THE INFLUENCE OF TOXIC METALS ON SMALL FOR GESTATIONAL AGE

by

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A thesis submitted to the graduate program in Epidemiology
In conformity with the requirements for
the degree of Master of Science

Queen’s University
Kingston, Ontario, Canada
(July, 2014)

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Abstract

**Background/Purpose/Objectives:** The purpose of this research is to determine the relationship between metal exposure during the first and third trimesters of pregnancy and the risk of small for gestational age (SGA). Size for gestational age is an important predictor of early childhood morbidity and mortality. Genetic polymorphisms in glutathione s transferase omega 1 (GSTO1) and pi 1 (GSTP1) were explored as possible effect modifiers of this relationship. **Study Design/Methods:** This research is nested within a larger study called The Maternal-Infant Research on Environmental Chemicals (MIREC) Study. The MIREC study is a biomonitoring study being conducted by Health Canada, the Sainte Justine hospital in Montreal, and clinical researchers from various other cities. The study population is a sample of 2000 pregnant women, recruited during the first trimester of pregnancy from across Canada between 2007 and 2011. Blood lead, cadmium, mercury and arsenic were all measured in the first and third trimesters of pregnancy. Arsenic in urine was measured in the first trimester. A log binomial regression model was used to investigate the relationship of interest. Product terms were used to explore the gene polymorphisms. **Results:** No association was found between blood lead, cadmium or arsenic and risk for SGA. Increased risk for SGA was observed between the $\leq 0.8 \mu g/L$ and the $\geq 1.6 \mu g/L$ exposure groups for mercury (RR=1.56; 95% CI = 1.04-2.58) and, between the $\geq 22.56 \mu g/L$ and $\leq 0.75 \mu g/L$ exposure groups for arsenobetaine (RR= 1.65; 95% CI = 1.10-2.47) after adjustment for the effects of parity and smoking. A marginally significant interaction was observed between the GSTP1 polymorphism and blood lead level in relation to risk for SGA ($p$ for interaction =0.06). No other SNPs were found to modify the relationship between any metal exposure and SGA risk.

**Conclusions:** These results suggest that increased levels of mercury in a pregnant woman’s blood or of arsenobetaine in a pregnant woman’s urine, are associated with giving birth to an infant that is SGA. These results are suggestive of increased risk for an adverse effect of low-level lead exposure during pregnancy on fetal size in those with the GSTP1A114V variant genotype.
Co-Authorship

This thesis is the work of Shari Thomas in collaboration with her supervisors, Dr. Tye E. Arbuckle and Dr. Will King. The MIREC study was designed by Dr. Tye Arbuckle and her research team at Health Canada. Shari Thomas was responsible for developing specific thesis hypotheses, data management, statistical analyses, interpretation of results and writing the manuscript with guidance and revisions provided by Dr. Arbuckle and Dr. King.
Acknowledgements

This thesis could not have been completed without the guidance and assistance of many people. I would like to begin by thanking my supervisors for their mentorship and support throughout this process. This study benefitted from their competent guidance and overall encouragement and assistance I would like to thank Dr. Tye Arbuckle for her timely revisions and providing the opportunity for me to be involved with this project. Special thanks to Dr. Will King for his patience, talent for providing clarity, and his way of making everything seem within reach. I have grown a great deal as a researcher over the course of this degree, and much of that can be attributed to my frequent discussions with Dr. King.

I wish to acknowledge the MIREC participants at Health Canada, Ottawa for donating their time to research. I am also grateful to Dr. King’s lab group (Viki, Sarah and Janet) for always being there for help and guidance whenever I asked. Their kind assistance and cooperation are much appreciated.

My acknowledgement is also extended to the Department of Public Health Sciences, Queens for providing a supportive learning environment throughout my master’s degree. My thanks to my classmates for the constant sense of support, togetherness and community that I have felt over the past two and a half years. The MSc. Class of 2013 is an amazing group of young women and I am very fortunate to have come out of this experience with some lifelong friends and colleagues. A special thanks to Lidija Latifovic and Erin Liu for trying to share their love of running with me, and for their willingness to lift weights with me at the gym. These two always found the right balance between providing a distraction and contributing helpful opinions on schoolwork.

My appreciation and acknowledgment is extended to Health Canada for financial support provided and the Empire Life of Canada for funding the study via the Empire Life Fellowship in Child Health Research.
Finally, I would like to thank my parents, my sister and Pat for their continued support of my love for learning. Their patience and well-timed distractions were critical to the completion of this work, and I am lucky to have such wonderful people behind me.
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<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGA</td>
<td>Appropriate for Gestational Age</td>
</tr>
<tr>
<td>ALA</td>
<td>Aminolevulinic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>As$^{3+}$</td>
<td>arsinite</td>
</tr>
<tr>
<td>As$^{5+}$</td>
<td>arsenate</td>
</tr>
<tr>
<td>ASTDR</td>
<td>Agency for Toxic Substances and Disease Registry</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>CEPA</td>
<td>Canadian Environmental Protection Act</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CMHS</td>
<td>Canadian Health Measures Survey</td>
</tr>
<tr>
<td>CMP</td>
<td>Chemicals Management plan</td>
</tr>
<tr>
<td>CHU</td>
<td>Centre hospitalier de l'Université de Montréal</td>
</tr>
<tr>
<td>DMA</td>
<td>Dimethylarsinic Acid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione S- transferase</td>
</tr>
<tr>
<td>GSTO1</td>
<td>Glutathione S- transferase omega 1</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Glutathione S- transferase pi 1</td>
</tr>
<tr>
<td>Hg</td>
<td>Mercury</td>
</tr>
<tr>
<td>HWE</td>
<td>Hardy Weinberg Equilibrium</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>INSPQ</td>
<td>Institut National de Santé Publique du Québec</td>
</tr>
<tr>
<td>IUGR</td>
<td>Intrauterine Growth Restriction</td>
</tr>
<tr>
<td>LBW</td>
<td>Low Birthweight</td>
</tr>
<tr>
<td>LMP</td>
<td>Last Menstrual Period</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>MIREC</td>
<td>Maternal Infant Research on Environmental Chemicals</td>
</tr>
<tr>
<td>MMA</td>
<td>Monomethylarsonic acid</td>
</tr>
<tr>
<td>N</td>
<td>Sample number</td>
</tr>
<tr>
<td>nmol/Kg</td>
<td>Nanomoles per kilogram</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Nanomoles per litre</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>Pb</td>
<td>Lead</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>r</td>
<td>Spearman’s correlation coefficient</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive Nitrogen Species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for Gestational Age</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>TDI</td>
<td>Tolerable daily intake</td>
</tr>
<tr>
<td>TE</td>
<td>Tris EDTA buffer</td>
</tr>
<tr>
<td>ug/dL</td>
<td>Micrograms per deciliter</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>ug/L</td>
<td>Micrograms per litre</td>
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Chapter 1

General Introduction

1.1 Introduction

Neonatal health outcomes are some of the most indicative of a population’s health. The ability to care for this susceptible subpopulation mirrors the quality of a country’s public health infrastructure (MacDorman & Mathews, 2009). Toxic metals are ubiquitous in the environment and have many known health effects on various human systems. Humans can be exposed to these metals through various sources including food, water, air and cigarette smoke. Pregnant women and their fetuses are a group that is potentially susceptible to these effects, and are also commonly overlooked in national biomonitoring surveys. Toxicokinetics for various chemicals can be altered during pregnancy because of changes in a woman’s digestive, cardiovascular, pulmonary and renal systems (Mattison, Blann, & Malek, 1991). While these changes are designed to increase nutrient availability and to remove waste from the fetus, physiological differences can cause the absorption, distribution and metabolism of many xenobiotics to change (Mattison et al., 1991). Fetuses are highly susceptible to the harmful effects of toxic metals because of their physiologic immaturity. Their developing organ systems are sensitive to environmental insults during critical periods of development in utero because of changing metabolic capacities and periods of rapid cell proliferation (Šrám, Binková, Dejmek, & Bobak, 2005). Environmental insults and inadequate nutrition can impair fetal growth and put the fetus at risk for a variety of undesirable birth outcomes, including being small for gestational age (SGA). SGA is an important public health issue as children born SGA are at increased risk for birth complications and life-long health issues. (Clayton et al., 2007; Kingwell, Heart, & Road, 2011; Nardozza et al., 2012; Reinehr, Kleber, & Toschke, 2009). Inter-individual differences in genetic capacity to detoxify harmful exposures make certain individuals more susceptible to the adverse effects of metal exposure than others. The purpose of this study is to examine the relationship between exposure to toxic metals during pregnancy and the risk of giving
birth to an infant that is SGA. It will also consider genetic factors that may modify this relationship.

1.2 Rationale

Canadians are exposed to low levels of toxic metals daily. It is currently unknown what health effects, if any, these levels could present. These potential unknown effects are especially important to measure in pregnant women. Pregnant women and their fetuses are a susceptible group that is often excluded from biomonitoring efforts such as the Canadian Health Measures Survey. The literature in this area is scarce and the studies that have been done have yielded inconclusive results. To date, it has been uncommon to study gene-environment interaction in this context. Identification of potential genetic susceptibility would help identify those at greatest risk for adverse pregnancy outcomes due to metal exposure. This research will generate knowledge that will contribute to assessments of risks for various levels of metals in biological tissues or fluids. It may also reveal a need to focus on monitoring pregnant women to identify those with elevated body burdens.

1.3 Overview of Study methods

This thesis is nested within the Maternal-Infant Research on Environmental Chemicals (MIREC) study - a multi year cohort study that recruited women during the first trimester of pregnancy. Approximately 2000 women from ten sites across Canada were recruited to participate in the MIREC study. The MIREC study collected maternal and cord blood, maternal urine, maternal hair samples, breast milk and meconium to be analysed for environmental contaminants. Only maternal blood and maternal urine were used for this study. Blood for toxic metal analysis was drawn during the first and third trimester. Blood for genotyping was drawn during the first trimester, and the urine sample was also taken during the first trimester. Exposures for this thesis were estimated as the average of first and third trimester measures in whole blood.
1.4 Objectives

The specific objectives of this thesis are as follows:
1. To determine the relationship between toxic metal exposure during pregnancy and risk of SGA. It was hypothesized that elevated levels of toxic metals in maternal blood and urine will be associated with increased risk of SGA birth.

2. To explore interaction between toxic metal exposure and polymorphisms in glutathion transferase (GST) gene in relation to risk of an SGA birth. It was hypothesized that the effect of metals on SGA will be more pronounced in individuals who have the variant genotype for the GST polymorphisms.

1.5 Context of Research

The Maternal-Infant Research on Environmental Chemicals (MIREC) study is a national-level biomonitoring study and a key deliverable under the Government of Canada's Chemicals Management Plan (CMP). The student investigator developed the specific research objectives for this project, and developed as well as implemented the data analysis strategy. The student was also responsible for deriving variables and merging datasets. Student funding was provided by the Empire Life fellowship for research in child health as well as Health Canada. Information on the exposure variables, covariates and pregnancy outcomes were provided by the larger study.

1.5 Thesis Outline

This thesis is organized into six chapters. The relevant literature is reviewed in chapter two. A review of the general methods (study design, data collection and statistical analysis) is provided in chapter 3. In particular, methods that are not discussed in the manuscript will be elaborated upon here. A manuscript that addresses objective one, the relationship between toxic metals and SGA is presented in chapter 4. Chapter 5 presents the results of the exploratory gene-environment interaction analysis in the context of toxic metal exposure. Chapter 6 summarizes the findings of the study, discusses
methodological issues in study design and presents conclusions to be drawn from the study findings.

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Chapter 2

Background and Literature Review

2.1 Introduction

This research investigates the relationship between exposure to current low levels of toxic metals during pregnancy and the risk of subsequent small for gestational age (SGA) birth. It also explores the potential effect of genetic predisposition on this relationship. Identification of exposure to specific toxic metals as a risk factor for SGA, and genes that modify the relationship between this exposure and outcome will provide evidence for the further risk management of these chemicals and may help to distinguish the underlying biologic mechanism of how these exposures can lead to SGA.

2.2 Fetal Growth

Fetal growth trajectories and fetal size are important indicators of fetal health (Mayer & Joseph, 2012). Adequate fetal growth is heavily dependent on genetic, nutritional and hormonal factors, among others (Nardozza et al., 2012). The placenta serves the hepatic, and renal functions of the fetus, and as such placental function is paramount to fetal development (Mayer & Joseph, 2012). The ability of the placenta to meet fetal demands diminishes from mid-pregnancy onwards (Konje, Kaufmann, Bell, & Taylor, 2001; Rurak & Bessette, 2013). The fetal growth trajectory is monitored throughout the pregnancy using various ultrasound techniques and customized equations to calculate estimated fetal weight (Nardozza et al., 2012). Abnormalities in fetal growth are commonly diagnosed using criteria based on weight for age percentiles (such as SGA) and weight (such as low birth weight).
2.3 Small for Gestational Age

The outcome of interest for this thesis is giving birth to an infant that is SGA, defined as a singleton live birth weighing less than the 10th percentile of birth weights for the same sex and gestational age. The reference population for assessing SGA is Canadian male and female singletons born between 1994 and 1996, as this is the most recent Canadian reference population (M S Kramer et al., 2001).

Size relative to gestational age is an important predictor of early childhood morbidity and mortality, as well as chronic conditions during adulthood (Rahman et al., 2009). Babies who are born SGA can have many complications at birth such as meconium aspiration, cognitive dysfunction, cerebral palsy, metabolic and hematological disorders (Nardozza et al., 2012). They are also prone to excessive weight gain in early adulthood (Clayton et al., 2007). Common adverse health outcomes associated with SGA infants that develop later in life include cardiovascular diseases (Clayton et al., 2007; Kingwell et al., 2011), metabolic syndrome (Reinehr et al., 2009) and insulin resistance syndrome (Clayton et al., 2007). Furthermore, mothers who give birth to SGA infants are also at increased risk of developing cardiovascular disease later in life (Bukowski, Davis, & Wilson, 2012).

Low birth weight (LBW) (less than 2500 grams) is frequently used as an indicator of perinatal health; however a major limitation to its use is that infants who are classified as LBW are not a homogenous group. Being low birth weight can be a result of intrauterine growth restriction, preterm birth, or a combination of the two (Canadian Institute for Health Information, 2009). Furthermore, preterm birth and SGA can occur across the entire spectrum of birth weights and are not limited to infants who are LBW. There are differences in the health outcomes and factors associated with infants who are preterm and those who are SGA. Because of this, SGA is the preferred measure for assessing fetal growth restriction (the failure to attain optimal fetal growth) (Canadian Institute for Health Information, 2009). It is important to note that although the SGA label implies fetal growth restriction, not all fetuses who are SGA are growth restricted, just as not all fetuses who are appropriate for gestational age (AGA) have necessarily had optimal growth (Canadian Institute for Health Information, 2009; Mayer & Joseph,
Among fetuses that are classified as SGA, some will be normal fetuses that are simply constitutionally small with no pathologic growth restriction.

### 2.4 Epidemiology of SGA

In 2006-2007, the prevalence of SGA in Canada was 8.3% of live births (Canadian Institute for Health Information, 2009). The highest provincial SGA prevalence was 8.9% for Ontario and 8.7% for Alberta, while Newfoundland and Labrador (5.9%) and Prince Edward Island (6.8%) had the lowest provincial rates. Neighbourhoods in the highest income quintile experienced the lowest rates of SGA. SGA rates were significantly higher in urban settings than in rural areas 8.7% versus 7.0%, and these differences are even more pronounced at a provincial level. Here, communities with a population of greater than 10,000 are classified as urban, and less than 10,000 as rural (Canadian Institute for Health Information, 2009).

Initially, the definition of SGA (live births weighing less than the 10th percentile of the same sex and gestational age) may appear to be suggestive of a fixed rate of SGA at 10%, however this is not the case. An infant’s size for gestational age is determined by comparing them to the 10th percentile of a growth standard. The rate of infants falling below this standard can vary from year to year. The provincial averages of around 8% imply that infants are larger now than they were in 1994-1996 (The years for which all live births were used to develop the Canadian growth standard.)

### 2.5 Risk Factors for SGA

Many modifiable and non-modifiable risk factors for conditions representing fetal growth restriction (intrauterine growth restriction (IUGR), SGA, and LBW) have been identified and evaluated in scientific literature. Risk factors for SGA birth include maternal characteristics, environmental exposures, maternal lifestyle factors, maternal medical conditions, fetal characteristics, pregnancy complications, obstetric history, and paternal factors. This review summarizes the relationship between established risk factors for fetal growth restriction (including LBW, SGA and IUGR) on the basis of review papers of independent risk factors for fetal growth restriction in humans, published in the last ten
years (Ashdown-Lambert 2005; Bonzini et al. 2007; Han et al. 2011; Henderson et al. 2007; McCowan and Horgan 2009; Newburn-Cook and Onyskiw 2005; Patra et al. 2011; Valero De Bernabé et al. 2004). All available systematic reviews, meta-analyses and review papers that outlined possible risk factors for SGA, LBW or IUGR are included in this review. All systematic reviews and meta-analyses included searched Pubmed and Embase among other scientific databases. Newburn-Cook et. al. had very stringent criteria for study inclusion in their systematic review. The quality of the included studies was assessed based on criteria related to inclusion criteria, appropriateness of the statistical analysis, measurement of risk and outcome, study design control of potential confounders, and discussion of study limitations. Each category was given a rating of not reported, unsatisfactory, or satisfactory. The sum of these ratings was used as the overall validity rating. Only validity ratings of 11 or greater were included in this review.

Bonzini et. al. and Patra et. al. used two independent abstractors to decide if studies met the inclusion criteria for the systematic review, while others followed pre-determined quality assessment methodology such as the MOOSE consensus statement (Han et al. 2011), or the Newcastle-Ottawa Quality assessment scale (Henderson et al. 2007). The inclusion and exclusion criteria for the review papers (McCowan and Horgan 2009; Valero De Bernabé et al. 2004) is less clear and not reproducible, making these of inferior quality to the meta analyses and systematic reviews that are included in this review.

The nature of these risk factors is such that there is a great deal of mutual confounding between them. This review focuses on the independent, un-confounded effects for each risk factor.

### 2.5.1 Maternal Characteristics

Maternal characteristics that have an established relationship with fetal growth outcomes include maternal age, ethnicity, height, and socio-economic status.

Women at both extremes of reproductive life are at increased risk of SGA birth. Advanced maternal age (≥35 and ≥40 years) has been shown to be a risk factor for SGA (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004). Very young mothers...
(teenagers) are also more likely to give birth to infants that are SGA (Valero De Bernabé et al., 2004). A Systematic review from 2005 concluded that there is not enough evidence to determine if maternal age is a true independent risk factor for SGA, or if it is a proxy for other age-related confounders (Newburn-Cook & Onyskiw, 2005).

It has been consistently shown that black women have a higher incidence of LBW than white women (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004) however customized centiles would help to determine if these perceived ethnic differences persist after accounting for other factors (McCowan & Horgan, 2009).

The literature is inconsistent regarding the independent influence of socioeconomic status on SGA. Socioeconomic status is closely tied to many other factors that influence overall health, making it difficult to quantify the risk of SGA that is attributable to this factor (Valero De Bernabé et al., 2004). Some of the variation in the literature regarding the effect of this risk factor is explained by the fact that different studies use different measures of socioeconomic status, including but not limited to maternal education, social class or social deprivation (McCowan & Horgan, 2009).

Short women have increased risk of SGA birth, and women with a pre-pregnancy body mass index (BMI) under 20 are twice as likely to give birth to a child that is SGA (Ashdown-Lambert, 2005; McCowan & Horgan, 2009). A meta-analysis reports a pooled RR of 1.48 (95%CI: 1.29-1.68) for the risk of underweight women having a LBW birth in developed countries (Han et al., 2011). Maternal birth weight is another risk factor for SGA with mothers who were born SGA themselves having 4.7 times increased rate of SGA births (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004).

2.5.2 Maternal Lifestyle Factors

Fetal growth can be influenced positively and negatively by maternal lifestyle factors such as smoking behaviours, diet, drug use, caffeine consumption or physical exertion.

Maternal smoking has a causal and dose dependent relationship with SGA and is the single most important risk factor for SGA in developed countries (Ashdown-Lambert, 2005; Maulik, 2006; McCowan & Horgan, 2009; Valero De Bernabé et al., 2004).
The literature on alcohol consumption during pregnancy is inconclusive. A systematic review on low-to moderate alcohol consumption reviewed 7 studies with data on SGA. Of these, only one found an association with SGA. Three studies included in the review found that alcohol consumption was mildly protective against SGA (Henderson et al., 2007). Since this systematic review, the generation R study of 7141 subjects found that one or more drinks in any trimester of pregnancy was not significantly associated with SGA but three or more was associated with a 3 fold increase in SGA (McCowan & Horgan, 2009). The pooled OR of 11 studies of alcohol consumption and SGA that controlled for confounders was 1.11 (95% CI: 0.95–1.30). The pooled RR for this risk factor and LBW was 1.06 (95% CI: 0.99–1.13). Moderate pre-pregnancy alcohol consumption was found to be protective against SGA/LBW (Patra et al., 2011).

Other suspected lifestyle risk factors for SGA include illicit drug use, caffeine intake, and maternal diet. The data on marijuana use (the most commonly used drug during pregnancy) and birth weight is inconclusive. A study of 12000 women found that the infants born to those who used marijuana at least weekly before and during pregnancy were lighter than those with mothers who did not use marijuana. (p=0.056). Another, US study found that marijuana use was not a significant risk factor after adjusting for other factors (McCowan & Horgan, 2009). Despite the fact that cocaine use during pregnancy is uncommon, cocaine has been identified as the drug with the strongest independent effect on infant birth weight OR=2.24 (95% CI: 1.72–2.91) (McCowan & Horgan, 2009).

Caffeine consumption has also been suspected of having a role in determining fetal size. A study of 2635 low-risk pregnant women reported increased risk of SGA in women who consumed 200-299mg of caffeine daily, (RR =1.5 (95% CI 1.1-2.1)) and >300 mg daily (RR=1.4 (95% CI 1.0-2.0)) (McCowan & Horgan, 2009).

Protective odds ratios ranging from 0.4 to 0.5 have been reported for the risk of SGA birth after consumption of milk and leafy green vegetables during pregnancy. Fruit consumption at 28 weeks gestation has also been shown to have a protective effect on fetal size (McCowan & Horgan, 2009). The data on maternal fish consumption and birthweight is inconclusive. Some studies have found that increased levels of maternal fish intake is protective against SGA while others have found that it is a risk factor for SGA (McCowan & Horgan, 2009).
Some occupational activities have been suspected of having an adverse effect of pregnancy outcomes. A systematic review of studies examining working hours and physical activities during pregnancy demonstrates that the literature in this area is conflicting. Odds ratios ranging from null to 2.1 have been reported for the effect of working longer than 40 hours a week on the risk of SGA. Lifting, standing and physical workload have all also had inconsistent associations with risk of LBW (Bonzini et al., 2007).

2.5.3 Environmental exposures

Environmental exposure to tobacco smoke, indoor and outdoor air pollution and water disinfection byproducts have all been suspected of playing a role in fetal size.

A recent summary of meta-analyses of environmental tobacco smoke reports pooled risk estimates of 1.2-1.3 for the effect of this risk factor on SGA (Nieuwenhuijsen, Dadvand, Grellier, Martinez, & Vrijheid, 2013). A meta-analysis of outdoor air pollution and LBW found an association but it did not reach statistical significance. A different meta-analysis found indoor air pollution form solid fuel use to increase risk of LBW (OR: 1.38 (95% CI: 1.25, 1.52)) (Nieuwenhuijsen et al., 2013). Finally, a meta-analysis of eight studies found marginal risk of SGA in women exposed to trihalomethanes (water disinfection by-products) during their third trimester of pregnancy (Nieuwenhuijsen et al., 2013).

2.5.4 Maternal Medical Conditions

Maternal medical history can influence fetal size. Both chronic and acute conditions can increase the risk of SGA.

Chronic hypertension both with pre-eclampsia (RR: 2.30 (95% CI: 1.85–2.84)), (OR: 5.6(95% CI: 1.8–16)) and without (OR: 2.9 (95% CI: 1.6–5.0)) have been associated with increased risk of SGA (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004). Odds ratios as high as 10.4 have been reported for the risk of SGA in diabetic women with vascularopathy (nephropathy, retinopathy of pre-existing hypertension) (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004) While renal
disease during pregnancy is rare, it is very strongly associated with SGA (OR=5.3(95% CI 2.8-10.0)) (McCowan & Horgan, 2009). Maternal uterine malformations, history of lupus, antiphospholipid syndrome, asthma, uncontrolled hyperthyroidism, disorders involving hypoxemia, glucose metabolism disorders and malaria are other medical conditions that are also associated with increased SGA or LBW (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004).

Infection with various microorganisms during pregnancy has been associated with elevated risk of LBW. Untreated gonorrhea and syphilis, as well as Chlamydia, b-hemolytic streptococcus, Ureoplasma urealyticum, Mycoplasma, Trichomonas, Staphylococcus aureus have been associated with LBW (Valero De Bernabé et al. 2004).

2.5.5 Fetal Characteristics

Some characteristics of the fetus itself, such as multiple pregnancies, female sex of the fetus, or genetic conditions, can influence fetal growth outcomes.

Multiple births are more susceptible to a variety of perinatal adverse events including growth restriction. Morbidity and mortality increase as the number of fetuses increases (Valero De Bernabé et al., 2004). Arbuckle et. al. reports that up to 25% of twins in Canada are born growth restricted (Valero De Bernabé et al., 2004). Fetal sex is also a determinant of fetal size. Female fetuses are typically smaller than male fetuses (Ashdown-Lambert, 2005). Aneuploidy, gene deletions and duplications can all result in syndromes that result in fetal growth restriction. Turner’s syndrome, Down’s syndrome, trisomy 18 and trisomy 13 are all associated with increased rates of growth restriction (Mayer & Joseph, 2012). Syndromes that cause multiple malformations, anencephaly and fetal heart diseases are often associated with growth restriction (Valero De Bernabé et al., 2004).

2.5.6 Pregnancy Complications

During pregnancy, a condition may develop that influence fetal growth. Pregnancy complications including heavy bleeding, placental abruption, preeclampsia and inadequate weight gain can all increase the risk of SGA. Heavy bleeding during early pregnancy is associated with elevated risk of SGA. SGA risk is also increased with
placental abruption (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004).

Gestational weight gain is a predictor of fetal size. Low weight gain during pregnancy is associated with smaller infants (Ashdown-Lambert, 2005), however there is no definitive data on optimal gestational weight gain (Valero De Bernabé et al., 2004).

2.5.7 Obstetric History

A woman’s history of prior pregnancies can be an important predictor of outcomes of the current pregnancy. Parity, inter-pregnancy interval and prior SGA birth are all independent risk factors for SGA birth.

Women in their first pregnancy are at an increased risk of delivering an infant that is SGA compared to women in their second or third pregnancy, with odds ratios from 1.3-2.1 being reported for this risk factor and SGA (McCowan & Horgan, 2009). High parity is also a risk factor for growth restriction. Second and third children are generally larger than the first child, but this trend begins to reverse with the fourth, and subsequent pregnancies (Valero De Bernabé et al., 2004). History of previous LBW/SGA births may be the strongest predictor of risk in the current pregnancy (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004).

The optimal inter-pregnancy interval is 18-23 months. Inter-pregnancy intervals both shorter and longer than this have been found to be associated with increased risk of SGA, with the most pronounced risks seen with intervals shorter than 6 months (OR=1.26 (95% CI 1.18-1.33)) and greater than 60 months (OR=1.29 (95% CI 1.20-1.39)) (McCowan & Horgan, 2009). Two studies have found that maternal history of 2 or more previous miscarriages was a risk factor for SGA (OR=1.5 (95%CI 1.4-1.6)) while one found no effect on the risk for SGA (McCowan & Horgan, 2009).

2.5.8 Paternal Factors

Factors related to fetal paternity can also have an effect on SGA risk. A relationship between change in paternity and risk of SGA has been reported (OR=1.91 (95% CI 1.28-2.87))(McCowan & Horgan, 2009), this risk factor appears to reduce the benefit of multiparity (parity greater than one) on SGA. An association between a father
being born SGA and their child also being born SGA has been described (OR=3.47 (95% CI 1.17-10.27))(McCowan & Horgan, 2009).

2.6 Toxic Metals of Interest

In Canada, efforts to minimize population exposure to toxic metals have been successful (Health Canada, 2002); however, as toxic metals are present throughout the environment, eliminating exposure entirely is impossible. The intrauterine period of development is one of the most sensitive stages in the biological development process. Low levels of environmental pollutants, including toxic metals, which may otherwise be considered benign, may be harmful during this vulnerable developmental period (Rahman et al., 2009). Lead, cadmium, mercury and arsenic are the most common toxic metals to which Canadians are exposed. The following sections define each metal of interest, sources of exposure, and metabolism. For each metal, the epidemiologic literature referring to exposure and fetal growth outcomes is briefly reviewed. Relevant literature was identified using Pubmed and embase through the Queen’s University web proxy. Key words for this search included “metal exposure and fetal growth”, “metal exposure and IUGR”, “metal exposure and fetal growth restriction”, “metal exposure and birthweight”, “metal exposure and LBW”. These combinations of terms were also repeated for each metal independently (i.e. “lead and fetal growth etc.). Only studies examining lead, cadmium, mercury or arsenic were selected.

This review of epidemiologic evidence will consider only relevant fetal growth outcomes (SGA and LBW). Where studies evaluated multiple fetal growth outcomes, this review will focus on birthweight and size for gestational age. Studies conducted prior to 1990 will not be considered in this review.

2.6.1 Lead

Lead has several stable isotopes that exist in nature. It is the most ubiquitous of the toxic metals (Health Canada, 2004), and Canadians can be exposed to it through contaminated food, drinking water, jewelry, soil and household dust (Health Canada, 2004). In adults, 3-10% of ingested lead is absorbed into blood. In children this number
can be up to 50% (Health Canada, 2002). Lead is a cumulative general poison, with children and fetuses being at greatest risk for adverse health effects (World Health Organization, 2011b). Lead is classified as group 2A, probably carcinogenic to humans by the International Agency for Research on Cancer (IARC) (IARC 2006), and is on schedule 1 of the List of Toxic Substances under the Canadian Environmental Protection Act (CEPA), 1999 (Canada, 1999).

The majority of the body’s lead is stored in the bones. As the body’s calcium demands change during pregnancy and lactation, this lead can be mobilized from the bones into the maternal blood stream and transferred to the fetus through cord blood (Gulson et al., 1997). Blood lead levels decrease with parity, implying that the greatest risk is during the first pregnancy (Manton et al., 2003). Exposure to low levels of lead in early life can compromise the integrity of the central nervous system and cause deficits in neurobehavioural development (Hammond & Dietrich, 1990).

Epidemiologic Studies of Lead and Fetal Growth

Of the toxic metals under examination in this thesis, lead has been the most extensively studied with regard to its effect on fetal development. Prior to 1990, at least 20 studies have examined the relationship between lead exposure and fetal growth outcomes (Andrews, Savitz, & Hertz-Picciotto, 1994). Despite this, there have been a limited number of studies conducted to examine low-level lead exposure, (as currently experienced in most developed countries such as Canada) and SGA/LBW. The epidemiologic literature concerning the relationship between maternal blood lead levels and SGA/LBW is summarized in table 2.1. Much of the prior work on this area has been in populations exposed to higher levels of lead (mean exposure >10 ug/dL) or with occupational exposure. (P.-C. Chen, Pan, & Wang, 2006; Kristal-Boneh et al., 1998; Salpietro et al., 2002). Based on the literature available at that time, a review has concluded that there is limited evidence for an association between prenatal lead exposure and fetal growth outcomes like low birth weight and intrauterine growth restriction (Wigle et al., 2008). Some studies report a significant dose-dependent relationship (P.-C. Chen et al., 2006; Jelliffe-Pawlowski, Miles, Courtney, Materna, & Charlton, 2006; J O Odland, Nieboer, Romanova, Thomassen, & Lund, 1999). Others
simply report a significant relationship (Berkowitz, Price-Green, Bove, & Kaye, 2006; González-Cossío et al., 1997; Gundacker et al., 2010; Xie et al., 2013; Zhu, Fitzgerald, Gelberg, Lin, & Druschel, 2010); While others report no association (Jones et al., 2010; Shirai, Suzuki, Yoshinaga, & Mizumoto, 2010; Sowers et al., 2002). Sample size was less than 1000 in at least 7 analyses (González-Cossío et al. 1997; Gundacker et al. 2010; Jelliffe-Pawlowski et al. 2006; Odland et al. 1999; Osman et al. 2000; Shirai et al. 2010; Sowers et al. 2002) reducing these studies’ power to detect small effects.
### Table 2.1 Epidemiologic Studies of Lead and Fetal growth

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Population</th>
<th>N</th>
<th>Exposure matrix /Concentration</th>
<th>Timing of exposure measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhu et al. 2010</td>
<td>Upstate New York mothers 15-49 years old who had singleton live births</td>
<td>43,288</td>
<td>Maternal blood range: 0 - 9.9 μg/L</td>
<td>Maternal blood before or at the delivery date</td>
<td>Statistically significant association between blood lead and birth weight, no association with preterm delivery or SGA</td>
</tr>
<tr>
<td>Osman et al. 2000</td>
<td>Swedish women</td>
<td>106</td>
<td>Maternal blood lead range: 10–230 nmol/L, median: 55 nmol/L</td>
<td>Maternal blood lead at 36 weeks gestation</td>
<td>Maternal blood lead was not associated with LBW</td>
</tr>
</tbody>
</table>
| Chen et al. 2006 | Infants born to parents identified from Taiwan’s occupational blood-lead notification system | 1611  | Maternal blood lead arithmetic mean: 10.1 μg/L (SD10.4) Maternal blood maximum: 62.8 μg/L | Maternal blood lead levels were examined in the period of 1 year before fertilization | RR of SGA in elevated (>/>=20 μg/dl) maternal blood lead =2.15; (95% CI, 1.15–3.83)  
Higher risk (RR=2.22; 95% CI, 1.06–4.26) for LBW in the medium level of maternal blood lead (10–19 mg/dL) |
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Description</th>
<th>Sample Size</th>
<th>Blood Lead Details</th>
<th>Blood Lead Collection</th>
<th>Outcomes</th>
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<tr>
<td>Odland et. al. 2004</td>
<td>Mother-infant pairs in Finnmark, Norway or western arctic/subarctic regions of Russia</td>
<td>50</td>
<td>Maternal blood lead range: (0.02 μmol/L-0.65 μmol/L)</td>
<td>Maternal blood was collected upon patient presentation to hospital delivery departments</td>
<td>No association between maternal blood lead and LBW</td>
</tr>
<tr>
<td>Bjerregaard et. al. 2000.</td>
<td>Pregnant women from the five municipalities of the Disko Bay area in western Greenland</td>
<td>136</td>
<td>Maternal blood range: 34.5-12.1μg/L</td>
<td>Not stated</td>
<td>No significant association between maternal blood lead and birth weight</td>
</tr>
<tr>
<td>Jelliffe-Pawlowski et. al. 2006.</td>
<td>Pregnant women in California between 14 and 45 years old</td>
<td>262</td>
<td>Maternal blood range: ≤1 to 130.0 μg/dL</td>
<td>Whole blood lead testing done between day 1 of pregnancy and day of delivery</td>
<td>Increased risk of SGA in women with blood lead levels ≥ 10 μg/dl vs. those with blood lead levels ≤10 μg/dL (OR=4.2, 1.3–13.9)</td>
</tr>
<tr>
<td>González-Cossío et al. 1997.</td>
<td>Women from hospitals in Mexico City</td>
<td>272</td>
<td>Maternal blood arithmetic mean: 8.9 μg/dL; Maternal blood median=8.1 μg/dL; SD (4.1)</td>
<td>Maternal venous blood samples were obtained at delivery and 1-month postpartum</td>
<td>No association was found between maternal blood lead levels and birthweight</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>N</td>
<td>Blood Samples</td>
<td>Collection Period</td>
<td>Findings</td>
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<tr>
<td>Gundacker et al. 2010</td>
<td>Women recruited at the General hospital of Vienna</td>
<td>52</td>
<td>Maternal blood median: 25 μg/L Cord blood median: 13 μg/L</td>
<td>Maternal blood samples were collected between gestational week 34 and 38.</td>
<td>Low Pb exposure can result in lower birth weight (impotance coefficient= 0.487, rank =1)</td>
</tr>
<tr>
<td>Sowers et. al. 2002.</td>
<td>Women 12-34 years old in Camden, New Jersey</td>
<td>705</td>
<td>Maternal blood geometric mean: 1.2 μg/dL SE (0.03)</td>
<td>Blood samples were collected during each trimester of pregnancy, at delivery, and at the first postpartum visit</td>
<td>No association with birthweight or SGA was found</td>
</tr>
<tr>
<td>Xie et. al. 2013</td>
<td>Women 18 or older residing in the Laizhou Bay of the Bohai Sea in Shandong Province, China</td>
<td>252</td>
<td>Maternal blood median: 3.20 μg/dL</td>
<td>Maternal blood obtained within 3 days before delivery)</td>
<td>Increasing maternal blood lead exposure was associated with decreasing birth weight</td>
</tr>
</tbody>
</table>
Levels of Lead Exposure in Canada

Lead has been measured in urine, bone and teeth, however blood is the most desirable matrix for human biomonitoring (ASTDR (Agency for Toxic Substances and Disease Registry), 2007b). The elimination half-life of lead in blood is 30 days, meaning that it is a cumulative toxicant, and that previous exposure can be detected after long periods of non-exposure (Bernard, 1995). A proportion of lead in the blood may be present because of bone remodeling mobilizing lead that has been stored from previous exposure (Smith, Hernandez-Avila, Téllez-Rojo, Mercado, & Hu, 2002). The geometric mean blood lead level of Canadian females age 20 to 39 during the last round of the Canadian Health Measures Survey (CHMS), cycle 2 (2009-2011) was 0.85 (95% CI .074-0.98) µg/dL (Health Canada, 2013a).

2.6.2 Cadmium

Cadmium occurs in nature in a variety of inorganic forms; organo-cadmium compounds are very rare and have not been detected in the environment (Health Canada, 1986). The main sources of non-occupational cadmium exposure are cigarette smoke, contaminated soil, food grown in contaminated soil and drinking water. The human body has no mechanism for cadmium excretion and so it accumulates in the body’s tissues (Jomova & Valko, 2011). High concentrations have been detected in placental tissue (Lin, Doyle, Wang, Hwang, & Chen, 2011). Dietary cadmium absorption into the bloodstream is dependent on various components of an individual’s diet, however average absorption was estimated to be 5% in men and 10% or higher in women (CDC, 2009). Cadmium’s IARC classification is group 1 – Carcinogenic to humans (IARC (International Agency for Research on Cancer), 2012) and is also a schedule 1 substance on Canada’s List of toxic substances (Canada, 1999).

Epidemiologic Studies of Cadmium and Fetal Growth

The prenatal risks of cadmium exposure are not well understood. Studies examining the association between maternal and cord blood cadmium levels and fetal growth have yielded conflicting results. Such studies have only been conducted in areas with considerable cadmium exposure; there have been no studies examining low-level
exposure. The epidemiologic literature regarding maternal blood cadmium levels and SGA/LBW is summarized in table 2.2. Studies conducted in Asian countries reported a relationship between cadmium exposure and outcomes representing fetal growth restriction (Tian et al. 2009; Lin et al. 2011; Shirai et al. 2010), while in Sweden, France and Arctic regions, no relationship was found (Menai et al., 2012; J O Odland et al., 1999; Osman et al., 2000). One study done in Mexico reports a marginally significant relationship between cord blood cadmium levels and birthweight (p<0.06) (Galicia-García, Rojas-López, Rojas, Olaiz, & Ríos, 1997). The sample sizes for these studies were also relatively small. The largest study of maternal cadmium exposure and birth size was conducted in Bangladesh (n=1616). The exposure matrix in this study was urinary cadmium with an exposure range of 0.04 µg/L to 7 µg/L. This study reports an association with SGA in females but not in males (Kippler et al., 2011). The exposure levels in these regions were high compared to the Canadian geometric mean blood concentration of 0.33 (95% CI 0.25-0.44) µg/L for females 20 – 39 years of age (Health Canada, 2013a). Placenta and cord blood have been popular matrices for measuring cadmium levels in the context of perinatal biomonitoring. Many studies have included measures of cord and maternal blood samples finding the two to be correlated, with cord blood levels consistently higher than maternal blood levels (Bjerregard et.al. 2000; Odland et.al. 1999; Lin et.al. 2011).
<table>
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<tr>
<th>Reference</th>
<th>Study Population</th>
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<th>Exposure matrix /Concentration Range</th>
<th>Timing of exposure measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odland et.al. 1999</td>
<td>148 Russian women and 114 Norwegian women</td>
<td>262</td>
<td>Median maternal blood-cadmium in Russian mothers: 2.2 nmol/L</td>
<td>Immediately post partum</td>
<td>No association between cadmium and birthweight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median maternal blood-cadmium in Norwegian mothers: 1.8 nmol/L</td>
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<td></td>
</tr>
<tr>
<td>Galicia-Garcia et. al. 1997;91:57–61</td>
<td>Mother-infant pairs in Mexico City</td>
<td>49</td>
<td>Maternal blood range: 0.8-2.9 μg /L -2.1 μg/L</td>
<td>Not stated</td>
<td>Cord blood was found to have a marginally significant (p&lt;0.06) correlation with birthweight</td>
</tr>
<tr>
<td>Tian LL et. al. 2009</td>
<td>Pregnant women from Da-Ye county of Hubei Province in Central China</td>
<td>109</td>
<td>Maternal blood range: 0.43-25.25 μg/L</td>
<td>Maternal blood was collected within 1 week before delivery</td>
<td>No association between maternal blood cadmium and SGA/LBW</td>
</tr>
<tr>
<td>Reference</td>
<td>Study Population</td>
<td>N</td>
<td>Exposure matrix /Concentration Range</td>
<td>Timing of exposure measure</td>
<td>Results</td>
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<tr>
<td>Menai et. al. 2012</td>
<td>Women from 18- to 45-years old who presented before 24 weeks of gestation for prenatal care at two clinics in France</td>
<td>901</td>
<td>Maternal blood: 0.88 μg/L (0.59)</td>
<td>Maternal blood was collected between 24 and 28 weeks of gestation</td>
<td>OR = 1.41; 95% CI: 1.00–1.99 \nFor blood cadmium concentration (&lt;1μg/L vs. 1.5 μg/L)</td>
</tr>
<tr>
<td>Osman et. al. 2000</td>
<td>Swedish women</td>
<td>106</td>
<td>Maternal blood range: 0.12–18 μg/L \nMaternal blood median: 1.4 μg/L</td>
<td>Maternal venous blood was collected at 36 weeks gestation</td>
<td>No association between blood cadmium concentrations and birth size</td>
</tr>
<tr>
<td>Bjerregaard et. 2000</td>
<td>Pregnant women from the five municipalities of the Disko Bay area in western Greenland</td>
<td>136</td>
<td>Maternal blood range: 1.2-0.02μg/l \nMaternal blood arithmetic mean: 0.9μg/l</td>
<td>Not stated</td>
<td>No significant association between maternal blood cadmium and birth weight was found</td>
</tr>
</tbody>
</table>
Levels of Cadmium exposure in Canada

Cadmium can be measured in multiple human tissues including hair, feces, liver, and urine, however blood measures reflect both cumulative and recent exposure (CDC, 2009). The elimination half life of cadmium in blood is 100 days, meaning that it is a cumulative toxicant and that previous exposure can be detected after long periods of non-exposure (Bernard, 1995). Blood cadmium concentrations are twice as high in smokers compared to non-smokers (ASTDR (Agency for Toxic Substances and Disease Registry), 2008). According to cycle 2 CHMS results, the geometric mean blood cadmium concentration of Canadian females 20 – 39 years of age was 0.33 (95% CI 0.25-0.44) µg/L (Health Canada, 2013a).

2.6.3 Mercury

Mercury is able to form organic and inorganic compounds. Methylmercury, the organic form, is the form that the greatest proportion of the general population is exposed to through the consumption of some fish and seafood. Chronic mercury exposure can adversely affect the nervous, reproductive and gastrointestinal systems. Prenatal exposure can cause developmental and intellectual delays and may interfere with the development of the fetal nervous system (Wong & Lye, 2008a). Methylmercury is easily absorbed through the gut and deposited throughout the body. Mercury concentrates in the brain, liver, kidneys, placenta and fetus. In the fetus, mercury concentrates in the brain, peripheral nerves and bone marrow. Approximately 95% of orally ingested organic mercury is absorbed through the gastrointestinal tract (ASTDR (Agency for Toxic Substances and Disease Registry), 1999b). Methylmercury compounds are in group 2B according to the IARC-possibly carcinogenic to humans (IARC 1993). Mercury and its compounds are toxic substances on schedule 1 of the CEPA (Canada, 1999).

Epidemiologic Studies of Mercury and Fetal Growth

The epidemiologic literature regarding maternal blood mercury levels and SGA/LBW is summarized in table 2.3. The impact of low-level mercury exposure on fetal growth is uncertain. Only one study has found a significant association between mercury and birth weight (B.-E. Lee et al., 2010). All other studies using birthweight as
the outcome of interest have concluded that there is no association with mercury (Bjerregaard & Hansen, 2000; Daniels, Rowland, Longnecker, Crawford, & Golding, 2007; Ding et al., 2013a; Hujoel et al., 2005; Lederman et al., 2008; Lucas et al., 2004). Only one study to date has reported on size for gestational age as an outcome. The results were suggestive of an effect on fetal growth (Ramón et al., 2009) but the study lacked statistical power (Karagas et al., 2012). Lederman et. al., Bjerregaard and Hansen, Ding et. al. and Lee et. al. were the only studies to use maternal blood as the exposure matrix. Cord blood and tissue were used in all other studies.
<table>
<thead>
<tr>
<th>Reference</th>
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<th>Timing of exposure measure</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Lee et al. 2010</td>
<td>Korean mother-infant pairs</td>
<td>417</td>
<td>Geometric mean mercury maternal blood: 3.30 μg/L</td>
<td>12–20 gestational weeks and 28–42 gestational weeks.</td>
<td>Inverse relationship between birth weight and maternal and cord blood Hg levels.</td>
</tr>
<tr>
<td>Lederman et al. 2008</td>
<td>Non-smoking pregnant women between 18-39 years of age</td>
<td>329</td>
<td>Arithmetic mean maternal blood: 2.29 μg/L (2.33)</td>
<td>within 24 hours of delivery</td>
<td>No significant association between maternal blood mercury and birth weight was found</td>
</tr>
<tr>
<td>Bjerregaard and Hansen, 2000</td>
<td>Pregnant women from the five municipalities of the Disko Bay area in western Greenland</td>
<td>136</td>
<td>Maternal blood range: 13.6-1.9μg/l , Maternal blood arithmetic mean: 12.8μg/l</td>
<td>Not stated</td>
<td>No significant association between maternal blood mercury and birth weight was found</td>
</tr>
<tr>
<td>Ding et al. 2013</td>
<td>Women 18 or older residing in</td>
<td>258</td>
<td>Maternal blood geometric mean: 0.84</td>
<td>obtained within 3 days</td>
<td>No significant association between</td>
</tr>
<tr>
<td>the Laizhou Bay of the Bohai Sea in Shandong Province, China</td>
<td>μg/L</td>
<td>prior to delivery.</td>
<td>maternal blood mercury and birth weight was found</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Levels of Mercury Exposure in Canada**

Hair, blood and urine are all matrices that have been used to measure mercury exposure in humans (ASTDR (Agency for Toxic Substances and Disease Registry), 1999b). The elimination half-life of mercury in blood is 18 days, meaning that it is a cumulative toxicant with the possibility of detection of previous exposure after long periods of non-exposure (Bernard, 1995). This makes blood a good choice for examining mercury exposure. The geometric mean blood mercury level of Canadian women aged 20 to 39 from the second cycle of the CHMS is 0.66 (95% CI (0.47 - 0.92) µg/L (Health Canada, 2013a).

### 2.6.4 Arsenic

Arsenic is a potent toxicant and carcinogen that is able to form both organic and inorganic compounds in the environment and the human body. The majority of organic arsenic is present in forms that are biologically unavailable (M Vahter, Marafante, & Dencker, 1983), or in forms that are rapidly excreted from the body (Health Canada, 2006), therefore inorganic arsenic is the form of greatest concern to human health. Arsenic occurs naturally in rocks and can contaminate drinking water (Rahman et al., 2009) and food, most notably fish and meat (Gartrell, Craun, Podrebarac, & Gunderson, 1986). Up to 95% of ingested inorganic arsenic is absorbed into the bloodstream (ASTDR (Agency for Toxic Substances and Disease Registry), 2007a) Arsenic and arsenic compounds are carcinogenic to humans (IARC 2012) and are also listed as schedule 1 on the list of toxic substances under the CEPA (Canada, 1999).

**Epidemiologic Studies of Arsenic and Fetal Growth**

The epidemiologic literature concerning maternal blood and urinary arsenic levels and SGA/LBW is summarized in table 2.4. Two studies have suggested an association between arsenic exposure and LBW, but these studies were ecologic in design and therefore subject to certain biases (Hopenhayn, Ferreccio, et al., 2003; Yang et al., 2003). Most studies of arsenic exposure and fetal growth outcomes have been conducted in regions with high-level exposure, and therefore the health effects of arsenic levels likely to be seen in Canada remain unknown. The majority of studies of arsenic exposure and
fetal growth outcomes focus on outcomes such as stillbirth, fetal loss or spontaneous abortion and therefore will not be included in this review. A cohort study in Bangladesh reported that infant birth weight decreased by 1.68g for each 1-μg/L increase of arsenic in urine (Rahman et al., 2009). Shirai et al. found no association between urinary arsenic levels and SGA in a study of 78 mother-infant pairs (Shirai et al., 2010).
Table 2.4 Epidemiologic Studies of Arsenic and Fetal Growth

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Population</th>
<th>N</th>
<th>Exposure matrix /Concentration Range</th>
<th>Timing of exposure measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rahman et. al. 2009</td>
<td>Mother infant pairs in Matlab, Bangladesh</td>
<td>1578</td>
<td>Urinary arsenic range: 6 –978 μg/L</td>
<td>Urine sample collected around gestational week 8 and 30</td>
<td>Birthweight decreased by 1.68g (SE, 0.62) for each 1-μg/L increase of arsenic in urine at arsenic exposure (&lt;100 μg/L)</td>
</tr>
<tr>
<td>Hopenhayn et. al. 2003</td>
<td>Pregnant women aged 18-45 living in Antofagasta, Chile and Valparaiso, Chile</td>
<td>844</td>
<td>Mean urinary arsenic in Antofagasta: 40 μg /L &lt;1 μg /L</td>
<td>Urine samples were collected between 16–35 weeks gestation</td>
<td>High (&lt;50 μg/L) exposure group had lower mean birth weight (-57 g; 95% confidence interval = -123 to 9)</td>
</tr>
<tr>
<td>Shirai et. al. 2010</td>
<td>Pregnant women in Tokyo, Japan</td>
<td>78</td>
<td>Urinary arsenic range: 9.81–1603 μg /L urinary arsenic geometric mean: 76.9 μg /L SD (2.33)</td>
<td>Urine sample was done at 9 to 40 gestational weeks.</td>
<td>No association between arsenic and birth size</td>
</tr>
</tbody>
</table>
Levels of Arsenic Exposure in Canada

Arsenic has been measured in hair, nails, blood and urine (World Health Organization, 2011a). Measurement of urinary arsenic levels is generally accepted as the most reliable indicator of recent arsenic exposure, given that arsenic absorbed from the lungs or the gastrointestinal tract is excreted in the urine within one to two days, while arsenic is cleared from blood within a few hours (ASTDR (Agency for Toxic Substances and Disease Registry), 2007a). However some papers have shown very good correlation between urinary arsenic and blood arsenic (Hall et. al, 2006).

The half-life of inorganic arsenic in humans is estimated to be between two and 40 days (Pomroy et al. 1980). A considerable proportion of inorganic arsenic is eliminated from the body by the rapid urinary excretion of non-methylated arsenic in both trivalent and pentavalent forms and by sequential methylation of arsenic (+3) to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in the liver (World Health Organization, 2001). The geometric mean concentration of DMA in urine for Canadians women age 20-39 is 6.4 (95% CI 4.8 – 8.7) µg/L (Health Canada, 2013a).

2.7 Risk Factors for Elevated Toxic Metal Body Burden

While exposure to elevated levels of toxic metals is uncommon in Canada at present, the levels to which the general population is exposed varies regionally and demographically. Canadians with the lowest household incomes, born in certain countries outside of Canada, or living in houses that were built over 50 years ago have the highest blood lead levels (Statistics Canada 2011). Smokers are at the greatest risk of elevated cadmium body burden. Non-smokers with the highest cadmium exposure risk are women with low iron stores and people consuming a diet high in fiber and shellfish (Järup, Berglund, Elinder, Nordberg, & Vahter, 1998). Those working in certain industries, having dental amalgam fillings, or who consume a diet high in marine animals, fish and shellfish are at the greatest risk for having elevated levels of blood mercury. This is especially true of people living in the most northern climates who may consume meat from marine animals like whales or seals as these animals are at or near
the top of their food chain. (ASTDR (Agency for Toxic Substances and Disease Registry), 1999a) Individuals living in an area with contaminated groundwater are at the greatest risk for arsenic exposure, however for the vast majority of Canadians, the greatest source of arsenic exposure is through food (Health Canada, 2006). Arsenic in food is mainly in organic form and is therefore of low toxicity (World Health Organization, 2001).

2.8 Biologic Mechanism for Toxic Metals and SGA

The most prominent hypothesis as to how toxic metals may contribute to SGA pathology is through oxidative stress (Myatt, 2006). This process is outlined in figure 1.1. Specifically, when toxic metals are present in the maternal bloodstream, they may cause abnormal placental function and impair nutrient transport to the fetus through the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These free radicals include but are not limited to: singlet oxygen, hydrogen peroxides, hydroperoxides the superoxide radical, hydroxyl radical and nitric oxide (Jomova & Valko, 2011) that cause oxidative stress by reacting with macromolecules and damaging them. When the vascular endothelium is damaged, endothelins are released, causing the smooth muscle around the blood vessels to contract. This vasoconstriction causes maternal blood pressure to increase and decreases the blood flow to the fetus (Pollock & Pollock, 2005). This reduced blood flow may cause ischemic damage, which could lead to fetal growth restriction and subsequent low birthweight (Llanos & Ronco, 2009).

The toxic metals being studied are understood to be redox inactive, meaning that they are unable to form ROS directly. Instead, these metals can induce oxidative stress through various indirect methods (Ercal, Gurer-Orhan, & Aykin-Burns, 2001).
2.8.1 Experimental Animal and *In Vitro* Studies

The current understanding of about the biologic mechanism involved in of how these metals the functioning of these metals inside living organisms is largely learned from *in vitro* and in experimental animal studies. The following review details what is known about how these metals induce oxidative stress.

**Lead**

Lead can induce oxidative stress in rats by acting directly on cell membranes. It can alter membrane integrity by causing membrane components to deteriorate (Gurer & Ercal, 2000). Lead also interacts with oxyhemoglobin to induce oxidative stress. Lead can generate superoxide and peroxide radicals by causing a buildup of Aminolevulinic acid (ALA), which then undergoes aerobic oxidation (Monteiro, Abdalla, Faljoni-Alário, & Bechara, 1986). Furthermore, numerous rat studies have shown that lead can increase oxidative stress by depleting the cell’s antioxidant pools, most importantly, glutathione (GSH) (Gurer and Ercal 2000; Korsrud and Meldrum; Monteiro et al. 1985). The sulfhydryl groups of GSH bind to toxic metals like arsenic and mercury, therefore lead exposure may enhance the toxicity of other metals (Zhu et al., 2010).
Cadmium

Studies in rats have been used extensively to inform understanding of cadmium toxicokinetics. Alterations in GSH levels have been observed in cadmium toxicity. Cells respond to oxidative stress by increasing levels of GSH. If the oxidative stress continues and GSH synthesis cannot meet the demand, GSH depletion occurs (Kamiyama et al., 1995; Rana & Boora, 1992; Rana & Verma, 1996; Shaikh, Vu, & Zaman, 1999). Cadmium enhances lipid peroxidation (Yiin, Sheu, & Lin, 2001) and can have a deleterious effect on cellular enzymes (Shaikh et al., 1999).

Arsenic

Little is known about the exact mechanism through which arsenic may induce oxidative stress. An in vitro study by Lee and Ho shows that arsenic compounds can deplete cell antioxidant defense systems (T. C. Lee & Ho, 1995), but there is also evidence from a study in mice for direct arsenic-induced free radical formation (Liu et al., 2001).

Mercury

Mercury can induce oxidative stress by binding to and causing irreversible excretion of GSH molecules. In vitro studies show that mercury increases production of \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) by impairing electron transport and oxidative phosphorylation in the mitochondria (Lund, Miller, & Woods, 1991; Nath, Croatt, Likely, Behrens, & Warden, 1996). Studies in rats suggest that mercury displaces copper and iron from their binding sites, accelerating ROS formation (Huang, Cheng, & Lin, 1996; Sarafian, 1999) Rat studies have also shown that mercury induces oxidative stress through induction of lipid peroxidation, depleting cells antioxidant pools and interference with calcium homeostasis (Huang et al., 1996; Lund, Miller, & Woods, 1993).

2.9 Genetic Polymorphisms

Genetic variability in metabolism of potentially toxic exposures plays an important role in environment-health relationships. There are several polymorphic genes that may be
relevant to the metabolism and detoxification of toxic metals such as the metallothionein and cytochrome P450 families (Sasaki et al., 2006; Tekin, Kayaalti, Aliyev, & Soylemezoglu, 2012). This review focuses on 3 particular genetic polymorphisms because they were the most relevant to metal metabolism and SGA that were measured in the MIREC study.

Genetic polymorphisms of human glutathione S-transferases (GSTs) affect the metabolism and excretion of toxic metals, and may be important in terms of individual susceptibility to the harmful effects of toxic metal exposure. GSTs are phase 2 xenobiotic metabolizing enzymes that play a role in catalyzing conjugation reactions of multiple electrophilic compounds (including toxic metals) with glutathione (Sheehan, Meade, Foley, & Dowd, 2001).

2.9.1 GSTP1

GSTP1-rs1138272 (C>T) and rs1695 (A>G) are two polymorphisms that have been associated with diminished enzyme activity of several classes of substrates (García-González et al., 2012). In Caucasian populations, the genotype distribution for rs1138272 is: CC 80%, TC 20%, TT 0% (Cariaso & Lennon, 2012) and for rs1695 is: AA 35%, AG 55%, GG 10% (Cariaso & Lennon, 2012). Epidemiological and in vitro studies have suggested that GSTP1 may play a role in mercury and arsenic toxicokinetics (Custodio et al., 2004; Goodrich & Basu, 2012; Gundacker et al., 2010; Marcos et al., 2006).

2.9.2 GSTO1

GSTO1- rs4925 (C>A) is a nonsynonymous polymorphism that has been shown to affect the functional activity of the enzyme (Punia et al., 2011). The physiological role of the GSTO1 enzyme is not fully understood, however in vitro studies have identified GSTO1 as the rate-limiting step in arsenic biotransformation (Zakharyan et al., 2001). In Caucasian populations, the genotype distribution is as follows: CC 42%, AC 48%, AA 10% (Cariaso & Lennon, 2012). Thioltransferase activity has been shown to be significantly lower for the rs4925 variant than the wild type (Rodrigues et al., 2012), thus influencing individual susceptibility to oxidative stress (Punia et al., 2011). Rodrigues et al.’s findings indicate that thioltransferase activity may play a role in arsenic metabolism or that this variant is highly
correlated with another single-nucleotide polymorphism (SNP) involved in arsenic metabolism (Rodrigues et al., 2012).

2.10 Methodologic Issues in Previous Epidemiologic Studies

While all types of study designs have been used to investigate the relationship between toxic metal exposure and fetal growth outcomes, the vast majority of studies in this area have been cohort studies. Growth restriction is difficult to measure in utero, so proxy measures are always used. Among the proxies of growth restriction available, SGA has historically been one of the less common outcome measures studied in the context of metal exposure during pregnancy. Most studies of metal exposure and fetal growth outcomes representing IUGR use birthweight, or LBW as an outcome, and few account for gestational age.

Exposure matrices in cohort studies of metal exposure and fetal growth restriction tend to be either cord blood, or a combination of cord blood and maternal blood, however placental, urinary, hair and bone measures have also been used. Many studies of arsenic exposure and fetal growth have used arsenic concentrations in well water to quantify exposure, with the limitations of potential misclassification of maternal exposure. Cord blood and placental exposure measures are always taken at birth. It has not been common practice to collect maternal blood and urine samples at any specific period of gestation corresponding to a proposed biologic window of interest. These samples have been collected during all stages of pregnancy in the literature.

There are many recent US studies of lead exposure and fetal growth with exposure levels similar to those that would be expected in Canada. With the exception of lead, the majority of studies of toxic metal exposure and fetal growth outcomes are conducted in areas where a large proportion of the sample is exposed to relatively high levels of the metal being studied. Finally, most studies in this area have been relatively small, the majority having 500 subjects or less. All prior studies have controlled for the major known predictors of fetal size in some way.

2.11 Summary

Given the current body of evidence, it is difficult to say what the role of prenatal toxic metal exposure at current Canadian levels is with respect to fetal growth. If the hypothesized
oxidative stress biological mechanism is correct, then the use of cord blood as an exposure matrix is questionable. The oxidative stress mechanism suggests that SGA is a result of vasoconstriction in maternal blood vessels, meaning that maternal blood may be the matrix of interest.

Many previous studies of toxic metal exposure during pregnancy report on birthweight but only few account for gestational age. The GSTP1 and GSTO1 SNPs in this study have never been studied in relation to toxic metal detoxification and fetal growth. Furthermore, many of the previous studies of these kinds of relationships have had small sample sizes, making them able to detect only very large effects. This study is needed to explore genetic factors influencing the oxidative stress pathway as few studies have overtly studied toxic metals and SGA in this context. Only a handful of studies had considered possible gene environment interactions in the relationship between prenatal toxic metal exposure and risk for SGA.

2.12 References


Chapter 3

Design and Methods

3.1 Introduction

This chapter summarizes the study objectives and addresses the methods key to this thesis that are not discussed in the manuscript section.

3.2 Study Objectives and Hypotheses

1. To determine the relationship between toxic metal exposure during pregnancy and risk of SGA. It was hypothesized that elevated levels of toxic metals in maternal blood and urine are associated with increased risk of SGA birth.

2. To explore whether or not GST polymorphisms are an effect modifier of the relationship between metal exposure and risk of an SGA birth. It was hypothesized that the effect of toxic metals on SGA is more pronounced in individuals who have the variant genotype for the GST polymorphisms.

3.3 Study Design

This study was nested within a larger cohort study called the Maternal-Infant Research on Environmental Chemicals (MIREC) study that was conducted by Health Canada and the Ste. Justine Hospital along with other clinical and academic researchers. The study’s co-principal investigators are Dr. Tye Arbuckle and Dr. William Fraser. The MIREC study has multiple primary aims which include; 1) To determine whether contemporary non-occupational-level metal exposure as measured by maternal and fetal body burdens is related to elevated maternal blood pressure, hypertension and fetal growth retardation. 2) To obtain national-level data on maternal and neonatal exposure to priority environmental contaminants. 3) To obtain Canadian data on smoking behaviour and exposure to tobacco smoke (active and passive) in pregnancy. 4) To obtain contemporary levels of priority environmental chemicals, food packaging and food processing related chemicals, selected nutrients and relevant immunoprotective endpoints and antioxidative markers in mature human milk 5) To obtain
contemporary levels of maternal hair-mercury. 6) To characterize dietary exposure of breastfed infants ages 2-10 weeks.

3.3.1 Sampling

Women were recruited into a national level convenience sample during the first trimester of pregnancy and followed up to ten weeks after delivery. An information poster and pamphlet were used to recruit women into the study. These materials were placed in physician offices as well as other key locations that were identified by the clinic sites. Information about the study was also communicated to appropriate levels of government and non-government organizations. A convenience sample was used because acquiring a probabilistic sample of women in early pregnancy would be inefficient and very resource intensive. There were ten study sites across Canada: Toronto, Ottawa, Kingston, Montreal, Edmonton, Winnipeg, Sudbury, Vancouver, Hamilton, and Halifax. Sites were selected to represent different geographical regions of Canada, and on the basis of their having pre-existing clinical obstetrical research infrastructure in place. Women were approached and screened for eligibility at early prenatal clinics. Only women able to consent and to communicate in English or French, age 18 years or older, planning on delivering at a local hospital, and agreeing to participate in the cord blood collection component of the MIREC study were eligible to participate.

3.4 Detectable Effects

It was anticipated that exposure categories would be created based on observed distributions. Given the cohort size, the thesis plan was to create three exposure categories. The power calculation to follow assumed an equal number of subjects in each category. Statistical power will be reduced (the detectable effect will increase) if the distribution is such that tertiles are not an appropriate categorization. The main comparison is based on exposure tertiles, the highest exposure group against the lowest exposure group. Based on this comparison, this study is able to detect relative risks of 1.67 and greater with 80% power at a significance level of 0.05 with a sample of 1800 participants, a ratio of exposed to unexposed of 1, and with an overall rate of SGA of 7%.
The comparisons in the exploratory gene-environment analysis are based on dichotomous genotype distributions. The genotype distribution for GSTP1 A114V is: CC 80%, TC + TT 20% for GSTP1 I105V is: AA 35%, AG + GG 65%, and for GSTO1 A104A is: CC 42%, AC + AA 58% (Cariaso & Lennon, 2012).

The investigation of gene-environment interactions had limited statistical power. The focus of the detectable effect estimates to follow was the detectable relative risk in the genotype category where the effect of metal exposure on SGA was hypothesized to be strongest. For GSTP1 A114V, 20% of the population is anticipated to be in the variant genotype category (n=360). For a dichotomous comparison of the highest tertile versus the lower two thirds of exposure, with an event rate of 6% in the unexposed, statistical significance of 0.05 and 80% power, the detectable relative risk is 2.56 For GSTP1 I105V (65% (n=1170) in the variant genotype category) and GSTO1 A104A (58% (n=1044) in the variant genotype category) the detectable relative risk for metal exposure in relation to SGA in the at risk genotypes with all other factors the same as in the prior calculation are 1.75 and 1.80 respectively.

3.5 Data Collection

The data collection methods are summarized in the manuscript section of this thesis. More detail on the methods in the underlying MIREC study can be found in the paper by Arbuckle et. al.(Arbuckle et al., 2013)

3.6 SGA

Measurement of infant weight and gestational age are detailed in the manuscript section of this thesis. Details on size for gestational age calculation and on SGA categorization can also be found here.
3.7 Data Analysis

3.7.1 Data Management

The student investigator was responsible for identification of relevant variables for this analysis using two data dictionaries; one for questionnaire data and another for lab data.

There were 4000 variables in the questionnaire dataset, containing both neonatal and maternal information. Many of these variables had to be manipulated to derive the variables used in this analysis. Each derived variable was created in a smaller dataset that was made using the master dataset. These smaller datasets were then all merged into one large dataset containing all of the questionnaire data relevant to this analysis.

The lab dataset originally contained data for each test done in the MIREC study and contained 266,671 records. The data was organized so that each participant’s test results were contained in 103 records. The student investigator extracted the lab results for each relevant lab test and, with results below the detection limit being assigned a value of half of the detection limit. The student investigator transposed the data so that each record contained all of the information for one participant by creating a series of small datasets and then merging them together by a unique identifier.

Once both a lab and a questionnaire dataset were created, the same unique identifier had to be created in the questionnaire dataset to allow for the relevant lab data and relevant questionnaire data to be merged into one master dataset containing all of the information that would be necessary to explore the relationships between the exposures and outcome of interest while considering important predictors of the outcome.

Following this merge, subjects with no lab data corresponding to their questionnaire data were removed. The clean dataset before exclusions were made contained 1983 records, corresponding to 1983 subjects. For the purpose of this thesis, multiple births were excluded as they have different criteria for being categorized as SGA and are much more likely to be SGA than singletons. After all appropriate exclusions were made; the final dataset contained 1835 records for 1835 subjects. The exclusion process is detailed in the data tree figure 1.

Genotyping data for the three genes in question was extracted from a third Health Canada data source. This information was then merged into the larger dataset by the unique identifier that had previously been created. No further subjects were removed from the dataset, however
genotyping data was missing from some of the subjects, such that there were 1821 women with genotyping information for GSTP1A114V, 1820 with information for GSTP1I105V and 1816 with information for GSTO1 in the final dataset. 44 subjects did not have a measure of urinary arsenic metabolites.

Figure 3.1 Data Tree

5108 Eligable participants contacted

2001 consented

166 ineligible
• 18 withdrawals
• 51 multiple births
• 9 stillbirths
• 32 Spontaneous abortions

N=1835
3.7.2 Variable conceptualization

*Exposure variables*

Exposure variable conceptualization for the main analysis is described in detail in the manuscript sections of this thesis. For the exploratory analysis, an average measure of exposure between the first and third trimester measures was calculated. When only one value was available, that value was used as the value for average metal exposure. Metal exposure then was dichotomized into a high and a low exposure group such that each group contained between ~30-60% of the sample.

*Interaction with GST polymorphisms*

In order to explore possible genetic influence on the relationship between metal exposure and SGA, maternal genotyping data was extracted from a Health Canada database. All polymorphisms were dichotomized as wild type homozygous vs. variant allele positivity, assuming a dominant model. This assumption was based on a-priori evidence of gene functionality (Ali-Osman, Akande, Antoun, Mao, & Buolamwini, 1997; Hu et al., 1997; Parker et al., 2008). This resulted in the following categorization:

**GSTP1 A114V polymorphism:** CC vs. CT + TT  
**GSTP1 I105V polymorphism:** AA vs. AG + GG  
**GSTO1 polymorphism:** CC vs. CA + AA

3.7.3 Descriptive statistics

Descriptive statistics were calculated for all variables that were considered in the analyses. Frequency tables were generated to describe the demographic characteristics of the sample. T-tests were performed to determine if mothers of SGA infants had different geometric mean blood concentrations of each metal than mothers of infants who were not SGA. Correlation coefficients were calculated for the relationship between the first and third trimester measures of each metal in blood. Crude odds ratios were calculated using contingency tables and logistic regression. Log-binomial regression and contingency tables were used to calculate crude relative risks (McNutt, Wu, Xue, & Hafner, 2003).
The bivariate relationship between each metal exposure variable and each covariate was evaluated using t-tests (for dichotomous covariates) and ANOVA (for categorical covariates). Chi square tests were done to verify that each gene being studied was in Hardy Weinberg Equilibrium. Bivariate relationships between each SNP and SGA were also evaluated with contingency tables.

3.7.4 Statistical modeling-Main effects

Log-binomial regression was used to estimate the relative risks associated with toxic metal exposure adjusting for potential confounders that have strong established relationships with fetal growth. The modeling strategy employed was to control for the strongest predictors of SGA by building a parsimonious, predictive model. Data came from ten study centres and therefore represent a cluster sample. The variable “centre” and a random effects parameter were introduced into the model in order to evaluate concern over data clustering due to the sampling method used. The result of this analysis did not change the width of the confidence intervals, and so there was no need to introduce a random effects parameter into the regression models. The remaining details of the statistical modeling used in this analysis are detailed in the manuscript sections of this thesis.

3.8 Exploratory gene-environment interaction analysis

DNA extraction

DNA was extracted from blood aliquots using the DNeasy blood and tissue kit (QIAGEN) following the manufacturer’s protocol. Samples were rinsed in 200ul AE buffer. Genotyping was done at CHUL Research Center (CRCHUL) Sequencing and Genotyping Laboratory, Center Hospitalier Universitaire de Québec (CHUQ), Université Laval

PicoGreen DNA quantification

DNA concentration was measured with the Quant-it PicoGreen assay (Invitrogen). Briefly, PicoGreen dye was added to each well. Following this, the fluorescent signal of the
sample was measured and plotted against the standard DNA concentration used to make a standard curve.

3.9 Gene-environment interaction analysis

The distribution of alleles and genotypes were examined and frequencies were tested using a likelihood ratio test chi-square statistic to compare observed and expected counts according to principles of Hardy-Weinberg equilibrium (HWE).

Each main analysis was stratified into two groups distinguished by either wild type (homozygous) or variant (heterozygous and homozygous variant) genotype, thus assuming dominant effects. A log binomial regression model containing the genotype of interest, and a product term between that metal and the genotype variable was used to generate crude relative risks based on the distribution of high and low exposure with and without SGA. This allowed for an interaction p-value to be produced. All analysis was performed using SAS enterprise guide version 4.2.

3.10 References


4.1 Abstract

Background: Lead, mercury, cadmium and arsenic are some of the most common toxic metals to which Canadians are exposed. The effect of exposure to current low levels of toxic metals on fetal growth restriction is unknown.

Objective: The aim of this study is to examine relationships between exposure to lead, mercury, cadmium and arsenic during pregnancy, and risk of small for gestational age (SGA) birth.

Methods: Mean lead, mercury, cadmium and arsenic levels were measured in blood samples from the first and third trimesters in 1835 pregnant women from across Canada. Arsenic species in first trimester urine were also assessed. Relative risks controlling for important covariates were estimated using log-binomial regression.

Results: Lead, cadmium, mercury and arsenic were detectable in blood of 100%, 99.4%, 92.9% and 97.5% of participants respectively. Dimethylarsinic acid (DMA) and Arsenobetaine/Arsenocholine were detectable in 85.4% and 48.8% of participants. No association was found between blood lead, cadmium or arsenic and risk for SGA. Increased risk for SGA was observed between the ≤0.8 µg/L and the ≥1.6 µg/L exposure groups for mercury (RR=1.56; 95% CI = 1.04-2.58) and, between the ≥22.56 µg/L and ≤7.75 µg/L exposure groups for arsenobetaine (RR= 1.65.; 95% CI = 1.10-2.47) after adjustment for the effects of parity and smoking.

Conclusions: These results suggest that exposure to mercury and arsenobetaine at levels observed in the Canadian population are associated with increased risk of giving birth to an SGA infant. These findings, combined with previous research underscore the need to reduce mercury and arsenic exposure as much as possible, especially for women of child-bearing age.
Keywords: biomonitoring, pregnancy cohort study, small for gestational age, mercury, cadmium, lead, arsenic

4.2 Introduction

Pregnant women and their fetuses are especially susceptible to the effects of exposure to a variety of environmental toxicants including lead, mercury, cadmium, and arsenic which are some of the most common toxic metals to which humans are exposed. Metals are ubiquitous in the environment, and exposure occurs through ingestion of food, water, soil, or dust; inhalation from air; and through direct contact with contaminated products. Some known consequences of high level exposures to these metals include: neurological deficits, cancer, renal impairment, convulsions, coma, and bone disease (Duruibe, J.O, Ogwuegbu, M.O.C and Egwurugwu, 2007; Jarup, 2003). There are presently no established “safe” levels of toxic metal exposure and it is unknown what health effects, if any, are produced by exposure to the low levels of metals to which most of the general Canadian populations are exposed.

Physiologic changes that occur during pregnancy alter toxicokinetics and toxicodynamics of environmental chemicals, such as metals, in the pregnant woman’s body. Increased maternal plasma volume, body fat, and body water, coupled with increased excretion, are designed for optimal nutrient supply and waste removal from the fetus (Mattison et al., 1991). Little is known about the impact of these changes on metabolism and excretion of toxicants during pregnancy and lactation, but likely will differ depending on the chemical class or nature of the substance. The entire pregnancy is a period of dynamic growth and change for the developing embryo and fetus, thus, any insults to the in utero environment could result in suboptimal fetal development (Rice & Barone, 2000).

Growth restricted fetuses fail to reach their full genetic growth potential because of reduced tissue deposition due to a decreased nutrient supply from the utero placental circulation (Cetin et al., 2004). Intrauterine growth restriction (IUGR) manifests as small for gestational age (SGA), defined as an infant weighing less than the 10th percentile for their gestational age and sex (Canadian Institute for Health Information, 2009). Size relative to
gestational age is an established predictor of early childhood mortality and morbidity (Rahman et al., 2009). Children who are born SGA are more likely to have birth complications and are at increased risk for excessive weight gain, cardiovascular disease and insulin resistance syndrome later in life (Clayton et al., 2007). Metals are potential risk factors for SGA births, and are hypothesized to induce growth restriction through oxidative stress mediated pathways (Myatt, 2006).

The effects of lead, cadmium, mercury and arsenic have all been studied to varying degrees in the context of their effect on fetal growth outcomes representing fetal growth restriction. In general, the literature regarding metal exposure and fetal growth is inconclusive. Some studies report associations (P.-C. Chen et al., 2006; B.-E. Lee et al., 2010; J O Odland et al., 1999; Xie et al., 2013; Zhu et al., 2010) while others report no association (Daniels et al., 2007; Ding et al., 2013b; Salpietro et al., 2002; Shirai et al., 2010; Tian et al., 2009). Few studies of this type have examined low-level exposure as currently experienced in most developed countries such as Canada. Currently, there no established “safe levels of toxic metal exposure. It remains unknown what health effects, if any at all, can result from the levels that most Canadians are currently exposed to. The aim of this study is to examine relationships between exposure to lead, mercury, cadmium and arsenic during pregnancy; and risk of small for gestational age (SGA) birth.

4.3 Methods

4.3.1 Study Population

The Maternal-Infant Research on Environmental Chemicals (MIREC) Study is a prospective cohort study conducted by scientists at Health Canada, CHU Sainte-Justine’s in Montreal, and other clinical and academic institutions. Subject accrual methods are described in detail elsewhere (Arbuckle et al., 2013) and will only be summarized here. Between 2008-2011, 2,001 pregnant women were recruited in the first trimester of pregnancy from ten study sites across Canada: Toronto, Ottawa, Kingston, Montreal, Edmonton, Winnipeg, Sudbury, Vancouver, Hamilton, and Halifax. Exclusion criteria for participation in the MIREC study included: inability to communicate and consent in either French or English, being greater than 14 weeks gestation at the time of recruitment, being less than 18 years of age, or unwilling to
provide an umbilical cord blood sample at birth. Women with a diagnosed fetal anomaly and those with a history of major chronic disease were also not eligible for participation in MIREC. Excluded from this analysis are: 18 women who withdrew during the study, 51 women who gave birth to multiples, 9 stillbirths, 32 spontaneous abortions, 13 therapeutic abortions, 28 with no metal exposure data, and 15 with no infant sex, weight, or gestational age recorded. The final sample size for this analysis was 1,835 mother-infant pairs.

4.3.2 Metals Exposure

Maternal blood was collected during the first and third trimesters of pregnancy and analyzed for total lead, cadmium, mercury, and arsenic concentrations. Speciated levels of dimethylarsinic acid (DMA) and arsenobetaine/arsenocholine, used to represent exposure to organic arsenic were measured in urine samples collected during the first trimester. Samples were analyzed using inductively-coupled plasma mass spectrometry by the Toxicology Laboratory of the Institut National de Santé Publique du Québec (INSPQ). The detection limits for this study were as follows: Blood Pb (0.02 µg/dL), Cd (0.04 µg/L), total Hg (0.10 µg/L), As (0.23 µg/L), and 0.075 µg/L for all As species. To remain consistent with other Canadian studies (The Canadian Health Measures Survey (Health Canada, 2013a) and the Northern Contaminants Program (Aboriginal Affairs and Northern Development Canada, 2009) results with levels below the analytical limit of detection (LOD) were assigned a value of half of the LOD. Exposure indices based on blood were created by calculating an average of the first and third trimester measures for each metal. When data for only one trimester was available, that value was used as the exposure index. Categorical exposure representations were based on creating three equidistant exposure categories such that each contained at least 10% of the sample.

Arsenate, arsenite, and monomethylarsenic acid (MMA) were also measured in urine; however, these species were not included in the analysis of SGA because they were not detectable in >85% of the study population. Urinary arsenic species were adjusted for specific gravity, to account for variation in urine dilution due to variation in sampling, temperature, physical activity and fluid intake (Suwazono, Åkesson, Alfvén, Järup, & Vahter, 2008) by including specific gravity as a covariate in the regression model when generating risk
estimates for these exposures. Specific gravity adjustment is preferable to creatinine adjustment because it is less influenced by nutrition and muscle mass (Suwazono et al., 2008).

4.3.3 Birth outcomes

Each baby’s length (cm) and weight (g) at birth were abstracted from a medical chart review. Gestational age (weeks) was derived using both the woman’s last menstrual period (LMP) and ultrasound dating. For this analysis, LMP is the preferred method for estimating gestational age since ultrasound methods rely on fetal size to estimate gestational age (Nardozza et al., 2012). Gestational age was determined by LMP if the ultrasound and woman’s report of the first day of her LMP dates differed by ≤7 days. If the two methods differed by >7 days, then gestational age was determined using ultrasound due to concerns over recall and reliability of the LMP estimate. SGA status was determined by comparing infant’s weight and gestational age to cutoff weights specific to the week of gestation at birth, based on Canadian singletons born between 1994 and 1996 (M S Kramer et al., 2001). SGA births were identified as those weighing less than the 10th percentile of this reference population based on the same completed week of gestation and infant sex.

4.3.4 Covariates

Information on important predictors of SGA and possible confounders of the relationships being studied was obtained from answers to questionnaires administered by research staff during both the first and third trimester. Most covariates were such that they would not change over the course of the study (ethnicity, household income, education, age etc.) and so the responses to the first trimester questionnaire were used. A first trimester measure was used for modifiable lifestyle covariates (smoking and alcohol consumption). This analysis considered the effects of: age (<29, 30-35, 36+ years), parity (0, >1), ethnicity (white, non-white), country of origin (Canadian born, foreign born), household income, education (college diploma or less, undergraduate degree, graduate degree), smoking (never smoker, former smoker, current smoker (a current smoker was defined as a women having smoked at any time during the pregnancy)), alcohol consumption (no alcohol vs. any alcohol), pre-pregnancy BMI (underweight or normal, overweight or obese), and marital status (married or common law, not married).
4.3.5 Statistical Analysis

Spearman’s correlation coefficients were calculated to examine the relationship between the first and third trimester measures and between the average levels of the metals. Many of the covariates were collapsed into categories of only two or three levels to ensure model convergence. Relative risks and 95% confidence intervals controlling for important covariates were estimated using log binomial regression. A parsimonious covariate model predicting SGA was created using backward stepwise deletion at a p-value of 0.15. Relative risks for each toxic metal were adjusted for this covariate model. All analysis was performed using SAS enterprise guide version 4.2.

4.4 Results

Lead, cadmium, mercury and arsenic were detectable in the blood of 100%, 99.4%, 92.9% and 97.5% of participants respectively. DMA and Arsenobetaine/arsenocholine were detectable in 85.4% and 48.8% of the sample, while arsenate, arsenite, and MMA were not detectable in >85% of the study population. First and third trimester levels of lead, mercury and cadmium in maternal blood showed good correlation with coefficients ranging from 0.63-0.80 and the correlation coefficient for arsenic in blood between first and third trimester was 0.21 (data not presented).

Table 1 presents the distribution of metal exposure in the study population. All metals had a right-skewed distribution. Samples with metal values that were below the limit of detection (LOD) were assigned a value of 1/2 the LOD. No lead samples were below the LOD.

a. Limits of detection in blood: lead (0.02 µg/dL), cadmium (0.04 µg/L), mercury (0.10 µg/L), arsenic (0.23 µg/L); and in urine: DMA (0.75 µg/L) and arsenobetaine/arsenocholine (0.01 µg/L).

b. Adjusted for specific gravity
4.4.1 Factors associated with SGA

Table 2 describes the characteristics of the study population, presents bivariate analysis between the covariates and SGA, and presents the relationships with covariates included in the final parsimonious model. Among the births to this cohort, 5.8% were SGA. Mothers in this study were mostly white, never smokers, Canadian born and of higher socioeconomic status. 52.7% of the infants were male and 47.3% were female. Independently of other factors, only parity and smoking were associated with SGA. Parous women had a decreased risk for a SGA birth (RR = 0.51, 95% CI = 0.35-0.74) compared to nulliparous women. Smoking at any time during pregnancy was associated with a 72% increased risk of SGA birth (RR 1.72, 95% CI 1.07-2.77). Pre-pregnancy BMI was associated with decreased risk of SGA (RR 0.61, 95% CI 0.39-0.96); however, this association was no longer significant when other factors were considered.

4.4.2 Relationship between metal exposure and SGA

Table 3 presents the main results for the relationship between lead, mercury, or arsenic exposure and SGA risk. Mercury and arsenobetaine/arsenocholine exposures were each associated with increased risk for SGA with relative risk for highest versus low exposure of 1.56 (95% CI 1.04-2.58) and 1.65 (95% CI 1.10-2.47) respectively. No relationship was observed between blood lead or (total) arsenic and SGA risk. The relative risk for moderate and high exposure to cadmium were close to the null value when adjusted for smoking (RR 0.15-0.3 µg/L 0.97 (95% CI: 0.62-1.52); RR >0.3 µg/L 1.03 (95% CI: 0.60-1.78).

Because cigarette smoke is a major source of cadmium exposure, and an important predictor of low birthweight, the effect of cadmium on SGA was analyzed in three different ways. When smokers are included in the analysis, there is no association with SGA between the middle and low exposure groups, and a small but statistically insignificant relationship with SGA between the high and low exposure categories. This is true both before and after controlling for the effect of smoking. When smokers are excluded from the analysis, the risk
estimate between the middle and low exposure groups increases. In all cases, there is a dose-
dependent relationship, but it does not reach statistical significance.

4.5 Discussion

In this Canadian cohort of pregnant women from 10 cities across Canada detectable levels of lead, cadmium, mercury and arsenic were found in first and third trimester blood samples for over 90% of women. This is an indication of the sensitivity of the analytical method used and not an indication of any increased risk. The mean levels of metals detected in this sample of women were consistently lower than those found in the general population of Canadian women aged 20-39 (Health Canada, 2013a). Increased exposure to mercury and arsenobetaine/arsenocholine were associated with increased risk of giving birth to a SGA infant in this sample of pregnant women. Exposures to lead, cadmium or arsenic at the levels observed in this study were not associated with increased risk of SGA.

Lead has been extensively studied using a variety of matrices, with most of the more recent studies of the relationship with SGA using maternal blood, cord blood and placenta. Multiple studies have found an association with SGA (Berkowitz et al., 2006; P.-C. Chen et al., 2006; González-Cossío et al., 1997; Gundacker et al., 2010; Jelliffe-Pawlowski et al., 2006; J O Odland et al., 1999; Xie et al., 2013) while others report no relationship (Jones et al., 2010; Shirai et al., 2010; Sowers et al., 2002). The lead exposure in the Taiwanese study (P.-C. Chen et al., 2006) was very high, with a maximum exposure of 62 µg/dL. Studies conducted in the U.S. (Bellinger et al. 1991; Berkowitz et al. 2006; Jelliffe-Pawlowski et al. 2006; Jones et al. 2010; Sowers et al. 2002; Zhu et al. 2010) and in Nordic countries (Jon Oyvind Odland et al., 2004; Osman et al., 2000) had levels of exposure closer to those measured in the MIREC study, with maximum exposure levels ranging from ~10-12 µg/dL (still considerably higher than the levels in the MIREC cohort). Only the Taiwanese study had a sample size greater than 1000 (P.-C. Chen et al., 2006). Two studies have had sample sizes greater than 4000 (Bellinger et al., 1991; Berkowitz et al., 2006).

There have been no studies of associations between individual urinary arsenic metabolites and SGA. Rahman et. al. found a linear relationship between total arsenic
exposure and birthweight (Rahman et al., 2009) while Xu et. al. observed a relationship between total urinary arsenic levels and SGA in male infants but not in females (Xu et al., 2011). The levels of arsenic exposure in the MIREC cohort were much lower than levels in these studies.

Maternal blood, cord blood, placental tissue and urine are all matrices that have been used to study the association between cadmium exposure and fetal growth. Two studies found no effect of cadmium on fetal growth outcomes (Loiacono et al., 1992; J O Odland et al., 1999) while others found relationships with birth weight or length (Galicia-García et al., 1997; Kippler et al., 2011; Nishijo et al., 2004; Tian et al., 2009). Most studies of cadmium exposure and SGA have similar exposure ranges as the MIREC sample, however 2 studies in Taiwan (Lin et al. 2011; Tian et al. 2009) and one in Bangladesh (Kippler et al., 2011) had exposure ranges higher than those observed in this study. Many of these studies had very small sample sizes; only one analysis (Kippler et al., 2011) was larger than 1000 subjects.

Of the studies examining the relationship between mercury and SGA, four used measures of mercury in both cord blood and maternal blood (Bjerregaard and Hansen 2000; Guo et al. 2013; Lederman et al. 2008; Lee et al. 2010). The remaining studies of mercury and SGA looked at mercury in cord blood, cord tissue, or amalgam fillings, and did not use maternal blood. Only three studies have reported an association between mercury and birthweight (Foldspang & Hansen, 1990; B.-E. Lee et al., 2010; Ramón et al., 2009). However, later analysis of a more complete dataset disproved Foldspang and Hansen’s original findings (Bjerregaard & Hansen, 1996), and the analysis by Ramon et. al. lacked statistical power. Only two of the previous studies of mercury exposure and SGA have had sample sizes greater than 1000 (Daniels et al., 2007; Kippler et al., 2011), and all but two of these samples had geometric mean blood mercury levels of at least 3.3µg/L, which is higher than the exposure levels seen in the MIREC sample. MIREC is the largest study to date examining current levels of these metals in a Canadian setting.

We found mercury to be associated with increased risk for SGA infants. In vitro studies have shown that mercury can interfere with nutrient transfer across the placenta (Urbach, Boadi, Brandes, Kerner, & Yannai, 1992). Mercury has a very high affinity for fetal hemoglobin (Jedrychowski et al., 2006) and methylmercury can quickly be transported into
the fetal bloodstream through the neutral amino acid carrier system (Kajiwara, Yasutake, Adachi, & Hirayama, 1996).

We also found arsenobetaine/arsenocholine levels to be associated with risk of SGA. Organic arsenic is not typically considered to be of much toxicological significance (Marie Vahter, 2009). And there is no literature to suggest that it would be related to fetal size independent of other arsenic metabolites. It is possible that this association was found due to chance, or that urinary arsenobetaine/arsenocholine levels are a proxy for the compound that is truly associated with SGA.

There are several potential reasons for the differing observed effects on SGA among the metals. First, it could be that the metals in this study have different effect thresholds and that these thresholds were reached for some, but not all, of the metals or species in this study population. Also, it could be that there were not enough women with high enough exposures or with SGA infants to detect effects in our sample. Another important possibility is that the mechanism through which these metals act is not identical. Different mechanisms of action are plausible given that high-level exposures to these metals do not produce identical health effects. A recent epidemiologic study reported findings that imply different modes of action for placental transfer of different toxic metals (Gundacker et al. 2010).

Smoking is the single most important known (or ‘identified’) predictor of SGA, (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004) and so it is important factor to include when creating a statistical model that is predictive of SGA; however, this analysis is complicated by the fact that cigarette smoke is also a major source of cadmium exposure. Including smoking in a model used to assess the effect of cadmium exposure on SGA may control away the effect that we are trying to quantify. This was accounted for by modeling the effect of cadmium in three different ways: first without controlling for the effect of smoking, then re-analysing the data while controlling for smoking, and finally by excluding smokers from the analysis. When smokers are included in the analysis, there is no significant effect on SGA both before and after controlling for the effect of smoking. When smokers are removed from the dataset, there are very few people in the high exposure category. This is to be expected as smokers have much higher blood cadmium levels than nonsmokers. Because of this, the risk estimate between the highest and lowest exposure groups in this analysis has
such a wide confidence interval that this risk estimate and dose response pattern are difficult to interpret. The results of these three analyses do not support the case for an association between cadmium exposure and SGA, independent of smoking status.

4.5.1 Methodological Considerations

Comparison to Canadian birth statistics reveal that this sample is older, more educated, greater proportion are married or common law, had fewer smokers and higher household income than the general Canadian pregnant population from 2009 (Public Health Agency of Canada 2009; Arbuckle et al. 2013).

There is inherent misclassification in the use of SGA as a surrogate measure of fetal growth restriction. Specifically, not all infants who are SGA are pathologically growth restricted, and not all infants who are pathologically growth restricted are SGA (Mayer & Joseph, 2012). This misclassification is likely to be non-differential based on infants’ toxic metal exposure in utero.

There is possible uncontrolled confounding in this analysis due to lack of information on maternal diet. Dietary factors such as milk and leafy green consumption have been shown to have a positive effect on fetal growth outcomes (Mayer & Joseph, 2012). Maternal under-nutrition is associated with IUGR (Wu, Imhoff-Kunsch, & Girard, 2012), but this is likely not an issue in this study of Canadian women of relatively high socioeconomic status. Of more relevance is maternal over-nutrition, or obesity during/prior to pregnancy, which can also result in IUGR (Wu et al., 2012). While this analysis does not account for nutritional factors, it does examine pre-pregnancy BMI, which may capture some of the variation caused by individual differences in diet. Furthermore, the trace element deficiencies that are associated with IUGR are not typical of people living in developed countries, especially in our sample of pregnant women who are older, more educated, and of higher socioeconomic status. All of these characteristics make them more likely to start prenatal care earlier, to have more prenatal visits, and to take prenatal dietary supplements (Public Health Agency of Canada, 2009).

4.5.2 Implications
The results indicate that neither blood lead, cadmium or arsenic at the levels typical of Canadian exposure, have a significant measurable effect on birth size by gestational age. The major source of mercury and of organic arsenic is seafood consumption (Shiomi, Sugiyama, Shimakura, & Nagashima, 1995). The positive association with mercury and the positive association with arsenobetaine/arsenocholine found in this study further underscore the importance of limiting the intake of certain seafoods during pregnancy. Regular consumption of US barracuda, marlin, escolar, bigeye tuna, fresh tuna, canned albacore tuna, swordfish, sea bass or shark can result in pregnant women exceeding the tolerable daily intake (TDI) for methylmercury (Health Canada, 2007) of 0.2 µg methylmercury/kg bw/day that has been set by the World Health Organization (World Health Organization, 2003). Upon routine sampling, these species have been found to have the potential to contain total mercury levels that approximate or exceed the Canadian standard (Health Canada, 2007) of 1.0 ppm (Canadian Food Inspection Agency, 2012).

Balancing the risk of mercury and arsenobetaine/arsenocholine exposure in fish with the benefits of fish consumption on fetal growth may prove difficult, as the largest source of mercury exposure and of organic arsenic exposure is fish and seafood. Fish contain high levels of omega 3 fatty acids (Marques et al., 2013) and other essential nutrients that help the fetus to grow, so in some instances the protective effect of fish consumption may outweigh the deleterious effect of low-level mercury contamination and low level organic arsenic exposure on fetal size.

4.6 Conclusions

Increased exposure to mercury and arsenobetaine/arsenocholine were associated with increased risk of giving birth to a SGA infant in this cohort of Canadian women. These findings, in addition to previous work in this area, underscore the need to monitor contaminants in fish and encourage women of childbearing age to comply with fish and seafood advisories.
4.7 Acknowledgements

The authors thank the MIREC participants, site and coordinating center staff and the MIREC Study Group for their contributions to the success of this study. The MIREC Study was funded by Health Canada’s Chemicals Management Plan, the Canadian Institute of Health Research (grant # MOP - 81285) and the Ontario Ministry of the Environment. ASE was supported by NIEHS K01-ES014907.

4.8 References


**4.9 Tables**

Table 1: Distribution of metal exposure in study population (n = 1,835)

<table>
<thead>
<tr>
<th>Metal</th>
<th>Below LOD</th>
<th>Minimum</th>
<th>25&lt;sup&gt;th&lt;/sup&gt; percentile</th>
<th>50&lt;sup&gt;th&lt;/sup&gt; percentile</th>
<th>75&lt;sup&gt;th&lt;/sup&gt; percentile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Lead (µg/dL)</td>
<td>0 (0)</td>
<td>0.17</td>
<td>0.43</td>
<td>0.59</td>
<td>0.81</td>
<td>4.04</td>
</tr>
<tr>
<td>Cadmium (µg/L)</td>
<td>11 (0.6)</td>
<td>&lt;LOD</td>
<td>0.13</td>
<td>0.20</td>
<td>0.30</td>
<td>4.65</td>
</tr>
<tr>
<td>Mercury (µg/L)</td>
<td>141 (7.1)</td>
<td>&lt;LOD</td>
<td>0.32</td>
<td>0.64</td>
<td>1.19</td>
<td>6.80</td>
</tr>
<tr>
<td>Arsenic (µg/L)</td>
<td>48 (2.5)</td>
<td>&lt;LOD</td>
<td>0.51</td>
<td>0.75</td>
<td>1.13</td>
<td>33.00</td>
</tr>
<tr>
<td>Urine DMA(µg/L)</td>
<td>261 (14.6)</td>
<td>&lt;LOD</td>
<td>1.128</td>
<td>2.4064</td>
<td>4.5872</td>
<td>1880</td>
</tr>
<tr>
<td>Arsenobetaine</td>
<td>917 (51.2)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>3.384</td>
<td>1579.2</td>
</tr>
<tr>
<td>/arsenocholine (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMA (µg/L)</td>
<td>1654 (92.35)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>11.28</td>
</tr>
<tr>
<td>Arsenate (µg/L)</td>
<td>1506 (84.09)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>24.06</td>
</tr>
<tr>
<td>Arsenite (µg/L)</td>
<td>1762 (98.38)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>22.56</td>
</tr>
<tr>
<td>DMA (µg/L)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>261 (14.6)</td>
<td>&lt;LOD</td>
<td>1.57</td>
<td>2.42</td>
<td>3.91</td>
<td>2036.66</td>
</tr>
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<td>Arsenobetaine</td>
<td>917 (51.2)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>1.03</td>
<td>37.88</td>
<td>892.59</td>
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<td>/arsenocholine (µg/L)&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>MMA (µg/L)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1654 (92.35)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>7.72</td>
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<td>Arsenate (µg/L)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1506 (84.09)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.81</td>
<td>&lt;LOD</td>
<td>24.20</td>
</tr>
<tr>
<td>Arsenite (µg/L)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1762 (98.38)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>15.64</td>
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</table>

Table 2: Association of maternal characteristics with SGA

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>SGA (%)</th>
<th>Total births</th>
<th>RR (95% CI)</th>
<th>RR (95%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;29</td>
<td>36 (6.3)</td>
<td>568</td>
<td>Reference</td>
<td></td>
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<tr>
<td>30-35</td>
<td>33 (5.1)</td>
<td>644</td>
<td>0.81 (0.51-1.28)</td>
<td></td>
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<tr>
<td>36+</td>
<td>37 (5.9)</td>
<td>623</td>
<td>0.94 (0.60-1.46)</td>
<td></td>
</tr>
<tr>
<td>Risk Factor</td>
<td>SGA (%)</td>
<td>Total births</td>
<td>RR (95% CI)</td>
<td>RR (95%)†</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------</td>
<td>--------------</td>
<td>-------------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
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<td></td>
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<tr>
<td>White</td>
<td>83 (5.6)</td>
<td>1,496</td>
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<tr>
<td>Not White</td>
<td>23 (6.8)</td>
<td>339</td>
<td>1.22 (0.78-1.91)</td>
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<td><strong>Country of birth</strong></td>
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<tr>
<td>Foreign</td>
<td>25 (7.2)</td>
<td>348</td>
<td>Reference</td>
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<tr>
<td>Canadian</td>
<td>81 (5.5)</td>
<td>1487</td>
<td>0.76 (0.49 – 1.17)</td>
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<tr>
<td><strong>Parity</strong></td>
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<tr>
<td>0</td>
<td>65 (8.1)</td>
<td>802</td>
<td>Reference</td>
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<tr>
<td>1+</td>
<td>41 (4.0)</td>
<td>1,031</td>
<td>0.49 (0.34-0.72)</td>
<td>0.51 (0.35-0.74)</td>
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<tr>
<td><strong>Education</strong></td>
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</tr>
<tr>
<td>College/trade diploma or less</td>
<td>38 (5.5)</td>
<td>691</td>
<td>Reference</td>
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</tr>
<tr>
<td>Undergraduate degree</td>
<td>36 (5.4)</td>
<td>673</td>
<td>0.97 (0.62-1.52)</td>
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<tr>
<td>Graduate Degree</td>
<td>32 (6.8)</td>
<td>469</td>
<td>1.24 (0.79-1.96)</td>
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<tr>
<td>** Household Income**</td>
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<td>$100 000 or more</td>
<td>36 (5.1)</td>
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<td>$70 001- $100 000</td>
<td>31 (6.0)</td>
<td>514</td>
<td>1.19 (0.74-1.89)</td>
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<td>$40 001- $70 000</td>
<td>19 (6.2)</td>
<td>305</td>
<td>1.23 (0.71-2.10)</td>
<td></td>
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<tr>
<td>&lt; $40 000</td>
<td>13 (5.8)</td>
<td>222</td>
<td>1.15 (0.62-2.13)</td>
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</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married or Common law</td>
<td>97 (5.5)</td>
<td>1,747</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>9 (10.2)</td>
<td>88</td>
<td>1.84 (0.96-3.52)</td>
<td></td>
</tr>
<tr>
<td>** Pre-pregnancy BMI**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight or normal</td>
<td>72 (6.6)</td>
<td>1,085</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Overweight or obese</td>
<td>25 (4.1)</td>
<td>613</td>
<td>0.61 (0.39-0.96)</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking during pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>59 (5.2)</td>
<td>1,129</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>27 (5.5)</td>
<td>487</td>
<td>1.06 (0.68-1.65)</td>
<td>1.08 (0.70-1.67)</td>
</tr>
<tr>
<td>Current</td>
<td>20 (9.2)</td>
<td>218</td>
<td>1.80 (1.08-2.85)</td>
<td>1.72 (1.07-2.77)</td>
</tr>
<tr>
<td><strong>Alcohol Consumption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No alcohol</td>
<td>76 (5.5)</td>
<td>1,379</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Any Alcohol</td>
<td>22 (7.6)</td>
<td>289</td>
<td>1.38 (0.87-2.18)</td>
<td></td>
</tr>
</tbody>
</table>
†This column contains results for covariates included in the final model using backward selection (p-value <0.15)

Table 3: Association between metal exposure and SGA

<table>
<thead>
<tr>
<th></th>
<th>SGA n (%)</th>
<th>Total births N</th>
<th>Crude RR (95%)</th>
<th>Adjusted RR (95%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lead</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.52 µg/L</td>
<td>35 (4.7)</td>
<td>744</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>0.52-1.04 µg/L</td>
<td>57 (6.6)</td>
<td>862</td>
<td>1.41 (0.93-2.12)</td>
<td>1.33 (0.88-1.99)</td>
</tr>
<tr>
<td>&gt;1.04 µg/L</td>
<td>14 (6.1)</td>
<td>229</td>
<td>1.30 (0.71-2.37)</td>
<td>1.19 (0.65-2.18)</td>
</tr>
<tr>
<td><strong>Mercury</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.8 µg/L</td>
<td>59 (5.4)</td>
<td>1,086</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>0.8-1.6 µg/L</td>
<td>23 (4.8)</td>
<td>479</td>
<td>0.88 (0.55-1.41)</td>
<td>0.89 (0.55-1.42)</td>
</tr>
<tr>
<td>&gt;1.6 µg/L</td>
<td>24 (8.9)</td>
<td>270</td>
<td>1.64 (1.04-2.56)</td>
<td>1.56 (1.04-2.58)</td>
</tr>
<tr>
<td><strong>Cadmium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.15 µg/L</td>
<td>31 (5.2)</td>
<td>593</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>0.15-0.3 µg/L</td>
<td>44 (5.5)</td>
<td>804</td>
<td>1.05 (0.67-1.64)</td>
<td>1.00 (0.64-1.56)</td>
</tr>
<tr>
<td>&gt;0.3 µg/L</td>
<td>31 (7.1)</td>
<td>438</td>
<td>1.35 (0.84-2.19)</td>
<td>1.26 (0.78-2.04)</td>
</tr>
<tr>
<td><strong>Arsenic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.525 µg/L</td>
<td>30 (6.0)</td>
<td>498</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>0.525-1.05 µg/L</td>
<td>42 (5.1)</td>
<td>818</td>
<td>0.85 (0.54-1.34)</td>
<td>0.85 (0.54-1.34)</td>
</tr>
<tr>
<td>&gt;1.05 µg/L</td>
<td>34 (6.6)</td>
<td>519</td>
<td>1.09 (0.68-1.75)</td>
<td>1.08 (0.68-1.74)</td>
</tr>
<tr>
<td><strong>DMA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.88 µg/L</td>
<td>19 (4.2)</td>
<td>453</td>
<td>0.77 (0.43-1.37)</td>
<td>0.74 (0.42-1.32)</td>
</tr>
<tr>
<td>1.88-3.76 µg/L</td>
<td>43 (5.9)</td>
<td>726</td>
<td>1.23 (0.69-2.22)</td>
<td>1.18 (0.66-2.12)</td>
</tr>
<tr>
<td>&gt;3.76 µg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Arsenobetaine</strong>/arsenocholine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.75 µg/L</td>
<td>46 (4.8)</td>
<td>958</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>0.75-22.56 µg/L</td>
<td>9 (5.1)</td>
<td>177</td>
<td>1.14 (0.56-2.28)</td>
<td>1.20 (0.60-2.41)</td>
</tr>
<tr>
<td>&gt;22.56 µg/L</td>
<td>47 (7.2)</td>
<td>656</td>
<td>1.64 (1.10-2.47)</td>
<td>1.65 (1.10-2.47)</td>
</tr>
</tbody>
</table>

†Adjusted for the effect of smoking and parity

* Analysis of Cadmium exposure is not adjusted for smoking
Chapter 5

Results of Exploratory Gene-Environment Interaction Analysis

5.1 Introduction

Variant alleles in the GSTP1 and GSTO1 genes reduce enzyme activity and lessen the effective capacity for detoxification (Custodio et al., 2004; Goodrich & Basu, 2012; Gundacker et al., 2010; Marcos et al., 2006). As a result, it is hypothesized that the relative risk for SGA associated with metal exposure would be higher in those with variant forms of these polymorphisms.

This chapter details the results of the exploratory gene-environment interaction analysis that was performed as a secondary objective of this thesis. First, the subjects and the exposure characteristics of the study cohort are described. Following this, the results of the exploratory analysis are presented, and finally an interpretation of these results is provided.

5.2 Genotype distributions

The distribution and relative risks for the polymorphisms investigated are presented in table 1. For each polymorphism, the heterozygous and homozygous variant are grouped together to form a strata with presumed decreased enzyme activity (and reduced detoxification capacity). All genotype and allele frequencies were in hardy Weinberg equilibrium (HWE), with p-values as follows: GSTP1A114V p=0.92, GSTP1 I105V p=0.20, GSTO1 A104A p=0.16. None of the genotypes were significantly associated with the risk of SGA in this analysis of crude main effects.
Table 5.1 Genotype distribution of study sample

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SGA (%)</th>
<th>Not SGA (%)</th>
<th>RR  SGA (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTP1 A114V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>88 (5.6)</td>
<td>1490 (94.4)</td>
<td></td>
</tr>
<tr>
<td>TC+TT</td>
<td>20 (8.2)</td>
<td>223 (91.8)</td>
<td>1.48 (0.93-2.35)</td>
</tr>
<tr>
<td>GSTP1 I105V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>49 (6.2)</td>
<td>748 (93.9)</td>
<td></td>
</tr>
<tr>
<td>AG+GG</td>
<td>59 (5.8)</td>
<td>964 (94.2)</td>
<td>0.94 (0.65-1.35)</td>
</tr>
<tr>
<td>GSTO1 A104A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>53 (5.8)</td>
<td>863 (94.2)</td>
<td></td>
</tr>
<tr>
<td>CA+AA</td>
<td>55 (6.1)</td>
<td>845 (93.9)</td>
<td>1.06 (0.73-1.52)</td>
</tr>
</tbody>
</table>

5.3 Main Analysis

Table 2 presents the relative risk for metal exposure in strata defined by genotype, and tests for multiplicative interaction. Average metal exposure between the first and third trimester was calculated for each metal in blood. When data for only one trimester was available, that value was used as the exposure index. Metal exposure was then dichotomized into a high and a low exposure group such that each exposure group contained 40-60% of the sample. Relative risks are adjusted for the covariate model identified in chapter 4 (i.e. smoking for cadmium exposure, and smoking and parity for all other exposures), and are estimated using log-binomial regression.

The relative risks of SGA for DMA exposure differed in across strata defined by GSTO1 A104A (p-value interaction 0.02). The relative risk for those with a variant allele was in the direction of increased risk (RR=1.48 95% CI 0.87 to 2.51), while the relative risk for those with a homozygous wildtype genotype was in the direction of a protective effect
(RR=0.56 95% CI 0.29 to 1.08). There is a marginally significant interaction between lead exposure and the GSTP1 A114V SNP (p=0.06). No other interactions approached statistical significance.
Table 5.2 Gene-environment interaction in relation to risk for SGA

<table>
<thead>
<tr>
<th></th>
<th>GSTP1 A114V = CC</th>
<th></th>
<th>GSTP1 A114V = TC + TT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA (%)</td>
<td>Not SGA (%)</td>
<td>Adjusted RR</td>
</tr>
<tr>
<td>Pb&gt;0.08µg/DL</td>
<td>24 (5.3)</td>
<td>430 (94.7)</td>
<td>0.90 (0.57-1.41)</td>
</tr>
<tr>
<td>Pb≤0.08µg/DL</td>
<td>62 (5.6)</td>
<td>1050 (94.4)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd&gt;0.30µg/L</td>
<td>24 (5.9)</td>
<td>383 (94.1)</td>
<td>1.06 (0.67-1.68)</td>
</tr>
<tr>
<td>Cd≤ 0.30µg/L</td>
<td>62 (5.4)</td>
<td>1097 (94.7)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg &gt; 1.20µg/L</td>
<td>31 (7.7)</td>
<td>372 (92.3)</td>
<td>1.65 (1.08-2.52)</td>
</tr>
<tr>
<td>Hg ≤ 1.20µg/L</td>
<td>55 (4.7)</td>
<td>1108 (95.3)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As &gt;0.79 µg/L</td>
<td>36 (4.8)</td>
<td>711 (95.2)</td>
<td>0.77 (0.51-1.17)</td>
</tr>
<tr>
<td>As≤0.79 µg/L</td>
<td>50 (6.1)</td>
<td>769 (93.9)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMA &gt; 1.50µg/L</td>
<td>24 (4.8)</td>
<td>472 (95.2)</td>
<td>0.87 (0.55-1.38)</td>
</tr>
<tr>
<td>DMA≤1.50µg/L</td>
<td>58 (5.6)</td>
<td>977 (94.4)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AsBe &gt;5.64µg/L</td>
<td>41 (6.4)</td>
<td>598 (93.6)</td>
<td>1.43 (0.94-2.18)</td>
</tr>
<tr>
<td>AsBe ≤5.64µg/L</td>
<td>41 (4.6)</td>
<td>851 (95.4)</td>
<td>Reference</td>
</tr>
</tbody>
</table>

P for interaction = 0.06

P for interaction = 0.35

P for interaction = 0.59

P for interaction = 0.45

P for interaction = 0.39

P for interaction = 0.66
Table 5.2 Gene-environment interaction in relation to risk for SGA (cont.)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SGA (%)</th>
<th>Not SGA (%)</th>
<th>Adjusted RR</th>
<th>Gene</th>
<th>SGA (%)</th>
<th>Not SGA (%)</th>
<th>Adjusted RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTP1 I105V = AA</td>
<td></td>
<td></td>
<td></td>
<td>GSTP1 I105V = AG+GG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb&gt;0.08µg/DL</td>
<td>17 (7.3)</td>
<td>217 (92.7)</td>
<td>1.22 (0.69-2.15)</td>
<td>Pb&gt;0.08µg/DL</td>
<td>16 (5.7)</td>
<td>266 (94.3)</td>
<td>0.95 (0.54-1.66)</td>
</tr>
<tr>
<td>Pb≤0.08µg/DL</td>
<td>31 (5.6)</td>
<td>526 (94.4)</td>
<td>Reference</td>
<td>Pb≤0.08µg/DL</td>
<td>42 (5.7)</td>
<td>691 (94.3)</td>
<td>Reference</td>
</tr>
<tr>
<td>Pb for interaction=0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd&gt;0.30µg/L</td>
<td>17 (8.3)</td>
<td>188 (91.7)</td>
<td>1.51 (0.86-2.66)</td>
<td>Cd&gt;0.30µg/L</td>
<td>14 (5.5)</td>
<td>243 (94.5)</td>
<td>0.90 (0.50-1.61)</td>
</tr>
<tr>
<td>Cd≤ 0.30µg/L</td>
<td>31 (5.3)</td>
<td>555 (94.7)</td>
<td>Reference</td>
<td>Cd≤ 0.30µg/L</td>
<td>44 (5.8)</td>
<td>714 (94.2)</td>
<td>Reference</td>
</tr>
<tr>
<td>Cd for interaction=0.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg &gt; 1.20µg/L</td>
<td>20 (9.6)</td>
<td>188 (90.4)</td>
<td>1.98 (1.15-3.43)</td>
<td>Hg &gt; 1.20µg/L</td>
<td>16 (6.6)</td>
<td>227 (93.4)</td>
<td>1.21 (0.69-2.11)</td>
</tr>
<tr>
<td>Hg ≤ 1.20µg/L</td>
<td>28 (4.8)</td>
<td>555 (95.2)</td>
<td>Reference</td>
<td>Hg ≤ 1.20µg/L</td>
<td>42 (5.4)</td>
<td>730 (94.6)</td>
<td>Reference</td>
</tr>
<tr>
<td>Hg for interaction=0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As &gt;0.79µg/L</td>
<td>23 (5.9)</td>
<td>366 (94.1)</td>
<td>0.93 (0.54-1.60)</td>
<td>As &gt;0.79µg/L</td>
<td>22 (4.8)</td>
<td>437 (95.2)</td>
<td>0.73 (0.44-1.22)</td>
</tr>
<tr>
<td>As≤0.79µg/L</td>
<td>25 (6.2)</td>
<td>377 (93.8)</td>
<td>Reference</td>
<td>As≤0.79µg/L</td>
<td>36 (6.5)</td>
<td>520 (93.5)</td>
<td>Reference</td>
</tr>
<tr>
<td>As for interaction=0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMA &gt; 1.50µg/L</td>
<td>17 (6.9)</td>
<td>231 (93.2)</td>
<td>1.23 (0.70-2.19)</td>
<td>DMA &gt; 1.50µg/L</td>
<td>15 (4.6)</td>
<td>310 (95.4)</td>
<td>0.76 (0.43-1.35)</td>
</tr>
<tr>
<td>DMA≤1.50µg/L</td>
<td>30 (5.7)</td>
<td>497 (94.3)</td>
<td>Reference</td>
<td>DMA≤1.50µg/L</td>
<td>40 (6.0)</td>
<td>624 (94.0)</td>
<td>Reference</td>
</tr>
<tr>
<td>DMA for interaction=0.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AsBe &gt;5.64µg/L</td>
<td>24 (7.4)</td>
<td>299 (92.6)</td>
<td>1.54 (0.89-2.67)</td>
<td>AsBe &gt;5.64µg/L</td>
<td>28 (6.8)</td>
<td>381 (93.2)</td>
<td>1.46 (0.88-2.43)</td>
</tr>
<tr>
<td>AsBe ≤5.64µg/L</td>
<td>23 (5.1)</td>
<td>429 (94.9)</td>
<td>Reference</td>
<td>AsBe ≤5.64µg/L</td>
<td>27 (4.7)</td>
<td>553 (95.3)</td>
<td>Reference</td>
</tr>
<tr>
<td>AsBe for interaction=0.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2 Gene-environment interaction in relation to risk for SGA (cont.)

<table>
<thead>
<tr>
<th>GSTO1 A104A = CC</th>
<th>GSTO1 A104A = CA+AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA (%)</td>
</tr>
<tr>
<td>Pb&gt;0.08µg/DL</td>
<td>15 (5.7)</td>
</tr>
<tr>
<td>Pb≤0.08µg/DL</td>
<td>36 (5.6)</td>
</tr>
<tr>
<td>P for interaction = 0.54</td>
<td></td>
</tr>
<tr>
<td>Cd&gt;0.30µg/L</td>
<td>18 (7.9)</td>
</tr>
<tr>
<td>Cd≤ 0.30µg/L</td>
<td>33 (4.8)</td>
</tr>
<tr>
<td>P for interaction =0.14</td>
<td></td>
</tr>
<tr>
<td>Hg &gt; 1.20µg/L</td>
<td>19 (8.0)</td>
</tr>
<tr>
<td>Hg ≤ 1.20µg/L</td>
<td>32 (4.8)</td>
</tr>
<tr>
<td>P for interaction =0.66</td>
<td></td>
</tr>
<tr>
<td>As &gt;0.79µg/L</td>
<td>22 (5.1)</td>
</tr>
<tr>
<td>As≤0.79µg/L</td>
<td>29 (6.1)</td>
</tr>
<tr>
<td>P for interaction =0.83</td>
<td></td>
</tr>
<tr>
<td>DMA &gt; 1.50µg/L</td>
<td>11 (3.6)</td>
</tr>
<tr>
<td>DMA≤1.50µg/L</td>
<td>38 (6.5)</td>
</tr>
<tr>
<td>P for interaction =0.02</td>
<td></td>
</tr>
<tr>
<td>AsBe &gt;5.64µg/L</td>
<td>23 (6.2)</td>
</tr>
<tr>
<td>AsBe ≤5.64µg/L</td>
<td>26 (5.0)</td>
</tr>
<tr>
<td>P for interaction =0.38</td>
<td></td>
</tr>
</tbody>
</table>

Models for Cadmium analysis control for the effect of parity
Models for all other exposures control for the effect of parity and smoking
5.4 Interpretation of results

This analysis explored whether SNPs in GSTP1 and GSTO1 modify the relationship between lead, cadmium, mercury or arsenic on SGA. Stronger relative risks were hypothesized in genotype categories with decreased detoxification capacity.

A statistically significant interaction between the GSTO1 A104A polymorphism and urinary DMA levels was found. DMA is an intermediary metabolite of inorganic arsenic detoxification. This interaction supports that individuals with the GSTO1A104A wild type genotype (CC) are more efficient at metabolizing inorganic arsenic than those with a variant genotype. The risk of SGA in those with high urinary DMA concentrations and the variant genotype is 1.48, however the risk estimate for those with high urinary DMA and the wild type is in the opposite direction (0.56). This finding is of particular interest, as the main analysis did not find any association between DMA levels and SGA.

It is also interesting to note the marginal significance of the interaction between lead exposure and GSTP1 A114V (p=0.06). The relative risk between lead exposure and SGA in the wild type group is 0.90, while the RR in the variant group is 2.25. There was no evidence that any other metal-fetal growth relationships were modified by any of the SNPs being studied.

To the student investigator’s knowledge, GSTO1 has never been studied in a fetal growth context before, however the findings of this analysis do support evidence that GSTO1 is involved in arsenic metabolism (Zakharyan et al., 2001). These findings should be interpreted in the context that arsenic metabolism changes during pregnancy, with pregnant women producing a higher proportion of methylated arsenic species relative to inorganic arsenic than women who are not pregnant (Hopenhayn, Huang, et al., 2003). The interpretation of this finding is further complicated by the fact that it is unknown if this increase in methylation observed during pregnancy represents an increased risk of exposure to highly toxic trivalent urinary arsenic metabolites, or an increase in capacity for arsenic detoxification (Concha, Vogler, Lezcano, Nermell, & Vahter, 1998).

The results of this study may help to explain the variation in conclusions about lead exposure and fetal growth that are presented in the literature. If the effect of lead on
SGA is real, but only some subpopulations are at risk for this effect, then genetic variation may be the reason that some studies report significant relationships while others do not. One study found GSTP1 to have no effect on fetal growth (Yamada, 2004), however the study simply compared frequencies using a fisher’s exact test, and genotype was analysed as the exposure rather than an effect modifier of a relationship with an environmental exposure.

Several considerations must be employed when interpreting the relationships between GSTO1 and GSTP1. Glutathione mediated metal detoxification may be influenced by several factors including glutathione availability and oxidation state, exposure (type, intensity, duration, dose), exposures to other compounds with similar chemical structures (Goodrich & Basu, 2012).

There is also the possibility of an unknown linkage disequilibrium between GSTP1A114V and another gene, or between GSTO1A140A and another gene that is the true gene modifying the relationship.

5.5 Methodological Considerations

Although a parsimonious log-binomial regression model was used to control for the effect of possible confounders of the effect of heavy metals on SGA, assessing confounding in interactions is an evolving and complicated area of epidemiology. Factors that do not confound in a regular regression analysis can still affect the magnitude of effect estimates in gene-environment interactions (Wang et al., 2002).

A ratio of urinary arsenic metabolites relative to one another would have been useful in gaining a better understanding of the relationship between inorganic arsenic exposure and SGA in the context of fetal growth and of gene-environment interaction (Chung et al., 2012). It was not possible to do this accurately, as the other urinary arsenic metabolites were mostly non-detectable in this population.

The study results could have been strengthened with a haplotype analysis. Combinations of genes may give a truer representation of an individual’s detoxification capacity. Looking at these relationships in the context of a single SNP at a time may be an oversimplification of the true biologic mechanisms taking place.
The results of this analysis may also have been strengthened if mother-infant genotyping were available, however the magnitude of the role of fetal genotypes in these types of relationships remains to be determined. In theory, maternal genotype determines the biological dose of toxicant that reaches the fetus, and fetal genotype determines fetal capacity to detoxify this biological dose (Wang et al., 2002). Duarte-Salles et. al. examined the relationship between benzo (a) pyrene and SGA, and found the association to be strongest when 105val allele was present in both mother and child (Duarte-Salles et al., 2012).

Finally, this analysis of 6 exposures and 3 different genotypes resulted in 18 comparisons. The role of chance is an important consideration when interpreting these results. More specifically, as the number of comparisons increases, so does the likelihood that a significant effect will be found due to chance. It is possible that the associations described in this analysis of 18 comparisons were found due to chance and not because of any underlying biologic relationship.

5.6 References


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Chapter 6

General Discussion and Conclusions

6.1 Review of Objectives

The metals lead, mercury, cadmium, and arsenic are widely dispersed in the environment and can be toxic at high levels (Jarup, 2003). The Canadian Health Measures Survey evaluated blood levels of several metals in the Canadian population and in general, population exposures are low (Wong & Lye, 2008b). For example, fewer than 1% of Canadians have lead or mercury blood concentrations above the Health Canada guidance value of 10µg/dL (Health Canada, 2013b; Wong & Lye, 2008b). Pregnant women are an important population who were excluded from the Canadian Health Measures Survey. These women and their fetuses are a potentially susceptible sub-population, so it is important to have accurate information on their exposure to toxic substances, and an indication of how these substances might impact their health. The primary objective of this study was to determine the relationship between exposure to lead, cadmium, mercury, or arsenic, and the risk for giving birth to an infant that is SGA in a Canadian population. The secondary objective explored effect modification of this relationship according to polymorphisms in genes with a functional role in the metabolism and excretion of toxic metals. Specifically, the interaction with three polymorphisms in glutathione S-transferases genes (GSTO1 and GSTP1) were investigated.

6.2 Summary of Key Findings

Increased risk for SGA was observed between concentrations of ≥1.6 µg/L and ≤0.8 µg/L for blood mercury exposure (RR=1.56. 95%CI=1.04–2.58) and between concentrations of ≥22.56 µg/L and ≤.75 µg/L for urinary arsenobetaine/arsenocholine exposure (RR=1.65. 95%CI=1.10–2.47) after adjusting for the effects of smoking and parity. No association was found between SGA and levels of any other metals measured in whole blood or between SGA and dimethylarsinic acid (DMA) measured in urine. There was a statistically significant interaction with the GSTO1 polymorphism (A104A
SNP) in the relationship between DMA exposure and SGA (p-value for interaction=0.02). The GSTP1 polymorphism (A114V) had a marginally significant interaction in the relationship between blood lead and SGA (p-value for interaction =0.05). No other SNPs had statistically significant interactions between metal exposure and SGA.

6.3 Strengths

The major strength of this study is the utilization of biomarkers to measure exposure rather than an ecological approach or extrapolation from a questionnaire. This approach provides greater accuracy in exposure assessment over some previous studies (Ahmad et al., 2001; Börzsönyi, Bereczky, Rudnai, Csanady, & Horvath, 1992; P.-C. Chen et al., 2006; Kristal-Boneh et al., 1998; Rahman et al., 2007; Salpietro et al., 2002; Von Ehrenstein et al., 2006).

With the exception of blood arsenic levels, the biomarkers used in this study have been validated as representative of cumulative exposures, and as such are expected to provide accurate representations of the fetus’ in utero exposure (Sakamoto et al., 2007; Murphy et al., 2010). It is debated how reflective a single exposure measurement of an environmental toxicant is of fetal exposure at critical times during pregnancy (Murphy et al., 2010). A strength of this study in this regard is that exposure was measured at two time points during gestation. A further strength is the availability of information on several potential risk factors for SGA in this cohort. Finally, investigating this research question in a Canadian context is a strength of this study, as the findings are relevant to exposure levels occurring in Canada.

6.4 Limitations

This Canadian sample population does not facilitate large contrasts in exposure compared to a studies conducted in a geographic areas with much more variation in exposure levels and prevalence. Most subjects had very low exposure levels and very few had high exposure levels. As a result, the potential to observe strong relationships is somewhat limited.
This study uses SGA a proxy for growth restriction. There is inherent misclassification in the use of SGA as an endpoint. Specifically, not all infants who are SGA have pathological growth restriction, and not all infants who are pathologically growth restricted are SGA. This misclassification is likely to be non-differential based on infants’ metal exposure in utero and would therefore tend to bias risk estimates towards the null (Rothman, Greenland, & Lash, 2008). Another consideration regarding the use of SGA as an outcome is that the SGA group may not a homogenous disease entity. There are 2 major types of growth restriction, symmetrical and asymmetrical. Symmetrical growth restriction is less common and begins earlier in gestation. A fetus with symmetrical growth restriction misses one or more cycles of cell division and is therefore smaller than expected for its gestational age. All organs, including the brain, are affected equally. Asymmetrical growth restriction begins later on in the pregnancy when growth is less dependent on cell division and more on adequate nutrition and oxygen supply. These fetuses have regular sized heads and smaller bodies as blood is preferentially diverted to the brain at the expense of other organs (Cox & Marton, 2009).

The data collected does not allow for a distinction to be made between symmetrical and asymmetrical growth restriction. The effect of investigating a heterogeneous outcome is the potential to mask or miss relationships that might exist between metal exposures and a specific type of fetal growth restriction.

A final potential limitation of this study is that the Canadians most likely to have elevated body burdens of metal exposure (northern populations and low socioeconomic status) are not included or very underrepresented in the sample. This limits the generalizability of estimates of exposure prevalence and limits the exposure contrasts represented in risk analysis.

6.5 Exposure Distribution

The exposure levels detected in blood in this sample are compared to the biomonitoring results in the second cycle of the CHMS for women 20-39 in figures 6.1-6.3. Blood arsenic was not measured in the CHMS cycle 2 and so it is not presented here.
The geometric mean levels of all other metals in blood of this sample are consistently lower than those detected in the CHMS.

**Figure 6.1 Comparison of mean blood lead concentrations: MIREC vs. CHMS**

**Figure 6.2 Comparison of mean blood cadmium concentrations: MIREC vs. CHMS**
Five urinary arsenic metabolites were measured by the MIREC study, however this analysis only considered DMA and arsenobetaine/arsenocholine. This was largely...
due to the fact that the other urinary arsenic metabolites (As$^{3+}$, As$^{5+}$ and Monomethylarsenic acid (MMA)) were not detectable in >85% of the sample and as such a meaningful analysis could not be conducted using these exposures.

This thesis examined the relationship between exposure to four different toxic metals and SGA. Although the underlying hypothesized mechanism for each metal’s effect is via oxidative stress – these individual metals have different metabolic pathways and potentially different effects on intermediate events (oxidative stress) and on fetal growth. It is also possible that the different metals act via mechanisms other than oxidative stress. Furthermore, SGA is not a homogeneous outcome. Both facts imply that there could be multiple causal pathways for the effects observed. As a result, this research did not create a summary measure of metal exposure.

Metals were detectable in the blood of at least 92.9% of subjects. This is an indication of the robustness of the detection method and not suggestive on its own of any health effects due to exposure. In the case of lead for example, the detection limit in this study is 0.02 µg/dL. The current Health Canada guidance value for blood lead concentration is 10µg/dL. While health effects have been seen below this level, fewer than 1% of Canadians have blood lead levels exceeding 10µg/dL, and fewer than 5% have blood lead levels greater than 5µg/dL (Health Canada, 2013b).

6.6 SGA in the study cohort

In this cohort, 5.8% of the births in this sample were SGA, compared to the Canadian SGA rate of 8.3% in 2006-2007 (Canadian Institute for Health Information, 2009). In general, trends of older maternal age, diabetes and hypertension have all acted to increase fetal growth restriction rates. This is countered however by increases in pre-pregnancy weight and BMI, reduction in smoking behaviours and increases in gestational weight gain, which have had the opposite effect (Mayer & Joseph, 2012).

An important consideration is that the data for this study was collected over multiple years, and that there is a general trend for birthweight to increase (and therefore the rate of SGA to decrease) over time (Michael S Kramer et al., 2002). Some of the changes in the observed prevalence of SGA are due to improvements in the accuracy of
gestational age assessment (Michael S Kramer et al., 2002). This cohort was of higher socioeconomic status, and had lower rates of smoking during pregnancy than the Canadian pregnant population, all of which may explain the lower rate of SGA. Furthermore, SGA is more common in teenage mothers (X.-K. Chen et al., 2007; Valero De Bernabé et al., 2004), who were excluded from this sample. The population did however have an overrepresentation of older mothers, who are also more likely to give birth to a child that is SGA.

6.7 Comparison of observed relationships to the literature

The ability to reproduce established relationships with risk factors for SGA provides an indication of the validity of outcome and covariate measures.

The sample consisted of mostly middle aged and older women. Maternal age is an important predictor of SGA (McCowan & Horgan, 2009; Newburn-Cook & Onyskiw, 2005; Valero De Bernabé et al., 2004), however, it was not significantly associated with the outcome in this sample. Parity was found to have a protective effect against SGA and this finding is consistent with the literature (Canadian Institute for Health Information, 2009; McCowan & Horgan, 2009; Valero De Bernabé et al., 2004). Higher Pre-pregnancy BMI was protective against SGA in the crude analysis of this data, but this effect was no longer observed after controlling for other factors. Maternal smoking was found to be the strongest predictor of SGA, which is consistent with the literature regarding SGA (McCowan & Horgan, 2009; Valero De Bernabé et al., 2004). While ethnicity, country of origin, education, maternal alcohol consumption and marital status have all been reported to have some association with SGA, none of these risk factors were associated with SGA in this sample. There is no evidence of a relationship between the SNPs that were explored in this thesis and SGA in the current published literature.

All metals other than blood arsenic showed good correlation ($r \geq 0.63$) between first and third trimester measures. This indicates that the levels of these metals in subject’s blood remained relatively stable over the course of the study. A somewhat low correlation was observed between first and third trimester arsenic ($r=0.21$)– this may be in part due to the short half-life of blood arsenic and resulting sensitivity of this measure
to recent exposures (ASTDR (Agency for Toxic Substances and Disease Registry), 2007a).

6.8 Comparison of findings to existing literature

Direct comparison of these results with the existing literature is complicated by differences in exposure metrics, levels of exposure, variation in exposure, socioeconomic status, and statistical approaches to analysis (Karagas et al., 2012). The fetal growth literature is suggestive of a possible relationship between lead and birth size (Berkowitz et al., 2006; P.-C. Chen et al., 2006; González-Cossío et al., 1997; Gundacker et al., 2010; Jelliffe-Pawlowski et al., 2006; J O Odland et al., 1999; Xie et al., 2013; Zhu et al., 2010) however no relationship was observed in this cohort. Most previous literature on arsenic and pregnancy outcomes have examined fetal loss (Ahmad et al., 2001; Börzsönyi et al., 1992; Milton et al., 2005; Rahman et al., 2007; Von Ehrenstein et al., 2006). Only one study of arsenic exposure done in Bangladesh used birthweight as an outcome. This study found a 1.68g decrease in birthweight for every 1 μg/L increase of arsenic in urine (Rahman et al., 2009). This thesis did not observe a relationship between arsenic and SGA, possibly because of the much lower arsenic exposure in this Canadian cohort – in contrast to the Banglandesh study (Rahman et al., 2009) where much higher exposures are prevalent.

A relationship between mercury exposure and SGA was observed in this Canadian cohort. The literature on mercury and SGA is somewhat inconsistent, where only two studies (B.-E. Lee et al., 2010; Ramón et al., 2009) have reported a relationship and six others have reported null results (Bjerregaard & Hansen, 2000; Daniels et al., 2007; Ding et al., 2013b; Hujoel et al., 2005; Lederman et al., 2008; Lucas et al., 2004).

The literature concerning cadmium and fetal size is inconclusive. While five studies have reported a relationship (Nishijo et al. 2004; Tian et al. 2009;Lin et al. 2011; Shirai et al. 2010; Salpeirto et al. 2002), two have had inconclusive or marginally significant results (Galicia-García et al., 1997; Kippler et al., 2011), and three others have reported no relationship (Menai et al., 2012; J O Odland et al., 1999; Osman et al., 2000).
Among these studies, none included the effect of smoking in their statistical models, however Salpeirto et. al. excluded smokers from their sample and Menai et. al. stratified their analysis by smoking status.

This study was novel in that the polymorphisms that were explored in the gene-environment exploratory analysis have never been investigated in prior published epidemiologic studies in the context of fetal growth.

6.9 Methodology

6.9.1 Selection Bias

Because of the nature of the sampling frame, probabilistic sampling was not possible. Pregnancy only lasts for a brief period of time, making it inefficient to arrive at a final cohort using a random sampling method. For this reason, a volunteer sample was used.

This sample is older; more educated, and has a higher household income than the Canadian pregnant population (Public Health Agency of Canada, 2009). There was also a higher proportion of never smokers and women who were married or living as married than the general population of pregnant women in Canada (Public Health Agency of Canada, 2009).

Despite the fact that the sample was not representative of the Canadian population of pregnant women, the findings of this study are generalizable to this, and other pregnant populations with similar metal exposure. The relationship being studied is a biologic one and thus it should hold in a population that is not demographically identical. However, it would be incorrect to make any general statements about rates of SGA or any other pregnancy outcomes based on what was observed in this sample.

6.9.2 Controlling for Confounding

In analyzing this dataset, many continuous variables were converted into categorical variables. The advantages of this approach are that it allows calculation of relative risks for different exposure categories, and it avoids misspecification of the model. This is
because categorization of the exposure does not assume that the underlying exposure-disease relationship has a particular distribution.

In grouping continuous covariates to form categorical ones, there is a loss of information and the potential for residual confounding. Ideally, we want to group people with like characteristics together and make sure that the different groups have important differences between them, however this has to be balanced with the need to keep the number of categories small because of the need for adequate sample size within categories. For example, within the group of women identified as smokers, there is likely to be differences in frequency and amount of cigarettes smoked.

When building the parsimonious regression model used in the analysis, a liberal p-value (0.15) was used to ensure that factors accounting for only a small proportion of SGA risk would still be controlled for in the model.

The level of detail in certain important covariates that were used in this analysis (such as alcohol consumption and smoking during pregnancy) was imprecise. Information on gestational diabetes and other important predictors of SGA (such as maternal weight gain during pregnancy) were missing for a large proportion of the sample and as a result uncontrolled confounding is a concern.

6.10 Multiple comparisons

The role of chance is an important consideration in this analysis. More specifically, the issue is that of multiple comparisons. The concern here is that looking for multiple relationships (i.e. having 6 main exposures and then another 18 comparisons for the gene-environment exploratory analysis) increases the chance of making a type 1 error, finding an association where there is none. One way to correct for this is to inflate the p-value using a bonferonni correction, however some argue that this approach is too conservative.

6.11 Exposure Windows

In conducting this analysis, first and third trimester measures of metal concentrations in blood were averaged to create an average exposure measure. For many
fetal outcomes and fetal growth in general, it may be that certain exposure windows are more important than others. If it is the case that a specific exposure window is critical in determining size for gestational age – then it is possible that taking an average exposure measure may have led to misclassification of exposure in this regard.

6.12 Study Power

The initial detectable effects reported in the proposal phase of this study were based on a prevalence of SGA of 8%. The prevalence of SGA in this sample was only 5.8%, so the study had less power to detect an effect than initially anticipated.

6.13 Causation

Associations between mercury exposure and SGA, as well as arsenobetaine levels and SGA were reported in this thesis. There was also a significant interaction between GSTO1 A104A and the effect of DMA and SGA however; these associations on their own are not enough to imply that metal exposure causes SGA. The relationship between metal exposure and SGA is temporal and biologically plausible, however the dose response relationship is not strong, and the literature is inconsistent in reporting relationships between these exposures and this outcome. Furthermore the risk estimates reported in this thesis are not strong enough to imply causation. Based on the criteria for causation, low-level exposure to some of the metals investigated in this study is associated with SGA, but is not a cause of SGA.

6.14 Contribution of Research

This research adds to the existing body of knowledge about the relationship between in utero exposure to metals and size relative to gestational age at birth. This is one of only a few studies to examine genetic factors in relation to metal exposure during pregnancy and small for gestational age birth. Exploring possible gene environment interactions adds another dimension to the understanding of the relationship of interest. Interactions
are important relationships that need to be reported and understood in order to identify the groups who are most vulnerable to the effects of the exposure.

6.15 Conclusions

The results of the main analysis imply that maternal exposure to mercury at ≥1.6 µg/L vs ≤0.8 µg/L ((RR=1.56.; 95% CI = 1.04-2.58) or arsenobetaine/arsenocholine in maternal urine at ≥22.56 µg/L vs ≤.75µg/L ((RR= 1.65.; 95% CI = 1.10-2.47) are possible risk factors for SGA birth. The results of the gene-environment interaction analysis point to an interaction between GSTO1 A104A and urinary DMA levels (p=0.02). They are suggestive of a potential interaction between the effect of lead on SGA and the variant genotype in the GSTP1 A114V SNP (p=0.06). Because fish and seafood are the major dietary sources of human exposure to mercury and arsenobetaine, these findings emphasize the importance of avoidance of certain types of seafood during pregnancy. The interaction between GSTO1 A104A and urinary DMA highlights the fact that certain genetic sub populations are at risk for harmful effects of exposures that may be benign to other subpopulations. In the context of this study, individuals with the variant form of this gene may be at increased risk for SGA birth associated with metal exposure.

6.16 Future Directions

Ensuring that fetuses attain adequate size prior to birth is an important public health concern, as there is a growing body of evidence that even a small downward shift of birthweight within normal range is associated with adverse population health outcomes (McCowan & Horgan, 2009). The relationships described in this thesis need to be reproduced by other researchers before they can be confirmed. Future studies in this area should include those Canadians at highest risk for increased exposure to toxic metals, as these relationships are most relevant for them. These are also perhaps the populations in which the greatest positive impact can be made with public health interventions.
6.17 References


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Exposure. *Environmental Health Perspectives, 120*, 799–806. doi:10.1289/ehp.1104494


### Appendix A

**Additional Tables**

**Table A1. Correlation (Spearman’s r) Between average levels of Metals in Maternal Blood**

<table>
<thead>
<tr>
<th></th>
<th>Lead</th>
<th>Mercury</th>
<th>Cadmium</th>
<th>Arsenic</th>
<th>Urinary arsenic</th>
</tr>
</thead>
<tbody>
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<td>Lead</td>
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<td>p=&lt;0.01</td>
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</tr>
<tr>
<td>Mercury</td>
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<td>1.00</td>
<td>-0.02</td>
<td>0.25</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>p=&lt;0.01</td>
<td>p=0.43</td>
<td>p=&lt;0.01</td>
<td>p=0.01</td>
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</tr>
<tr>
<td>Cadmium</td>
<td>0.13</td>
<td>-0.02</td>
<td>1.00</td>
<td>-0.01</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td>p=&lt;0.01</td>
<td>p=0.43</td>
<td>p=0.65</td>
<td>p=0.46</td>
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</tr>
<tr>
<td>Arsenic</td>
<td>0.08</td>
<td>0.25</td>
<td>-0.01</td>
<td>1.00</td>
<td>0.34</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>Urinary</td>
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<td>0.06</td>
<td>-0.02</td>
<td>0.34</td>
<td>1.00</td>
</tr>
<tr>
<td>arsenic</td>
<td>p=&lt;0.01</td>
<td>p=0.01</td>
<td>p=0.46</td>
<td>p=&lt;0.01</td>
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</table>
Table A2. The effect of blood cadmium (Cd) exposure on SGA

<table>
<thead>
<tr>
<th></th>
<th>SGA</th>
<th>Total number of births</th>
<th>Cd Only RR (95% CI)</th>
<th>Adjusted for parity RR (95% CI)</th>
<th>Adjusted for parity and smoking RR (95% CI)</th>
</tr>
</thead>
<tbody>
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<td><strong>All participants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.15 µg/L</td>
<td>31 (5.2)</td>
<td>593</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>0.15-0.3 µg/L</td>
<td>44 (5.5)</td>
<td>804</td>
<td>1.05 (0.67-1.64)</td>
<td>1.00 (0.64-1.56)</td>
<td>0.97 (0.62-1.52)</td>
</tr>
<tr>
<td>&gt;0.3 µg/L</td>
<td>31 (7.1)</td>
<td>438</td>
<td>1.35 (0.84-2.19)</td>
<td>1.26 (0.78-2.04)</td>
<td>1.03 (0.60-1.78)</td>
</tr>
<tr>
<td><strong>Non-smokers only</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.15 µg/L</td>
<td>21 (4.8)</td>
<td>438</td>
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<td>Reference</td>
<td></td>
</tr>
<tr>
<td>0.15-0.3 µg/L</td>
<td>29 (5.4)</td>
<td>540</td>
<td>1.12 (0.65-1.94)</td>
<td>1.06 (0.61-1.82)</td>
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<tr>
<td>&gt;0.3 µg/L</td>
<td>9 (6.0)</td>
<td>151</td>
<td>1.24 (0.58-2.65)</td>
<td>1.17 (0.55-2.49)</td>
<td></td>
</tr>
<tr>
<td><strong>Smokers and former smokers only</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.15 µg/L</td>
<td>10 (6.5)</td>
<td>155</td>
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<tr>
<td>0.15-0.3 µg/L</td>
<td>15 (5.7)</td>
<td>264</td>
<td>0.88 (0.41-1.9)</td>
<td>0.85 (0.39-1.84)</td>
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</tr>
<tr>
<td>&gt;0.3 µg/L</td>
<td>22 (7.7)</td>
<td>287</td>
<td>1.19 (0.58-2.44)</td>
<td>1.11 (0.54-2.29)</td>
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</tr>
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</table>
Appendix B

Ethics Approval

QUEEN’S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD-DELEGATED REVIEW
December 17, 2012

Miss Shari Thomas
Department of Community Health and Epidemiology
Queen’s University

Dear Miss Thomas

Study Title: EPID-402-12 Influence of Heavy Metals on Small for Gestational Age
File # 6007541
Co-Investigators: Dr. W. King and Dr. T.E. Arbuckle

I am writing to acknowledge receipt of your recent ethics submission. We have examined the protocol for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of the Chair's signature below. This approval will be reported to the Research Ethics Board. Please attend carefully to the following listing of ethics requirements you must fulfill over the course of your study:

Reporting of Amendments: If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval. Please use event form: HSREB Multi-Use Amendment/Full Board Renewal Form associated with your post review file # 6007541 in your Researcher Portal (https://eservices.queensu.ca/romeo_researcher/)

Reporting of Serious Adverse Events: Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information. Serious Adverse Event forms are located with your post-review file 6007541 in your Researcher Portal (https://eservices.queensu.ca/romeo_researcher/)

Reporting of Complaints: Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

Annual Renewal: Prior to the expiration of your approval (which is one year from the date of the Chair's signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

[Signature]

Chair, Research Ethics Board
December 17, 2012

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete
Queen's University, in accordance with the "Tri-Council Policy Statement 2, 2010" prepared by the Interagency Advisory Panel on Research Ethics for the Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada and Social Sciences and Humanities Research Council of Canada requires that research projects involving human participants be reviewed annually to determine their acceptability on ethical grounds.

A Research Ethics Board composed of:

**Dr. A.F. Clark**, Emeritus Professor, Department of Biomedical and Molecular Sciences, Queen's University (Chair)
**Dr. H. Abdollah**, Professor, Department of Medicine, Queen's University
**Dr. C. Cline**, Assistant Professor, Department of Medicine, Director, Office of Bioethics, Queen's University, Clinical Ethicist, Kingston General Hospital
**Dr. R. Brison**, Professor, Department of Emergency Medicine, Queen's University
**Dr. M. Evans**, Community Member
**Ms. J. Hudacin**, Community Member
**Mr. D. McNaughton**, Community Member
**Ms. P. Newman**, Pharmacist, Clinical Care Specialist and Clinical Lead, Quality and Safety, Pharmacy Services, Kingston General Hospital
**Ms. S. Rohland**, Privacy Officer, ICES-Queen's Health Services Research Facility, Research Associate, Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute
**Dr. A. Singh**, Professor, Department of Psychiatry, Queen's University
**Dr. J. Walia**, Assistant Professor and Clinical Geneticist, Department of Paediatrics, Queen's University and Kingston General Hospital
**Ms. K. Weisbaum**, LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)

has reviewed the request for renewal of Research Ethics Board approval for the project “Influence of Heavy Metals on Small for Gestational Age” as proposed by **Miss S. Thomas** of the Department of Public Health Sciences, at Queen's University. The approval is renewed for one year, effective December 17, 2013. If there are any further amendments or changes to the protocol affecting the participants in this study, it is the responsibility of the principal investigator to notify the Research Ethics Board. Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other adverse events must be reported within 15 days after becoming aware of the information.

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**Dr. A.F. Clark**
Chair, Health Sciences Research Ethics Board

Date: March 31, 2014

Assistant to the Chair

Renewal 1 [x] Renewal 2 [ ] Extension [ ] Code# EPID-402-12 Romeo file# 6007541