impact birth outcome. Inflammation, hormonal changes and other such biological processes have been strongly associated with influencing pregnancy and birth outcomes. To conclude, this review may aid in identifying subtle, but clinically important effects which may not have been apparent in individual studies. Further research is required to assess the various infant-related birth outcomes in relation to diverse maternal mechanistic pathways, maternal physiological parameters and maternal biochemical changes.

5.6 Limitations

Genetic biomarkers, alongside biomarkers in other biological samples such as, amniotic fluid, saliva, urine, cord blood or cervicovaginal fluid were not included, the scope of this review was limited to biomarkers found in maternal blood. Likewise, only neonatal outcomes were investigated and maternal outcomes such as preeclampsia and gestational diabetes were not explored. Consequently, information on other biomarkers found in maternal samples that may have potentially shown significant relationships with adverse birth outcomes are not covered by this systematic review. Second, a number of studies were excluded because they did not report enough information to calculate pooled odds ratios, which may have resulted in a loss of potentially relevant data. Thirdly, although noted in the summary or articles, this study could not factor in the potential role of race and ethnicity, pregnancy stage, co-morbidities and maternal age into the assessment of outcome association due to a lack of information provided by the included studies. Finally, matrix metalloproteinases, among several other biomarkers, are still very novel and the resulting number of studies was too small to draw definitive conclusions. This review and meta-analysis attempts to broaden the understanding of maternal blood biomarkers in hopes of aiding the discovery of mechanistic pathways underlying pregnancy and birth complications. This study reveals that biomarkers are complex in nature and all markers, are in some way connected, influencing one another, so further research should be conducted to investigate the interdependencies among markers. Future work should investigate maternal outcomes such as preeclampsia or gestational diabetes along with inclusion of multiple biological samples as well.

5.7 Conclusion

Research has reported that preterm birth is a growing concern in developed countries and that

billions of dollars are spent annually to treat the 540,000 premature infants that are delivered each year.¹⁴ Preterm birth has become one of the leading causes of perinatal mortality and morbidity since a shortened gestational age and decreased birth weight may lead to increased disease burden and growth restriction.¹⁵ Despite significant research being conducted and compelling evidence to suggest several underlying pathogenic pathways and factors, the specific aetiologies of PTB and SGA remain largely elusive. Over the past ten years, it has become increasingly clear that infection and inflammation demonstrate strong associations with undesirable pregnancy outcomes¹⁹. Up-regulated pro-inflammatory biomarkers such as CRP, TNF- α , IFN- γ , IL-1 β , IL-2, and IL-8, have all exhibited positive associations with adverse infant outcomes such as LBW and PTB. Likewise, anti-inflammatory biomarkers such as IL-6R and IL-10 have also exhibited associations with negative birth outcomes.

It is important to predict adverse birth outcomes because it could allow for the identification of women at highest risk, for whom appropriate, risk-specific interventions could be developed.¹⁹ The ability to predict cases may also help acquire important insights into the mechanisms or pathways leading to a preterm birth or small for gestational age infants. As a result, the study of maternal biomarkers has become a growing interest in improving the diagnosis of PTB and IUGR cases along with the possibility of achieving a better understanding of the events leading to negative infant outcome manifestation.

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Contribution to Authorship: All authors conceived and designed the study. FA extracted and analyzed the data, interpreted the results, and drafted the manuscript. The other authors (AB and JG) critically revised the draft. The final version of the paper was approved by all authors.

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TABLE 14. SEARCH TERMS USED FOR INITIAL SEARCH FOR SYSTEMATIC LITERATURE REVIEW.

Biomarkers	Outcome	Inclusion	Exclusion
Cytokine	Preterm	Maternal blood	Therapy
Chemokine	Birth weight	Maternal serum	Treatment
Inflammatory markers	Preterm birth	Maternal plasma	Therapeutic
IFN	Small for gestational age	In utero	Infection
VEGF	SGA		Pulmonary
VCAM	Gestational age		Bronchopulmonary
ICAM			Cervicovaginal
TNF alpha			Urinary
GM CSF			Urine
CRP			Placenta
MMP			
MCP-1			
MIP-1			
Endothelin			
Interleukin			

TABLE 15. SUMMATIVE QUALITY SCORES.

#	Author, Year	Reporting	External Validity	Internal Validity		Total
				Bias	Confounding	
1	Bakalis et al., 2012	4	1	4	2	11
2	Bhat et al., 2014	4	1	4	2	11
3	Botsis et al., 2006	3	1	4	1	9
4	Brou et al., 2012	4	1	4	2	11
5	Curry, Thorsen et al., 2009	4	1	4	2	11
6	Curry, Vogel et al., 2007	4	1	4	2	11
7	Ekelund et al., 2008	4	1	4	2	11
8	Ernst et al., 2011	4	1	5	2	12
9	Ferguson et al., 2014	4	1	4	2	11
10	Georgiou et al., 2011	4	1	4	1	10
11	Goldenberg, Andrews et al., 2000	4	1	5	2	12
12	Goldenberg, Iams et al., 2001	4	1	5	2	12
13	Hee et al., 2011	4	1	4	2	11
14	Jeliffe-Paulowski et al., 2014	4	1	4	2	11
15	Laudanski et al., 2012	4	1	4	2	11
16	Lohsoonthorn et al., 2007	4	1	5	1	11
17	Moghaddam Banaem et al., 2012	4	1	4	2	11
18	Ozgu-Erdinc et al., 2014	4	1	4	2	11
19	Paternoster et al., 2002	4	1	5	1	11
20	Pearce, Garvin et al., 2008	4	1	5	1	11
21	Pearce, Grove et al., 2009	4	1	4	2	11
22	Pearce, Nguyen et al., 2016	4	1	5	1	11
23	Pitiphat et al., 2005	4	1	4	2	11
24	Ruiz et al., 2012	4	1	4	2	11
25	Tency et al., 2012	4	1	4	2	11
26	Tsiartas et al., 2000	4	1	5	2	12
27	Turhan et al., 2000	4	1	4	2	11
28	Von Minckwitz et al., 2000	4	1	5	1	11
29	Whitcomb et al., 2009	4	1	4	1	10
30	Dane et al., 2013	4	1	4	2	11
31	Kirkegaard et al., 2011	4	1	4	2	11
32	Goetzinger et al., 2010	4	1	4	2	11
33	Spencer et al., 2008	4	1	4	2	11
34	Sorokin et al., 2010	4	2	5	1	12
35	Van Ravenswaaij	4	2	5	2	13
36*	Cemgil Arkan et al., 2012	4	1	4	1	10
37*	Coussons-Read et al., 2012	4	1	5	2	12
38*	De Steenwinkel et al., 2013	4	0	5	2	11
39*	Dibble et al., 2014	4	1	5	1	11
40*	Fransson et al., 2011	3	1	4	2	10
41*	Haedersdal et al., 2013	4	2	5	1	12
42*	Karampas et al., 2014	4	2	4	1	11
43*	Keith et al., 2000	4	1	4	1	10
44*	Kim et al., 2011	4	1	4	2	11
45*	Koucky et al., 2010	4	1	5	1	11
46*	Kuč et al., 2010	3	1	5	1	10
47*	Lyon et al., 2010	3	1	5	2	11
48*	Makhseed et al., 2003	3	1	4	1	9
49*	Mattos et al., 2011	4	1	4	1	10
50*	Murtha et al., 1998	3	1	5	3	12
51*	Poon et al., 2009	4	2	5	1	12
52*	Sattar et al., 2004	4	2	4	2	12
53*	Sesso et al., 2010	4	1	4	2	11
54*	Visentin et al., 2014	4	1	4	1	10

* Not included in meta-analysis due to insufficient data or odds ratios, confidence intervals and p-values were not reported

** Used modified Black & Downs checklist as see in supplementary material

TABLE 16. RELATIONSHIP BETWEEN BIOMARKERS AND ADVERSE BIRTH OUTCOMES IDENTIFIED IN THE LITERATURE SEARCH (N=54).

Biomarker		Number of Studies	PTB	LBW	SGA	IUGR
Inflammatory-related biomarkers	IL-1 β	6 ^{20,22,34,51,61,63}	+++++		+	
	IL-2	5 ^{22,24,32,51,64}	+++++			
	IL-6	19 ^{20,23,24,26,27,32,45,47,49,50,51,58,61,63,64,66,67,70,74}	+++++	+	+	
	IL-8	5 ^{21,22,26,51,65}	+++	+	+	
	IL-12	4 ^{34,49,61,65}	++	+	+	
	IL-17	2 ^{36,49}	++			
	IL-18	3 ^{33,49,61}	+++			
	IL-4, -5, -7, -9, -13	6 ^{34,49,51,56,64,65}	+++	+	++	
	TGF- α/β	2 ^{49,61}	++			
	IFN- γ	8 ^{24,26,32,34,49,56,61,65}	+++++	+	++	
	TNF- α/β	13 ^{20,22,23,24,32,37,45,49,51,61,63,66,67}	+++++	+	+	
	ICAM/VCAM	2 ^{27,39}	++			
	CRP	18 ^{20,25, 27,31,40,42,45,46,50,53,58,65,67,68,69,70,71,72}	+++++	+++	+++	+
	GM-CSF	6 ^{24,32,42,48,52,73}	+++++	+	+	
	G-CSF	4 ^{27,34,52,61}	+++		+	
	Ferritin	3 ^{27,43,44}	++			+
	MMP-2	3 ^{60,70,77}	++		+	
	MMP-3/7	2 ^{22,48}	++			
	MMP-8	2 ^{70,77}	++			
	MMP-9	9 ^{38,48,49,58,60,62,70,76,77}	+++++	+	++	
	TIMP-1, -2, -4	3 ^{22,48,77}	+++			
	Eotaxin	2 ^{34,39}	+		+	
	MIP-1	4 ^{21,22,39,49}	++++			
	MCP-1/3	3 ^{21,34,49}	++		+	
	MIF	3 ^{45,49,57}	+++			
	IL-1R	3 ^{22,34,47}	++		+	
	TNF-R1	4 ^{21,22,49,62}	++++		+	
	IL-6R	3 ^{21,22,49}	+++			
	IL-10	10 ^{20,27,45,47,49,56,61,63,66,70}	+++++	+	++	
Growth factor/hormone-related biomarkers	CRH	2 ^{27,45}	++			
	BDNF	2 ^{39,49}	++			
	NT-3/4	1 ⁴⁹	+			
	β -hCG	5 ^{41,53,54,55,75}	+++		++	
	PAPP-A	6 ^{41,53,54,55,62,75}	++++		+++	
	Cortisol	2 ^{27,45}	++			
	FGF/VEGF	1 ²²	+			
	IGFBP-1	1 ³⁹	+			
	ANGPT2	2 ^{21,22}	++			

+ indicates a single study which addresses the specific biomarker and outcome of interest.

Underlined study: excluded from the meta-analysis due to insufficient data.

TABLE 17. SUMMARY OF FINDINGS FOR INFLAMMATORY-RELATED MATERNAL BLOOD BIOMARKERS.

Outcome	Biomarker	Odds Ratio	95%CI LL	95%CI UL	Time of Sampling	Study
SGA	IL-10	0.18	0.01	3.04	Unknown	Pearce, Nguyen et al.
	IL-12	3.62*	1.74	7.54	Unknown	Georgiou et al.
	IL-13	0.11*	0.02	0.66	Unknown	Pearce, Nguyen et al.
	IL-1Ra	5.32*	2.40	11.79	Unknown	Georgiou et al.
	IFN- γ	3.72*	1.54	9.03	Unknown	Georgiou et al.
	IFN- γ	0.06*	0.01	0.75	Unknown	Pearce, Nguyen et al.
	CRP	1.76	1.00	3.11	<18w	Ernst et al.
LBW	Eotaxin	2.32	0.98	5.46	Unknown	Georgiou et al.
	CRP	1.05	0.51	2.17	<18w	Ernst et al.
PTB <37w	IL-1 β	1.00	0.83	1.20	Unknown	Ferguson et al.
	IL-1 β	3.74*	1.31	10.70	Delivery	Brou et al.
	IL-1 β	1.25	0.37	4.23	Delivery	Von Minckwitz et al.
	IL-1Ra	2.55*	1.24	5.24	22-24w	Ruiz et al.
	IL-1Ra	3.08*	1.16	8.20	Delivery	Brou et al.
	IL-2	1.20	0.93	1.55	<16w	Curry, Thorsen et al.
	IL-2	0.99	0.73	1.33	<24w	Curry, Vogel et al.
	IL-2	4.02*	1.53	10.52	Delivery	Brou et al.
	IL-2	2.37	0.12	47.54	Delivery	Von Minckwitz
	IL-4	0.90	0.50	1.70	Delivery	Tsiartas et al.
	IL-4	0.32	0.01	16.79	Delivery	Von Minckwitz et al.
	IL-6	1.29*	1.07	1.56	Unknown	Ferguson et al.
	IL-6	1.15	0.89	1.48	<16w	Curry, Thorsen
	IL-6	1.45	0.93	2.28	22-24w	Ruiz et al.
	IL-6	1.31	0.99	1.75	<24w	Curry, Vogel et al.
	IL-6	1.50	0.80	2.90	<24w	Pearce, Grove et al.
	IL-6	1.27*	1.02	1.59	Delivery	Turhan et al.
	IL-6	30.80*	3.93	241.20	Delivery	Von Minckwitz et al.
	IL-6R	2.72*	1.15	6.48	Delivery	Bhat et al.
	IL-6R	2.02	0.71	5.70	Delivery	Brou et al.
	IL-6R	2.70*	1.20	5.80	Delivery	Tsiartas et al.
	IL-8	3.75*	1.22	11.50	Delivery	Bhat et al.
	IL-8	1.50	0.58	3.87	Delivery	Brou et al.
	IL-8	5.25*	1.14	24.27	Delivery	Von Minckwitz et al.
	IL-10	2.52	0.50	12.78	22-24w	Ruiz et al.
	IL-10	2.30*	1.20	4.50	<24w	Pearce, Grove et al.
	IL-10	4.60*	2.20	9.70	Delivery	Tsiartas et al.
	IL-12	0.60	0.20	1.65	Delivery	Tsiartas et al.
	IL-17	1.80	0.80	3.80	Delivery	Tsiartas et al.
	IL-18	0.50	0.20	1.20	Delivery	Tsiartas et al.
	TGF- β	1.70	0.80	3.40	Delivery	Tsiartas et al.
	IFN- γ	0.84	0.64	1.10	<16w	Curry, Thorsen et al.
	IFN- γ	1.36*	1.03	1.81	<24w	Curry, Vogel et al.
	IFN- γ	1.60	0.80	3.10	Delivery	Tsiartas et al.
	TNF- α	1.16	0.81	1.66	Unknown	Ferguson et al.
	TNF- α	1.32*	1.03	1.70	<16w	Curry, Thorsen et al.
	TNF- α	1.10	0.83	1.47	<24w	Curry, Vogel et al.
	TNF- α	2.40*	1.30	4.70	<24w	Pearce, Grove et al.
	TNF- α	6.86*	2.42	19.42	Delivery	Brou et al.
	TNF- α	1.60	0.80	3.20	Delivery	Tsiartas et al.
	TNF- α	3.09	0.06	159.83	Delivery	Von Minckwitz et al.
	TNF- β	2.90*	1.40	6.00	Delivery	Tsiartas et al.
	TNF-R1	1.21	0.45	3.28	Delivery	Bhat et al.
	TNF-R1	6.42*	2.24	18.41	Delivery	Brou et al.
	TNF-R1	3.70*	1.60	8.50	Delivery	Tsiartas et al.
	CRP	1.07	0.84	1.37	Unknown	Ferguson et al.
	CRP	2.04*	1.13	3.69	Unknown	Lohsoonthorn et al.
	CRP	8.96*	4.60	17.43	Unknown	Moghaddam Banaem et al.
	CRP	1.34	0.67	2.66	<18w	Ernst et al.
	CRP	1.56	0.89	2.75	<19w	Pitiphat et al.
	CRP	1.90	1.00	3.70	<24w	Pearce, Grove et al.
	GM-CSF	1.35*	1.05	1.75	<16w	Curry, Thorsen et al.
	GM-CSF	1.11	0.83	1.48	<24w	Curry, Vogel et al.
	GM-CSF	0.40*	0.20	0.80	Delivery	Tsiartas et al.

*p<0.05

TABLE 17. (CONT'D)

Outcome	Biomarker	Odds Ratio	95%CI LL	95%CI UL	Time of Sampling	Study
PTB <37w	G-CSF	1.50	0.60	3.90	22-24w & 28-29w	Goldenberg, Andrews et al.
	Ferritin	1.05*	1.00	1.09	16-22w	Ozgu-Erdinc et al.
	Ferritin	2.12	0.60	7.50	24w	Paternoster et al.
	MMP-7	1.62	0.63	4.20	Delivery	Brou et al.
	MMP-9	33.75*	5.84	194.80	Unknown	Botsis et al.
	MMP-9	6.00*	2.90	12.70	Delivery	Tsiartas et al.
	TIMP-1	2.31	0.89	6.04	Delivery	Brou et al.
	Eotaxin	3.16*	1.13	8.84	Unknown	Laudanski et al.
	MIP-1a	2.62	0.95	7.21	Unknown	Laudanski et al.
	MIP-1a	1.36	0.49	3.79	Delivery	Bhat et al.
	MIP-1a	2.98*	1.13	7.86	Delivery	Brou et al.
	MIP-1a	1.70	0.80	3.40	Delivery	Tsiartas et al.
	MIP-1b	2.50*	1.20	5.10	Delivery	Tsiartas et al.
	MCP-3	4.44*	1.58	12.46	Delivery	Brou et al.
	MCP-1	0.50	0.20	1.20	Delivery	Tsiartas et al.
	MIF-1	4.16*	1.83	9.48	Unknown	Pearce, Garvin et al.
PTB <35w	MIF-1	3.50*	1.80	6.70	<24w	Pearce, Grove et al.
	MIF-1	4.70*	2.20	9.90	Delivery	Tsiartas et al.
	IL-6	1.10	0.48	2.50	20w & 24w	Goldenberg, Iams et al.
	IL-10	0.40	0.12	1.20	20w & 24w	Goldenberg, Iams et al.
	ICAM-1	1.90	0.89	4.05	20w & 24w	Goldenberg, Iams et al.
	CRP	1.20	0.50	3.11	20w & 24w	Goldenberg, Iams et al.
PTB <34w	G-CSF	1.00	0.40	2.49	22-24w & 28-29w	Goldenberg, Andrews et al.
	Ferritin	1.50	0.62	3.63	20w & 24w	Goldenberg, Iams et al.
	Ferritin	4.85*	1.60	14.70	24w	Paternoster et al.
	IL-2	1.13	0.85	1.49	<16w	Curry, Thorsen et al.
	IL-2	1.02	0.74	1.41	<24w	Curry, Vogel et al.
	IL-6	1.11	0.84	1.47	<16w	Curry, Thorsen
	IL-6	1.07	0.78	1.48	<24w	Curry, Vogel et al.
	IL-18	3.40*	1.20	9.80	Delivery	Ekelund et al.
	IFN- γ	1.00	0.76	1.33	<16w	Curry, Thorsen et al.
	IFN- γ	1.25	0.91	1.70	<24w	Curry, Vogel et al.
	TNF- α	0.97	0.73	1.29	<16w	Curry, Thorsen et al.
	TNF- α	1.13	0.82	1.54	<24w	Curry, Vogel et al.
	CRP	1.06	0.41	2.71	11-13w	Bakalis et al.
	GM-CSF	1.09	0.82	1.45	<16w	Curry, Thorsen et al.
	GM-CSF	1.03	0.75	1.42	<24w	Curry, Vogel et al.
	MMP-3	1.02	0.78	1.35	Delivery	Tency et al.
PTB <33w	MMP-9	2.21*	1.59	3.07	Delivery	Tency et al.
	TIMP-1	0.89*	0.84	0.95	Delivery	Tency et al.
	TIMP-2	0.78*	0.73	0.84	Delivery	Tency et al.
	TIMP-4	1.08	0.97	1.19	Delivery	Tency et al.
	IL-17	0.37	0.11	1.26	19w	Hee et al.
	G-CSF	1.42*	1.01	1.99	Unknown	Whitcomb et al.
PTB <32w	IL-6	2.43*	1.29	4.59	Unknown	Sorokin et al.
	IL-6	1.30	0.32	5.07	20w & 24w	Goldenberg, Iams et al.
	IL-10	0.50	0.11	1.99	20w & 24w	Goldenberg, Iams et al.
	ICAM-1	4.90*	1.30	18.81	20w & 24w	Goldenberg, Iams et al.
	CRP	3.71*	1.99	6.91	Unknown	Sorokin et al.
	CRP	1.70	0.39	7.71	20w & 24w	Goldenberg, Iams et al.
	G-CSF	0.80	0.20	3.10	22-24w & 28-29w	Goldenberg, Andrews et al.
	Ferritin	8.00	0.94	67.46	20w & 24w	Goldenberg, Iams et al.
	Ferritin	1.20	0.20	7.10	24w	Paternoster et al.
	MMP-9	0.96	0.46	2.00	Unknown	Sorokin et al.
PTB <30w	IL-2	0.94	0.58	1.52	<16w	Curry, Thorsen et al.
	IL-2	1.35	0.72	2.51	<24w	Curry, Vogel et al.
	IL-6	0.98	0.61	1.56	<16w	Curry, Thorsen
	IL-6	1.15	0.62	2.13	<24w	Curry, Vogel et al.
	IFN- γ	0.61	0.36	1.03	<16w	Curry, Thorsen et al.
	IFN- γ	1.08	0.57	2.04	<24w	Curry, Vogel et al.
	TNF- α	0.37	0.11	1.26	Unknown	Jelliffe-Pawlowski et al.
	TNF- α	0.73	0.44	1.21	<16w	Curry, Thorsen et al.
	TNF- α	1.29	0.71	2.35	<24w	Curry, Vogel et al.
	GM-CSF	1.09	0.69	1.74	<16w	Curry, Thorsen et al.
	GM-CSF	1.37	0.76	2.46	<24w	Curry, Vogel et al.

*p<0.05

TABLE 18. SUMMARY OF FINDINGS FOR GROWTH FACTOR/HORMONE-RELATED MATERNAL BLOOD BIOMARKERS.

Outcome	Biomarker	Odds Ratio	95%CI LL	95%CI UL	Time of Sampling	Study
SGA	low β -hCG	1.31*	1.05	1.63	Unknown	Van Ravenswaj et al.
	low β -hCG	1.91*	1.21	3.02	8-13w	Kirkegaard et al.
	low PAPP-A	0.54*	0.50	0.57	Unknown	Spencer et al.
	low PAPP-A	2.30*	1.91	2.77	Unknown	Van Ravenswaj et al.
PTB <37w	CRH	3.20*	1.70	6.20	<24w	Pearce, Grove et al.
	BDNF	3.16*	1.13	8.84	Unknown	Laudanski et al.
	BDNF	3.70*	1.60	8.20	Delivery	Tsiartas et al.
	NT-3	2.30*	1.10	4.70	Delivery	Tsiartas et al.
	NT-4	0.40*	0.20	0.85	Delivery	Tsiartas et al.
	low β -hCG	1.50	0.95	2.38	8-13w	Kirkegaard et al.
	low PAPP-A	1.95*	1.39	2.73	8-13w	Kirkegaard et al.
	Cortisol	1.20	0.60	2.30	<24w	Pearce, Grove et al.
	FGF basic	0.49	0.18	1.29	Delivery	Brou et al.
	VEGF	3.83*	1.46	10.09	Delivery	Brou et al.
	IGFBP-1	2.41	0.88	6.61	Unknown	Laudanski et al.
	ANGPT2	0.32*	0.12	0.89	Delivery	Brou et al.
PTB <35w	CRH	1.40	0.62	3.21	20w & 24w	Goldenberg, Iams et al.
	low β -hCG	1.40	0.80	2.40	Unknown	Goetzinger et al.
	low PAPP-A	2.00*	1.00	3.80	Unknown	Goetzinger et al.
	Cortisol	1.70	0.73	3.87	20w & 24w	Goldenberg, Iams et al.
PTB <34w	low β -hCG	2.00	0.25	16.30	<12w	Dane et al.
	low PAPP-A	7.00*	1.80	27.70	<12w	Dane et al.
PTB <32w	CRH	2.70	0.49	14.45	20w & 24w	Goldenberg, Iams et al.
	low β -hCG	2.00	0.99	3.80	Unknown	Goetzinger et al.
	low PAPP-A	2.70*	1.10	6.40	Unknown	Goetzinger et al.
	Cortisol	5.40	0.61	48.40	20w & 24w	Goldenberg, Iams et al.

***p<0.05**

FIGURE 2: STUDY SELECTION PROCESS.

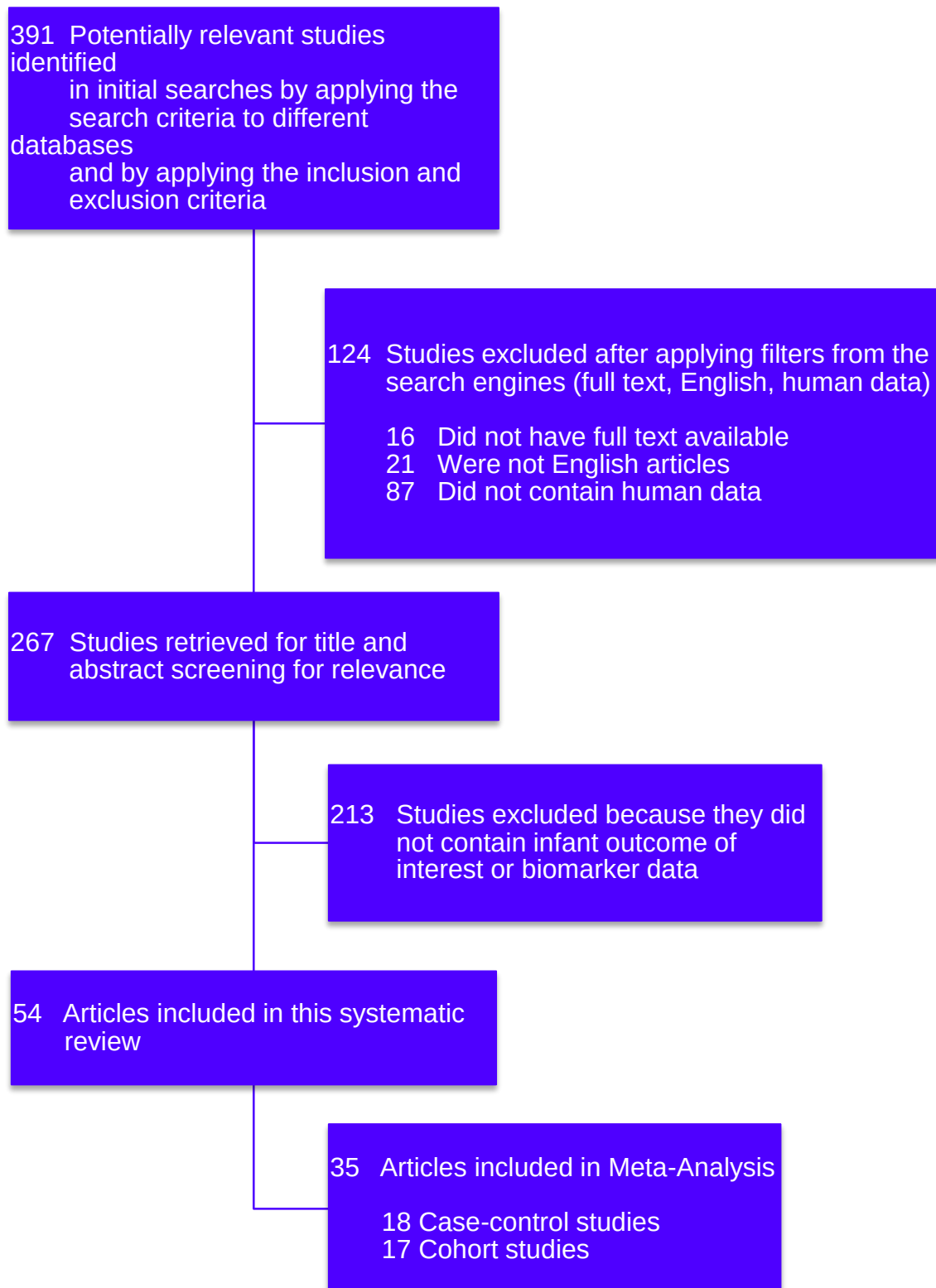


FIGURE 3: BIOMARKERS RELATED TO ADVERSE BIRTH OUTCOMES IDENTIFIED IN LITERATURE.

1. Inflammatory-related biomarkers

Interleukin-1 β , -2, -4, -5, -6, -7, -8, -9, -12, -13, -17 and -18
Transforming growth factor- α and - β
Interferon- γ
Tumour necrosis factor- α
Intercellular cell adhesion molecule
Vascular cell adhesion molecule
C-reactive protein
Granulocyte macrophage colony-stimulating factor
Granulocyte colony-stimulating factor
Ferritin
Matrix metalloproteinase-2, -3, -7, -8, and -9
Tissue inhibitor metalloproteinase-1, -2, and -4
Eotaxin
Macrophage inflammatory protein -1
Monocyte chemotactic protein-1 and -3
Macrophage migration inhibitory factor
Interleukin-1RA
Tumour necrosis factor receptor-1
IL-6R and -10

2. Growth factor/hormone-related biomarkers

Corticotropin releasing hormone
Brain derived neurotrophic factor
Neurotrophin-3 and -4
 β -human chorionic gonadotropin
Pregnancy-associated Plasma Protein A
Cortisol
Fetal growth factor
Vascular endothelial growth factor
Insulin-growth-factor-binding protein-1
Angiopoietin2

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5.9 Supplemental Material

TABLE 19. STUDY QUALITY CHECKLIST.

Factors for Evaluation of Study Quality	Scores
a) Reporting	
1. Is the hypothesis/aim/objective of the study clearly described?	/1
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	/1
3. Are the exposure measures clearly mentioned and distinguished? (case vs. control)	/1
4. Have actual probability values been reported for the main outcomes except where the probability value is less than 0.001?	/1
Subtotal for Reporting	/4
b) External Validity	
5. Were the study subjects recruited to participate in the study representative of the entire population of interest?	/1
6. Were those subjects who participated in the study similar to those who did not and were they representative of the entire population?	/1
Subtotal for External Validity	/2
c) Internal Validity – Bias	
7. Were the research subjects and the interviewers blinded when conducting the study?	/1
8. Were the results of this study based on data dredging or re-analyzed data?	/1
9. Were the time periods between exposure and outcome consistent and constant for the subjects?	/1
10. Were the statistical methods appropriate for the study?	/1
11. Were the exposures determined reliably and were clearly defined and based on pre-existing or documented information?	/1
12. Were the outcome measures accurately defined and valid?	/1
Subtotal for Internal Validity – Bias	/6
d) Internal Validity - Confounding	
13. Were the different exposure groups and unexposed groups recruited from the same population?	/1
14. Were the different exposure groups recruited at the same time?	/1
15. Were confounders accounted for and adequate adjustments made for confounding in the data analysis process?	/1
Subtotal for Internal Validity – Confounding	/3
Total Score	/15

CHAPTER 6: ADDITIONAL RESULTS

As described in **Chapter 2, 3 and 4**, metals play a potential role in adverse birth effects as well as inflammation-related biomarkers. As shown in **Figure 4** below, metal exposure, biomarkers and adverse birth outcomes are indeed related in a complex relationship. **Chapter 3** solidified the presumption of metals and their impacts on maternal, particularly regarding blood plasma biomarkers. MMPs, as mentioned in **Chapter 2**, contain a metal-binding domain so the potential for interruptions to normal function due to environmental toxicants is highly suspect. **Chapter 5** noted the importance of inflammatory regulation during pregnancy and several biomarkers were identified as potential predictors of adverse birth outcome. Due to the novelty of matrix metalloproteinases, their specific functional pathways are not well understood and very little research has been done to assess their potential interactions with other biomarkers and triggering a cascade of reactions resulting in adverse birth outcomes.

FIGURE 4: CONCEPTUAL RELATIONSHIP MODEL.

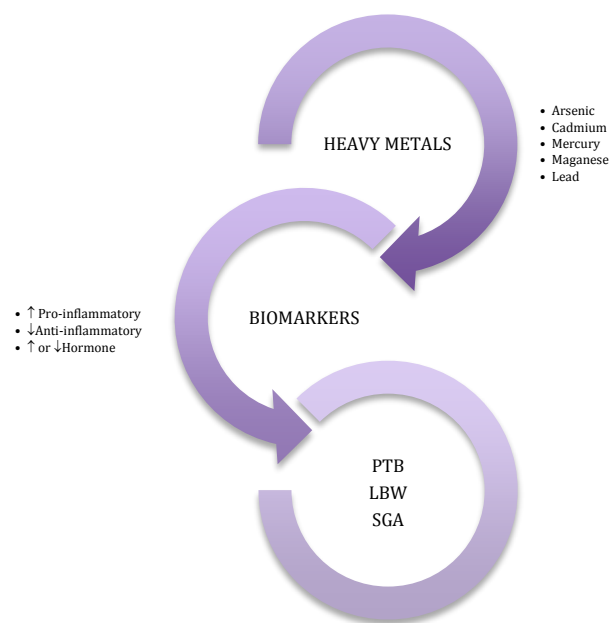


Table 20 describes the unadjusted odds of adverse birth outcomes (preterm birth, low birth weight and small-for-gestational-age infants) associated with low ($\leq 10^{\text{th}}$ percentile) and high ($\geq 90^{\text{th}}$ percentile) third trimester maternal blood plasma MMP concentrations. Likewise, **Table 21** reports the odds ratios for third trimester maternal blood plasma MMP levels associated with adverse infant birth outcomes and adjusted for maternal age, pre-pregnancy body mass index, and second trimester blood pressure (both systolic and diastolic).

TABLE 20. UNADJUSTED ODDS RATIOS FOR ASSOCIATIONS BETWEEN MATERNAL PLASMA BIOMARKER LEVELS AND BIRTH OUTCOMES IN THE MIREC STUDY COHORT (N=1533).⁺

BIOMARKER		PTB OR (95%CI)	LBW OR (95%CI)	SGA OR (95%CI)
MMP-1	Low	2.24 (1.37-3.66)*	0.90 (0.27-3.01)	0.71 (0.32-1.58)
	High	1.84 (1.11-3.03)*	1.30 (0.50-3.43)	0.85 (0.41-1.80)
MMP-2	Low	1.23 (0.72-2.11)	0.89 (0.26-3.03)	0.74 (0.33-1.65)
	High	1.20 (0.71-2.04)	1.47 (0.58-3.72)	0.88 (0.42-1.82)
MMP-7	Low	0.56 (0.31-1.03)	1.41 (0.52-3.82)	1.17 (0.58-2.35)
	High	1.11 (0.67-1.84)	1.28 (0.47-3.47)	0.70 (0.31-1.58)
MMP-9	Low	1.31 (0.75-2.29)	0.96 (0.29-3.23)	1.22 (0.61-2.44)
	High	1.36 (0.82-2.27)	1.61 (0.64-4.10)	1.21 (0.61-2.39)
MMP-10	Low	0.97 (0.55-1.71)	0.92 (0.27-3.11)	0.80 (0.36-1.78)
	High	1.12 (0.64-1.96)	1.83 (0.74-4.52)	1.23 (0.62-2.42)

*p<0.05

⁺ Odds ratios are relative to the group including percentiles from 10th to 90th of MMPs

TABLE 21. ADJUSTED ODDS RATIOS FOR ASSOCIATIONS BETWEEN MATERNAL PLASMA BIOMARKER LEVELS AND BIRTH OUTCOMES IN THE MIREC STUDY COHORT (N=1533).⁺

BIOMARKER		PTB OR (95%CI)	LBW OR (95%CI)	SGA OR (95%CI)
MMP-1	Low	2.38 (1.44-3.92)*	1.01 (0.30-3.43)	0.75 (0.34-1.69)
	High	1.78 (1.06-2.98)*	1.37 (0.51-3.67)	0.83 (0.38-1.82)
MMP-2	Low	1.32 (0.77-2.26)	1.02 (0.30-3.48)	0.78 (0.35-1.75)
	High	1.07 (0.62-1.87)	1.66 (0.64-4.27)	0.84 (0.39-1.81)
MMP-7	Low	0.57 (0.31-1.05)	1.16 (0.38-3.50)	1.09 (0.52-2.29)
	High	1.09 (0.64-1.85)	1.38 (0.50-3.81)	0.64 (0.27-1.51)
MMP-9	Low	1.42 (0.80-2.50)	1.12 (0.33-3.82)	1.17 (0.56-2.42)
	High	1.47 (0.88-2.47)	1.82 (0.71-4.71)	1.19 (0.58-2.42)
MMP-10	Low	1.04 (0.59-1.85)	1.05 (0.31-3.58)	0.87 (0.39-1.96)
	High	1.19 (0.68-2.09)	1.98 (0.79-5.00)	1.36 (0.68-2.70)

*p<0.05

⁺ Odds ratios are relative to the group including percentiles from 10th to 90th of MMPs adjusted for maternal BMI, maternal age and 2nd trimester systolic and diastolic blood pressure.

CHAPTER 7: DISCUSSION

This chapter begins with a discussion of how the two manuscripts presented in **Chapter 4**, **Chapter 5**, and the additional results presented in **Chapter 6** relate to one another. Next, this section will touch on the significance and implications of the research findings and will be followed by a limitations section.

7.1 Discussion and Integration of Results

When combining the results from both articles, the broad scope of this study can truly be appreciated. There is great potential for the application of this study in determining the mechanistic pathways impacting adverse obstetrical outcomes.

7.1.1 Maternal Heavy Metal Exposures: Predictors of MMP Activity?

Quantitative analysis in this project helped us understand that heavy metal exposure during pregnancy can affect the mother's normal bodily functions. Researchers have hypothesized that metals possess immunotoxic properties and although the exact mechanisms are not fully understood, heavy metals have been linked with oxidative stress and negative impacts on pregnancy and birth (Dietert et al., 2004; Luebeke et al., 2006; Watson, 2015). In utero exposure to certain environmental toxicants may be associated with altered levels of immunological biomarkers and can trigger unwanted immune responses (Ashley-Martin, Levy, et al., 2015). Since pregnancy is rooted in inflammatory and immunological pathways, any alterations to normal biomarker concentrations could prove detrimental to birth outcome (Coussons-Read et al., 2012).

Cytokines are important in immune responses and a change in cytokine balance is necessary for human conception, pregnancy, delivery and early fetal life, and since the onset of labour

resembles an inflammatory reaction, the regulation of these cytokines is crucial to normal birth (Lyon et al., 2010; Vogel et al. 2005). The MMP family is especially important to this project because the zinc atom in the active site highlights the presence of a highly conserved metal-binding domain. Heavy metals have been associated with several pregnancy-associated biomarkers as well as adverse neonatal outcomes (Ashley-Martin and Dodds et al., 2015; Ashley-Martin and Levy et al., 2015; Watson, 2015).

High maternal blood concentrations of lead have been associated with MMP-2 and MMP-9 (Barbosa et al., 2006; Gonzalez-Puebla et al., 2012). Meanwhile, high levels of arsenic were associated with high MMP-2 and MMP-9 (Islam et al., 2015). It was reported that MMPs are also thought to modulate the effects of heavy metals, especially mercury, which a few studies have linked with increased MMP-2 and MMP-9 expression (Watson, 2015; Jacob-Ferreira, 2009; Jacob-Ferreira, 2010; Jacob-Ferreira, 2011). Manganese, important to manganese superoxide dismutase, was linked to MMP-1, MMP-2, MMP-3 and MMP-9 (Raganathan et al, 2001; Zhang et al, 2002; Liu, et al., 2009; Chebassier et al., 2004). Cadmium, another metal found in the environment, has been linked with increased MMP-2 and MMP-9 and cadmium has even been reported to substitute the zinc ion in certain MMPs to inhibit MMP activity (Bertini et al., 2009; Puerta et al., 2006).

As seen in **Chapter 4**, both low and high levels of different metals affect MMP activity. Statistical testing with the MIREC Study cohort revealed similar findings with published literature. Our research reported both that both arsenic and cadmium were positively associated with MMP-2, similar to literature findings (Islam et al., 2015; Bertini et al., 2009). We also found that high concentrations of mercury increased the odds of high MMP-9 activity, which is reported by several studies (Watson, 2015; Jacob-Ferreira, 2009; Jacob-Ferreira, 2010).

Likewise, both manganese and lead were associated with MMP-9 (Chebassier et al., 2004; Barbosa et al., 2006).

7.1.2 Maternal MMP Activity: Predictors of Adverse Birth Outcomes?

Although few studies have evaluated matrix metalloproteinases and, while they are strongly associated with pregnancy and the onset of labour, the predictive benefits of individual MMPs still remains largely unknown. MMPs have been thought to play a role in vascular dysfunction and higher levels of MMP-2 and MMP-9 have been observed in SGA infants, which may be the result of a poor intrauterine environment caused by endothelial dysfunction (Sesso and Franco, 2010). Increased MMP-9 concentrations have been correlated with decreased gestational age and increased risk for PTB (Karampas, 2014; Tency et al., 2012; Poon et al, 2009; Kuc, 2010; Biggio et al., YEAR). Decreased MMP-2 was associated with decreased gestational age and decreased MMP-7 concentrations were associated with decreased risk of PTB (Koucky, 2010; Kuc, 2010; Brou et al., 2012).

As reported in **Chapter 5** and **Chapter 6**, MMPs are statistically significant to the prediction of LBW, PTB and SGA. Pregnancy is largely mediated by inflammatory and immunological pathways and is suggested to be very sensitive to oxidative stress, infection and chronic inflammation. PTB, LBW and SGA infants have been strongly correlated with imbalances of cytokines, chemokines and other inflammatory markers. Because enhanced oxidative stress increases the expression of and activates MMP-2 and MMP-9, it is possible that increased oxidative stress in Pb-exposed people results in increased MMP's activities (Barbosa et al., 2006).

In **Chapter 6**, the statistical analyses of the MIREC Study cohort explored the associations between maternal plasma MMP concentrations and adverse birth outcome (PTB, LBW, & SGA).

Our results revealed that high MMP-1 concentrations increased the risk of PTB and LBW and high MMP-2 concentrations increased the risk of PTB, LBW as well as SGA. The significant statistical findings for MMP-2 contradicted literature findings as most of the studies that assessed MMP-2, with the exception of Sesso and Franco (2010), reported low concentrations as being indicative of PTB (Koucky et al., 2010; Kuc et al., 2010). It was also interesting to note both low and high MMP-9 levels in the MIREC Study cohort were associated with a decreased risk of PTB, while low MMP-9 concentrations was associated with an increased risk of SGA. Again, this conflicts with the literature findings, which indicate high MMP-9 as a marker for PTB and SGA (Karampas, 2014; Tency et al., 2012; Poon et al, 2009; Kuc, 2010; Biggio et al., YEAR).

7.1.3 The Nature of Relationships Among Maternal Blood Metals, MMPs, and Adverse Birth Outcomes

Cytokines have been repeatedly linked with pregnancy in relation to inflammatory and immune-mediated pathologies and have been noted as crucial proponents to biomonitoring during pregnancy (Lyon et al., 2010). Additionally, cytokines have been cited to stimulate uterine contractions and perhaps cause preterm cervical ripening and premature rupture of membranes via the stimulation of MMPs. Subsequently, it can be determined that disruption of normal cytokine balance may result in adverse neonatal outcomes and changes in these biomarkers can be considered to possible markers of SGA and other adverse birth outcomes (Sesso and Franco, 2010). Still, current research suggests that the relationships between biomarkers are not linear and direct, but various players are involved to affect any given pathway.

Studies have identified MMPs as one of the many important players during pregnancy and labour and we are only beginning to understand the intricate network of relationships between biomarkers, metals and birth outcomes. A few studies have even implicated MMPs as players

associated with preterm birth and LBW infants as a result of prenatal exposure to heavy metals like lead and arsenic (Georgescu et al., 2011). So et al. (1992), reported TNA- α as a stimulator of MMP biosynthesis and IL-1 β was also reported to increase MMP production. Subsequently, it is understood that if any biomarkers are altered, there is a possibility other biomarkers could also be altered, which may lead to undesired birth outcome (Lyon et al., 2010). Similarly, CRP, a marker that is increased in concentration for infection and inflammation, has been positively associated with MMP-8, MMP-9 and negatively associated with MMP-2 (Sorokin et al, 2010; Kuc et al., 2010). MMP-9 was also strongly associated with TNFR1, which was associated with decreased PAPP-A, increased risk for PTB as well as SGA (Sesso and Franco, 2010; Poon et al., 2009). MMP-2 was found to be activated by an increase in MMP-14 and a decrease in TIMP-2 and served as a marker for PTB before 34 weeks of gestation (Kuc et al., 2010).

As previously mentioned, lead exposure increases MMP-2 and has also been linked to increased blood pressure (Rizzi et al., 2009). The imbalance of MMPs and TIMPs results in accelerated ECM breakdown and tissue destruction may be the underlying cause of hypertension during pregnancy and subsequently, premature ruptures of membranes or preterm birth (Sesso and Franco, 2010).

7.2 Significance, Implications and Future Plans

Prior to this thesis, there were not any reports on the link between environmental heavy metal exposures, maternal blood biomarkers and adverse infant birth outcomes to our knowledge. There is also a major gap in the literature on novel biomarkers such as MMPs and TIMPs and their contributions to pregnancy progression. The information we collected will add to the growing evidence surrounding the potential harm associated with exposure to toxic chemicals in today's environment amongst susceptible populations, such as pregnant women and their fetuses.

Specifically, this thesis will shine a light on how early life exposure to toxic chemicals may affect key endocrine sensitive endpoints resulting in adverse health outcomes. The information acquired through the research can be used to guide future studies that employ a longitudinal study design looking at how these endpoints change throughout childhood and adult years, and suggest how development at birth may serve as indicators of adverse conditions later on in life. Moreover, results from this research project may help strengthen health risk assessments, contributing to the improvement of public health, consumer products, and the environment. The information we collected can be used to affect policy changes and potentially aid in the identification of etiological pathways related to adverse pregnancy outcomes

This study has only examined a small subgroup of biomarkers and future work can be conducted to further establish mechanistic pathways to assess maternal health and birth outcomes as affected by alterations in expression of biomarkers, which in turn may be affected by exposure to heavy metals during pregnancy. This thesis hopes to stimulate further research in this field, in order to develop screening methods for metal toxicity as well as biomarkers for the early detection of adverse pregnancy outcomes. Future research should continue to look at dose-response relationships and attempt to identify protective factors.

7.3 Limitations

This research project suffered from several limitations, the first surrounding the MIREC Cohort dataset. While the subset of 1533 mother-infant pairs was a decent sample size, the comparisons and statistical analyses were conducted on the same cohort of women and infants, with the controls being selected based on normal distribution and percentile cut-offs. Though the study recruited women from all across Canada, in an attempt to obtain a varied sample, the mothers in this study may not represent the general Canadian population. Furthermore, the heavy

metal exposures stratified into quartiles for comparison with MMPs and outcomes may not be relatable in other countries or other regions in the world. Again, hindered by statistically analyzing the same subset of mother-infant pairs to identify cases and controls threatens the generalizability of the results. This study acknowledges that the control cases in this sample population may not be considered normal in other populations and more case-control studies should be conducted to further examine relationships between environmental toxicant exposures, maternal biomarkers and adverse infant outcomes.

Another limitation to this study is the scope of the research topic. This thesis project focused on matrix metalloproteinases, select heavy metals and adverse neonatal outcome, but excluded other environmental toxicants, biomarkers in other biological samples and maternal outcomes. Therefore, the conclusions obtained from this current study may not fully represent the complexity of the interdependencies and causal relationships. Furthermore, the systematic review and meta-analysis, albeit thorough, did not include dissertations, conference proceedings, and other grey-literature. As such, research studies involving populations from developing countries may have been omitted, resulting in an incomplete development of mechanistic pathways. Henceforth, additional synthesis of literature is necessary to address the relationships not studied in this project and achieve a more comprehensive understanding of pathways implicated in adverse birth outcomes (i.e. cervicovaginal fluid, urine or salivary biomarkers and the prediction of adverse birth outcomes, biomarkers and relationships with maternal health outcomes such as gestational diabetes and preeclampsia).

CHAPTER 8: CONCLUSIONS

This study was the first of its kind to examine the possible reproductive risks of exposure to environmental chemicals in a population of Canadian mothers and their infants and associate the findings to predictors of perinatal health. More specifically, it was the first study to examine the association between prenatal, maternal exposure to heavy metals and MMP-1, -2, -7, -9, -10. Furthermore, associations between maternal blood biomarkers and adverse infant outcomes were also investigated. We found that although there is significant correlation between trimesters for blood metals and biomarkers, there are still differences in MMP patterns, implying time of exposure-related effects on this class of enzymes.

The results suggest an association between maternal biomarker and adverse birth outcomes. However, the results of this study indicate that prenatal, maternal heavy metal exposures are significantly associated with biomarkers found in maternal blood, namely matrix metalloproteinases. The biological predictors of exposure that we identified can guide pregnancy-screening initiatives in order to reduce adversities caused by environmental pollutants. This information can be very informative and may serve as guidelines for consumers, risk assessors, policy makers, and health care providers alike, although further research should be conducted to include longitudinal analysis as well as analysis on cord blood and other maternal biological samples.

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APPENDENCIES

Appendix 1: Informed Consent for the MIREC Study



CHU Sainte-Justine
*Le centre hospitalier
universitaire mère-enfant*

Pour l'amour des enfants



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:

Co- principal investigators:

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Obstetrician, CHU Ste-Justine

Tye Arbuckle

Epidemiologist, Health Canada

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Local Site Investigators: Peter von Dadelszen (Vancouver), Denise Hemmings and Jingwei Wang (Edmonton), Michael Helewa and Shayne Taback (Winnipeg), Mathew Sermer (Toronto), Warren G. Foster (Hamilton), Greg Ross and Paul Fredette (Sudbury), Graeme Smith (Kingston), Mark Walker (Ottawa), Roberta Shear (Montreal), William D. Fraser (Montreal) and Linda Dodds (Halifax).

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.

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 ½ 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 (Collection of Biological Samples)



CHU Sainte-Justine
*Le centre hospitalier
universitaire mère-enfant*

Pour l'amour des enfants



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



MIREC
Maternal-Infant Research
on Environmental Chemicals

Co- principal investigators:

Dr William D. Fraser
Tye Arbuckle

Obstetrician, CHU Ste-Justine
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 access the 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

<u>Spouse of the participant:</u> 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

Appendix 3: Informed Consent for the MIREC-ID Study



INFORMED CONSENT

Maternal-Infant Research on Environmental Chemicals - Infant Development - (MIREC-ID)

Birth and 6-month visit

Investigators:

Team Leader: Gina Muckle, Ph.D. (Laval University)

Co- principal investigators: Tye Arbuckle Ph.D. (Health Canada), William D Fraser MD. (University of Montreal, Sainte-Justine Research Center), Gina Muckle, Ph.D. (Laval University), Jean R Séguin, Ph.D. (University of Montreal, Sainte-Justine Research Center), Bruce Lanphear MD. (Simon Fraser University, British Columbia Children's Hospital).

Co-investigators: Dave Sainte-Amour, Ph.D. (U of Montreal, Sainte-Justine Research Center), Éric Dewailly, MD. (Laval University), Patricia Monnier, MD. (University of Montreal Sainte-Justine Research Center), Benoit Jutras, Ph.D. (University of Montreal, Sainte-Justine Research Center), Christine Till, Ph.D. (York University), Michel Boivin, Ph.D. (Laval University), Ginette Dionne, Ph.D. (Laval University), Zhong Cheng Luo, MD (University of Montreal, Sainte-Justine Research Center), Shu Qin Wei, MD. (University of Montreal, Sainte-Justine Research Center), Warren G. Foster, Ph.D. (McMaster University), Linda Dodds, Ph.D. (Dalhousie University), Belkacem Abdous, Ph.D. (Laval University), Pierre Ayotte, Ph.D. (Laval University), Mark Walker, MD. (University of Ottawa, Ottawa Health Research Institute), Gideon Koren, MD (Hospital for Sick Children, Toronto).

Local Site Investigator: Dr. William D. Fraser, MD.

Funding agencies and research partners: Health Canada

1. Why is the MIREC-ID study being done?

We thank you for your participation in the Maternal-Infant Research on Environmental Chemicals (MIREC) study. The information that we have collected during your pregnancy will help us to determine what levels of various chemicals are found in the Canadian population and the effects, if any, of exposure to metals such as lead and mercury, and to persistent organic pollutants such as, polychlorinated biphenyls (PCBs), on pregnancy outcomes.

Now we are inviting you to participate in the MIREC-Infant Development study (MIREC-ID). These research activities will take place at birth and when your infant is 6 months of age. **The purpose of the MIREC-ID study is to examine whether there is a link between prenatal exposure to environmental chemicals, as collected in the MIREC study, and growth, sensory and sexual development, cardiac variability, and general development and behaviour during infancy.**

We know that not everyone who experiences the same risks will develop a certain health problem. In the MIREC study you agreed to let us test for characteristics that may make you and your baby more or less resistant to any harmful effects of environmental exposures (i.e. genomic testing to identify genes that could affect reactions to certain chemicals or diseases; nutritional status; socioeconomic characteristics). In MIREC-ID, we would like to determine if any nutritional, socioeconomic and psychosocial characteristics combined with environmental chemical exposure affects the risk of adverse infant health, developmental and behavioural outcomes.

Please read this consent form to decide whether you are interested in participating in this follow up study. Please take your time to make your decision, carefully read the following information and ask any questions you may have.

2. Number of participants in this study

We are inviting 1000 women across Canada who participated in the MIREC study to be part of this follow up. We expect to enrol between 100 and 200 infants *at CHU Sainte Justine*.

3. How will this study be conducted?

There are two phases where we will do some assessments of your baby: (A) within 24 to 48 hours of birth of your child before you leave the hospital and (B) when your baby reaches 6 months of age. The latter assessments will also be conducted at the hospital. Data obtained from your participation in the MIREC study will help us to determine in MIREC-ID, if exposure to environmental chemicals is associated with infant growth, development and behaviour.

A: At Birth

If you agree to participate in this new phase, a trained research nurse or a trained research assistant will conduct three additional procedures on your baby. These procedures are non

invasive and involve limited discomfort for your baby and will take about 20 to 30 minutes. You will be present during these procedures. The research nurse or the research assistant will audio record his/her measurement results to ease the data recording procedures.

If your baby shows any signs of discomfort or cries during any of the procedures/measurements, the research nurse or research assistant will allow you to calm your baby, and try the procedure/measurements again with your permission. If at any time you are uncomfortable with the procedures/measurements, you may choose to stop the procedure or withdraw from that particular assessment.

1. **Neonatal blood spot:** (Newborn blood sample) As part of standard medical care, a blood sample is routinely taken from your baby by pricking your baby's heel or by using a needle into a vein of your baby's arm or hand before you leave the hospital. At the same time that this is being done, we will obtain 1 to 2 extra blood drops (around half teaspoon) on a filter paper, which will be stored in the MIREC Biobank and later used to measure markers that may be important in understanding the potential role, if any, of environmental chemicals on infant health. No additional needle prick will be done to obtain our blood sample.
2. **Growth measurements:** Your baby will be weighed and measured at birth including length, head and arm circumferences, and skin fold thickness.
3. **Genital exam:** This includes the review of your baby's medical chart and a general examination of the breast and genitals, including the position of testis and pigmentation (skin color) of the genitals, measurements of the penis size (if applicable) and anogenital distance (distance from the anus to the base of the penis or the clitoris).

The color of the skin will be measured with a machine called a Mexameter. A probe attached to this machine will be applied gently onto the bottom of the back, the coloured skin area around the nipple, and the genital area of your baby to measure the skin color at these places.

We will also take a cell sample from the vagina of girls for analysis. This sampling is conducted by a gentle application of a cotton swab onto the opening of the vagina for a period of 10 to 15 seconds.

These tests and measurements are indicators of potential effects of prenatal exposure to environmental chemicals on your baby's hormones (i.e. steroid hormones). Your baby may experience a little discomfort during these measures but they do not pose any risk to her/him. This is a relatively new area of research and as such we will not be able to interpret what any of these assessments mean to your baby's health.

B: At 6 months

The research nurse or the research assistant will call you to schedule a 2 to 4 hour visit at the hospital. At the beginning of this visit, we will place tiny sensors on your child's chest in

order to measure his or her heartbeat with a small portable instrument. Then, we will conduct a short eye examination and test the visual acuity (sharpness) of your infant by presenting cards. We will also do auditory (hearing) testing and examine brain activity while your child is looking at pictures on a computer monitor or listening to sounds. These tests will use special sensors placed on your child's head to record brain activity.

Following these assessments, your baby will be weighed and measured including length, crown-rump length, length of fingers, circumferences of the head and arm, and skin fold thickness. Additional assessments will consist of measuring the pigmentation (skin color) of the genitals of your baby. The research nurse or the research assistant may audio record his/her measurement results to ease the data recording procedures.

Lastly, to assess your baby's behaviour, your baby will be placed in a sitting position and the research nurse or the research assistant will gently hold your baby's forearms on each side of the trunk for 3 minutes while kneeling with her head down. Your baby will be able to see you if he/she turns their head. If your infant cries more than 20 seconds, we will end this procedure.

Your child's assessments at 6 months will be video recorded. This recording will be reviewed by our senior child tester to provide feedback to the research nurse or the research assistant on the quality of the assessments and for coding of the child's reaction during the arm restraint task.

You will also be interviewed about your background (e.g. education, occupation), lifestyles (e.g. smoking, alcohol consumption and drug use), prenatal stress, well-being, interaction with your partner and support you may receive from your family and neighbourhood. Other questions will be related to your baby's sleep, nutrition, health, childcare, general development and behaviour.

Finally, in a self-administered questionnaire you will be asked to answer some questions about how you feel, your relationship with your current partner, relationships between you and your baby, about your childhood, your adolescence and your adulthood. Some of the questions may be sensitive or upsetting for you. In that case, you may contact the study nurse or the study research assistant who will recommend appropriate resources to meet your needs. You will only be identified on this questionnaire by an identification code and all information will be kept confidential.

4. What advantages and benefits can I expect?

Your participation in this follow-up study may not directly benefit you or your baby. **The testing we will do as part of this study does not replace the regular medical follow-up of your baby by his or her own doctor.** However, your participation will help us to understand whether or not exposure to environmental chemicals is affecting the health of Canadian infants. If any health-related problems requiring medical consultation are found with your child during the 6 months visit, we will inform you so you can consult your baby's doctor. In case you do not have a medical doctor, we will refer you to the pediatrician of the research group, Dr Anne-Monique Nuyt.

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

There are minimal risks to you and to your baby. For the additional blood drops, extra pricking should not be necessary. If an extra pricking is required, your baby's discomfort will last a few seconds more than what he/she may usually experience during the blood sampling procedure routinely done at birth. All other procedures are non-invasive and your infant should experience none to minimal discomfort. If your baby becomes irritable during any part of the exams we will stop the measurements to allow your baby to calm down and try the measurements again. If your baby remains upset we will stop the assessments. You may also ask us to stop the assessments at any time.

Some questions you will be asked are of a personal nature and may be of some discomfort to you, however, you can at any time refuse to answer any questions. The 6 month visit and questionnaire may take up to 2-4 hours and this may be an inconvenience to you. Should a situation of emotional, family or social difficulties arise and you do not have access to appropriate services, we will consult Dr. Martin St-André, psychiatrist of the team and we will recommend appropriate resources to meet your needs.

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

All information collected about you and your child during the course of this study will be kept confidential unless otherwise authorized by you or required by law. To achieve this end, you and your child will only be identified in these research records by an ID number. The research data, neonatal blood spots will be kept secured after the end of the study in the MIREC Data and Biobank for as long as Dr. William D. Fraser of the CHU Sainte-Justine Research Centre or someone appointed by him can guarantee their proper management, after which they will be destroyed.

Only the study team members at this site will have access to your personally identified records. However, for the purposes of auditing the proper conduct of the study and ensuring your protection, it is possible that a delegate from the CHU Sainte-Justine's Research Ethics Board and representatives from Health Canada will consult the research data file. Furthermore, the study's findings may be published or released at a scientific meeting, but no identifiable information about you or your child will be given at that time.

7. 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 nurses, (Susanne Andersen, or Julie Savaria at 514-345-4931, extension 4334)*. For all information regarding your rights as a research project participant you can contact hospital's Complaints Commissioner and the quality of services at 514- 345-4749.

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

At the six month visit, you will be provided with \$50 for expenses such as child care, parking fees and lunch and your child will receive a small gift.

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

Taking part in this study is voluntary. You can choose to take part in the study and later change your mind and withdraw from the study. In that case, if you request it, your data will be destroyed. You are also free to refuse to answer any questions or to stop any procedures or interview before it is over. Your decision will not change any present or future relationships with your health care providers or any other services you and your child are entitled to receive.

Even though you volunteered to participate, to be eligible for this study, your infant must be a singleton without major congenital birth defects, or neurological disorders. In this situation, the investigator may decide not to include your baby in the study.

10. Investigator's responsibility

In the unlikely event of problems resulting from a procedure during this study, your baby will receive all the care and medications needed by your health status and covered by the provincial health insurance plan. By signing this consent form, you are not, in any way, giving up your legal rights. Moreover you are not freeing the investigators from their legal and professional responsibilities.

11. Participant Follow-up

If future funds become available, the researchers are interested in continuing to follow you and your baby as he or she continues to grow and develop. We would like to do this in order 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:

- I agree that the study has been explained to me, all my questions were answered to my satisfaction and the possible risks of the study have been described to me.
- I understand that I can refuse to participate, to withdraw at any time and to not answer specific questions without consequence to continuing medical care.
- I have read the informed consent, and a copy has been given to me. I agree to participate with my child in this research project.

	Yes	No	<u>Initials</u>
I agree to be contacted after this follow up to see if I am interested in further research on the health of my baby.	☐	☐	
I agree to the storage of my child's dried blood in the MIREC Biobank for future research.	☐	☐	

Name of participant parent

Age

Participant 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