Metal Exposure During Pregnancy: Trends, Predictors, Associations with Birth Outcomes and the Modifying Effect of Maternal Psychosocial Stress

by

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Dedication

I dedicate this dissertation to my mom, زیاخان هیسامدین (Ziyahan Hisamidin).

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Abstract

Preterm birth is a significant public health concern as a leading cause of infant mortality; it also contributes substantially to childhood and adult morbidity. Other adverse birth outcomes including low birthweight are associated with later comorbidities. There is growing evidence that the underlying contributors to adverse birth outcomes may include environmental contaminants (like metals), but these factors are understudied. Puerto Rico has one of the highest preterm birth rates of all U.S. states and territories. Moreover, the population in Puerto Rico is exposed to higher levels of many environmental chemicals because of heavily contaminated hazardous industrial sites. Even though prenatal exposure to heavy metals has been well investigated, our knowledge of the threats to the fetus at low levels of exposure remains rather limited. From animal studies, few data are available on the effects of excessive exposure from essential trace elements on adverse pregnancy outcomes. As humans are continuously exposed to a mixture of environmental toxicants, and typically not to single agents in isolation, there is a pressing need to study the relationship of exposures both individually and as mixtures. This dissertation investigates the predictors of environmental metal exposures among pregnant women, and the potential of metal exposures measured in different media to increase the risk of adverse birth outcomes. The interactions between psychosocial stress and the exposure biomarkers on adverse birth outcomes are also explored.

The four aims of this dissertation examine a subset of participants from the "Puerto Rico Testsite for Exploring Contamination Threats (PROTECT)" cohort. Aim 1 of this dissertation identifies levels, trend, and predictors of prenatal exposure for 14 metals. Aim 2 investigates the individual and collective effects of metals on adverse birth outcomes. Of all the metals assessed, blood lead at low levels, and potentially below current reference levels, was the most strongly associated with increased risk of preterm birth and decreased gestational length. Findings in Aim 2 also showed that lead, zinc, and manganese may contribute to adverse birth outcomes. Aim 3 explores the modifying effect of maternal psychosocial stress on the association between the metal exposure

biomarkers and adverse birth outcomes. Presence of "poor" psychosocial status strengthened the adverse associations between Mn and preterm birth, indicating that prenatal psychosocial stress may modify vulnerability to metal exposure. Finally, Aim 4 examines the mixture predictive performance of urine and blood metal biomarkers, and integrated multimedia biomarkers incorporating both matrices, in association with preterm birth. Metal mixtures measured in urine (specific gravity corrected), blood, and integrated biomarkers had comparable performance in associations with preterm birth, indicating that using urine or blood may be an equally good approach to evaluate the metals as a mixture, but only when urine measurements of metal account for urinary dilution.

Overall, these results broaden our understanding of the effects of metal mixtures on birth outcomes. We identify dietary and behavioral predictors of metal exposures which could inform exposure reduction strategies, and potentially result in an eventual reduction in preterm birth rates. Furthermore, our novel study design underscores the importance of considering the performance of exposure biomarkers measured in different media, and modifying effects of non-chemical exposures, when evaluating the relationship between chemical exposures and birth outcomes. Further studies are needed to substantiate these findings to advance our knowledge on the impact of environmental chemicals on pregnancy.

Chapter I

Introduction

Adverse birth outcomes and environmental exposures in Puerto Rico

Preterm birth (prematurity) is defined as delivery <37 completed weeks of gestation and is the leading cause of newborn death [1, 2]. Survivors are at risk for many adverse health consequences, including neuro-developmental delays, disability, chronic respiratory, vision impairment, and hearing impairment [1, 3, 4]. In addition to the health consequences of preterm birth, the emotional and economic impact of preterm birth on families are high. Therefore, preterm birth and its consequences constitute a major public health problem in the United States and worldwide [5]. The Institute of Medicine estimates the annual societal economic burden (medical, educational, and lost productivity) associated with preterm birth in the United States to be at least $26.2 billion in 2005 [5]. Other important adverse birth outcomes including low birthweight (<2500g) and being small for gestational age (SGA) may result directly from preterm labor and/or growth restriction and also contribute substantially to childhood and adult morbidity [6-8].

Thus, there is a need to identify risk factors to adverse birth outcomes and find ways to prevent preterm birth and low birthweight. This is especially important in a region like Puerto Rico, which has one of the highest incidences of preterm births among all US jurisdictions. In addition, Puerto Rico has higher rates of childhood obesity and asthma [9-11] as well as adult obesity, metabolic syndrome, and diabetes [12, 13] compared to the rest of the U.S., all of which have been associated with adverse birth outcomes. Moreover, the traditional risk factors, such as mother's age and use of tobacco and alcohol, do little to explain this high rate of preterm birth and associated consequences [14]. Even though there is growing evidence that environmental factors may play a key role, these factors remain understudied and underappreciated.

Puerto Rico, a self-governing dependent territory of the United States, has a long-standing history of contamination with environmental chemicals, as there are sixteen active Superfund sites and

200+ hazardous waste sites [15]. Many of these sites lie on unlined landfills that overlie Karst aquifers, which creates pathways for toxic substances to contaminate groundwater. Therefore, the risk of human exposure to contamination is high. For example, previous research within this area suggests that pregnant women in Puerto Rico may have higher exposure to certain phenols [16, 17] and phthalates [18], compared to women of reproductive age in the U.S. general population. Our preliminary analysis on metals also showed a higher level of exposure among Puerto Rican pregnant women compared to the general U.S female population.

The Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) study was launched in 2010 with funding from the NIEHS Superfund Research Program and is conducted in Puerto Rico because of its high preterm birth rate and the extent of hazardous waste contamination [16, 17]. A prospective cohort of more than 2000 pregnant women has been recruited since 2010 and followed until delivery. PROTECT aims to explore environmental, clinical, demographic, behavioral factors contributing to preterm birth risk in Puerto Rico. The project also provides information on the predictors and sources of exposure among pregnant women as well as the potential mechanistic pathways involved in preterm birth. Particular attention is paid to chemicals commonly found at Superfund sites, including phthalates, phenols, and metals, suspected to be associated with high preterm birth rates [19-21].

Exposures to metals

Metals occur naturally in the environment and enter the human body through ingestion of food, supplements, and water, and through inhalation and skin contact of metal-containing products [22, 23]. In the United States, reports from the National Health and Nutrition Examination Survey (NHANES) showed that children and adults have detectable concentrations of a range of metals in their bodies [24], including pregnant women and their fetuses because of the trans-placental metal transfer [25-27].

Some of these metals are essential for human health and required for fetal growth, such as cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn). At the same time, excess or insufficient exposure poses risks to pregnancy. Others can be toxic even at low concentrations [22]; some are reproductive toxicants and

neurotoxicants, such as lead (Pb) and mercury (Hg), while others are known as human carcinogens, including cadmium (Cd) and arsenic (As). The toxic metals have been shown to induce oxidative stress, which plays an important role in the development of many adverse health outcomes, including cardiovascular, metabolic, and renal disease [28, 29]. Several metals are also suspected endocrine disruptors [30-33].

Prenatal metal exposure and adverse birth outcomes

Adverse birth outcomes, including pregnancy and fetal growth outcomes, encompass overall and spontaneous preterm birth, low birth weight, and small and large for gestational age, etc. [34]. Pregnant women and developing fetuses have increased vulnerability to the toxicological consequences of environmental exposures to metals. Prenatal exposures to metals have been suspected risk factors for adverse pregnancy outcomes, including the following:

(1) Pregnancy outcomes (gestational length, preterm and spontaneous preterm birth): Although exposure to several metals is highly prevalent [35], with the exception of some heavy metals (Pb, Cd, Hg, and As) human studies of exposure and pregnancy outcomes are quite limited:

Pb: Pb is generally present in water, food, air, soil, and dust. Pb is a neurotoxicant [36] and is harmful to reproductive health [37]. Pb readily crosses the placenta by passive diffusion, and therefore easily enters the fetus from the mother. From studies conducted in the US, Mexico, Japan, Indian, China, there is strong evidence for an association between high-level lead exposure during pregnancy and significant decreases in gestational length and increased risk of preterm birth [38-41]. In addition, limited studies have found evidence of the effect of low Pb exposure on pregnancy outcome [42, 43], and one study conducted in upstate New York with more than 43,288 mother-infant pairs, with an average blood Pb concentration of 2.1 μg/dL, found no association of maternal blood Pb with preterm birth but did find a relationship between Pb exposure and decreased birth weight [44]. As for the mechanism, Pb may induce oxidative stress by producing reactive oxygen species (ROS) that alters the placental functions possibly leading to preterm birth [45, 46]. Numerous experimental studies have also indicated that Pb increases the parameters of oxidative stress in the placenta [47, 48].

Cd: Cd is a well-known environmental toxic pollutant, and it is accumulated in food, air, and water. Once absorbed into the body, Cd can diffuse through the blood to the placenta and pose reproductive and fetal toxicity. In early studies, the reports on associations of Cd and preterm birth were mixed. Fagher et al. reported associations with higher blood Cd levels and preterm delivery among a small cohort of Polish and Swedish women (n=30) [49], while another small cohort of women in China (n=44) and an ecologic study conducted among women (n=38,718) in southern Sweden both found no association [50, 51]. However, caution must be taken since those studies either were ecologic studies or had a small sample size. Recently, large cohort studies from China [52, 53] and Japan [54] have reported that maternal serum/urine levels of Cd during pregnancy are positively associated with higher risk of preterm births. Studies have indicated that Cd can disturb normal fetus growth and pregnancy outcomes by altering placental functions, such as the transfer of essential metals (calcium and zinc), and by reducing blood flow in the placental tissue [52, 53, 55-57].

Hg: Hg is a non-essential and toxic metal in the human body. Inorganic Hg does not readily cross the placental barrier to enter the fetus; however, elemental and organic Hg are lipid soluble and therefore can cross the placenta and cause developmental toxicity to the fetus. Among two studies that evaluated the effects of Hg exposure measured in maternal blood on birth outcomes, one showed that higher maternal blood Hg was associated with higher risk of preterm birth and low birth weight [25], while the other observed a null association [58]. One study conducted in Michigan found a positive association between maternal Hg levels in hair and risk of very preterm (between 28 to 32 weeks) delivery [59].

As: As is identified as a human carcinogen by the International Agency for Research on Cancer [60]. Among the various routes of As exposure, drinking water and food, especially contaminated rice, are the largest sources of As. Both inorganic and methylated organic forms of As (the inorganic form is more toxic but also rarer) can easily cross the placenta [55]. A number of epidemiological studies have reported that As exposure from drinking water is associated with spontaneous abortion, stillbirth, and preterm birth [61-63]. In contrast, a study conducted in Taiwan suggested a null association between As levels in well-water in the residence and preterm delivery [64]. Animal studies and in vitro studies have proposed that the

mechanism of action for As and Hg is similar to Pb. Those metals may activate the oxidative perceptive signaling pathways by either forming free radicals or inhibiting anti-oxidative enzyme processes, which in turn damage the placental cell and eventually cause preterm delivery [65-67]

To date, existing research has focused primarily on the reproductive effects of heavy metals, with most reports involving high doses not commonly encountered by pregnant women and fetuses [68]. More recently, a growing body of evidence is suggesting that certain essential trace metals, including Cu [69, 70] and Ni [71], are associated with an increased risk of preterm delivery. However, most of the studies investigating the association between metals and preterm birth were cross-sectional. Given the recent evidence, it is imperative to study how both essential and non-essential metals are affecting pregnancy, at low doses found in everyday environments.

(2) Fetal growth outcomes (birthweight, small or large for gestational age): Low birth weight may result directly from preterm labor or growth restriction due to detrimental factors occurring during pregnancy, such as lack of nutrition, maternal infection, and exposure to environmental toxicants [72]. Small for gestational age (SGA), a manifestation of intrauterine growth restriction (IUGR), results when fetuses fail to reach their full genetic growth potential relative to their gestational age [73]. Low birth weight and small for gestational age (SGA) correlates with infant mortality, but also increased risk of chronic disease and cancer later in life [6-8]. Newborn babies that weigh more than usual relative to their gestational age are termed large for gestational age (LGA) and are at higher risk of long-term health consequences [74]. Accumulation of toxic metals in the placenta may result in altered growth patterns and adverse fetal growth outcomes [57, 75, 76]. As stated previously, many human and animal studies have elucidated the effects associated with heavy metals (Pb, Cd, Hg, and As) on fetal growth outcomes, mainly on low birth weight and SGA. The reports are inconsistent, as described below:

Pb: Pb in maternal blood [44, 77, 78], cord blood [79, 80], and the placenta [55, 81] has been significantly negatively correlated with birth weight. While additional evidence of the effects of Pb on SGA births were reported [82-84], a few other studies showed lack of association between maternal exposure to Pb and SGA [85-88].

Cd: Reports on associations of Cd and fetal growth outcomes have been mixed. One study from an e-waste recycling town in China reported no significant correlations between placental Cd and birth weight [89]. Urinary Cd was negatively associated with birthweight among pregnant women in Tokyo [87] and Toyama [90], Japan, while a similar inverse association was found with cord blood Cd in Mexico [91] and Italy [92], and placenta Cd in Chile [55]. A different study from Bangladesh found significant inverse associations between maternal Cd exposure and birth weight only in girls but not in boys [93].

Hg: A few studies have suggested a negative correlation between maternal blood Hg levels and newborn birth weight [58, 59, 94, 95] while several other studies reported a null association between elevated Hg levels and birth weight [96-98]. A Canadian birth cohort with 1835 pregnant women reported a small increase in risk for SGA in infants born to women with higher exposure to Hg and As. [85]

As: There is considerable evidence for associations between maternal As exposure and low birth weight [55, 64, 99, 100] and increased risk of SGA. A causal pathway analysis in a Bangladesh population suggested that the toxicological effect of As on fetal growth was the result of As exposure decreasing gestational length and maternal weight gain during pregnancy [101].

Recent studies on metals and fetal growth outcomes have also paid more attention to essential trace metals; a few studies reported inverted U-shaped dose-response curves for the associations between birth weight and maternal metal exposures, including cobalt (Co) [81] and manganese (Mn) [102, 103]. These results indicate that both too low and too high metal concentrations may affect mechanisms underlying fetal growth. However, other studies found no evidence of nonlinearity between trace metals and birth weight [104, 105]. Reports of other metal concentrations in relation to birth weight are none to very limited. In addition, most previous reports on the effects of metals on pregnancy are from studies usually involving high doses (e.g., studies on Pd before the elimination of Pb in paint and gasoline), that are not commonly encountered by pregnant women today [38, 68]. However, due to the widespread exposure of

humans and known toxicity of these metals, concern is growing that low-level exposure may also adversely affect birth outcomes, and several birth cohorts have evaluated the health effects of low-level exposure to metals during pregnancy [103, 106-110]. Therefore, there is a pressing need to study the effects of excessive exposure to essential trace elements on adverse pregnancy outcomes.

In summary, caution must be taken while comparing studies evaluating metals in relation with adverse birth outcomes as there were potentially important differences between the studies: 1) Study populations and study designs vary across those studies; and 2) Metal concentrations were measured in various media (blood, urine, cord blood etc.). In addition, most studies were cross-sectional and included biological samples from a single time point during pregnancy.

Interaction between metals and psychosocial status during pregnancy

There is a growing interest in the combined effect of chemical and non-chemical exposures in the environment on human health, among which evaluating the interactions and cumulative effects of chemicals and stress has been identified as a key research need [111, 112]. Prenatal maternal psychosocial status has been found to be associated with an increased risk of adverse pregnancy outcomes—psychological distress, perceived stress, anxiety, depression symptoms, and low social support among pregnant women were associated with an increased risk of pre-eclampsia [113], preterm birth [114-119], and low birth weight [114, 118]. The majority of epidemiologic studies in this area to date have evaluated the impact of individual chemical and non-chemical exposures. However, pregnant women are exposed to both environmental chemical and psychosocial stressors, and psychosocial factors may influence how a particular environmental chemical is experienced or what the physical response to it may be. Recently, there has been a general acknowledgment that there is likely to be joint effects of environmental chemicals (e.g. phthalates, black carbon, lead [Pb]) and psychosocial stress exposure on pregnancy and child development outcomes [112, 120-127]. Similarly, when looking at the effect of metals on maternal and children health, psychosocial factors are important to consider in deepening our understanding on how the environment impacts humans. Ultimately, the identification of modifiable psychosocial factors may lead to interventions during pregnancy to reduce the harmful effects of metals on birth outcomes.

Studying metals as mixtures

We have summarized inconsistent results from studies assessing the deleterious effect of individual metals on adverse birth outcomes. This discrepancy in the literature may be due, in part, to the fact that humans are exposed to hundreds of metals and other chemicals simultaneously. Moreover, human biomonitoring data have shown the presence of a mixture of metals in the prenatal environment [24-26]. Given that variety, we need to characterize associations between metals and adverse birth outcomes, not only individually, but also collectively. A few studies published recently have specifically focused on the general impact of collective metal exposure effects in health outcomes [69, 128-136], and a few have explored metal mixtures in relation to adverse birth outcomes [69, 133-136]. PROTECT has one of the largest numbers of toxic and essential trace metal analytes measured to date, which enables us to investigate the effects of metal(loid)s on adverse birth outcomes both individually and as mixtures.

Challenges in metal mixture exposure assessment

Epidemiologic studies aiming to determine the effects of environmental chemical mixtures on human health are growing rapidly. Due to limiting factors such as the financial cost and methodologic challenges, mixture studies based on biomarkers typically use a unified human specimen, such as blood or urine to determine exposure to various chemicals [23, 47, 137-139]. Although this approach applies well to chemicals with similar structure and pharmacokinetics, it is challenging to accurately describe metal mixtures using one unified medium. Each metal exhibits unique physiochemical properties and toxicokinetics, such as half-life, storage, or elimination rate from the body. As such, the preference for either blood or urine concentration as a better indicator is different across metals. For example, urinary concentration of As has often been used as an indicator of recent exposure because urine is the main route of excretion of most arsenic species [140, 141]. In contrast, blood is the preferred specimen for Pb as blood Pb has a longer half-life and subsequently lower variability in the body compared to urine [142]. As for other metals such as Mn, Cu, and Cr, there is a lack of consensus in the literature as to which biomarker is the most consistent and valid. Previous mixture studies on prenatal metal exposures and birth outcomes measured metals in different media including urine [143-146], whole blood [135, 147], cord blood [148], toenails [133], and teeth [149]. As mentioned above, each medium biomarker depicts levels in a particular body compartment that may have differential biological

relevance and may not fully represent the best measure of internal dose for all the metals. Therefore, it is imperative that we understand how the choice of different media can impact the performance of analyzing chemical mixtures in relation to a certain health outcome.

In conclusion, pregnancy and birth are time periods when the health of women and children is most vulnerable to the exposure to chemicals, including metals. While many human and animal studies have elucidated the effects associated with non-essential metals and have reported mixed effects, less attention has been given to other metals. Moreover, as humans are continuously exposed to a mixture of environmental toxicants, which are often correlated, there is a pressing need to study the relationship of exposures both individually and as mixtures. We also have a limited understanding of how metals interact with psychosocial stress during pregnancy. Investigating the sources, predictors, and effects of metal mixtures, their interaction with stress, and identifying the specific metal(s) that is/are most critical to adverse pregnancy outcomes are paramount for understanding how environmental chemicals impact preterm birth. Characterizing modifiable factors, including sources and psychosocial modifiers of metal exposure, could have huge public health impact as it potentially leads to contaminant remediation strategies and eventually a reduction in preterm birth rates.

Specific Aims

This dissertation advances our understanding of the effects of metal mixtures on birth outcomes by exploring the potential of metal exposures measured in different media to increase the risk of adverse birth outcomes. A conceptual diagram outlining the specific aims of this dissertation is illustrated in **Figure I.1**. This dissertation draws upon a prospective birth cohort, The Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) study. PROTECT launched in 2010 with funding from the Superfund Research Program and conducted in Puerto Rico because of its high preterm birth rate and the extent of hazardous waste contamination. PROTECT aims to explore environmental, clinical, demographic, behavioral factors contributing to preterm birth risk in Puerto Rico. The center also aims to provide information on the predictors and sources of exposure among pregnant women as well as the potential mechanistic pathways involved in

preterm birth. Particular attention is paid to chemicals commonly found at Superfund sites, including phthalates and metals, suspected to be associated with high preterm birth rates.

Specific Aim 1: I characterized metal exposures among pregnant women and to identify predictors of prenatal metal exposure. Specifically, I analyzed the repeated measurements from up to three study visits of urine and blood levels of metals for their distributions, trends, and correlations within and between urine and blood biomarkers. I then explored the associations between metal concentrations and potential predictors (demographic variables, personal care products, food, and water usage) using linear mixed models (LLM) with random intercepts.

Hypothesis 1: The blood and urine concentrations of metals will be correlated to the different degrees for different metals, and the levels of urine, blood metals, and urine/blood ratio are comparable to similar cohorts (NHANES) and reports from recent literature.

Hypothesis #2: Reported demographics/household characteristics and use of certain personal care products will be predictive of concentrations of urinary and blood metals.

Specific Aim 2: I investigated the associations between blood concentrations of metals and birth outcomes (preterm and spontaneous preterm birth, gestational age, birthweight, small for gestational age, and large for gestational age). First, I applied multivariate linear and logistic regression analyses to assess single pollutant associations between average exposure and each birth outcome. Differences in associations between study visits and infant sex were also tested. Upon evaluating the results, I utilized two distinct mixtures analysis methods, environmental risk score and Bayesian Kernel Machine Regressions (BKMR), to determine the cumulative effect of multiple metals and identify the most predictive metals.

Hypothesis #3: Increased metal concentrations in blood will be associated with birth outcomes and the collective effect of metal mixtures will have greater association with the adverse birth outcomes compared to individual metals.

Specific Aim 3: I examined the extent to which maternal psychosocial status modifies the associations between the metal biomarkers and adverse birth outcomes. Using K-means clustering, I categorized pregnant women into one of two groups: “good” and “poor” psychosocial status, based on overall psychosocial well-being characterized by depression, perceived stress, social support, and life events. I then evaluated whether the effect of blood metals (geometric average) on adverse birth outcomes varies between two clusters of women.

Hypothesis #4: The associations between metal exposure biomarkers and adverse birth outcomes will be stronger in the presence of "poor" psychosocial status.

Specific Aim 4: I assessed the mixture predictive performance of urine and blood metal biomarkers, and integrated multi-media biomarkers, in association with preterm birth. For each metal, I integrated exposure estimates from paired urine and blood biomarkers into multi-media biomarker (MMB). I then built Environmental risk scores (ERSs) of the metal mixtures to evaluate the performance of urine, blood, and multi-media biomarkers by examining the association between ERSs and preterm birth, using logistic regressions.

Hypothesis #5: The use of urine, blood, and the integrated metal mixtures will demonstrate different performance when modeling adverse birth outcomes.

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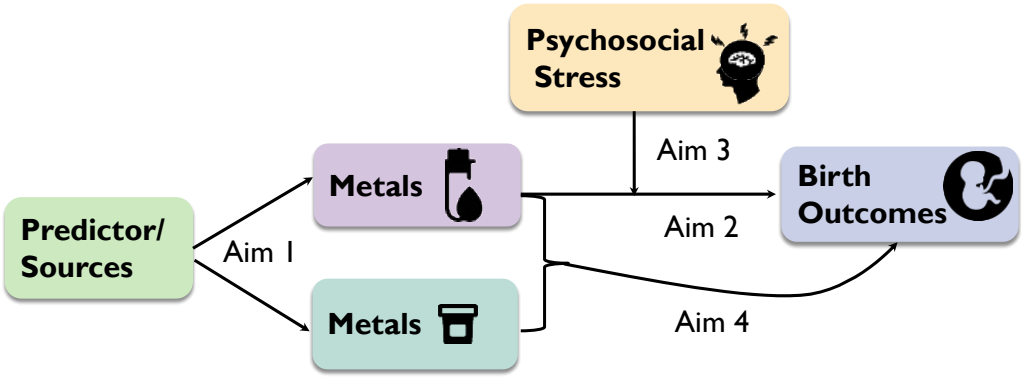
Figure I.1 Conceptual diagram of dissertation aims



Chapter II

Predictors of Urinary and Blood Metal(loid) Concentrations Among Pregnant Women in Northern Puerto Rico

Abstract

Background: Given the potential adverse health effects related to toxic trace metal exposure and insufficient or excessive levels of essential trace metals in pregnant women and their fetuses, the present study characterizes biomarkers of metal and metalloid exposure at repeated time points during pregnancy among women in Puerto Rico. **Methods:** We recruited 1,040 pregnant women from prenatal clinics and collected urine, blood, and questionnaire data on demographics, product use, food consumption, and water usage at up to three visits. All samples were analyzed for 16 metal(loid)s: arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), cesium (Cs), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), titanium (Ti), uranium (U), vanadium (V), and zinc (Zn). Urine samples were additionally analyzed for molybdenum (Mo), platinum (Pt), antimony (Sb), tin (Sn), and tungsten (W). **Results:** Mean concentrations of most metal(loid)s were higher among participants compared to the general US female population. We found weak to moderate correlations for inter-matrix comparisons, and moderate to strong correlations between several metal(loid)s measured within each biological matrix. Blood concentrations of Cu, Zn, Mn, Hg, and Pb, and urinary concentrations of As, Ni, and Co, were shown to reflect reliable biomarkers of exposure. For other metals, repeated samples are recommended for exposure assessment in epidemiology studies. Predictors of metal(loid) biomarkers included fish and rice consumption (urinary As), fish and canned food (blood Hg), drinking public water (blood Pb), smoking (blood Cd), and iron/folic acid supplement use (urinary Cs, Mo, and Sb). **Conclusions:** Characterization of metal(loid) biomarker variation over time and between matrices, and identification of important exposure sources, may inform future epidemiology studies and exposure reduction strategies.

1. Introduction

Metals and metalloids occur naturally in the environment and enter the human body through ingestion of food, water, and supplements, and the use of metal-containing products via inhalation, dermal absorption, and incidental ingestion [1-5]. In the United States, reports from the National Health and Nutrition Examination Survey (NHANES) show that children and adults have detectable concentrations of a range of metal(loid)s in their bodies [6], including pregnant women and their fetuses because of trans-placental metal(loid) transfer [7-9]. Some of these metals are essential for human health and required for fetal growth [10, 11], such as cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn). Excess or insufficient levels of these metals may pose risks to pregnancy [11, 12]. Other metal(loid)s do not play an essential physiologic role and can be toxic if present even at low concentrations [1, 13, 14]; some, including lead (Pb) and mercury (Hg), are reproductive toxicants and neurotoxicants, while others, such as cadmium (Cd) and arsenic (As), are known human carcinogens. Several metal(loid)s (Pb, Hg, Cd, As, Mn, Zn) are also suspected endocrine disruptors [15-18].

Puerto Rico has a long-standing history of contamination with environmental chemicals, with 200+ hazardous waste sites and 16 active Superfund sites (the hazardous waste lands identified by the EPA as a site for cleanup because it poses a risk to human health and/or the environment) [19]. Many contaminated sites are above unlined landfills that overlie Karst aquifers, creating pathways for contamination of groundwater and potential drinking water sources. Therefore, the risk of human exposure to metal(loid) contamination is high. However, little is known regarding the extent and specific sources of human metal(loid) exposure on the island. This is the first study to examine distributions, time trends, and predictors of urinary and blood metal(loid) biomarkers measured at multiple times during pregnancy among women living in Northern Puerto Rico. Characterizing relationships of metal(loid) biomarkers over time and between matrices, and identifying important exposure sources to metal(loid)s, may inform risk evaluations in epidemiology and targeted approaches to reduce metal(loid) exposure.

2. Methods

2.1 Study population

This study was conducted among pregnant women participating in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) project [20-23], an ongoing prospective birth cohort in the Northern Karst Region of Puerto Rico that began in 2010. PROTECT aims to explore environmental toxicants and other factors contributing to preterm birth risk and other adverse birth outcomes in Puerto Rico.

Study participants were recruited at approximately 14 ± 2 weeks of gestation at seven prenatal clinics and hospitals throughout Northern Puerto Rico and followed until delivery. The present analysis reflects 1,040 women recruited into the study thus far at 18 to 40 years of age. Details on the recruitment and inclusion criteria have been described previously [20, 21]. Spot urine samples were collected from women at three separate study visits (18 ± 2 weeks, 22 ± 2 weeks, and 26 ± 2 weeks of gestation) and blood samples were collected during the first and third visits. During the initial visit, questionnaires collecting demographic information were administered to participants. Information on housing characteristics, employment status, and family situation were collected during a second, in-home visit using a nurse-administered questionnaire. Household product, personal care product use, and water source and usage information were collected at each visit.

The research protocol was approved by the Ethics and Research Committees of the University of Puerto Rico and participating clinics, the University of Michigan, and Northeastern University. The study was described in detail to all participants, and informed consent was obtained prior to study enrollment.

2.2 Measurement of metal(loid)s

Spot urine was collected in sterile polypropylene cups and aliquoted within one hour after collection, while blood samples were collected in metal-free whole blood tubes. All samples were frozen and stored at -80°C and shipped on dry ice to NSF International (Ann Arbor, MI, USA) for analysis. Concentrations of 16 metals and metalloids (As) were measured in both urine and blood: As, barium (Ba), beryllium (Be), Cd, Co, chromium (Cr), cesium (Cs), Cu, Hg, Mn, Ni, Pb, titanium (Ti), uranium (U), vanadium (V), and Zn; an additional 5 metals and metalloids (antimony) were measured in urine only: Mo, platinum (Pt), antimony (Sb), tin (Sn), and tungsten (W). Metal(loid) concentrations were measured using inductively coupled plasma mass

spectrometry (ICPMS) as described previously [24]. Considering that biological samples have high levels of carbon and chloride in the matrix, the laboratory selected the appropriate isotopes for the requested elements to best avoid interferences where possible. The ICPMS was calibrated with a blank and a minimum of 4 standards for each element of interest. An R^2 value of >0.995 was the minimum criteria for an acceptable calibration curve. The calibration curves were verified by initial checks at three calibration points within the curve. Continuing calibration checks and blanks after every 10 samples were also utilized throughout the analytical run to ensure the ICPMS system was maintaining acceptable performance. Urinary specific gravity (SG) was measured at the University of Puerto Rico Medical Sciences Campus using a hand-held digital refractometer (Atago Co., Ltd., Tokyo, Japan) as an indicator of urine dilution.

2.3. Questionnaire

The product use questionnaire was adapted from questionnaires used in other studies of adults to capture information on potential exposure sources with which the pregnant women may have been in contact [20, 21]. At each visit, the questionnaire was administered by a study nurse to collect data on product and water use. The household/personal care product use section contained yes/no questions about the use of different products in the 48-h period preceding biological sample collection: bar soap, cologne/perfume, colored cosmetics, conditioner, deodorant, fingernail polish, hair cream, hairspray/ hair gel, laundry products, liquid soap, lotion, mouthwash, other hair products, shampoo, and shaving cream. In the water use section, participants were asked about the type of water utilized for drinking and cooking (municipal water, private well water, bottled/delivered water) as well as water storage behaviors (use of water cistern, filtration). In the second visit, participants also completed a food frequency questionnaire on the consumption of milk, cheese, fish, rice, yogurt, and other foods (never, <1 per month, 1 per month, 2–3 per month, 1 per week, 2 per week, 3–4 per week, 5-6 per week, 1 per day and 2 or more per day) as well as yes/no questions regarding supplement use (iron, folic acid, multivitamin, etc.).

2.4 Data pre-processing for statistical analyses

To account for urinary dilution, metal(loid) concentrations in urine were corrected for SG using the equation: $P_c = P[(SG_p - 1)/(SG_i - 1)]$; where P_c is the SG corrected biomarker concentration (ng/mL), P is the measured biomarker concentration, SG_p is the median urinary specific gravity in

this population (1.019), and SG_i is the individual's urinary specific gravity. Biomarker concentrations below the limit of detection (LOD) were replaced by LOD/√2. For statistical analysis, we included metal(loid)s with at least 50% of samples having concentrations above the LOD [25-27].

2.5 Descriptive statistics and comparison to NHANES

Descriptive statistics [geometric means (GM), geometric standard deviation (GSD), select percentiles] of urine and blood concentrations were calculated to describe distributions of metal(loid) concentrations among study participants and for comparison with previous reports. Using GM and selected percentiles, we compared concentrations measured in the present study with those measured in NHANES (2009-2010, 2011-2012, 2013-2014, 2015-2016), including women aged between 18 and 40 years (N for urine=1604, N for blood=3585).

2.6 Correlations between and within blood and urine concentrations

Spearman correlation coefficients and p values were calculated between blood and urine concentrations for 10 metal(loid)s (As, Cd, Co, Cs, Cu, Hg, Mn, Ni, Pb, and Zn) that were measured in both matrices and detected in >50% of samples; correlations were calculated using all samples that have measurements in both matrices. The ratio of urine concentration to blood concentration was constructed for each metal(loid) to further evaluate the relationship between the two biomarkers. Spearman rank correlations and p values were also calculated to assess relationships between different metal(loid)s within the same matrix; two sets of correlations were calculated using samples collected at each visit and using GM of metal(loid) concentrations over study visits.

2.7 Change in biomarkers across pregnancy (ICCs) and over time

To test for significant changes in biomarker concentrations across pregnancy (i.e., time points in gestation), linear mixed models (LMM) were used to account for repeated measurements from individuals. We also assessed the proportion of variance attributed to between-person variability across the three time points in pregnancy, using intra-class correlation coefficients (ICCs) and their 95% confidence intervals [28]. Ranging between 0 (no reproducibility) and 1 (perfect reproducibility), ICCs reflect a poor degree of reliability when below 0.40, a moderate to good

reliability when between 0.40 and 0.75, and an excellent reliability when above 0.75 [29]. Next, to examine the changes in urinary and blood metal(loid) concentrations over time (2011-2017), tests of linear trends across study period were conducted by modeling the GM for each individual's repeated measurements, including the year of visit as a continuous variable, and assessing statistical significance using the Wald test.

2.8 Predictor selection

Two approaches were taken to identify potential predictors of metal(loid) concentrations in urine and blood. Covariates (predictors) of interest (n=61) included demographic characteristics, 48-h recall of product use, dietary supplement intake, food consumption, and water use and sources. In the first approach, we regressed each covariate of interest against each measured biomarker, using linear mixed effects models (LMMs) with random intercepts. LMM accounts for the intra-individual correlation and variation of repeated measures over time and lead to smaller and more precise standard errors around means. With LMMs, we assessed log-transformed metal(loid) concentrations individually as continuous dependent variables; for urinary metal(loid)s, log-transformed concentrations were further corrected for SG. Potential predictors were modeled individually as independent variables. With the purpose of determining a subset of important predictor variables for each metal(loid), in the second approach, we fit multivariable LMMs with LASSO (least absolute shrinkage and selection operator) regularization (LMMLasso). LASSO regularization shrinks estimated regression coefficients corresponding to "weakly associated" covariates to zero, thereby embedding variable selection into the estimation procedure [30]. An optimal choice of the coefficient for the LASSO regularization (λ), corresponding with the lowest Bayesian Information Criterion (BIC), maximizes the probability of selecting the best model. In our analysis, for each metal, all predictor variables were entered in the LMMLasso models at the same time. The λ was identified using the R package glmmLasso version 1.3.3.

Furthermore, we analyzed associations between log-transformed metal(loid)s concentrations and food frequency questionnaire information collected at the second visit, using linear regression. To use the same/close time period for biomarkers and supplement use to assess these relationships, urine metal(loid) concentrations measured at the second visit and blood metal(loid) concentrations

measured at the third visit were used, as blood samples were not collected during the second visit. Data were analyzed using R version 3.2.2 and SAS 9.4 (SAS Institute Inc., Cary, NC)

3. Results

3.1 Descriptive statistics

A total of 1,285 urine samples and 1,183 blood samples from 1,040 women with measured metal(loid) concentrations in either blood and/or urine samples were included in this analysis. Among those 1040 women, 660 and 824 women provided urine and blood samples, respectively. Demographic characteristics of those women were described previously [23, 31] and are summarized in **Table II.1**. Most women in our study had private insurance, had an education above high school, were employed, and were married or in a domestic partnership. Nearly half of them had household incomes below $30,000/year. More than 80% of women never smoked while less than 2% smoked during pregnancy and 6% reported second-hand smoking exposure (>1 hour per day). Nearly all women reported no consumption of alcohol within the last few months. Demographic characteristics do not differ between women who provided urine samples (660 women) and blood samples (842 women).

Descriptive statistics (GM, GSD, select percentiles) are presented in **Table II.2**. Nearly all of the samples had detectable concentrations for most of the metals (98-100% > LOD), while a majority had detectable Cd (74.5% > LOD), Pb (72.1% > LOD), and Sb (90% > LOD) in urine and half had detected As (49% > LOD) and Cd (61% > LOD) in blood. 14 urinary metal(loid)s (As, Ba, Cd, Co, Cs, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Sn, and Zn) and 10 blood metal(loid)s (As, Cd, Co, Cs, Cu, Hg, Mn, Ni, Pb, and Zn) with at least 50% of samples having concentrations higher than LOD levels were included in the statistical analysis.

The comparisons with distributions among women 18 to 40 years old from NHANES 2009-10, 2011-12, 2013-14 and 2015-16 were included in **Table II.3** and **Table II.4**. In the NHANES cohort, some metals (Cu, Ni, and Zn) were not measured in urine samples and only Cd, Hg, Mn, and Pb were measured in blood samples. When comparing uncorrected urinary metal(loid) distributions with women of childbearing age enrolled in NHANES, women in our study had higher GM concentrations of all urinary metal(loid)s except for Cd, which were lower among

PROTECT women, and Pb, which were similar in the two cohorts. Median concentrations of As, Ba, Co, Hg, Mo, and Sb were 2-fold greater among women in this study compared to NHANES. PROTECT women had a median concentration of Mn and Sn that were 13 and 5 times greater than NHANES, respectively. For blood samples, PROTECT women had higher concentrations of Hg and Mn compared to NHANES while NHANES women had Cd and Pb concentrations (GM) that were twice as high as PROTECT women. Among women of childbearing age enrolled in NHANES, a small portion was pregnant (85 and 185 women in the urine and blood analysis, respectively) and the metal concentrations measured among these pregnant women were similar to the levels measured among other women included in our NHANES comparison.

3.2 Correlations between and within blood and urine concentrations

Spearman correlations between metal(loid)s within the same matrix did not differ when we calculated using GM of metal(loid) concentrations over study visit or using samples collected at each visit. Therefore, we presented the correlations between GM concentrations in **Figure II.1**. When looking across metal(loid)s measured in urine, there were some moderate to strong correlations [r=0.47 (Pb and Ba), 0.55 (Cd and Pb), 0.55 (Ni and Co), 0.59 (Ni and Ba)]. There were also weak to moderate (r = 0.30 to 0.45) but statistically significant ($p< 0.05$) correlations between several metal(loid)s. The correlations between metal(loid)s in blood were generally weaker compared to urinary metal(loid)s with only a few pairs being moderately correlated (Mn and Co, r=0.36; Cd and Co, r=0.33; As and Hg, r=0.32).

Spearman correlation coefficients for the same metal(loid)s across urine and blood matrices are presented in the last column of **Table II.2**. Most of the metal concentration in two matrices were significantly correlated, with Co (r=0.51) and Cs (r=0.43) having the highest coefficient followed by Hg (r=0.33) and As (r=0.27). Mn, Ni, Zn concentrations measured in urine and blood were not correlated.

3.3 Ratio

Distribution of urine/blood ratios for 10 metals are presented in **Figure II.2**. GM and median of urine/blood ratios were <1 for Cu, Zn, Pb, Mn, and Hg, indicating generally higher concentration measured in blood vs urine. Inversely, GM and median of urine/blood ratios were >1 for As, Ni,

Cs, and Co, indicating higher concentrations measured in urine vs blood. Cd concentrations were similar in two matrices (median urine/blood ratio of 1).

3.4 Change in biomarkers across pregnancy (ICCs) and over time

Figure II.3 and **II.4** show comparisons of urinary and blood concentration distributions for each biomarker between study visits. SG-corrected urinary concentrations of metal(loid)s were not significantly different between the three visits except for Co, Cs, Cu, Mo, and Zn ($p<0.05$ for all). First visit concentrations were higher compared to later visits for Cs, Mo, and Zn, while Co and Cu were higher at the third visit. Blood concentrations of Cs were higher at the first visit, while blood concentrations of Cd, Co, Cu, Mn, and Zn were lower, compared to the third visit.

ICCs for urine and blood metal(loid) concentrations and the urine/blood ratio are presented in **Table II.5**. Metals with a urine/blood ratio <1 (Cu, Zn, Pb, Mn, Hg) presented good to excellent reliability in blood with ICCs ranging from 0.54-0.78. Among the four metals with only urine measurements available, Sn had moderate reproducibility (ICC=0.55), whereas Mo, Sb, and Ba had weak reproducibility (ranging from 0.15 to 0.19). Reproducibility varied widely for the urine/blood ratio for each metal(loid), with ICCs ranging from 0.07 to 0.48.

Distributions of urinary and blood biomarker concentrations stratified by year are shown in SI **Figure II.5** and **II.6**. Results from linear trend tests indicated that the distributions of some biomarkers changed slightly over the course of our study period. For example, median levels of urine Ba, Cd, Cr, Cs, and blood Cs increased by 20-50% (P for trend<0.05) when comparing earlier and later years in the study period; while urinary Mn, Pb, Sb, Sn, and blood Ni and Pb were characterized by smaller, 20-30% decreases (P for trend<0.05).

3.5 Predictor selection

Variable selection analysis revealed several important predictors of urine and blood metal(loid) levels. Considering the concentrations of metal(loid)s measured in two matrices and reproducibility of different metal(loid)s in our analysis, we presented results for urinary concentrations of As, Co, Cs, Mo, and Sb (urine/blood ratio>1) and blood concentrations of Cu, Hg, Mn, Pb, and Zn (urine/blood ratio <1). Results from both urinary and blood concentrations of

Cd were included as the average urine/blood ratio was 1. No significant predictors were found for either blood or urine Ba, Ni, Sn (data not shown). Here we describe predictors identified by both univariable LMMs and multivariable LMMLasso, while **Figure II.7** shows all the variables selected through either approach. The two statistical approaches gave very similar effect estimates, therefore, **Figure II.7** presents effect estimates (β) and confidence intervals (CIs) obtained from the univariable LMMs. GM of urinary and blood metal(loid) concentrations in relation to different categories of demographic variables, self-reported product use, dietary supplement intake, food consumption, and water use are also shown in **Table II.6** and **Table II.7**.

3.5.1 Urine metals

As: Consuming fish 48 h prior to sample collection had the strongest relationship to urinary As concentration, while "other hair product" use, perfume use, and pesticide storage were negatively associated with As. **Cd:** We found strong positive associations between using a metal cistern to store water and urine Cd concentration, there was a 0.04 ng/ml difference on Cd concentration between women reporting the use of metal cistern and those who used plastic cistern or did not use cistern. Weak but significant positive associations were identified between urinary Cd concentration with age, parity, pre-pregnancy BMI, and use of perfume. **Co:** Smoking and consuming milk was associated with significantly higher urinary Co; self-reported use of other hair product was negatively associated with Co. **Cs:** Consumption of milk, spinach, folic acid supplement and drinking bottled water (vs public water) were positive predictors of higher Cs levels in urine. **Mo:** We found positive associations between self-reported folic acid, iron supplement, and peanut butter consumption and urine Mo concentration, while fish consumption and drinking filtered water were negatively associated with Mo concentration. **Sb:** Use of hair spray and consumption of folic acid were associated with higher Sb levels, while education and use of cosmetics were associated with lower Sb levels.

3.5.1 Blood metals

Cd: For Cd, smoking (ever, current vs never) was significantly associated with blood levels among pregnant women in the study, and the GM concentration difference between current smoker vs never smoker (0.13 ng/mL) was stronger than ever smoker vs never smoker (0.02 ng/mL). Cd concentrations were higher for women who consumed meat, tomatoes, or collards, and lower for

women who consumed multi-vitamins, compared to women who did not consume these items. **Cu:** Self-reported use of shaving cream and other hair product were important predictors of lower Cu levels; There was a trend for increasing concentration of Cu with increasing pre-pregnancy categories of BMI. **Hg:** Consuming fish, canned foods (e.g. canned tuna) and tomatoes were the strongest predictors of blood Hg levels. Hg concentrations were also higher among women with >12 years of education. **Mn:** Mn concentrations were associated with parity, where concentrations among women who had one or more children were significantly higher compared to women who had not yet had children. Blood Mn concentrations were lower among women who reported using shampoo and other hair products. Water treatment was also negatively associated with Mn concentration. **Pb:** Using bottled water as main drinking source was identified as the most significant predictor of lower Pb levels- participants who reported using bottled water as their main drinking source had significantly lower concentrations of Pb (0.30 µg/dL) compared to participants who drink public supply water (0.36 µg/dL). There were decreasing Pb concentrations associated with higher education levels. **Zn:** Pre-pregnancy BMI and using other hair products were negatively associated with blood Zn concentration.

3.6 Findings from the food frequency analysis

Our analysis of food frequency questionnaire information and metal(loid) concentrations found a trend for increasing concentrations of urinary As with increasing rice consumption frequency ($p<0.05$) (**Table II.8**). The geometric mean concentration of As was 2 fold higher among women who consumed rice once per day or more compared to women who consumed rice 2-3 times per month or less. Fish consumption frequency was negatively associated with urinary Cd and Pb concentrations (**Table II.8**). A similar trend was also observed for yogurt consumption frequency and urinary Sb concentration. In line with the results from the main predictor analysis above, there were positive linear trend relationships between meat consumption frequency and blood Cd, and fish consumption frequency and blood As and Hg levels (**Table II.9**). Blood Cs levels also increased with increased fish consumption.

4. Discussion

Our study provided much needed information on exposures to metal(loid)s among pregnant women in Northern Puerto Rico. We quantified levels of toxic and essential metal(loid)s in

maternal urine and blood, characterized variability of levels across pregnancy, and correlation between different metal(loid)s and matrices to better inform the use of metal(loid) biomarkers in epidemiology studies. We also identified important predictors of each metal(loid) in blood and urine which may suggest possible strategies and considerations for reducing exposure.

4.1 Comparison with other studies

Table II.10 provides an overview of reported metal(loid) concentrations in other studies of pregnant women. Urinary and blood concentrations of some essential metals such as Co, Cu, and Zn were within the range of what was reported in previous studies [32-37]. The concentrations of Cs in urine and blood were lower in this study compared with other studies of pregnant women in Australia and Spain [35, 38]. Urinary Mn concentrations (GM=1.2 ng/mL) in this study exceeded those seen in Australia [32] and Mexico [33], while blood Mn concentrations (GM=11.3 ng/mL) were comparable with those detected in other studies where the GM or median concentrations ranged from 6.5 to 16.1 ng/mL [32, 35, 37, 39].

Ba was only measured in urine and concentrations (GM=2.5 ng/mL) were lower in this study compared with Mexican pregnant women (GM=4.0 ng/mL) [33].The levels of Mo and Ni present in the urine samples from Puerto Rican pregnant women were similar to the levels reported in other studies [32-35]. Studies of Sb and Sn among pregnant women have been much more limited in number compared with other essential metals. The concentrations of urine Sb in our study, GM=0.1 ng/mL, were lower than the levels reported among Spanish pregnant women [35]. Sn levels measured in urine (GM=2.1 ng/mL) were one order of magnitude higher than the Japan study [34], where the GM was 0.2 ng/mL; however, this comparison needs to be interpreted cautiously given that Sn was only detected among 53% of the samples in the Japan study [34].

The urinary As concentration reported in our study was comparable to other studies of pregnant women while blood As concentrations were lower. The discrepancy between two matrices may be attributable to the fact that As in blood is more susceptible to variation as the half-life of inorganic As in blood is a few hours compared with a few days in urine [40]. Our study found that the GM blood Hg value among Puerto Rican pregnant women was 1.2 ng/mL with 3 participants having levels exceeding 5.8 µg/L, U.S. EPA's current reference dose for blood mercury [41].

Pregnant women in this study had lower urine and blood concentrations of Cd and Pb, compared to previous studies mentioned above. This is particularly significant where blood Pb concentrations among this population, with GM of 0.33 µg/dL, is the lowest when compared to women in NHANES (GM=0.64 µg/dL), and pregnant women in Australia (median= 0.37 µg/dL) [42], Japan (GM=0.64 µg/dL) [39], Ohio, US (GM=0.7 µg/dL) [43], Norway (two studies: median=2.5 µg/dL and GM=0.75 µg/dL) [37, 44], and South Africa (two studies: median=1.4 µg/dL and median= 2.3 µg/dL) [36, 45]. In epidemiological studies, higher Pb exposure may mask the effects of other exposures [46], therefore, having lower concentrations of Pb, this population may provide an opportunity to study the health effects of other metal(loid)s/exposures independent of Pb.

None of the blood samples in our study had Pb concentrations that exceeded the level of concern set by CDC, a blood level of 5 µg/dL for pregnant women [47]. However, concerns have been raised that even at low levels, prenatal Pb exposure may pose a toxic effect on fetal development [48-54].

These differences in metal(loid) concentrations among pregnant women could be mainly due to population differences, including different geographical and demographic environment, life style and dietary behaviors. The impact of demographic, dietary, and product use patterns during pregnancy on the variation of levels for metal(loid)s will be further discussed in this paper.

4.2 Variability of metal(loid) exposures

Limited studies have measured and/or compared metal(loid) concentrations at different times during pregnancy and mainly compared just a few metal(loid)s measured in blood or serum. As mentioned above, urinary concentrations of Co, Cs, Cu, Mo, and Zn among pregnant women in our study were statistically different between three visits. These different trends in concentration may due to an actual increase/decrease of metal(loid) concentrations in the body influenced by the change in fetal demand and maternal nutrient supply [55]. Metabolic changes during pregnancy, such as the change in glomerular filtration rate [56, 57] and plasma volume expansion [58] may also result in different filtration of metal(loid)s from blood into urine throughout pregnancy.

Our study reported a significant increase in blood Cd, Co, Cu, Mn, and Zn as gestation progresses. Similar increasing trends have been observed in previous studies considering concentrations of Co, Cu, and Mn in blood or serum [59-65]. The increasing levels of these metal(loid)s during pregnancy may be attributed to the increased intake and/or release of essential nutrients [66, 67]. For Cs, lower concentrations in the blood were observed during the third visit which may be explained by increasing plasma volume during pregnancy [58]. However, we would expect to see similar trends for all metals if the difference is due to metabolic changes during pregnancy.

We also found that urine/blood ratio remained constant for most of the metal over the course of pregnancy, except for Cd and Mn where the ratio was higher at the first visit and for Cu which had a higher ratio at the third visit (**Figure II.8**). These trends may reflect the absolute concentration changes of the metals in either matrix (the results are consistent with the single matrix results described above) and/or the different adjustments of toxicokinetics (distribution, excretion) of those metals throughout pregnancy.

Moderate to strong correlations were observed between urinary Pb and Ba **(**r=0.47) and Ni and Ba (r=0.59) (**Figure II.1**). Lewis et al also reported a strong correlation between urinary Pb and Ba (r=0.57) among Mexican pregnant women [33]. There were also a few blood metal(loid)s pairs that were moderately correlated (Mn and Co, r=0.36; As and Hg, r=0.32; Cd and Co, r=0.33) in our study (**Figure II.1**). Similar correlations between maternal blood Mn and Co, and As and Hg were reported among Norwegian pregnant women [37]. The correlation between As and Hg reflects the common source of exposures, seafood, which is consistent with results from our predictor analysis, whereas the pattern of correlations we observed between Pb and Ba, Ni and Ba, and Mn and Co concentrations could be due to combined use in products, demographic factors, and personal behaviors.

Urine and blood are commonly used to measure metal(loid)s in humans [68-70]. For most metal(loid)s examined in our study, weak to moderate correlations were observed between concentrations measured in both matrices. Most studies use a single human specimen (blood or urine) to determine exposure to various metal(loid)s. However, each metal(loid) exhibits unique physiochemical properties and toxicokinetics, such as half-life, storage, or elimination rate from

the body. As such, the preference for either blood or urine concentration as a better indicator for exposure to a given metal(loid) must be coordinated with the predicted toxicokinetics of the metal(loid) involved, the time between exposure and specimen collection, and the goals for a particular study (e.g. health outcome). For example, since As is excreted relatively rapidly via urine, urinary concentration of As is used as an indicator of recent exposure [71, 72]. In contrast, blood is the preferred specimen for Pb because Pb has a long biological half-life, resulting in less variability of blood concentrations over time [73]. Blood is also the preferred specimen to identify exposure to methyl-mercury, the most toxic form of Hg, whereas urine excretion represents inorganic Hg exposure [74-76]. For Cd, both urine and blood are useful for detecting exposures, as blood Cd primarily reflects recent exposure and urine Cd represents long-term exposure [46, 76].

Repeated measures of metal(loid) concentrations in both blood and urine samples enabled us to characterize metal(loid) exposures in different biological matrices, their interrelation, and variability during pregnancy, and select a better exposure indicator with higher reproducibility and abundance for each metal for application in epidemiology studies of pregnancy outcomes. Distributions of the ratio of urine/blood for non-essential metal(loid)s and ICCs for two matrices are consistent with previous knowledge; 1) the absolute concentrations of Pb and Hg were generally higher in blood than in urine (urine/ blood ratios<1 for most samples) and blood samples had good to excellent reproducibility (ICC for Pb=0.78, ICC for Hg=0.62); 2) concentrations of As were higher in urine (urine/blood ratio >1 for most samples); 3) concentrations of Cd were similar in both matrices (mean urine/blood ratio = 1). The concepts presented here for these non-essential metals can be applied to other metals with similar ratio and reproducibility. It is evident from **Figure II.2** that, metals with mean urine/blood ratio <1 (Cu, Zn, Mn, Hg, Pb) presented good to excellent reliability for blood measurements with ICC ranging from 0.54-0.78, this is consistent with studies indicating that blood Mn and Zn concentrations serve as a reasonable indicator of exposure [77-79]. The findings also indicate that repeated measurement of essential and non-essential metal(loid)s during pregnancy was necessary, particularly for most urinary biomarkers.

4.3 Predictors

Our predictor analysis revealed that some demographics, dietary factors, product use/water use behaviors can affect the distribution of various metal(loid)s. Smoking was the most significant predictor of blood Cd. We also found that the consumption of several food items (meat, tomato, collard) were additional predictors of Cd exposure. These results were somewhat expected given that diet and smoking are known sources of human Cd exposure [80]. In this population, we identified the consumption of fish as a significant predictor of As levels; rice consumption frequency was also positively associated with As levels. These findings are consistent with studies reporting increased exposure and possible health hazards associated with consuming As contaminated rice [81-85]. The forms of As found in rice are mostly inorganic and far more toxic than the organic form found in the environment and food like fish [86]. Fish was also one of the main predictors of blood Hg levels along with canned food and tomatoes. Fish and canned food (especially canned tuna) are food groups known to be potentially high in Hg [87-89]. However, our finding on tomato consumption and blood Hg are contrary to what was reported in previous studies where the consumption of tomato products and tropical fruits were associated with lower blood Hg [90-92]. The reported use of supplements during pregnancy, including folic acid and iron supplements, were significant and positive predictors of urinary Cs, Mo, and Sb concentrations. Cs and Mo are often in multi-vitamin and multi-mineral dietary supplements [93, 94]. It is also plausible that other specific supplementation that wasn't included in our questionnaires may contain those essential metal(loid)s and women in our study may be consuming those supplementations along with folic acid and iron supplements. Prenatal multi-vitamin use significantly decreased both blood and urine levels of Cd among this population, and this observation is supported by findings on the protective effect of vitamin E on heavy metal(loid)s absorption among animals [95, 96].

For blood concentrations of metal(loid)s, self-reported use of shaving cream and/or shampoo and/or other hair products were important predictors of lower Cu, Hg, Mn, and Zn levels. This inverse association may due to a higher frequency of washing behaviors (showering, face washing) which could help remove metals from the skin and reduce continued exposure.

While Pb concentrations in the study population were relatively low overall, we found that those whose drink AAA public water have higher levels of blood Pb compared to those who mostly

drink from bottled water. According to a report published in 2017 by the Natural Resources Defense Council, drinking water violations in Puerto Rico had the highest rate among all the U.S. jurisdictions with the presence of Pb and other pollutants in the water coming out of the taps during 2005-2015 [97]. Water treatment was inversely associated with blood Mn levels (among the questionnaire answers from women in our study, most treatments are referring to filtration). A study that assessed heavy metal(loid) concentrations in urban rivers of Puerto Rico found that Mn was the only metal found to exceed maximum contaminant levels established by the EPA for drinking water (US EPA: 5 μg/l) [98]. It is plausible that treatment of drinking water in homes may help reduce the levels of Mn in the water, therefore reducing exposure. Participants in our study who reported using metal cisterns to store water had elevated levels of urinary Cd. Various studies have found significantly higher levels of Cd in collected tank water and suggested that the main source of Cd in the tank water may be the corrosion of rooftop material since Cd is a common impurity in the Zn coating [99-101]. These findings suggest that proper and careful attention should be given to modifying household environments and water treatment behaviors when developing metal(loid) exposure remediation strategies.

4.4 Strengths and Limitations

To our knowledge, this is the first study to assess exposure to multiple metal(loid)s among pregnant women in Puerto Rico. PROTECT, a large prospective longitudinal cohort study in Puerto Rico, provides a unique opportunity to characterize metal(loid) exposure in this population. The study design allows for repeated collection of biological samples and questionnaire data to account for the varying levels of exposures during pregnancy, and LMM incorporated this full richness and structure of the data across pregnancy [21]. We measured a large panel of metal(loid)s in two biological matrices, urine and blood, which helps to inform future epidemiological analyses because different matrices may be more appropriate for assessing exposure to different metal(loid)s [35]. The study does have several limitations. We did not collect detailed information regarding the amount of personal product use, and the collection of maternal supplement use is not detailed as to specific ingredients and amount ingested. This may have caused non-differential misclassification and attenuated our results toward the null in the linear mixed models. Though our findings are possibly generalizable to the general pregnant population in Puerto Rico, they may not be generalizable to other pregnant women populations, considering that race/ethnicity,

personal care product use, dietary patterns, and toxicokinetics may be quite different compared to pregnant women in Puerto Rico.

5. Conclusion

In conclusion, we reported metal(loid)s exposure levels for 14 toxic and essential trace metal(loid)s in urine and blood samples from 1,040 pregnant women in Northern Puerto Rico. Exposure to many toxic and essential metal(loid)s are high among these women compared to women of reproductive age from the general US population. Blood concentrations of Cu, Zn, Mn, Hg, and Pb, and urinary concentrations of As, Ni, and Co, were shown to reflect reliable biomarkers of exposure. For other metal(loid)s, repeated samples are recommended for exposure assessment in epidemiology studies. We further examined a variety of predictors of prenatal metal(loid) exposure and found significant associations between potential predictors and biomarkers, including fish and rice consumption (urinary As), fish and canned food (blood Hg), drinking public water (blood Pb), smoking (blood Cd), and iron/folic acid supplement use (urinary Cs, Mo, and Sb). Improved understanding of biomarkers, sources, and pathways of metal(loid)s exposure can inform strategies to reduce exposure among Puerto Rico's residents.

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Table II.1 Demographic characteristics of n = 1,040 pregnant women from Puerto Rico (2011− 2017)

Variable	**Mean (SD)**	
Maternal Age	26.7 (5.5)	
Parity (# Live Births)	0.7 (0.8)	
Characteristic	**Category**	**Count (Percent)**
Insurance Type	Private	607 (58.4%)
	Public (mi salud)	351 (33.8%)
	Missing	82 (7.9%)
Maternal Education	<=High school/GED	214 (22.5%)
	Some college or technical school	359 (37.8%)
	College degree	312 (32.8%)
	Master's degree or higher	36 (3.8%)
	Missing	29 (3.1%)
Household Income	<$10,000	266 (25.6%)
	≥$10,000 to <$30,000	287 (27.6%)
	≥$30,000 to <$50,000	207 (19.9%)
	≥$50,000	117 (11.3%)
	Missing	163 (15.7%)
Marital Status	Single	206 (19.8%)
	Married or living together	801 (77%)
	Missing	33 (3.2%)
Gravidity (# Pregnancies)	0	415 (39.9%)
	1	363 (34.9%)
	>1	229 (22%)
	Missing	33 (3.2%)
Pre-pregnancy BMI (kg/m^2)	≤25	535 (51.4%)
	>25 to ≤30	309 (29.7%)
	>30	166 (16%)
	Missing	30 (2.9%)
Smoke During Pregnancy	Yes	9 (1.1%)
	No	787 (98.6%)
Employment Status	Unemployed	626 (62.6%)
	Employed	337 (33.7%)
	Missing	37 (3.7%)
Smoking	Never	852 (81.9%)
	Ever	144 (13.8%)
	Current	12 (1.2%)
	Missing	32 (3.1%)
Exposure to Second-hand Smoking	None	860 (82.7%)
	Up to 1 hour	43(4.1%)
	More than 1 hour	66(6.3%)
	Missing	71(6.8%)
Alcohol Consumption	None	511(49.1%)
	Before pregnancy	434(41.7%)
	Within the last few months	59(5.7%)
	Missing	36(3.5%)

Table II.2 Urinary and blood concentration of metal(loid)s (ng/ml) in 1,040 pregnant women from Puerto Rico in 2011–2017

Metal	Specimen	N (Sample)	LOD	% >LOD	GM	GSD	25%	50%	75%	95%	r[c]
As	Urine[a]	1285	0.3	100	10.9	2.5	6.1	10.8	19.0	46.4	0.27**
	Blood[b]	1183	0.3	48.9	0.34	1.8	0.21	0.21	0.48	0.99	
Ba	Urine[a]	1285	0.1	99.3	2.5	2.9	1.3	2.5	5.0	12.9	
	Blood[b]										
Cd	Urine[a]	1285	0.06	74.5	0.12	2.3	0.06	0.12	0.20	0.58	0.25**
	Blood[b]	1183	0.1	60.9	0.12	1.7	0.07	0.12	0.16	0.27	
Co	Urine[a]	1285	0.05	100	1.0	1.9	0.70	1.0	1.5	2.8	0.51**
	Blood[b]	1183	0.2	98.2	0.34	1.4	0.28	0.34	0.41	0.57	
Cs	Urine[a]	1285	0.01	100	4.9	1.7	3.7	5.3	7.1	10.7	0.43**
	Blood[b]	1183	0.04	99.9	1.1	1.4	0.94	1.2	1.4	1.9	
Cu	Urine[a]	1285	2.5	99.3	14.0	1.8	10.0	14.2	19.5	34.5	0.21**
	Blood[b]	1183	9	99.9	1552	1.3	1393	1562	1740	2096	
Hg	Urine[a]	1285	0.05	98.6	0.60	2.9	0.30	0.59	1.2	3.6	0.33**
	Blood[b]	1183	0.2	99.9	1.2	1.7	0.85	1.2	1.7	3.0	
Mn	Urine[a]	1285	0.08	100	1.2	1.6	0.95	1.2	1.6	2.3	0.01
	Blood[b]	1183	2	99.9	11.3	1.4	9.0	11.3	14.0	19.4	
Mo	Urine[a]	1285	0.3	100	58.9	2.0	38.9	62.9	92.2	166	
	Blood[b]										
Ni	Urine[a]	1285	0.8	98.9	5.4	2.0	3.5	5.5	8.5	15.5	0.06
	Blood[b]	1183	0.5	96.4	1.0	1.6	0.81	1.0	1.3	2.2	
Pb	Urine[a]	1285	0.1	72.1	0.25	2.7	<LOD	0.27	0.51	1.2	0.17**
	Blood[b]	1183	0.02	99.9	3.3	1.6	2.5	3.3	4.3	6.4	
Sb	Urine[a]	1285	0.04	90	0.09	1.9	0.06	0.08	0.12	0.22	
	Blood[b]										
Sn	Urine[a]	1285	0.1	100	2.1	3.0	1.0	1.9	4.0	14.0	
	Blood[b]										
Zn	Urine[a]	1285	2	100	266	2.5	155	300	498	947	0.07
	Blood[b]	1183	24	99.9	4682	1.3	4248	4752	5252	6055	

Abbreviations: arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn); limit of detection (LOD); geometric mean (GM); geometric standard deviation (GSD).

[a] Includes uncorrected urinary metal concentrations for up to 3 repeated samples per woman (n = 1285 samples)

[b] Includes blood metal concentrations for up to 2 repeated samples per woman (n = 1183 samples)

[c] Spearman correlation coefficient calculated for blood and urine metal concentrations; **P value for the Spearman test <0.01

Table II.3 Uncorrected urinary biomarker concentrations (ng/ml) in n = 660 pregnant women from Puerto Rico[a] in 2011− 2017 and comparison with U.S. population-based samples of women ages 18− 40 from NHANES[b]

	Cohort	N (Sample)	LOD	% >LOD	GM	GSD	25%	50%	75%	95%	P value[c]
As	PROTECT	1285	0.3	100	10.9	2.5	6.1	10.8	19.0	46.4	<0.01**
	NHANES	1562	0.26-1.25	97.6	7.3	3.2	3.3	6.4	13.3	62.3	
Ba	PROTECT	1285	0.1	99.3	2.5	2.9	1.3	2.5	5.0	12.9	<0.01**
	NHANES	1561	0.06-0.12	99.5	1.1	2.8	0.58	1.1	2.3	5.6	
Cd	PROTECT	1285	0.06	74.5	0.12	2.3	0.06	0.12	0.20	0.58	<0.01**
	NHANES	1561	0.036-0.056	85.7	0.14	2.8	0.06	0.15	0.28	0.75	
Co	PROTECT	1285	0.05	100	1.0	1.9	0.70	1.0	1.5	2.8	<0.01**
	NHANES	1561	0.023-0.048	99.6	0.44	2.5	0.25	0.41	0.81	1.8	
Cs	PROTECT	1285	0.01	100	4.9	1.7	3.7	5.3	7.1	10.7	<0.01**
	NHANES	1561	0.066-0.12	100	3.8	2.1	2.3	4.1	6.4	11.4	
Cu	PROTECT	1285	2.5	99.3	14.0	1.8	10.0	14.2	19.5	34.5	
	NHANES										
Hg	PROTECT	1285	0.05	98.6	0.60	2.9	0.30	0.59	1.2	3.6	<0.01**
	NHANES	1565	0.05-0.13	69.7	0.28	2.9	<LOD	0.25	0.55	1.8	
Mn	PROTECT	1285	0.08	100	1.2	1.6	0.95	1.2	1.6	2.3	<0.01**
	NHANES	1131	0.08-0.13	42.8	0.12	1.8	<LOD	<LOD	0.16	0.34	
Mo	PROTECT	1285	0.3	100	58.9	2.0	38.9	62.9	92.2	166	<0.01**
	NHANES	1560	0.8-0.99	100	36.1	2.5	19.6	39.4	69.8	139	
Ni	PROTECT	1285	0.8	98.9	5.4	2.0	3.5	5.5	8.5	15.5	
	NHANES										
Pb	PROTECT	1285	0.1	72.1	0.25	2.7	<LOD	0.27	0.51	1.2	0.11
	NHANES	1561	0.03-0.10	96.2	0.27	2.5	0.15	0.27	0.48	1.2	
Sb	PROTECT	1285	0.04	90	0.09	1.9	0.06	0.08	0.12	0.22	<0.01**
	NHANES	1561	0.022-0.041	69.4	0.05	2.3	<LOD	0.05	0.08	0.21	
Sn	PROTECT	1285	0.1	100.0	2.1	3.0	1.0	1.9	4.0	14.0	<0.01**
	NHANES	1130	0.09-0.22	86.2	0.43	3.2	0.18	0.38	0.88	3.3	
Zn	PROTECT	1285	2	100	266	2.5	155	300	498	947	
	NHANES										

Abbreviations: National Health and Nutrition Examination Survey (NHANES); arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn); limit of detection (LOD); geometric mean (GM); geometric standard deviation (GSD).

[a] Includes biomarker concentrations for up to 3 repeated samples per woman (n = 1,285 samples)

[b] Females 18−40 years of age; n = 1,604 for biomarkers measured in 2009-2010, 2011-2012, 2013-2014, and 2015-2016 NHANES

[c] P value for two sample t-test comparing geometric mean of chemical concentration in two cohorts; **P <0.01

Table II.4 Blood biomarker concentrations (ng/ml) in n = 842 pregnant women from Puerto Rico[a] in 2011− 2017 and comparison with U.S. population-based samples of women ages 18− 40 from NHANES[b]

	Cohort	N (Sample)	LOD	% >LOD	GM	GSD	25%	50%	75%	95%	P value[c]
As	PROTECT	1183	0.3	48.9	0.34	1.8	0.21	0.21	0.48	1.0	
	NHANES										
Cd	PROTECT	1183	0.1	60.9	0.12	1.7	0.07	0.12	0.16	0.27	<0.01**
	NHANES	3393	0.1-0.16	83.0	0.31	2.2	0.17	0.28	0.48	1.4	
Co	PROTECT	1183	0.2	98.2	0.34	1.4	0.28	0.34	0.41	0.57	
	NHANES										
Cs	PROTECT	1183	0.04	99.9	1.1	1.4	0.94	1.2	1.4	1.9	
	NHANES										
Cu	PROTECT	1183	9	99.9	1552	1.3	1393	1562	1740	2096	
	NHANES										
Hg	PROTECT	1183	0.2	99.9	1.2	1.7	0.85	1.2	1.7	3.0	<0.01**
	NHANES	3393	0.16-0.28	87.9	0.74	2.5	0.37	0.67	1.4	4.0	
Mn	PROTECT	1183	2	99.9	11.3	1.4	9.0	11.3	14.0	19.4	<0.01**
	NHANES	2174	0.99-1.06	100.0	10.7	1.4	8.4	10.6	13.6	19.2	
Ni	PROTECT	1183	0.5	96.4	1.0	1.6	0.81	1.0	1.3	2.2	
	NHANES										
Pb	PROTECT	1183	0.02	99.9	3.3	1.6	2.5	3.3	4.3	6.4	<0.01**
	NHANES	3393	0.7-2.5	98.8	6.4	1.8	4.4	6.1	9.0	16.9	
Zn	PROTECT	1183	24	99.9	4682	1.3	4248	4752	5252	6055	
	NHANES										

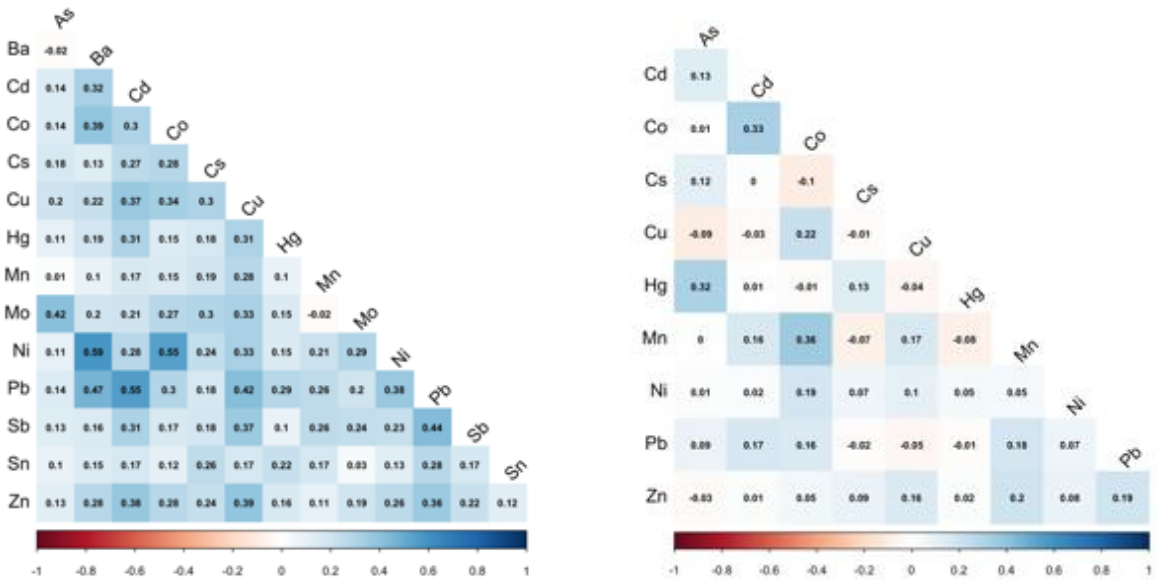
Abbreviations: National Health and Nutrition Examination Survey (NHANES); arsenic (As); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); nickel (Ni); lead (Pb); zinc (Zn); limit of detection (LOD); geometric mean (GM); geometric standard deviation (GSD).

[a] Includes biomarker concentrations for up to 2 repeated samples per woman (n = 1,183 samples)

[b] Females 18−40 years of age; n = 3,585 for biomarkers measured in 2009-2010, 2011-2012, 2013-2014, and 2015-2016 NHANES

[c] P value for two sample t-test comparing geometric mean of chemical concentration in two cohorts; **P <0.01

Figure II.1 Heat map of pairwise correlations between urine and blood GM concentrations among pregnant women in the PROTECT study[ab]



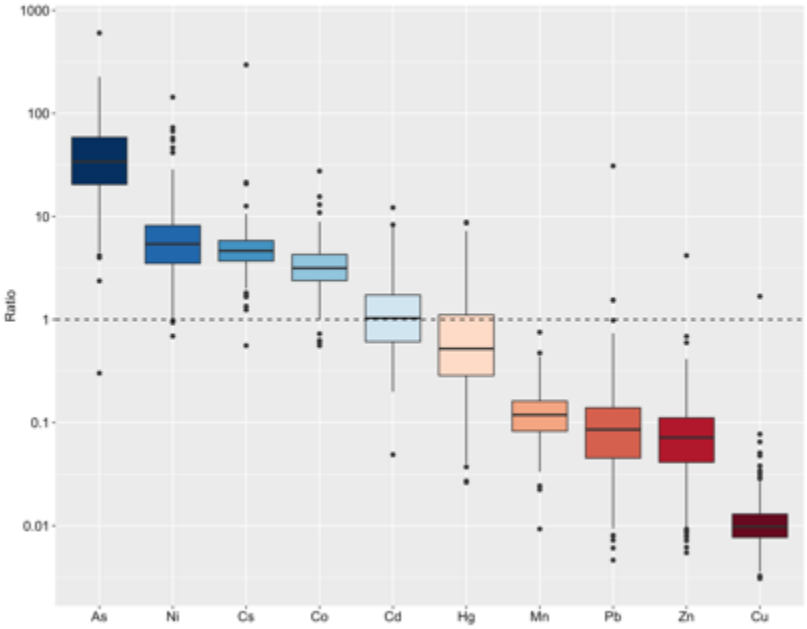
Urine **Blood**

Abbreviations: arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn).

[a] The correlation heat map was created using natural log-transformed urinary or blood metal(loid) concentrations

[b] All urinary concentrations were SG-corrected

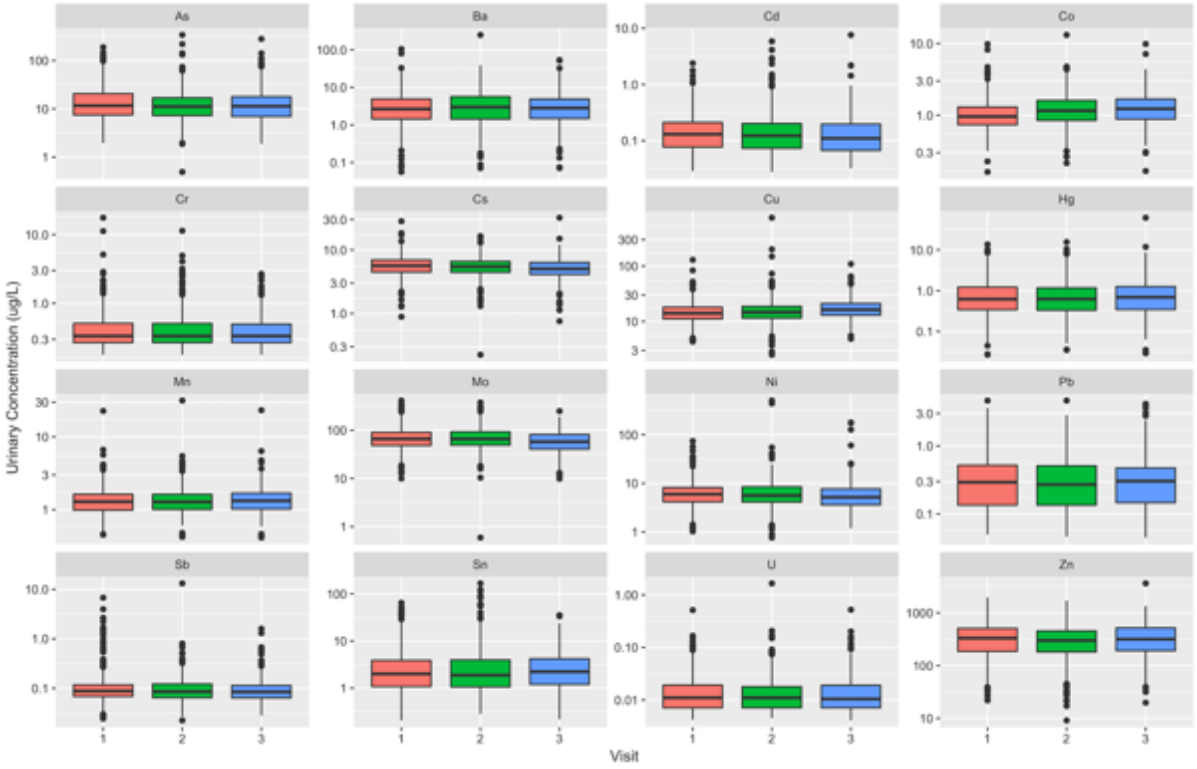
Figure II.2 Ratio of metal(loid) concentrations in urine and blood samples (n=509)[a]



Abbreviations: arsenic (As); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); nickel (Ni); lead (Pb); zinc (Zn).

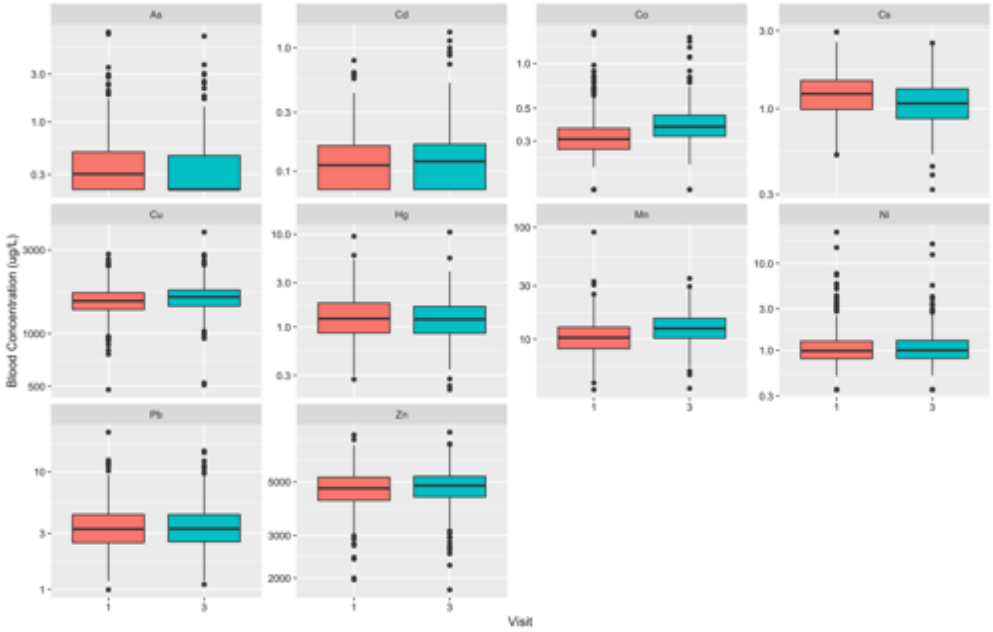
[a] All the urinary concentrations were SG-corrected

Figure II.3 SG-corrected urinary concentrations (ng/mL) of metal(loid)s by study visit (n=1285)[a]



Abbreviations: arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn).
[a] Number of participants in each visit were 500, 449, and 336 respectively

Figure II.4 Blood concentrations(ng/mL) of metal(loid)s by study visit (n=1183)[a]



Abbreviations: arsenic (As); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); nickel (Ni); lead (Pb); zinc (Zn).

[a] Number of participants in 1st and 3rd visits were 678 and 505, respectively

Table II.5 Intraclass correlation coefficients (ICCs) and 95% confidence for natural log-transformed urinary and blood concentrations of biomarkers and ratio of urine and blood concentrations

	Urine[ab]	Blood[c]	Urine/Blood Ratio[de]
biomarker	**ICC (95% CI)**	**ICC (95% CI)**	**ICC (95% CI)**
As	0.21 (0.15,0.29)	0.25 (0.17,0.36)	0.20 (0.08,0.42)
Ba	0.19 (0.13,0.28)	-	-
Cd	0.18 (0.12,0.26)	0.48 (0.41,0.56)	0.18 (0.07,0.39)
Co	0.27 (0.21,0.36)	0.16 (0.07,0.3)	0.07 (0.00,0.53)
Cs	0.31 (0.25,0.38)	0.77 (0.72,0.8)	0.40 (0.25,0.56)
Cu	0.21 (0.15,0.3)	0.68 (0.62,0.74)	0.22 (0.06,0.56)
Hg	0.51 (0.46,0.57)	0.62 (0.56,0.68)	0.43 (0.29,0.59)
Mn	0.13 (0.07,0.21)	0.54 (0.44,0.6)	0.31 (0.15,0.53)
Mo	0.15 (0.09,0.23)	-	-
Ni	0.13 (0.07,0.23)	0.13 (0.05,0.27)	0.22 (0.08,0.48)
Pb	0.08 (0.03,0.2)	0.78 (0.73,0.81)	0.22 (0.07,0.48)
Sb	0.17 (0.11,0.26)	-	-
Sn	0.55 (0.49,0.61)	-	-
Zn	0.39 (0.33,0.46)	0.75 (0.7,0.79)	0.48 (0.35,0.62)

Abbreviations: arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn).

[a] Among 660 women who had urine samples available, 184 had data from all three visits, 257 had data from two visits, and 219 had data from one visit

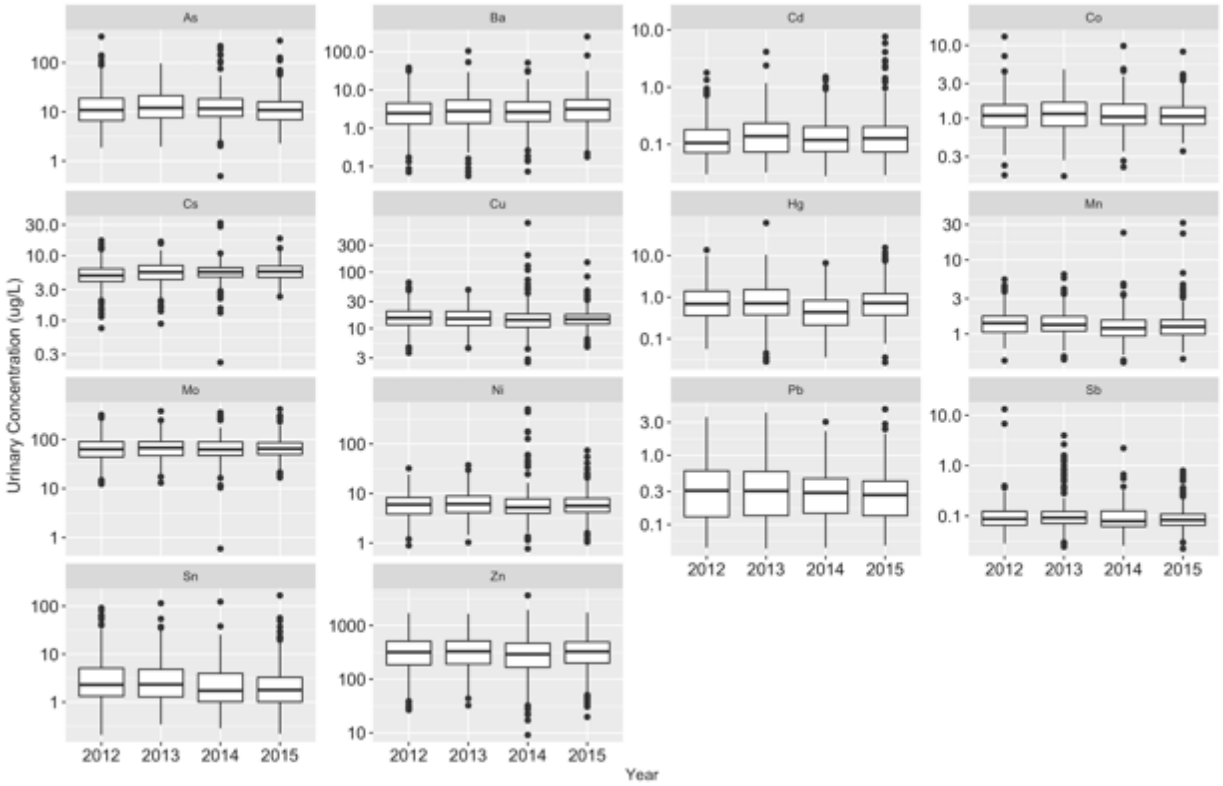
[b] specific gravity corrected concentration

[c] Among 842 women who had blood samples available, 341 had data from both visits, and 501 had data from one visit

[d] Among 403 women who had both urine and blood samples available, 106 had data from both visits, and 297 had data from one visit

[e] specific gravity corrected urinary concentration was used to calculate the ratio

Figure II.5 Distribution of urinary biomarker concentrations (ng/mL) among 660 pregnant women in Puerto Rico over study years (2011–2017)[ab]

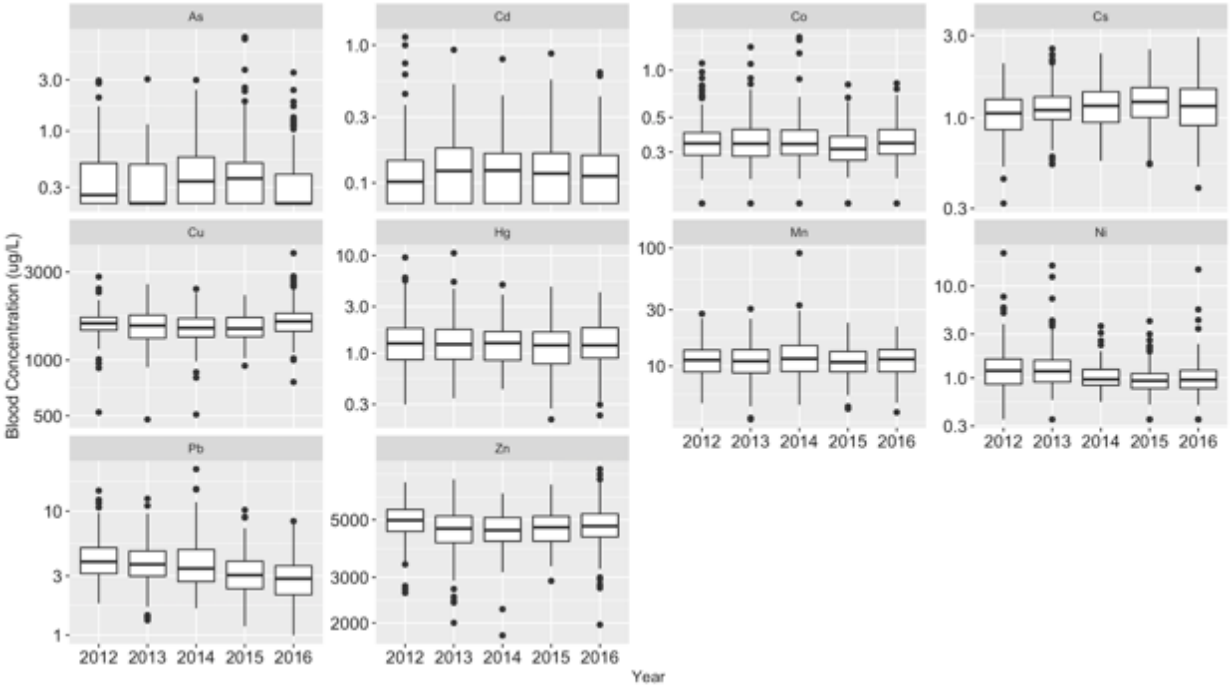


Abbreviations: arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn).

[a] 2011 and 2012, 2015 and 2016 are combined to have even numbers of samples in each box

[b] Number of participants in each year during 2011–2012, 2013, 2014, 2015-2016 were 302, 273, 196, and 434, respectively

Figure II.6 Distribution of blood biomarker concentrations (ng/mL) among 842 pregnant women in Puerto Rico over study years (2011–2017)[ab]

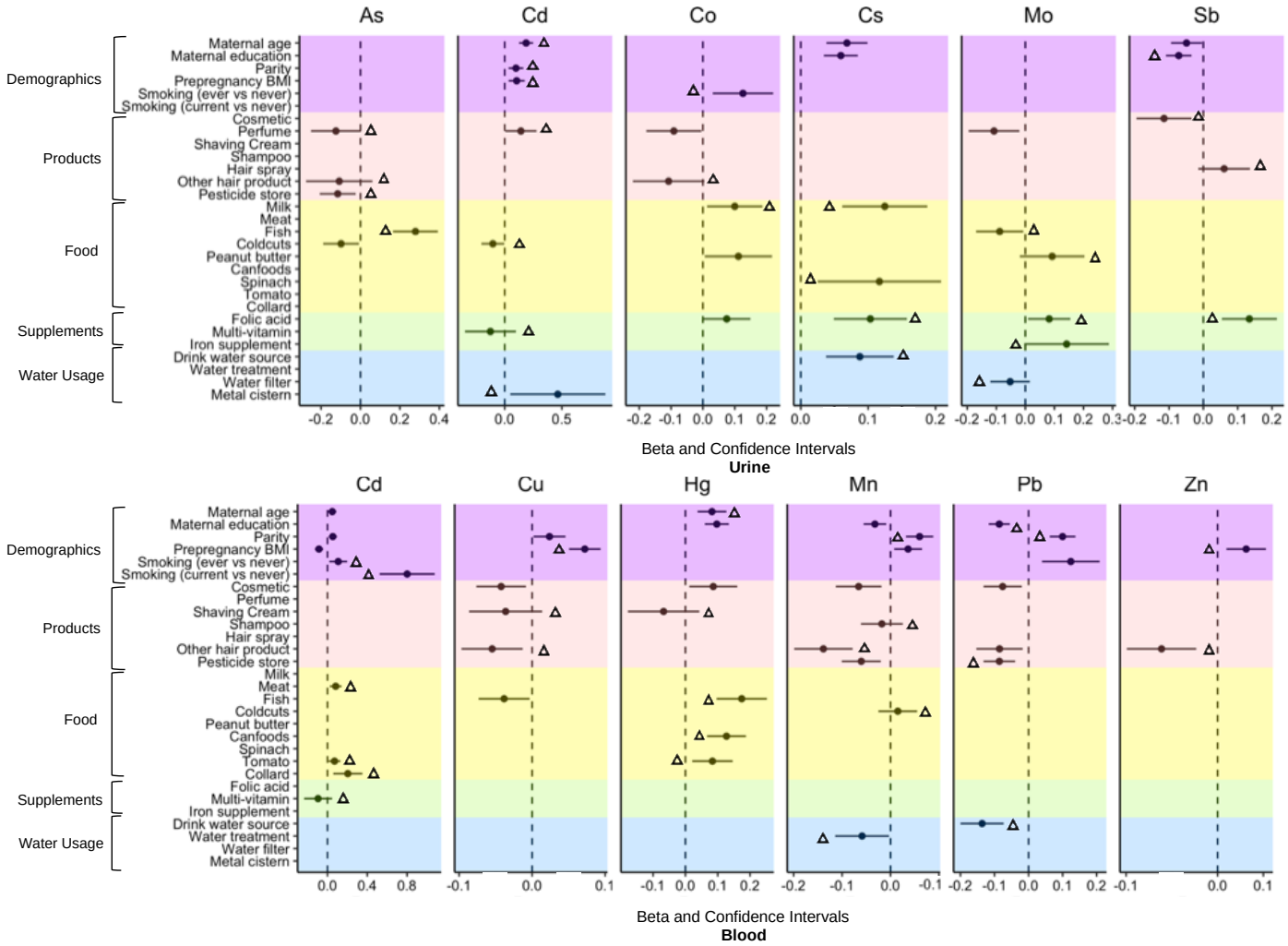


Abbreviations: arsenic (As); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); nickel (Ni); lead (Pb); zinc (Zn).

[a] 2011 and 2012, 2016 and 2017 are combined to obtain a balanced number of samples in each box

[b] Number of participants in each year during 2011–2012, 2013, 2014, 2015, and 2016-2017 were 212, 148, 147, 249, and 274, respectively

Figure II.7 Beta and confidence intervals extracted from individual linear mixed models for metal(loid) concentrations and potential predictors[ab]



Abbreviations: arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn).

Δ Variables also selected as predictors of metal(loid) exposure from multivariable LMMLasso models

[a] In this figure, covariates that were not associated with any metal(loid) concentrations in the univariable and multivariable analysis were not included in the y-axis

[b] Drinking water source: bottle water (1) vs AAA public water (0)

Table II.6 Geometric means of urinary (SG-corrected) and blood concentrations of metal(loid)s according to demographic, and maternal factors[ab]

	Urinary metal(loid)s						Blood metal(loid)s					
Variable	As	Cd	Co	Cs	Mo	Sb	Cd	Cu	Hg	Mn	Pb	Zn
overall	10.9	0.12	1.0	4.9	1.2	0.09	0.12	1552	1.2	11.3	3.3	4682
maternal age (years)												
<25		0.11		5.0		0.10	0.11		1.1			
25-30		0.13		5.4		0.09	0.11		1.3			
>30		0.17		5.8		0.09	0.13		1.3			
p value		**<0.001****		**<0.001****		**<0.001****	**0.02****		**<0.001****			
maternal education												
<=high school/ged				4.8		0.10			1.1	11.7	4.05	
some college or technical school				5.3		0.10			1.2	11.4	3.20	
college degree				5.6		0.08			1.3	10.9	2.99	
master's degree or higher				5.7		0.08			1.5	11.0	3.28	
p value				**<0.001****		**<0.001****			**<0.001****	**0.01****	**<0.001****	
parity (# pregnancies)												
0		0.12					0.11	1516		10.8	3.09	
1		0.13					0.11	1589		11.3	3.34	
>1		0.14					0.13	1560		12.2	3.75	
p value		**0.003****					**0.01****	**0.03****		**<0.001****	**<0.001****	
prepregnancy BMI (kg/m^2)												
≤25		0.12					0.12	1477		10.9		4585
>25 to ≤30		0.14					0.12	1603		11.7		4759
>30		0.14					0.10	1684		11.6		4825
p value		**0.004****					**<0.001****	**<0.001****		**0.01****		**0.005****
smoking												
never			1.1				0.11				3.23	
ever			1.2				0.13				3.75	
current			0.9				0.25				4.21	
p value (ever vs never)			**<0.001****				**<0.001****				**0.001****	
p value (current vs never)							**<0.001****					

Abbreviations: arsenic (As); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); lead (Pb); antimony (Sb); zinc (Zn).

[a] Results shown for food items with association detected

[b] p-values from linear mixed effects models accounting for within-person correlations; *P from 0.1 to 0.05, **P <0.05

Table II.7 Frequencies of product use, dietary supplement intake, food consumption, and water use and sources in the 48-h recall questionnaire and geometric mean urinary (SG-corrected) and blood concentrations of metal(loid)s (ng/mL) associated with self-reported use or non-use[ab]

		Urinary metal(loid)s								Blood metal(loid)s							
Variable	Use	n=660	N= 1285	As	Cd	Co	Cs	Mo	Sb	n= 842	N= 1183	Cd	Cu	Hg	Mn	Pb	Zn
Products																	
cosmetic	yes	435	845						0.09	568	787		1531	1.3	11.0	3.2	
	no	146	308						0.10	185	259		1604	1.1	11.7	3.6	
p value									**0.01****				**0.01****	**0.02****	**0.01****	**0.01****	
perfume	yes	484	965	11.5	**0.13**	1.1		62.7		628	868						
	no	97	188	13.1	**0.12**	1.2		69.9		124	176						
p value				**0.05***	**0.05***	**0.04****		**0.02****									
shaving cream	yes	48	94			1.1	5.5			67	90		1471	1.2			
	no	533	1060			1.1	5.4			687	957		1556	1.2			
p value						0.77	0.93						0.16	0.23			
shampoo	yes	409	815							541	743				11.0		
	no	172	338							213	302				11.6		
p value															0.10		
hairspray	yes	196	395						0.10	243	337						
	no	384	759						0.09	507	705						
p value									0.12								
other hair product	yes	81	81	10.2		1.0				109	112		1457		10.1	3.1	4366
	no	499	1073	11.9		1.1				645	934		1560		11.3	3.3	4716
p value				0.21		**0.06***							**0.01****		**<0.001****	**0.01****	**0.002****
store pesticide	yes	342	668	11.1						467	643				10.9	3.1	
	no	238	483	12.7						285	401				11.5	3.6	
p value				**0.01****											**0.004****	**<0.001****	

Table II.7 continued

		Urinary metal(loid)s								Blood metal(loid)s							
Variable	Use	n=660	N= 1285	As	Cd	Co	Cs	Mo	Sb	n= 842	N= 1183	Cd	Cu	Hg	Mn	Pb	Zn
Food items																	
milk	yes	485	979			**1.1**	**5.5**			622	873						
	no	96	174			**1.0**	**4.7**			133	175						
p value						**0.02****	**<0.001****										
meat	yes	360	729							470	654	0.12					
	no	221	424							285	394	0.11					
p value												**0.01****					
fish	yes	106	211	**14.99**				59.3		144	182		1485	1.5			
	no	475	942	**11.11**				64.8		611	866		1563	1.2			
p value				**<0.001****				**0.03****					**0.03****	**<0.001****			
cold cuts	yes	360	719	11.2	0.13					476	663				11.2		
	no	221	433	12.7	0.14					279	385				11.0		
p value				**0.04****	**0.05***										0.46		
peanut butter	yes	45	101			**1.2**		69.1		64	90						
	no	535	1051			**1.1**		63.3		690	957						
p value						**0.04****		0.10									
can foods	yes	303	601							393	557			1.3			
	no	277	551							362	491			1.1			
p value														**<0.001****			
spinach	yes	35	69				6.1			46	57						
	no	545	1083				5.3			709	991						
p value							**0.01****										
tomatoes	yes	218	442							293	409	0.12		1.3			
	no	362	710							462	639	0.11		1.2			
p value												**0.02****		**0.01****			
collard	yes	42	72							31	40	0.14					
	no	538	1080							724	1008	0.12					
p value												**0.01****					

Table II.7 continued

		Urinary metal(loid)s								Blood metal(loid)s						
Variable	Use	n=660	N= 1285	As	Cd	Co	Cs	Mo	Sb	n= 842	N= 1183	Cd	Cu	Hg	Mn	Pb	Zn
Supplements																	
folic acid	yes	172	344			1.2	5.7	67.4	0.10	213	298						
	no	434	864			1.1	5.2	62.3	0.09	606	856						
p value						**0.05***	**<0.001****	**0.03****	**0.001****								
multi-vitamin	yes	570	1145		0.13					775	1089	0.12					
	no	38	68		0.15					45	66	0.13					
p value					0.27							0.19					
iron supplement	yes	36	64					73.07		36	48						
	no	569	1142					63.19		779	1101						
p value								**0.06***									

Table II.7 continued

		Urinary metal(loid)s								Blood metal(loid)s						
Variable	Use	n=660	N= 1285	As	Cd	Co	Cs	Mo	Sb	n= 842	N= 1183	Cd	Cu	Hg	Mn	Pb	Zn
Water usage																	
water source for drinking **a**	bottled	236	479				5.7			361	507					3.0	
	public	382	742				5.2			446	629					3.6	
p value							**0.001****									**<0.001****	
water treatment	yes	147	299							162	231				10.7		
	no	471	923							650	913				11.3		
p value															**0.04****		
water filtration frequency **b**	~ never	235	469					65.7		289	398						
	~ 1/4 of the time	51	97					61.2		60	86						
	~ 1/2 of the time	51	93					60.3		66	99						
	~ 3/4 of the time	22	42					63.3		59	80						
	always	247	496					62.6		327	465						
p value (yes/no)								0.13									
cistern material	plastic	193	370		0.12					211	307						
	metal	10	18		0.16					12	18						
	other	13	32		0.13					14	21						
p value					**0.02****												

Abbreviations: number of participants (n); number of samples (N); arsenic (As); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); lead (Pb); antimony (Sb); zinc (Zn).

[a] Results shown for food items with association detected

[b] p-values are from linear mixed effects models accounting for within-person correlations: *P from 0.1 to 0.05, **P <0.05

Table II.8 Frequencies of selected food type consumption reported in second visit and sg-corrected urinary geometric mean concentrations of metal(loid) biomarkers (ng/ml) associated with self-reported frequency[a]

Food Item	Frequency category	n[c]	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Zn
fish	Never	22	11.3	2.9	0.22	1.3	5.2	14.8	0.63	1.2	76.9	5.9	0.34	0.10	2.0	349
	<1 per month	46	11.7	2.8	0.13	1.1	5.5	14.7	0.66	1.4	73.0	6.5	0.29	0.09	2.5	272
	1 per month	44	10.5	3.0	0.14	1.3	5.6	15.2	0.60	1.3	65.9	6.6	0.25	0.09	2.6	236
	2–3 per month	55	11.6	2.5	0.12	1.1	5.2	13.5	0.59	1.3	57.4	5.9	0.26	0.09	1.9	245
	1 per week	12	12.3	3.0	0.08	1.1	5.7	18.8	0.94	1.3	67.5	8.8	0.18	0.08	4.3	248
	3–4 per week and more	13	16.6	2.5	0.1	1.0	5.4	14.6	0.44	1.2	76.2	5.4	0.20	0.09	1.9	400
P value[b]			0.20	0.61	**0.02****	**0.06***	0.96	0.95	0.51	0.62	0.37	0.90	**0.04****	0.52	0.89	0.90
rice	2–3 per month or less	19	8.7	2.9	0.12	1.1	4.9	13.9	0.72	1.4	60.6	5.6	0.29	0.09	2.1	257
	1 per week	11	10.5	2.2	0.08	1.1	5.3	12.0	0.49	1.3	57.3	5.4	0.23	0.09	1.9	185
	2 per week	25	11.2	3.3	0.23	1.3	5.7	14.6	0.73	1.2	67.3	6.2	0.20	0.07	2.0	240
	3–4 per week	65	10.5	2.6	0.12	1.2	5.9	15.7	0.52	1.4	65.4	7.4	0.23	0.10	2.5	288
	5-6 per week	30	15.2	2.7	0.14	1.0	5.4	15.0	0.80	1.3	74.1	5.6	0.30	0.09	2.6	253
	1 per day and more	37	13.4	3.5	0.15	1.2	4.8	14.5	0.58	1.2	69.3	6.4	0.37	0.10	2.6	332
P value[b]			**0.02****	0.50	0.55	0.77	0.65	0.51	0.83	0.35	0.25	0.57	**0.09***	0.20	0.27	**0.09***

Abbreviations: arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn).

[a] Results shown for food items with association detected

[b] p-values are from linear mixed effects models accounting for within-person correlations: *P from 0.1 to 0.05, **P <0.05

[c] n=449 for total number of participants in second visit

Table II.9 Frequencies of selected food type consumption reported in second visit and blood geometric mean concentrations of metal(loid) biomarkers (ng/ml) measured in third visit associated with self-reported frequency[a]

Food item	Frequency category	n[c]	As	Cd	Co	Cs	Cu	Hg	Mn	Ni	Pb	Zn
Meat	<1 per month	40	0.40	0.11	0.38	1.0	1614	1.3	12.3	1.1	3.3	4638
	1 per month	41	0.31	0.12	0.36	1.1	1623	1.2	12.6	1.1	3.2	4815
	2–3 per month	89	0.31	0.11	0.39	1.1	1613	1.1	12.7	1.0	3.4	4776
	1 per week	32	0.32	0.14	0.36	1.1	1489	1.2	11.5	0.94	3.3	4698
	2 per week	29	0.31	0.13	0.38	1.2	1571	1.2	11.6	1.0	3.3	4681
	3–4 per week and more	26	0.31	0.14	0.43	1.0	1669	1.1	11.9	1.2	3.2	4628
P value[b]			0.16	**0.02****	0.27	0.35	0.78	0.26	0.26	0.80	0.76	0.69
fish	Never	51	0.29	0.11	0.40	1.0	1635	0.9	12.5	1.0	3.3	4856
	<1 per month	46	0.35	0.12	0.38	1.0	1596	1.1	12.3	1.1	3.4	4726
	1 per month	62	0.30	0.13	0.38	1.1	1667	1.2	12.7	1.0	3.3	4780
	2–3 per month	69	0.32	0.12	0.36	1.1	1547	1.3	11.6	1.0	3.1	4561
	1 per week	14	0.43	0.12	0.35	1.2	1525	1.3	12.6	1.0	3.4	4717
	3–4 per week and more	18	0.43	0.11	0.45	1.4	1514	1.4	13.5	0.94	3.8	4925
P value[b]			**0.02****	0.54	0.71	**<0.01****	0.06	**<0.01****	0.91	0.20	0.73	0.60

Abbreviations: arsenic (As); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); nickel (Ni); lead (Pb); zinc (Zn).

[a] Results shown for food items with association detected

[b] p-values are from linear mixed effects models accounting for within-person correlations: **P <0.05

[c] n=505 for total number of participants in third visit

Table II.10 Urinary and blood metal(loid) concentrations among pregnant women in PROTECT and previous studies[a]

Urinary Metal(loid)s Summary																			
Reference	**Country/Region**	**Year**	**n**	**As**	**Ba**	**Cd**	**Co**	**Cs**	**Cu**	**Hg**	**Mn**	**Mo**	**Ni**	**Pb**	**Sb**	**Sn**	**Zn**	**Correction[b]**	**GM /Median**	**Unit**
Present study	**Puerto Rico**	**2011-2017**	**1285**	**10.9**	**2.5**	**0.12**	**1.0**	**4.9**	**14.0**	**0.60**	**1.2**	**58.9**	**5.4**	**0.25**	**0.09**	**2.1**	**266**	**SG**	**GM**	**ng/mL**
Kalloo et al, 2018	Ohio, US	2003-2006	389	5.3	-	0.20	-	-	-	0.60	-	-	-	0.70	-	-	-	-	GM	ng/mL
Lewis et al 2018	Mexico	1997-2004	212	13.8	4.0	0.18	1.2	-	-	-	0.82	17.3	9.5	2.9	-	-	288	-	GM	ng/mL
Callan et al. 2013	Australia	2008-2011	157	13.2	-	-	1.2	-	10.4	-	0.53	-	2.3	-	-	-	396	Crt	Median	µg/g
Hinwood et al, 2013	Australia	2008-2011	157	-	-	0.78	-	-	-	<0.40	-	-	-	0.70	-	-	-	Crt	Median	µg/g
Hinwood et al, 2015	Australia	2008-2011	157	-	-	-	-	8.3	-	-	-	-	-	-	-	-	-	Crt	Median	µg/g
Birgisdottir et al, 2013	Norway	2003	184	79.6	-	0.16	-	-	-	1.2	-	-	-	-	-	-	-	Crt	Median	µg/g
Fort et al, 2014	Spain (1st trim)	2004-2006	489	32.0	-	0.61	0.45	8.0	12.0	-	-	-	3.9	3.8	0.36	-	256	Crt	Median	µg/g
	Spain (3rd trim)	2004-2006	489	35.0	-	0.54	1.3	6.8	15.0	-	-	-	3.9	3.9	0.28	-	290	Crt	Median	µg/g
Shirai et al, 2010	Japan	2007-2008	78	76.9	-	0.77	-	-	12.8	-	-	79.0	-	0.48	-	0.23	393	Crt	GM	µg/g

Table II.10 Continued

Blood Metal(loid)s Summary																			
Reference	**Country/Region**	**Year**	**n**	**As**	**Ba**	**Cd**	**Co**	**Cs**	**Cu**	**Hg**	**Mn**	**Mo**	**Ni**	**Pb[c]**	**Sb**	**Sn**	**Zn**	**Correction[b]**	**GM/Median**	**Unit**
Present study	**Puerto Rico**	**2011-2017**	**1183**	**0.34**		**0.12**	**0.34**	**1.1**	**1552**	**1.2**	**11.3**		**1.0**	**0.33**			**4682**	**-**	**GM**	**ng/mL**
Kalloo et al, 2018	Ohio, US	2003-2006	389	-		-	-	-	-	-	-			**0.70**			-	-	GM	ng/mL
Callan et al. 2013	Australia	2008-2011	172	1.3		-	0.28	-	1252	-	6.5		<2.0	-			2330	-	Median	ng/mL
Hinwood et al, 2013	Australia	2008-2011	172	-		0.38	-	-	-	0.46	-		-	0.37			-	-	Median	ng/mL
Hinwood et al, 2015	Australia	2008-2011	172	-		-	-	1.9	-	-	-		-	-			-	-	Median	ng/mL
Birgisdottir et al 2013	Norway	2003	184	5.9		0.45	-	-	-	4.0	-		-	2.5			-	-	Median	ng/mL
Hansen et al, 2011	Norway	2007-2009	211	1.4		0.15	0.10	-	1650	1.2	10.7		-	0.75			5110	-	GM	ng/mL
Mathee et al, 2014	South Africa	2010	307	8.0		0.20	-	-	-	0.60	-		-	1.4			-	-	Median	ng/mL
Rudge et al. 2009	South Africa	nr	62	0.57		0.15	0.60	-	1730	0.65	16.8		-	2.3			6290	-	Median	ng/mL
Nakayama et al, 2019	Japan	2011-2014	17997	-		0.71	-	-	-	3.8	16.1		-	0.64			-	-	GM	ng/mL

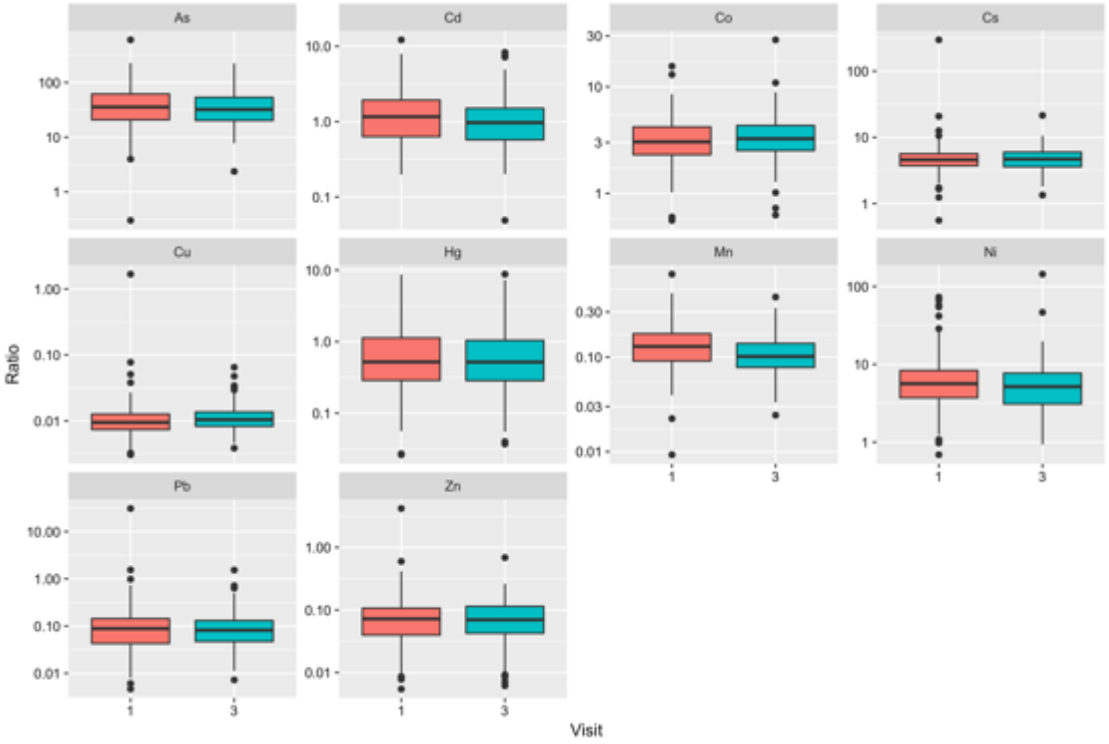
Abbreviations: sample size (n); arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn); geometric mean (GM); trimester (trim); not reported (nr).

[a] To allow for comparison on same scale, the urine concentrations were converted to ng/mL for unadjusted urine, µg/g for creatinine adjusted urine, and blood concentrations were converted to ng/mL

[b] - No correction applied, SG corrected for specific gravity Crt corrected for creatinine

[c] the unit for blood Pb concentration is µg/dL

Figure II.8 Urine/blood ratio distribution by study visit (n=509)[a]



Abbreviations: arsenic (As); barium (Ba); cadmium (Cd); cobalt (Co); cesium (Cs); copper (Cu); mercury (Hg); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); tin (Sn); zinc (Zn).
[a] Number of participants in 1st and 3rd visits were 309 and 200, respectively

Chapter III

Maternal Blood Metal and Metalloid Concentrations in Association with Birth Outcomes in Northern Puerto Rico

Abstract

Background: In previous studies, exposures to heavy metals such as Pb and Cd have been associated with adverse birth outcomes; however, knowledge on effects at low levels of exposure and of other elements remain limited. **Method:** We examined individual and mixture effects of metals and metalloids on birth outcomes among 812 pregnant women in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort. We measured 16 essential and non-essential metal(loid)s in maternal blood collected at 16–20 and 24–28 weeks gestation. We used linear and logistic regression to independently examine associations between geometric mean (GM) concentrations of each metal across visits and gestational age, birthweight z-scores, preterm birth, small for gestational age (SGA), and large for gestational age (LGA). We evaluated effect modification with infant sex*metal interaction terms. To identify critical windows of susceptibility, birth outcomes were regressed on visit-specific metal concentrations. Furthermore, average metal concentrations were divided into tertiles to examine the potential for non-linear relationships. We used elastic net (ENET) regularization to construct Environmental Risk Score (ERS) as a metal risk score and Bayesian Kernel Machine Regression (BKMR) to identify individual metals most critical to each outcome, accounting for correlated exposures. **Results:** In adjusted models, an interquartile range (IQR) increase in GM lead (Pb) was associated with 1.63 higher odds of preterm birth (95%CI=1.17, 2.28) and 2 days shorter gestational age (95% CI=-3.1, -0.5). Manganese (Mn) and zinc (Zn) were also associated with higher odds of preterm birth and shorter gestational age; the associations were strongest among the highest tertile for Mn and among females for Zn. Mercury (Hg) was associated with higher risk of preterm birth at the later window

of pregnancy. Ni measured later in pregnancy was associated with lower odds of SGA. ENET and BKMR models selected similar metals as "important" predictors of birth outcomes. The association between ERS and preterm birth was assessed and the third tertile of ERS was significantly associated with an elevated odds ratio of 2.13 (95% CI= 1.12, 5.49) for preterm birth compared to the first tertile. **Conclusion:** As the PROTECT cohort has lower Pb concentrations (GM=0.33 μg/dL) compared to the mainland US, our findings suggest that low-level prenatal lead exposure, as well as elevated Mn and Zn exposure, may adversely affect birth outcomes. Improved understanding on environmental factors contributing to preterm birth, together with sustainable technologies to remove contamination, will have a direct impact in Puerto Rico and elsewhere.

1. Introduction

Preterm birth (<37 completed weeks of gestation) is a significant public health concern as it is the leading cause of infant mortality [1-4]. Other important adverse birth outcomes including low birthweight (<2500g) and being small for gestational age (SGA), which may result directly from preterm labor and/or growth restriction due to detrimental factors occurring during pregnancy, also contribute substantially to childhood and adult morbidity [5-7].

Puerto Rico has one of the highest incidences of adverse birth outcomes among all US jurisdictions. In 2016, there were 3,248 preterm births in Puerto Rico, representing 11.5% of live births, compared to the national US average of 9.8% [8]. In addition, Puerto Rico has higher rates of childhood obesity and asthma [9-11] as well as obesity, metabolic syndrome, and diabetes in adults [12, 13] compared to the rest of the U.S., all of which have been associated with higher rates of preterm birth and/or low birthweight. Moreover, traditional risk factors do not explain this high rate of adverse birth outcomes and associated consequences in Puerto Rico. Even though there is growing epidemiological [14-17] and toxicological [18-21] evidence that environmental factors may play a key role, these factors remain understudied and underappreciated. Therefore, it is important to understand the role of environmental chemicals in adverse health outcomes and to develop new methods for reducing harmful exposures in Puerto Rico and beyond.

Ubiquitous in the environment, metals and metalloids have been widely detected among the U.S. population [22], including pregnant women and their fetuses because of the trans-placental metal transfer [23-25]. While many human and animal studies have focused on elucidating the effects associated with heavy metals cadmium (Cd), mercury (Hg), arsenic (As), and lead (Pb), less attention has been given to the other metals. However, a growing body of evidence suggests that certain essential or trace metals, including copper (Cu) [26] and Nickel (Ni) [27], may be associated with an increased risk of preterm delivery. A few other studies also reported inverted U-shaped dose-response curves for the associations between birth weight and maternal metal exposures, including cobalt (Co) [28] and manganese (Mn) [29, 30]. Therefore, there is a pressing need to study the effects of excessive exposure to essential trace elements on adverse pregnancy outcomes. In addition, most previous reports on the effects of metals on pregnancy are from studies usually involving high doses (e.g., studies on Pd before the elimination in paint and gas), not commonly encountered by pregnant women and fetus [31, 32]. Due to the widespread exposure of humans and known toxicity of these metals, concern is growing that low-level exposure may also adversely affect birth outcomes and several birth cohorts have evaluated the health effects of low-level exposure to metals during pregnancy [30, 33-37].

As humans are continuously exposed to a mixture of environmental toxicants, there is a pressing need to study the relationship of exposures both individually and as mixtures [38]. While most studies on metals have assessed metal exposures individually rather than in combination, a few have explored metal mixtures in relation to adverse birth outcomes [26, 39-42]. Compared to these earlier studies, our study has one of the largest numbers of toxic and trace metal analytes. Therefore, we investigated the effects of metal(loid)s on adverse birth outcomes both individually and as mixtures. Identifying modifiable environmental risk factors for adverse birth outcomes could have a positive public health impact if future exposures can be reduced through contaminant remediation or other exposure reduction strategies in an effort to reduce rates of preterm birth and other adverse health effects.

2. Methods

2.1 Study population

This study used data collected from 812 pregnant women participating in the ongoing prospective cohort project "the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT)" [43-46]. The PROTECT study launched in 2010 with funding from the NIEHS Superfund Research Program and conducted in Puerto Rico because of its high preterm birth rate and the extent of hazardous waste contamination on the island. PROTECT aims to explore environmental toxicants and other factors contributing to preterm birth risk and other adverse birth outcomes in Puerto Rico.

Study participants were recruited at approximately 14 ± 2 weeks of gestation at seven prenatal clinics and hospitals throughout Northern Puerto Rico and followed until birth. [43, 44]. Inclusion criteria for this study were: maternal age between 18 to 40 years; residence inside of the Northern Karst aquifer region; disuse of oral contraceptives within the three months prior to pregnancy; disuse of *in vitro* fertilization to become pregnant; and free of any major medical or obstetrical complications, including pre-existing diabetes. Each woman participated in a total of up to three study visits (18 ± 2 weeks, 22 ± 2 weeks, and 26 ± 2 weeks of gestation). Detailed information on medical and pregnancy history were collected at the initial visit. During an in-home visit (second visit), nurse-administered questionnaires were used to gather information on housing characteristics, employment status, and family situation. Blood samples were collected from women at the first and third visits. The present analysis reflects 812 women who delivered live singleton births in PROTECT and had metal biomarker measurements available.

The research protocol was approved by the Ethics and Research Committees of the University of Puerto Rico and participating clinics, the University of Michigan, Northeastern University, and the University of Georgia. The study was described in detail to all participants, and informed consent was obtained prior to study enrollment.

2.2 Measurement of Metals

Blood samples were collected in metal-free whole blood tubes and frozen at −80°C, and shipped on dry ice to NSF International (Ann Arbor, MI, USA) for analysis. Concentrations of 16 metals and metalloids: As, barium (Ba), beryllium (Be), Cd, Co, chromium (Cr), cesium (Cs), Cu, Hg, Mn, Ni, Pb, titanium (Ti), uranium (U), vanadium (V), and zinc (Zn) were measured in blood samples, using a Thermo Fisher (Waltham, MA, USA) ICAPRQ inductively coupled plasma mass spectrometry (ICPMS) and CETAC ASX-520 autosampler, as described previously [26]. Standards of known purity and identity were used during the preparation of the calibration, quality control, and internal standards. The ICPMS was calibrated with a blank and a minimum of 4 standards for each element of interest. The calibration curve response versus concentration was evaluated for goodness of fit. All validated analyte correlation coefficients (R) were ≥ 0.995.

2.3 Gestational Age and Preterm Birth Calculation

All the birth outcome data were extracted from medical records. The American Congress of Gynecologists (ACOG) recommendations for best obstetrical estimate to calculate the gestational age for complete pregnancies [47] were used in our study to as previously described [48, 49]. Per common practice, preterm birth (premature labor) was defined as delivery < 37 completed weeks of gestation. Based on the presentation of preterm delivery, preterm birth was further classified as spontaneous preterm birth (presentation of premature rupture of the membranes, spontaneous preterm labor, or both) and non-spontaneous preterm birth (preterm births with preeclampsia or with both artificial membrane rupture and induced labor). We included overall and spontaneous preterm birth as two of the birth outcomes in our analysis.

2.4 Birthweight calculations

Birthweight z-scores (defined as the number of standard deviations by which a birthweight is above or below the mean) are commonly used to compare individual birthweights with the cohort [50, 51]. Gestational age- and sex- specific birthweight z-score were constructed according to the INTERGROWTH-21st standards [52]. Small for gestational age (SGA) births were defined as below the 10th percentile of birthweight z-scores. Large for gestational age (LGA) births were defined as above the 90th percentile of birthweight z-scores.

2.5 Data pre-processing for statistical analyses

Biomarker concentrations below the limit of detection (LOD) were replaced by LOD/√2 (LOD). For statistical analysis, we included metal(loid)s with at least 70% of samples having concentrations above the LOD as continuous variables, and metal(loid)s with less than 70% of samples above the LOD (As and Cd) as binary variables (above vs below LOD). Metals with low detection rate (<30%) were excluded from the analyses. Descriptive statistics were calculated for all exposure and outcome variables. Log-transformed t-test was performed to compare the maternal metal concentrations between preterm and term births.

2.6 Single-Pollutant Models

Logistic regression models were used to examine the associations between metal exposure and binary adverse birth outcomes, including preterm birth (overall and spontaneous preterm birth), SGA, and LGA. As SGA and LGA may have similar complications, SGA models excluded LGA births, and LGA models excluded SGA births. Multiple linear regression was used to model metal exposures with continuous outcomes, gestational age and birthweight z-score. All outcomes are regressed on the geometric averages of participant concentrations across the two visits (when missing concentrations at one visit, the "average" concentration was equal to the single available concentration), with separate models for each exposure biomarker. Metal concentrations were natural log-transformed as they had right skewed distributions.

The crude models only included the geometric average blood metal concentration. The final set of covariates were selected in a stepwise procedure if they altered the beta coefficient of metal exposure by 10% or more. The covariates considered were maternal age, insurance type, maternal education level (an indicator of socioeconomic status), marital status, employment status, gravidity, pre-pregnancy BMI, smoking, exposure to second-hand smoking and alcohol consumption. The final models were controlled for maternal age, maternal education level, pre-pregnancy BMI, and exposure to second-hand smoking.

To assess potential windows of vulnerability in pregnancy, we fit separate multiple linear regression models for each visit using visit-specific metal concentrations. In another analysis, we divided average metal concentrations into tertiles to examine the potential for non-linear relationships. For non-essential metals, effect estimates were calculated for each of the top two tertiles in comparison to the lowest tertiles of exposure. For essential metals, effect estimates were calculated for the highest and lowest tertiles in comparison to the middle tertile of exposure [26]. Finally, to understand whether the effect estimates for metals on birth outcomes differed according to infant sex, all previously mentioned single-pollutant models were refitted with the addition of an interaction term between infant sex and metal concentration, and the interaction term coefficient was tested for significance.

The results were presented as change in days of gestational age and birthweight z-score (95% confidence intervals), and odds ratio of preterm birth, SGA and LGA (95% confidence intervals), per interquartile range (IQR) increase in metal concentrations. The alpha level was set at 0.05. We also considered significance after adjusting for multiple testing using the Benjamini-Hochberg method [53]. Since birth outcomes were correlated, we calculated *q* values (adjusted *p* values) treating each outcome as a family of tests (10 tests per outcome). A cutoff of 0.1 for *q* value was used to further interpret main results with greater confidence.

2.7 Mixture Analysis

In addition to analyzing each metal separately, we explored the effect of the metal mixture on birth outcomes with two approaches.

2.7.1 Elastic Net (ENET) and Metal Risk Score

In the first method, we constructed a metal risk score --Environmental Risk Score (ERS). An ERS is conceptualized as a weighted summary measure of the effects of multiple exposures where the weights are regression coefficients derived from a model of the association between chemical mixtures and the outcome of interest. We utilized elastic net (ENET) to identify the important metals that were driving the association with birth outcomes and to construct the ERS [54]. ENET

is a regularized regression method that combines the penalties of the least absolute shrinkage and selection operator (LASSO) and ridge regression [55]. The objective function for a continuous outcome can be expressed as:

$$\hat{\beta}_{ENET} = \underset{(\beta_0,\beta)\in\mathbb{R}^{p+1}}{\arg\min} \frac{1}{2n}\sum_{i=1}^{n}(y_i - \beta_0 - x_i^T\beta)^2 + \lambda\left(\frac{(1-\alpha)}{2}\|\beta\|_2^2 + \alpha\|\beta\|_1\right)$$

where $i = 1, \ldots, n$ indexes the subjects, $x_i^T \in \mathbb{R}^p$ is the vector of p covariates for the i-th subject, and y_i is the continuous health outcome for the i-th subject. ENET utilizes two tuning parameters (λ, α). Intuitively, $\lambda \in [0, \infty)$ controls the overall strength of the shrinkage while $\alpha \in [0,1]$ controls the tradeoff between automatic variable selection (L1 penalty) and stabilization of the solution path in the presence of collinear exposures (L2 penalty) [56]. Therefore, ENET is generally considered a useful penalized regression approach for variable selection in the presence of highly collinear predictor variables [54].

We fit ENET on all the metals (IQR standardized) in relation to each birth outcome of interest adjusted for the same covariates from the single-pollutant analysis (retained in the model, not subject to shrinkage). Tuning parameters were selected using 10-fold cross-validation. ERS was computed as a weighted sum of the selected non-zero predictor coefficients from each model. We further categorized ERS by tertiles and refit the regression models to examine the associations between categorical ERS and birth outcomes and compared results to those from individual tertile models.

2.7.2 Bayesian kernel machine regression (BKMR)

The second approach for conducting mixture analysis was Bayesian kernel machine regression (BKMR) [57], which enabled us to evaluate the joint effect of multiple metals, interactions between metals, and potential non-linear relationships between metals and outcomes of interest [58]. Because exposures in our study are correlated, we implemented BKMR with hierarchical variable selection (10,000 iterations by a Markov Chain Monte Carlo (MCMC) algorithm). This approach requires grouping of exposures based on correlations between exposures and similar

potential mechanisms of action (e.g. toxic metals vs essential metals). Therefore, we grouped As, Cd, Hg, and Pb into group 1 (toxic metals), Co and Mn into group 2 (correlated essential metals), and Cs, Cu, Ni, and Zn (essential metals) into group 3. Posterior inclusion probabilities (PIP) were extracted from each BKMR model, which provides a measure of variable importance for each exposure group (groupPIP) and how each exposure in that group is driving that group-outcome association (condPIP). To determine the importance of each group/exposure for each study outcome a threshold of PIP>0.5 was used [59, 60]. Data were analyzed using R version 3.6.2 and SAS 9.4 (SAS Institute Inc., Cary, NC)

3. Results

3.1 Descriptive statistics

Demographic characteristics of 812 women in our analysis were described previously [46, 48] and summarized in **Table III.1**. The mean age of participants was 26.7 and nearly half of the women had a BMI less than 25kg/m^2 prior to pregnancy. Approximately two-thirds of the women in our study had private insurance providers and were employed. 30% had reported graduating from college or higher. Nearly half of them had annual household incomes less than $30,000. 80% of the women never smoked while very few (6.8%) reported consumption of alcohol within the last few months. Mean gestational age was 38.8 (SD=2.1) weeks for 812 singleton births included in this analysis, among which 80 (10%) were preterm and 48 (6%) were spontaneous preterm; the rates of SGA and LGA were both 9%.

Descriptive statistics and Spearman correlations between different metals were previously reported elsewhere [61]. Briefly, 1) all metals were detected in the majority of samples, with the exception of As (49% > LOD) and Cd (61% > LOD) and Ba, Be, Cr, Ti, U, and V, which were detected in very few samples (<30%) (**Table III.2**); 2) mean concentrations of Cu, Mn, Pb, and Zn were higher for preterm birth cases compared to other births. 3) there were weak to moderate correlations between different blood metal concentrations (Mn and Co, r=0.36; As and Hg, r=0.32; Cd and Co, r=0.33, SI Figure S1); 4) the first visit had lower concentrations of Cd, Co, Cu, Mn, and Zn, and higher concentrations for Cs, when comparing to the third visit; and 5) Cu, Zn, Pb, Mn, and Hg

presented good to excellent reliability in repeated blood samples with intraclass correlation coefficients (ICCs) ranging from 0.54-0.78.

3.2 Single-pollutant Metal Analyses

The full models included 731 women who had complete data on the four demographic covariates (age, maternal education, pre-pregnancy BMI, and second-hand smoking). **Table III.3** presents the associations between average metal concentrations and birth outcomes, while **Figure III.1** and **Table III.4** show the visit specific associations.

Average Pb concentration was strongly associated with gestational age, for which an IQR increase was associated with 2 days (95% CI=-3.1, -0.5; *q* value=0.09) shorter gestational age; the effect estimates did not differ by study visit. Average Zn was also suggestively associated with decreased gestational age (∆/IQR= -0.7, 95% CI= -1.5, 0.2) and the association with third visit Zn remained significant, after stratification of the results by study visit (**Figure III.1** and **Table III.4**). No significant relationships were observed between birthweight z-score and average metal concentrations (data not shown). However, as shown in Figure 1, when stratified on study visit, third visit Co, Cs, Ni concentrations were significantly positively associated with birthweight z-score, with an increase of 0.14 (95% CI=0.03, 0.25), 0.14 (95% CI= 0.00, 0.28), and 0.11 (95% CI= 0.01, 0.21) in birthweight z-score per IQR increase in the metal concentrations, respectively (**Table III.4**).

In line with results from gestational age analysis, average Pb, Mn, and Zn concentrations were associated with elevated odds of preterm birth (both overall and spontaneous), with OR ranging from 1.32 to 1.83 per IQR increase in metal concentration (**Table III.3**). In the stratified analysis, Pb and Zn were associated with increased odds of spontaneous preterm birth at only visit 1 (Pb: OR/IQR= 1.75, 95% CI: 1.12, 2.73; Zn: OR/IQR= 2.04, 95% CI= 1.23, 3.39). In a sensitivity analysis where metal concentrations were entered in models as tertiles, the change in gestational age and odds of having a preterm birth was only significant among the highest tertile for Pb and Mn (**Figure III.2** and **Table III.5**).

Though geometric mean average models for Hg did not find any association with birth outcomes, Hg was associated with 1.5- and 2.3-fold increased odds of overall and spontaneous preterm birth at visit 3 (overall: OR/IQR= 1.46, 95% CI= 0.97, 2.19; spontaneous: OR/IQR= 2.30, 95% CI= 1.32, 4.02), respectively (**Figure III.1**); interestingly, this association appeared stronger when comparing women in lower two tertiles of exposure rather than higher two tertiles (**Figure III.2** and **Table III.5**). For SGA, Ni concentration was associated with decreased OR, although only significant when comparing the highest tertile to the middle tertile (OR/IQR= 0. 33, 95% CI= 0.16, 0.66). Visit specific analysis also revealed that higher Mn concentration at the third visit was associated with decreased odds of SGA (OR/IQR= 0.62, 95% CI= 0.42, 0.93). No metal concentrations were associated with LGA in average and visit stratified models (**Table III.4**).

After correcting for multiple testing, the associations of both average and first visit Pb and Zn with overall preterm birth, third visit Hg with spontaneous preterm birth, as well as the association of average Ni with SGA had *q*-values < 0.1 (**Table III.3**, **Figure III.1**), providing greater confidence in these associations.

3.3 Mixture analyses

Table III.6 shows the variable selection results from ENET models. The estimated weights (regression coefficients) presented in **Table III.6** are from models where metal concentrations were log transformed and IQR standardized. Preterm birth (overall) models had more than one metal with non-zero weights; Pb ($\beta = 0.057$, OR=1.06) and Zn ($\beta = 0.011$, OR=1.01) were selected as important predictors and all other metals were shrunk to zero. Therefore, we constructed ERS using estimated weights for Pb and Zn and regressed preterm birth by this score. The OR for preterm birth comparing the highest vs. the lowest tertiles of ERS was 2.13 (95% CI= 1.12, 5.49, *p*=0.02) (**Figure III.2**). In the BKMR hierarchical variable selection models for preterm birth, all three metal groups had posterior inclusion probabilities higher than 0.5 and the important metals selected from the groups included Zn (condPIP=0.83), Pb (condPIP=0.68), and Mn (condPIP=0.60) (**Table III.7)**. In a secondary analysis, we ran BKMR models regressing preterm

birth while only including Mn, Zn, Pb to explore the potential non-linearity and interaction between the predictors. The single metal-response curves in **Figure III.3 A** show that 1) Pb and Zn had a positive linear relationship with preterm birth at higher levels (the confidence intervals at lower distributions are wide due to sparse data); 2) the overall trend for Mn was also positive and generally linear. Further, the associations between each metal and preterm birth did not differ by varying quantiles of the other two metals, indicating a lack of interaction between different metals (**Figure III.3 B**).

3.4 Sex interaction

When interactions between infant sex and metal concentrations were added to single-pollutant models, the interaction terms were not statistically significant, except for the associations between Zn and gestational age (*p* value=0.01), and Cu and LGA (*p* value=0.03). Stratified analysis by infant sex showed that the effect of Zn on gestational age was only significant among female infants (*p* value=0.006) but not male infants (*p* value=0.62); one IQR increase in Zn was associated with 3 days (95% CI= -5.2, -0.9) shorter gestational age among women who delivered female infants. **Figure III.4** shows the interaction effect of infant sex on the association between the average Zn concentration and gestational age. The impact of Cu on LGA also varied by infant sex, where odds of LGA were reduced among female infants (OR/IQR= 0.62, 95% CI= 0.39, 0.99, *p* value=0.04). However, differences in associations between metals and birth outcomes by sex were not observed when we conducted the mixture analyses stratified by infant sex. Only Pb was identified as the important predictor of preterm birth/gestational age from ENET and BKMR models, among both female and male infants. The results from tertile analyses stratified by infant sex and study visit were similar to the main tertile analyses results we reported; sex-specific interactions were not observed.

4. Discussion

In this study, we evaluated the individual effects of prenatal essential and non-essential metal(loid) exposure on adverse birth outcomes among a Puerto Rico population. Our analyses demonstrated that maternal blood concentrations of Pb, Zn, Mn, and Hg were associated with shorter gestational

age and higher odds of preterm birth, while Ni was associated with higher birthweight and lower odds of SGA. Some associations were observed only when considering exposure at specific prenatal timepoints, which may reflect windows of exposure vulnerability. Additionally, we estimated the cumulative effect of metal mixtures using ENET and BKMR. ENET identified Pb and Zn as the most important predictors of preterm birth, while BMKR selected Pb, Zn, and Mn as most predictive of preterm birth. Findings from our study highlight that several metals are associated with adverse birth outcomes and stress the importance of assessing the effects of chemical mixtures on health outcomes, using multiple statistical methods and in comparison with single-pollutant models.

A few studies previously reported associations between prenatal metal mixtures and birth outcomes. Signes-Pastor et al. reported that Pb, Mn, and As combined were associated with reduced head circumference, weight, and length of newborns [39], Lee et al. found that the joint effects of Pb and Hg were related to birthweight reduction [42], and Luo et al. confirmed a negative association between Cd and As and birthweight [41]. In our study, negative effects were consistently observed for Pb in combination with other metals. Pregnant women in this Puerto Rico cohort had particularly low blood Pb concentrations (GM=0.33 μg/dL) when comparing across other studies of pregnant women, including studies conducted in Australia (median= 0.37 μg/dL)[62], Japan (GM=0.64 μg/dL) [63], Ohio, US (GM=0.7 μg/dL) [64], Norway (two studies: median=2.5 μg/dL and GM=0.75 μg/dL) [65, 66], South Africa (two studies: median=1.4 μg/dL and median= 2.3 μg/dL) [67, 68] and China (median=3.2 μg/dL) [69]. In addition, all blood samples in our study had Pb concentration lower than the level of concern set by CDC for pregnant women (5 μg/dL) [70]. However, our analysis revealed that maternal blood Pb, even at very low-levels, was the most strongly associated with increased risk of preterm birth and shorter gestational age of all the metals assessed. In recent years, concerns have also been raised that even at low levels, prenatal Pb exposure may pose toxic effects on fetal development [35-37, 71-75]. Our results are consistent with these studies and provide further evidence that blood Pb at low levels, and potentially below current reference levels, may be associated with preterm birth. However, we did not find an association between Pb and birthweight, whereas several previous studies reported

an inverse association with Pb and infant size when explored individually and in combination with other metals [40-42].

Zn was also a key exposure associated with birth outcomes in this study. Blood concentrations of Zn (GM=4682.4 ng/mL) were similar to levels reported in previous studies of pregnant women [66, 68, 76]. We found that associations between Zn and birth outcomes varied by infant sex, such that blood Zn was negatively associated with gestational age among female infants, whereas the association was not significant for male infants. It is unclear whether the infant sex-specific association we observed with Zn and gestational age could be indicative of vulnerability for women carrying a female fetus. The comparatively rapid growth of male fetuses may require more Zn compared to females, thus increased Zn may be less likely to produce adverse effects among males. Elevated blood Zn levels may also reflect the state of various processes in the body, including inflammation, oxidative stress and other key functions [77-79] that can play a role in gestation length.

Our negative findings with increased Zn are contrary to animal studies, observational and randomized trials on humans where maternal Zn deficiency is often associated with adverse birth outcomes, including preterm birth [80, 81]. Because one of many important biological functions of Zn is in the development and function of cells involved in the immune system, it is hypothesized that Zn deficiency may contribute to maternal or intrauterine infection and therefore affect premature birth [82]. Some reviews on the topic have suggested that the association observed between Zn deficiency and adverse birth outcomes could result from poor nutrition [82-85].

Mn is also an essential nutrient that plays a vital role in the body but can be toxic at excessive levels. In this population, Mn concentrations in blood (GM=11.3 ng/mL) were higher than those seen in Australian (GM=6.5 ng/mL) [76] and Norwegian pregnant women (GM=10.7ng/mL) [66]. We found that average Mn concentrations across pregnancy were associated with elevated odds of preterm birth and shorter gestational age; the association was significant for higher levels of blood Mn in the tertile analysis, indicating a potential threshold effect. BKMR graphs (Figure 3A) also

showed a generally linear relationship between Mn and preterm birth at higher levels. These findings are supported by reports on high dose Mn-related maternal and fetal toxicities [86-89] and the U-shaped Mn dose-response curve [29, 33] observed in animal studies and epidemiologic studies.

Mechanistically, the group of metals explored in our study likely impact various biological pathways associated with preterm delivery and fetal development. One leading hypothesis of the mechanism of action is through inducing oxidative stress, defined as the homeostatic imbalance between reactive oxygen species (ROS) formation and antioxidants [90]. Several in vivo and in vitro studies have linked metal toxicity to the formation of ROS [91, 92]. The excessive free radical species can induce oxidative stress and cause damage to lipids, proteins and DNA in the placental tissue that eventually lead to pregnancy complications [90, 93, 94]. Reproductive hormones also play an important role in maintaining pregnancy; in turn, disruption of the complex interplay between hormones may lead to adverse effects during gestation. A number of metals are reproductive toxicants and suspected endocrine disruptors [95-98]. Evidence suggests that metals can influence reproductive hormone levels through several pathways, including hormone synthesis, regulation, transport and metabolism, and/or interference with receptors [99-106], with potential implications for pregnancy outcomes.

Our approaches for analyzing the effects of combined metal exposures provide evidence that Pb, Zn, and Mn are likely key exposures during pregnancy contributing to adverse birth outcomes. The weak correlations between the three metals are not likely to reflect common sources of exposure. Although BKMR analysis did not suggest interaction between the three metals, future studies constructing mechanistically based exposure mixtures are needed.

Our study is the first to assess the impact of metals on birth outcomes among pregnant women in Puerto Rico. The PROTECT study, a large prospective longitudinal cohort study in Puerto Rico, provided an opportunity to study the relationships between environmental pollutants and adverse birth outcomes in an at-risk population. The innovative study design allows for repeated capture

of biological samples to account for the varying levels of exposures during pregnancy. Few epidemiology studies have evaluated metal exposures collectively in relation to birth outcomes, giving the proposed study a unique opportunity to test the impact of more realistic exposure profiles on birth outcomes.

The present study does have some limitations. The metal levels measured in blood depict circulating levels, which may not reflect levels in the uterine and fetal compartments that may be more biologically relevant. However, blood biomarkers may be indicative at least in part of the activity at the maternal-fetal interface, and collection of blood is much more feasible than placenta tissue or fluid samples from the uterus during pregnancy. We used the same data to calculate ERS and then to fit ERS models which has the potential for overfitting. Metal risk score needs to be validated in an independent cohort before being used as a prognostic tool by other studies. Another challenge with constructing ERS is that the ENET models assume a linear relationship between metals and the birth outcomes, and does not capture potential non-linear relationships and interactions between metals which maybe important when considering essential metals that may be toxic at high levels of exposure. However, BKMR analysis does allow for the possibility of nonlinearity and interactions between metals. There are also other statistical strategies for incorporating nonlinearity and interactions into constructing ERS that should be explored in future applications of this method [54, 107-109].

5. Conclusion

We considered different statistical methods to examine the effect of 10 toxic and essential trace metal(loid)s and metal risk score on various birth outcomes among pregnant women in Northern Puerto Rico. Although the PROTECT cohort has lower Pb concentrations (GM=0.33 μg/dL) compared to the mainland US and other studies of pregnant women in different countries, our findings suggest that low-level prenatal Pb exposure, as well as elevated Mn and Zn exposure, may adversely affect birth outcomes. These findings provide further support for the need to reduce Pb exposure as much as possible among pregnant women. Improved understanding of environmental and other factors that contribute to preterm birth, together with developing sustainable

technologies to remove contamination, will have a direct public health impact in Puerto Rico.

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Table III.1 Demographic characteristics of n = 812 pregnant women from Puerto Rico (2011− 2017)

Variable	**Mean (SD)**	
Maternal Age	26.7 (5.7)	
Characteristic	**Category**	**Count (Percent)**
Insurance Type	Private	478 (58.9%)
	Public (mi salud)	304 (37.4%)
	Missing	30 (3.7%)
Maternal Education	<=High school/GED	175 (21.6%)
	Some college or technical school	284 (35%)
	College degree	246 (30.3%)
	Master's degree or higher	95 (11.7%)
	Missing	12 (1.5%)
Household Income	<$10,000	226 (27.8%)
	≥$10,000 to <$30,000	230 (28.3%)
	≥$30,000 to <$50,000	156 (19.2%)
	≥$50,000	93 (11.5%)
	Missing	107 (13.2%)
Marital Status	Single	162 (20%)
	Married or living together	641 (78.9%)
	Missing	9 (1.1%)
Gravidity (# Pregnancies)	0	296 (36.5%)
	1	307 (37.8%)
	>1	98 (12.1%)
	Missing	111 (13.7%)
Pre-pregnancy BMI (kg/m^2)	≤25	413 (50.9%)
	>25 to ≤30	211 (26%)
	>30	145 (17.9%)
	Missing	43 (5.3%)
Infant Gender	Female	382 (47%)
	Male	426 (52.5%)
	Missing	4 (0.5%)
Employment Status	Unemployed	489 (60.2%)
	Employed	310 (38.2%)
	Missing	13 (1.6%)
Smoking	Never	678 (83.5%)
	Ever	115 (14.2%)
	Current	11 (1.4%)
	Missing	8 (1%)
Exposure to Second-hand Smoking	None	696 (85.7%)
	Up to 1 hour	25 (3.1%)
	More than 1 hour	51 (6.3%)
	Missing	40 (4.9%)
Alcohol Consumption	None	388 (47.8%)
	Before pregnancy	355 (43.7%)
	Within the last few months	57 (7%)
	Missing	12 (1.5%)

Table III.2 Blood biomarker concentrations (µg/L) in N = 812 pregnant women from Puerto Rico[a] in 2010− 2017 (stratified by preterm birth status) and comparison with U.S. population-based samples of women ages 18− 40 from NHANES[b]

Metals	LOD	%>LOD	Preterm Birth (n=112)			Term Birth (n=1034)			NHANES		
			GM	GSD	Median	GM	GSD	Median	GM	GSD	Median
Co	0.2	98.3	0.35	1.4	0.34	0.34	1.4	0.34			
Cs	0.04	99.9	1.2	1.4	1.2	1.1	1.4	1.2			
Cu*	9	99.9	1623	1.2	1620	1544	1.3	1556			
Mn*	2	99.9	11.9	1.4	12.0	11.2	1.4	11.2	10.7	1.4	10.6
Ni	0.5	96.4	1.0	1.7	1.0	1.0	1.6	1.0			
Zn*	24	99.9	5004	1.1	5030	4641	1.3	4712			
As	0.3	49.3	0.32	1.70	0.21	0.34	1.82	0.21			
Cd	0.1	60.8	0.11	1.61	0.11	0.12	1.66	0.12	0.31	2.2	0.28
Hg	0.2	99.9	1.3	1.7	1.3	1.2	1.7	1.2	0.74	2.5	0.67
Pb[c]*	0.02	99.9	0.39	1.6	0.36	0.32	1.5	0.32	0.64	1.8	0.61

Abbreviations: National Health and Nutrition Examination Survey (NHANES); cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); limit of detection (LOD); geometric mean (GM); geometric standard deviation (GSD).

[a] Includes biomarker concentrations for up to 2 repeated samples per woman (n = 1,146 samples)

[b] Females 18−40 years of age; n = 3,585 for biomarkers measured in 2009-2010, 2011-2012, 2013-2014, and 2015-2016 NHANES

[c] concentration unit for blood Pb is µg/dL

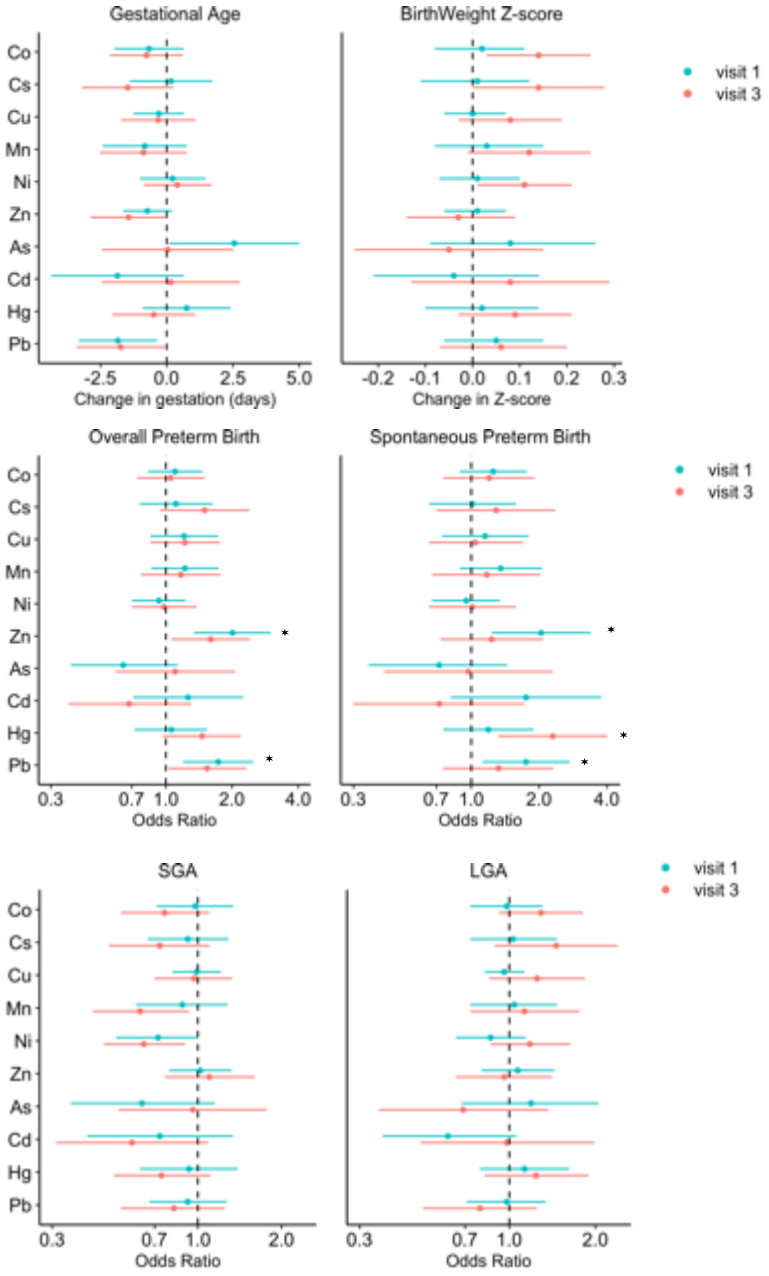
* metals with significantly higher concentration among women in the preterm birth subgroup compared to the term birth subgroup (log-transformed t-test was performed).

Table III.3 Change in gestational age, preterm birth, and SGA associated with average exposure biomarker concentration across two time points during pregnancy. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

Metals	**Gestational age (N=731)**		**Preterm Birth (overall, N=731)**		**Preterm Birth (spontaneous, N=700)**		**SGA (N=637)**	
	Change in days	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Co	**-1.1 (-2.3, 0.1)**	**0.06**	1.21 (0.93, 1.56)	0.16	**1.33 (0.96, 1.84)**	**0.09**	0.90 (0.68, 1.21)	0.49
Cs	-0.3 (-1.6, 1.1)	0.69	1.19 (0.84, 1.68)	0.33	1.10 (0.72, 1.69)	0.66	0.82 (0.62, 1.08)	0.15
Cu	-0.4 (-1.2, 0.4)	0.36	**1.32 (0.98, 1.78)**	**0.07**	1.20 (0.82, 1.76)	0.34	0.99 (0.83, 1.19)	0.94
Mn	-1.1 (-2.4, 0.3)	0.12	**1.32 (0.96, 1.80)**	**0.08**	**1.45 (0.98, 2.15)**	**0.07**	0.85 (0.62, 1.16)	0.30
Ni	0.8 (-0.3, 1.9)	0.15	0.85 (0.65, 1.11)	0.24	0.85 (0.60, 1.21)	0.36	**0.67 (0.49, 0.90)**	**0.01***
Zn	-0.7 (-1.5, 0.2)	0.11	**1.83 (1.28, 2.60)**	**0.001***	**1.53 (0.99, 2.38)**	**0.06**	1.00 (0.82, 1.21)	1.00
As[a]	1.8 (-0.4, 3.9)	0.10	0.72 (0.44, 1.18)	0.19	0.65 (0.34, 1.24)	0.19	0.75 (0.45, 1.25)	0.28
Cd[a]	-1.3 (-3.5, 0.9)	0.26	1.00 (0.60, 1.65)	0.99	1.24 (0.63, 2.43)	0.53	0.76 (0.45, 1.27)	0.29
Hg	0.8 (-0.6, 2.2)	0.27	1.05 (0.75, 1.48)	0.76	1.27 (0.82, 1.95)	0.28	0.86 (0.62, 1.21)	0.40
Pb	**-1.8 (-3.1, -0.5)**	**0.009***	**1.63 (1.17, 2.28)**	**0.004***	**1.53 (1.00, 2.35)**	**0.05**	0.91 (0.69, 1.20)	0.51

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); Odds ratio (OR); confidence interval (CI); interquartile range (IQR); small for gestation age (SGA).
[a] As, Cd were compared between two categories of above LOD and below LOD
**q* value (false discovery rate) <0.1

Figure III.1 Change in birth outcomes associated with visit specific exposure biomarker concentration. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentrationa. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking



Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); Odds ratio (OR); confidence interval (CI); interquartile range (IQR); small for gestation age (SGA); large for gestational age (LGA).

[a] As, Cd were compared between two categories of above LOD and below LOD

**q* value (false discovery rate) <0.1

Table III.4 Change in birth outcomes associated with exposure biomarker concentration at each visit during pregnancy. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

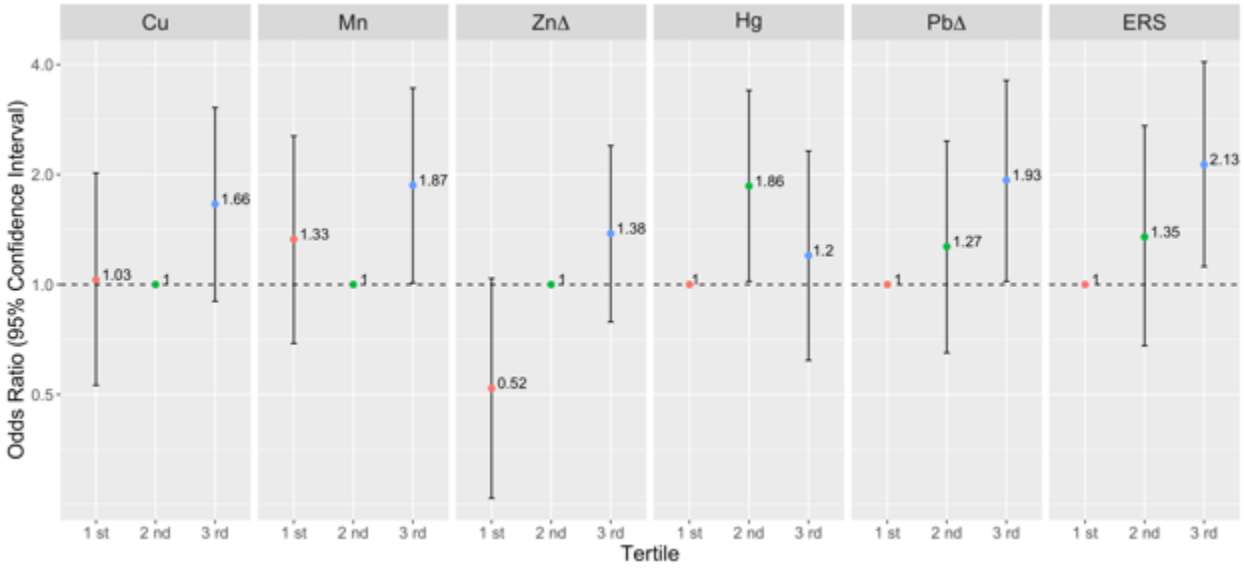
Metals	**Gestational age**				**Birthweight z-score**			
	Visit 1 (n=583)		**Visit 3 (n=453)**		**Visit 1 (n=566)**		**Visit 3 (n=442)**	
	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	Change in z-score (95% CI)	*p* value	Change in z-score (95% CI)	*p* value
Co	-0.7 (-2.0, 0.6)	0.32	-0.8 (-2.1, 0.6)	0.28	0.02 (-0.08, 0.11)	0.75	**0.14 (0.03, 0.25)**	**0.01**
Cs	0.2 (-1.4, 1.7)	0.84	-1.5 (-3.2, 0.3)	0.10	0.01 (-0.11, 0.12)	0.92	**0.14 (0.00, 0.28)**	**0.05**
Cu	-0.3 (-1.3, 0.7)	0.54	-0.3 (-1.7, 1.1)	0.66	0.00 (-0.06, 0.07)	0.91	0.08 (-0.03, 0.19)	0.14
Mn	-0.8 (-2.4, 0.8)	0.31	-0.9 (-2.5, 0.8)	0.29	0.03 (-0.08, 0.15)	0.57	0.12 (-0.01, 0.25)	0.08
Ni	0.2 (-1.0, 1.5)	0.72	0.4 (-0.9, 1.7)	0.53	0.01 (-0.07, 0.10)	0.74	**0.11 (0.01, 0.21)**	**0.03**
Zn	-0.7 (-1.7, 0.2)	0.13	**-1.4 (-2.9, 0.0)**	**0.05**	0.01 (-0.06, 0.07)	0.79	-0.03 (-0.14, 0.09)	0.62
As[a]	**2.5 (0.1, 5.0)**	**0.04**	0.0 (-2.4, 2.5)	0.98	0.08 (-0.09, 0.26)	0.35	-0.05 (-0.25, 0.15)	0.61
Cd[a]	-1.9 (-4.4, 0.6)	0.14	0.2 (-2.4, 2.7)	0.91	-0.04 (-0.21, 0.14)	0.70	0.08 (-0.13, 0.29)	0.45
Hg	0.7 (-0.9, 2.4)	0.38	-0.5 (-2.1, 1.1)	0.54	0.02 (-0.10, 0.14)	0.72	0.09 (-0.03, 0.21)	0.16
Pb	**-1.8 (-3.3, -0.4)**	**0.01**	**-1.7 (-3.4, -0.1)**	**0.04**	0.05 (-0.06, 0.15)	0.40	0.06 (-0.07, 0.20)	0.36
	Preterm birth (overall)				**Preterm birth (spontaneous)**			
	Visit 1 (n=583)		**Visit 3 (n=453)**		**Visit 1 (n=561)**		**Visit 3 (n=431)**	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Co	1.10 (0.83, 1.47)	0.50	1.05 (0.74, 1.50)	0.77	1.25 (0.89, 1.76)	0.20	1.20 (0.75, 1.91)	0.46
Cs	1.11 (0.76, 1.63)	0.59	1.50 (0.94, 2.40)	0.09	1.01 (0.65, 1.58)	0.96	1.29 (0.70, 2.36)	0.41
Cu	1.21 (0.85, 1.73)	0.30	1.22 (0.85, 1.76)	0.28	1.15 (0.74, 1.79)	0.52	1.04 (0.65, 1.69)	0.86
Mn	1.22 (0.86, 1.74)	0.26	1.17 (0.77, 1.77)	0.46	1.35 (0.89, 2.06)	0.16	1.17 (0.67, 2.02)	0.58
Ni	0.93 (0.70, 1.23)	0.59	0.98 (0.70, 1.38)	0.91	0.95 (0.67, 1.34)	0.77	1.01 (0.65, 1.58)	0.95
Zn	**2.01 (1.34, 3.00)**	**0.001***	**1.6 0(1.06, 2.41)**	**0.02**	**2.04 (1.23, 3.39)**	**0.01***	1.23 (0.73, 2.08)	0.44
As[a]	0.64 (0.37, 1.13)	0.12	1.10 (0.59, 2.07)	0.76	0.72 (0.35, 1.44)	0.35	0.97 (0.41, 2.30)	0.95
Cd[a]	1.26 (0.71, 2.25)	0.43	0.68 (0.36, 1.30)	0.25	1.75 (0.81, 3.77)	0.15	0.72 (0.30, 1.72)	0.45
Hg	1.06 (0.72, 1.54)	0.78	1.46 (0.97, 2.19)	0.07	1.19 (0.75, 1.89)	0.47	**2.3 0(1.32, 4.02)**	**0.003***
Pb	**1.73 (1.20, 2.50)**	**0.004***	**1.54 (1.03, 2.32)**	**0.04**	**1.75 (1.12, 2.73)**	**0.01***	1.32 (0.75, 2.31)	0.33
	SGA				**LGA**			
	Visit 1 (n=505)		**Visit 3 (n=403)**		**Visit 1 (n=517)**		**Visit 3 (n=394)**	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Co	0.98 (0.71, 1.34)	0.88	0.76 (0.53, 1.10)	0.15	0.98 (0.73, 1.31)	0.90	1.29 (0.92, 1.81)	0.15
Cs	0.92 (0.66, 1.29)	0.62	0.73 (0.48, 1.10)	0.13	1.03 (0.73, 1.47)	0.85	1.46 (0.89, 2.39)	0.13
Cu	0.99 (0.81, 1.21)	0.92	0.97 (0.70, 1.33)	0.84	0.96 (0.82, 1.13)	0.61	1.25 (0.85, 1.84)	0.25
Mn	0.88 (0.60, 1.28)	0.49	**0.62 (0.42, 0.93)**	**0.02**	1.04 (0.73, 1.47)	0.85	1.13 (0.73, 1.76)	0.58
Ni	**0.72 (0.51, 1.00)**	**0.05**	**0.64 (0.46, 0.90)**	**0.01**	0.86 (0.65, 1.14)	0.29	1.18 (0.86, 1.63)	0.31
Zn	1.02 (0.79, 1.32)	0.88	1.10 (0.76, 1.60)	0.60	1.07 (0.80, 1.44)	0.65	0.96 (0.65, 1.41)	0.84
As[a]	0.63 (0.35, 1.15)	0.14	0.96 (0.52, 1.77)	0.90	1.19 (0.68, 2.05)	0.54	0.69 (0.35, 1.37)	0.29
Cd[a]	0.73 (0.40, 1.34)	0.31	0.58 (0.31, 1.09)	0.09	0.61 (0.36, 1.06)	0.08	0.98 (0.49, 1.98)	0.96
Hg	0.93 (0.62, 1.39)	0.71	0.74 (0.50, 1.11)	0.14	1.13 (0.79, 1.62)	0.51	1.24 (0.82, 1.89)	0.31
Pb	0.92 (0.67, 1.27)	0.62	0.82 (0.53, 1.25)	0.35	0.98 (0.71, 1.34)	0.89	0.79 (0.50, 1.25)	0.32

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); Odds ratio (OR); confidence interval (CI); interquartile range (IQR); small for gestation age (SGA); large for gestational age (LGA).

[a] As, Cd were compared between two categories of above LOD and below LOD

**q* value (false discovery rate) <0.1

Figure III.2 Preterm birth (overall) odds ratio (95% confidence interval) associated with tertiles of geometric average exposure[ab] Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking



Abbreviations: copper (Cu); manganese (Mn); zinc (Zn); mercury (Hg); lead (Pb); environmental risk score (ERS).
[a] Referent levels were set at tertile 2 for essential metals (Cu, Mn, Zn)
[b] Referent levels were set at tertile 1 for non-essential metals (Hg, Pb), and ERS
Δ Individual metals that were selected from elastic net models to compose ERS

Table III.5 Change in birth outcomes associated with tertiles of average exposure[abc]. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

Metals	Gestational age (N=731)				Birthweight z-score (N=710)			
	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	Change in z-score (95% CI)	*p* value	Change in z-score (95% CI)	*p* value
Essential metals	Tertile 1 vs 2		Tertile 3 vs 2		Tertile 1 vs 2		Tertile 3 vs 2	
Co[a]	-1 (-3.6, 1.6)	0.46	-1.7 (-4.3, 0.9)	0.20	-0.04 (-0.24, 0.15)	0.66	0.03 (-0.17, 0.22)	0.79
Cs[b]	-0.7 (-3.3, 1.9)	0.61	-0.9 (-3.5, 1.8)	0.52	-0.04 (-0.23, 0.16)	0.72	0.08 (-0.12, 0.27)	0.45
Cu[a]	-1.6 (-4.2, 1.1)	0.24	-2.2 (-4.9, 0.5)	0.11	**-0.21 (-0.4, -0.01)**	**0.04**	**-0.23 (-0.43, -0.03)**	**0.02**
Mn[a]	-1.6 (-4.2, 1)	0.22	**-2.7 (-5.4, -0.1)**	**0.04**	0.07 (-0.13, 0.27)	0.49	**0.22 (0.02, 0.41)**	**0.03**
Ni[a]	0.9 (-1.8, 3.5)	0.51	2.2 (-0.4, 4.8)	0.10	-0.03 (-0.23, 0.16)	0.74	0.02 (-0.17, 0.21)	0.84
Zn[a]	1 (-1.6, 3.6)	0.47	-1.1 (-3.8, 1.5)	0.39	-0.01 (-0.2, 0.19)	0.94	-0.05 (-0.24, 0.15)	0.64
Non-essential metals	Tertile 2 vs 1		Tertile 3 vs 1		Tertile 2 vs 1		Tertile 3 vs 1	
Hg[c]	-1.2 (-3.8, 1.4)	0.38	1.4 (-1.3, 4)	0.31	0.17 (-0.02, 0.37)	0.08	0.06 (-0.14, 0.25)	0.58
Pb[c]	-0.2 (-2.9, 2.4)	0.86	**-2.9 (-5.5, -0.2)**	**0.03**	-0.12 (-0.32, 0.07)	0.23	0.09 (-0.11, 0.29)	0.38
Metals	**Preterm birth (overall, N=731)**				**Preterm birth (spontaneous, N=700)**			
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Essential metals	Tertile 1 vs 2		Tertile 3 vs 2		Tertile 1 vs 2		Tertile 3 vs 2	
Co[a]	0.81 (0.44, 1.51)	0.51	1.18 (0.65, 2.12)	0.59	0.59 (0.25, 1.41)	0.24	1.35 (0.65, 2.81)	0.42
Cs[b]	1.06 (0.58, 1.97)	0.84	1.25 (0.67, 2.3)	0.48	1.22 (0.55, 2.7)	0.62	1.19 (0.53, 2.65)	0.68
Cu[a]	1.03 (0.53, 2.02)	0.92	1.66 (0.9, 3.05)	0.11	1.12 (0.48, 2.62)	0.79	1.63 (0.74, 3.59)	0.23
Mn[a]	1.33 (0.69, 2.55)	0.40	**1.87 (1.01, 3.45)**	**0.05**	2.85 (1.07, 7.59)	**0.04**	**3.91 (1.52, 10.03)**	**0.005***
Ni[a]	0.89 (0.5, 1.57)	0.69	**0.49 (0.26, 0.93)**	**0.03**	0.96 (0.47, 1.97)	0.91	**0.4 (0.17, 0.95)**	**0.04**
Zn[a]	0.52 (0.26, 1.04)	0.06	1.38 (0.79, 2.4)	0.26	0.55 (0.24, 1.28)	0.17	1.02 (0.49, 2.11)	0.95
Non-essential metals	Tertile 2 vs 1		Tertile 3 vs 1		Tertile 2 vs 1		Tertile 3 vs 1	
Hg[c]	**1.86 (1.02, 3.4)**	**0.04**	1.2 (0.62, 2.32)	0.60	**3.23 (1.34, 7.78)**	**0.009***	1.9 (0.73, 4.92)	0.19
Pb[c]	1.27 (0.65, 2.47)	0.49	1.93 (1.02, 3.62)	**0.04**	0.69 (0.29, 1.66)	0.41	1.5 (0.71, 3.18)	0.28

Table III.5 Continued

Metals	SGA (N=637)				LGA (N=642)			
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	P value	OR (95% CI)	*p* value
Essential metals	Tertile 1 vs 2		Tertile 3 vs 2		Tertile 1 vs 2		Tertile 3 vs 2	
Co[a]	1.35 (0.72, 2.54)	0.34	1.15 (0.61, 2.19)	0.67	0.69 (0.38, 1.27)	0.23	0.79 (0.44, 1.42)	0.43
Cs[b]	1.5 (0.82, 2.75)	0.18	0.85 (0.44, 1.66)	0.63	1.08 (0.57, 2.03)	0.82	1.31 (0.72, 2.38)	0.37
Cu[a]	**2.13 (1.09, 4.18)**	**0.03**	1.83 (0.91, 3.69)	0.09	1.28 (0.71, 2.32)	0.41	0.81 (0.43, 1.54)	0.53
Mn[a]	0.85 (0.47, 1.54)	0.60	0.56 (0.29, 1.08)	0.08	0.92 (0.5, 1.72)	0.80	1.12 (0.62, 2.02)	0.71
Ni[a]	0.79 (0.45, 1.39)	0.41	**0.33 (0.16, 0.66)**	**0.002***	0.65 (0.35, 1.21)	0.18	0.72 (0.4, 1.28)	0.26
Zn[a]	1.08 (0.58, 2.02)	0.80	1.05 (0.56, 1.97)	0.88	0.72 (0.4, 1.3)	0.28	0.63 (0.34, 1.14)	0.13
Non-essential metals	Tertile 2 vs 1		Tertile 3 vs 1		Tertile 2 vs 1		Tertile 3 vs 1	
Hg[c]	0.63 (0.33, 1.2)	0.16	0.87 (0.48, 1.58)	0.64	1.59 (0.86, 2.93)	0.14	1.2 (0.63, 2.29)	0.58
Pb[c]	1.58 (0.88, 2.83)	0.12	0.62 (0.3, 1.26)	0.18	1.13 (0.63, 2.03)	0.67	0.74 (0.4, 1.4)	0.36

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); Odds ratio (OR); confidence interval (CI); interquartile range (IQR); small for gestation age (SGA); large for gestational age (LGA).

[a] Referent levels were set at tertile 2 for essential metals (Co, Cu, Mn, Ni, Zn).

[b] Cs is not regarded as essential to the health of plants or animals, nor does it present a hazard to them. For this analysis, Cs was considered as essential metal, therefore, referent levels were set at tertile 2.

[c] Referent levels were set at tertile 1 for non-essential metals (Hg, Pb).

**q* value (false discovery rate) <0.1

Table III.6 Estimated environmental risk score (ERS) weights (regression coefficient) for metals selected for each birth outcome. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

	Weights					
Metal	Gestational Age	Birthweight z-score	Preterm birth (overall)	Preterm birth (spontaneous)	SGA	LGA
Co						
Cs						
Cu						
Mn						
Ni						
Zn			0.011			
As						
Cd						
Hg						
Pb	-0.056		0.057	0.102		

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestation age (SGA); large for gestational age (LGA).

Table III.7 Posterior inclusion probabilities (PIPs) for group inclusion and conditional inclusion into birth outcome models, using Bayesian kernel machine regression (BKMR) model. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

Metal	Group	Gestational Age		Birthweight z-score		Preterm Birth (overall)		Preterm Birth (spontaneous)		SGA		LGA	
		groupPIP[a]	condPIP[b]	groupPIP[a]	condPIP[b]	groupPIP[a]	condPIP[b]	groupPIP[a]	condPIP[b]	groupPIP[a]	condPIP[b]	groupPIP[a]	condPIP[b]
As	1	0.64	0.01	0.44	0.24	0.64	0.19	0.57	0.14	0.50	0.29	0.36	0.25
Cd	1	0.64	0.17	0.44	0.23	0.64	0.06	0.57	0.12	0.50	0.30	0.36	0.35
Hg	1	0.64	0.04	0.44	0.27	0.64	0.07	0.57	0.18	0.50	0.16	0.36	0.19
Pb	1	**0.64**	**0.78**	0.44	0.26	**0.64**	**0.68**	**0.57**	**0.56**	0.50	0.25	0.36	0.21
Co	2	0.37	0.79	**0.57**	**0.63**	0.56	0.40	0.68	0.42	0.49	0.51	0.47	0.58
Mn	2	0.37	0.21	0.57	0.37	**0.56**	**0.60**	**0.68**	**0.58**	0.49	0.49	0.47	0.42
Cs	3	0.13	0.01	0.46	0.31	0.94	0.02	0.64	0.14	0.78	0.09	0.43	0.24
Cu	3	0.13	0.06	0.46	0.20	0.94	0.08	0.64	0.13	0.78	0.08	0.43	0.28
Ni	3	0.13	0.70	0.46	0.24	0.94	0.07	0.64	0.27	**0.78**	**0.76**	0.43	0.22
Zn	3	0.13	0.23	0.46	0.25	**0.94**	**0.83**	0.64	0.46	0.78	0.07	0.43	0.26

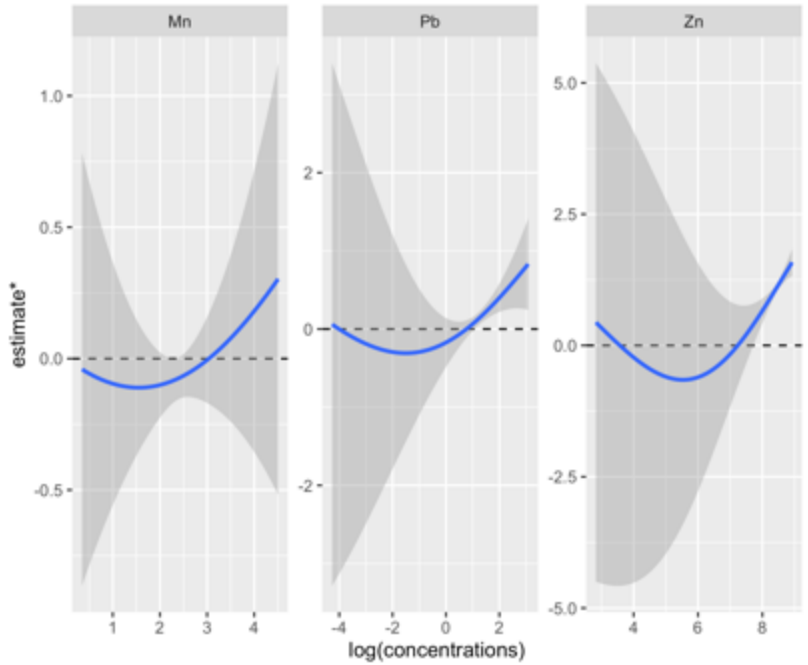
Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestation age (SGA); large for gestational age (LGA); Posterior Inclusion Probabilities (PIPs).

[a] GroupPIP provides group importance scores for pre-defined mutually-exclusive groups of variables

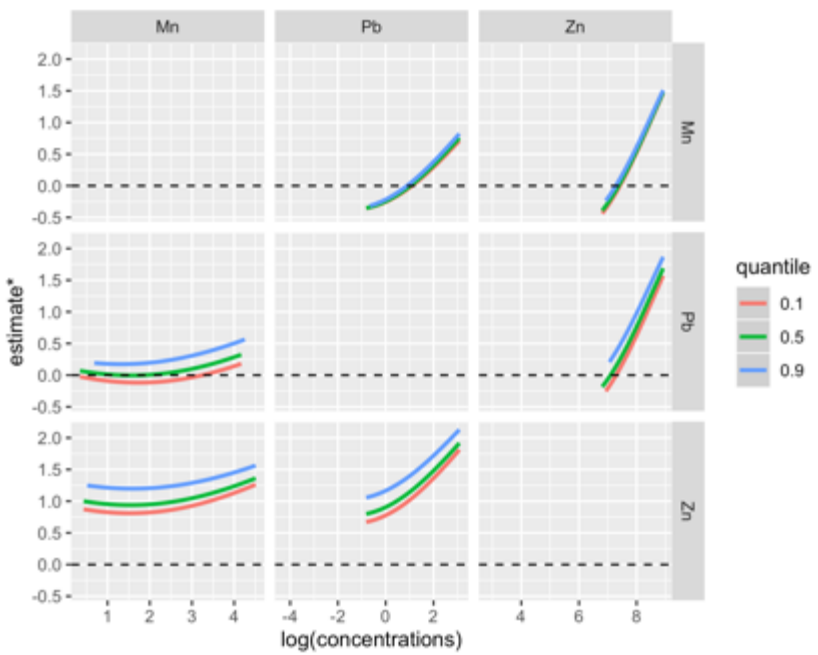
[b] CondPIP (conditional PIPs) estimates the importance of a metal given that the group that contains that metal is important.

Figure III.3 Univariate and bivariate predictor-response function for the effect of metal mixture (Mn, Pb, Zn) on preterm birth estimated by Bayesian Kernal Machine Regression (BKMR)[a]. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

A



B



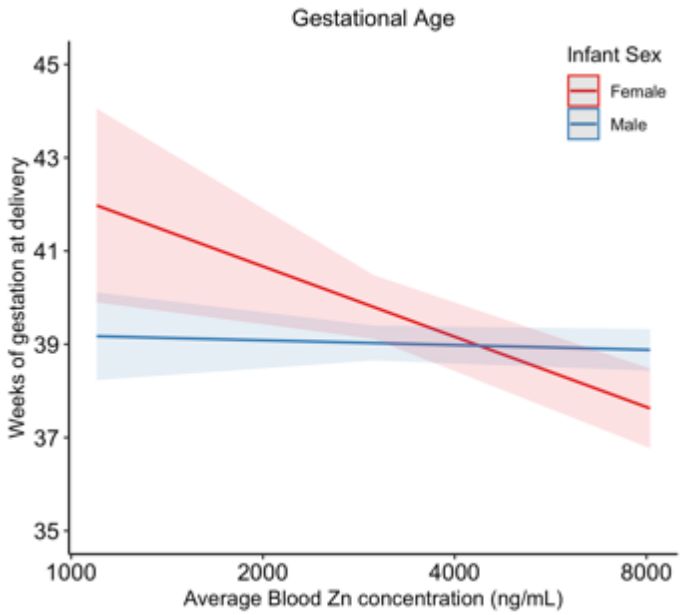
Abbreviations: manganese (Mn); zinc (Zn); lead (Pb);

[a] estimate* may be interpreted as a latent continuous marker of the binary outcome-overall preterm birth

A Univariate exposure–response functions and 95%confidence for each metal with the other pollutants fixed at the median

B Bivariate exposure–response functions for: Mn when Pb is fixed at either the 10th, 50th, or 90th percentile and Zn is fixed at the median (middle left panel); Mn when Zn is fixed at either the 10th, 50th, or 90th percentile and Pb is fixed at the median (bottom left panel); Pb when Mn is fixed at either the 10th, 50th, or 90th percentile and Zn is fixed at the median (top middle panel); Pb when Zn is fixed at either the 10th, 50th, or 90th percentile and Mn is fixed at the median (bottom middle panel); Zn when Mn is fixed at either the 10th, 50th, or 90th percentile and Pb is fixed at the median (top right panel); Zn when Pb is fixed at either the 10th, 50th, or 90th percentile and Mn is fixed at the median (middle right panel);

Figure III.4 Interaction effect of infant sex on the association between the average zinc (Zn) blood concentration and gestational age. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking



Chapter IV

Psychosocial Status Modifies the Effect of Maternal Blood Metal and Metalloid Concentrations on Birth Outcomes

Abstract

Background: Metal exposure and psychosocial stress in pregnancy have each been associated with adverse birth outcomes, including preterm birth and low birth weight, but no study has examined the potential interaction between them. **Objective:** We examined the modifying effect of psychosocial stress on the association between metals and birth outcomes among pregnant women in Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) birth cohort study. **Methods**: In our analysis of 682 women from the PROTECT study, we measured 16 essential and non-essential metals in blood samples at two time points. We administered questionnaires to collect information on depression, perceived stress, social support, and life experience during pregnancy. Using K-means clustering, we categorized pregnant women into one of two groups: "good" and "poor" psychosocial status. We then evaluated whether the effect of blood metals (geometric average) on adverse birth outcomes (gestational age, preterm birth [overall and spontaneous], birth weight z-score, small for gestation [SGA], large for gestation [LGA]) vary between two clusters of women, adjusting for maternal age, maternal education, pre-pregnancy body mass index (BMI), and second-hand smoke exposure. **Results**: Blood manganese (Mn) was associated with an increased odds ratio (OR) of overall preterm birth (OR/interquartile range [IQR] = 2.76, 95% confidence interval [CI] = 1.25, 6.12) and spontaneous preterm birth (OR/IQR: 3.68, 95% CI: 1.20, 6.57) only among women with "poor" psychosocial status. The association between copper (Cu) and SGA was also statistically significant only among women having "poor" psychosocial status (OR/IQR: 2.81, 95% CI: 1.20, 6.57). We also observed associations between nickel (Ni) and preterm birth and SGA that were modified by psychosocial status during pregnancy. **Conclusions**: Presence of "poor" psychosocial status intensified the adverse associations between Mn and preterm birth, Cu and SGA, and protective effects of Ni on

preterm. This provides evidence that prenatal psychosocial stress may modify vulnerability to metal exposure.

1. Introduction

Metals are ubiquitous in the environment, and they can enter the human body through ingestion of food and water, dietary supplement intake, contact with contaminated environments, and use of metal-containing products through inhalation, skin contact, and/or inadvertent ingestion [1-6]. Exposure to metals impacts various biological pathways that contribute to adverse birth outcome, including preterm delivery and low birthweight [1, 7-20]. Our recent analysis in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort study also suggested associations between elevated levels of maternal essential and non-essential metals and various birth outcomes [21]. We found a decrease in gestational length and increased odds of preterm birth in association with higher maternal blood lead (Pb) concentration; increased odds of preterm birth in association with blood copper (Cu), manganese (Mn), and zinc (Zn) concentrations; and that blood nickel (Ni) was associated with lower odds of small for gestational age (SGA). Prenatal psychosocial stress has also been found to be associated with an increased risk of adverse pregnancy outcomes—psychological distress, perceived stress, anxiety, depression symptoms, and low social support among pregnant women were associated with an increased risk of pre-eclampsia [22], preterm birth [23-28], and low birth weight [23, 27].

The majority of epidemiologic studies evaluate the impact of individual chemical and non-chemical exposures. However, pregnant women are exposed to both environmental chemicals and psychosocial stress, and psychosocial factors may influence how a particular environmental chemical is experienced or what the physical response to it may be. Recently, there has been a general acknowledgment that there is likely to be joint effects of environmental chemicals (e.g. phthalates, black carbon, lead [Pb]) and psychosocial stress exposure on pregnancy and child development outcomes [29-37]. The identification of modifiable psychosocial factors may lead to interventions during pregnancy to reduce the harmful effects of metals on birth outcomes. Therefore, the current study aimed to examine whether the psychosocial status of the mothers during pregnancy modifies the effects of metals on gestational length/preterm delivery and birthweight among pregnant women in Puerto Rico. We hypothesized that there would be stronger

associations between metal exposure and adverse birth outcomes in pregnant women with poorer psychosocial status compared to mothers with better psychosocial status.

2. Methods

2.1 Study population

This study used data collected from 682 pregnant women participating in the PROTECT study, an ongoing, prospective birth cohort [38-41]. The PROTECT study was launched in 2010 with funding from the National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program to investigate Puerto Rico's high preterm birth rate and the extent of hazardous waste contamination on the island. PROTECT aims to explore environmental exposures and other factors contributing to preterm birth risk and other adverse birth outcomes in Puerto Rico.

Study participants were recruited at approximately 14 ± 2 weeks of gestation at seven prenatal clinics and hospitals throughout Northern Puerto Rico and followed until delivery. [38, 39]. Inclusion criteria for this study were: maternal age between 18 to 40 years; residence inside of the Northern Karst aquifer region; planning to deliver in the collaborating hospitals. Exclusion of participants included the use of oral contraceptives within the three months prior to pregnancy; use of *in vitro* fertilization to become pregnant; or any major medical or obstetrical complications, including pre-existing diabetes. Each woman participated in a total of up to three study visits (18 ± 2 weeks, 22 ± 2 weeks, and 26 ± 2 weeks of gestation). Detailed information on medical and pregnancy history was collected at the initial visit. During an in-home visit (second visit), nurse-administered questionnaires were used to gather information on housing characteristics, employment status, and family situation. Questionnaires assessing the psychosocial status of study participants were administered at the second and third visits. Blood samples were collected during the first and third visits. The present analysis reflects 682 women who delivered a live singleton birth with measured metal(loid) concentrations in maternal blood and information on demographics and psychosocial variables available (**Figure IV.1**).

The research protocol was approved by the Ethics and Research Committees of the University of Puerto Rico and participating clinics, the University of Michigan, Northeastern University, and the

University of Georgia. The study was described in detail to all participants, and informed consent was obtained prior to study enrollment.

2.2 Measurement of metals

Blood samples were collected in metal-free whole blood tubes. Whole blood samples were frozen and stored at -80°C and shipped on dry ice. The analysis was performed at NSF International (Ann Arbor, MI, USA), where concentrations of 16 metals and metalloids were measured in blood: arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), cesium (Cs), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), Pb, titanium (Ti), uranium (U), vanadium (V), and zinc (Zn). Metal(loid) concentrations were measured using inductively coupled plasma mass spectrometry (ICPMS) as described previously [11]. Considering that biological samples have high levels of carbon and chloride in the matrix, the laboratory selected the appropriate isotopes for the requested elements to best avoid interferences where possible. The ICPMS was calibrated with a blank and a minimum of four standards for each element of interest. An R^2 value of >0.995 was the minimum criteria for an acceptable calibration curve. The calibration curves were verified by initial checks at three calibration points within the curve. Continuing calibration checks and blanks after every 10 samples were also utilized throughout the analytical run to ensure the ICPMS system was maintaining acceptable performance.

2.3 Birth outcomes

All birth outcome data were extracted from medical records. The American Congress of Gynecologists (ACOG) recommendations for gestational age at birth calculations [42] were used in our study as previously described [43, 44]. As per common practice, preterm birth was defined as delivery < 37 completed weeks of gestation. Based on the clinical presentation of preterm delivery, preterm birth was further classified as spontaneous preterm birth (presentation of premature rupture of the membranes, spontaneous preterm labor, or both) and non-spontaneous preterm birth (preterm births with preeclampsia, or with both artificial membrane rupture and induced labor). We included overall and spontaneous preterm birth as two of the birth outcomes in our analysis.

Birthweight z-scores (defined as the number of standard deviations by which a birthweight is above or below the mean) are commonly used to compare individual birthweights with the cohort

[45, 46]. Gestational age- and sex-specific birthweight z-score were constructed according to the INTERGROWTH-21st standards [47]. Small for gestational age (SGA) births were defined as below the 10th percentile of birthweight z-scores. Large for gestational age (LGA) births were defined as above the 90th percentile of birthweight z-scores.

2.4 Psychosocial variables and life events

Four questionnaires were administered in PROTECT to assess the psychosocial status of study participants [48-50]. They include (1) Center for Epidemiological Studies-Depression (CES-D), a 20-item score that measures depression symptoms according to the Diagnostic Statistical Manual-IV[51]; (2) Perceived Stress Scale (PSS) [52], a 10-item score that aims to determine the participants' perceived stress levels within the last month; (3) Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Social Support Instrument (ESSI), a 7-item score measuring functional social support [53, 54]; (4) Life Experiences Survey (LES) a 39-item score which provides information on positive and negative life events that the participants experienced since becoming pregnant [55]. The CES-D, PSS, and ESSI surveys were administered at the third visit in the clinic and the LES questionnaires were completed at the second visit at home; All questionnaires were translated and administered in Spanish. Previous studies among large and diverse samples of Hispanics/Latinos have evaluated the reliability and validity of the scales and recommended their use in Spanish among Hispanics/Latinos [56-58].

Responses from the four questionnaires were summed to create separate continuous measures of the four scales, depression (CES-D: range 0–48), perceived stress (PSS: range 0–40), social support (ESSI: range 0–34), and life events (LES: range -39 to 39). If the response to any individual question within a questionnaire was missing, the corresponding scale was coded as missing for that individual. The higher scores for CES-D and PSS corresponded to higher depression and stress level for all scales, whereas lower ESSI indicated low social support (i.e., high stress). A positive LES score represented the occurrence of relatively more positive events and vice versa.

2.5 Data pre-processing for statistical analyses

Metal concentrations below the limit of detection (LOD) were replaced by LOD/$\sqrt{2}$. For statistical analysis, we included metal(loid)s with at least 70% of samples having concentrations above the

LOD as continuous variables, and metal(loid)s with less than 70% of samples above the LOD (As and Cd) as binary variables (above vs below LOD). Metals with low detection rate (<30%), Ba, Be, Cr, Ti, U, and V were excluded from the analyses. Descriptive statistics were calculated for all exposure, modifier, and outcome variables. Distributions of all metals measured in blood were right-skewed and thus, were natural log-transformed for all analyses.

2.6 K-means Clustering

The four psychosocial scales we measured are correlated [48] and yet each reflects a unique aspect of the psychosocial well-being and together collectively represents a mothers' overall well-being. Therefore, in our main analysis, instead of assessing the modifying effect of each scale separately, we evaluated them simultaneously by grouping women based on their overall psychosocial well-being attributable to each scale. We used K-means clustering with the input of scores from the four psychosocial scales to identify subgroups of PROTECT participants with different overall measurements of psychosocial well-being. K-means clustering is one of the most commonly used unsupervised machine learning algorithms which allow us to split the dataset into k groups such that the observations in the same cluster are more similar than observations from different clusters [59]. Each cluster is represented by its center which corresponds to the mean of points assigned to the cluster. All four scales were standardized to make variables comparable. The number of optimal clusters (k) was determined based on (1) Elbow method [60] (2) average silhouette method [61], and (3) gap statistics method [62].

2.7 Main Analysis

In our previous work, we examined the associations between each birth outcome and each average metal exposure biomarker [21]. Logistic regression models were used to examine the associations between metal exposure and binary adverse birth outcomes, including preterm birth (overall and spontaneous preterm birth), SGA, and LGA, whereas multiple linear regression was used to model metal exposures with the continuous outcomes gestational age and birthweight z-score. In this analysis, we constructed separate regression models (n=k) for each association with an interaction term of (exposure biomarker* cluster indicator variable) to determine the effects of metal exposure on birth outcomes. We considered *interaction p* <0.1 as statistically significant. All outcomes were regressed on the geometric averages of participant concentrations across the two visits (when

missing concentrations at one visit, the "average" concentration was equal to the single available concentration), with separate models for each exposure biomarker. In the interaction models, the effect estimates of the covariates are still assessed using the whole dataset when a metal's effect is estimated within each cluster. The covariate selection process was described previously [21]. Briefly, a pool of potential confounders was selected based on *a priori* knowledge and the final set of covariates were selected in a stepwise procedure if their inclusion appreciably changed the beta coefficient of metal exposure. The final models were controlled for maternal age, maternal education level, pre-pregnancy BMI, and exposure to second-hand smoking.

In an effort to detect potential non-linear relationships between metals and birth outcomes within different clusters, we used generalized additive models (GAM) to graphically depict the metal and birth outcome associations within each cluster, adjusting for the same covariates.

2.8 Sensitivity Analysis

We ran additional linear models including income categories as an indicator of socio-economic status (SES) instead of maternal education categories. Another analysis was performed excluding women with reported maternal complications, preeclampsia (n=22) and gestational diabetes (n=14). To test whether the interactions between metal biomarkers and psychosocial clusters varied by study visits, we utilized indicator variables for study visits and included a three-way interaction term of (exposure biomarker* cluster indicator*study visit indicator) in the main models substituting the original two-way interaction. The interaction terms were tested for significance and the visit-cluster-specific metal effect estimates were also abstracted from the models. Finally, to further explore the contributions of individual psychosocial scales on effect modification, each of the psychosocial scale variables was evaluated separately as potential modifiers of the associations between metals and birth outcomes. For this analysis, binary psychosocial scale variables were created for CES-D, PSS, and ESSI score using cutoffs based on a priori knowledge and considering cluster balance. A score of 16 was used for CES-D as a cutoff (>=16 vs <16) as it is typically used to determine depression [63]. As there is no established cut-off for PSS, we set the cut-off for PSS at the 75th percentile (score of 18, maximum score= 39) [48]. Participants with scores >=18 were considered to have high perceived stress. Similarly, a cut off of 31 (25th percentile) allowed us to differentiate women with higher social support (>=31)

from women with lower social support (<31). The total LES score was categorized into three groups (labeled ‘‘negative”, ‘‘neutral” and ‘‘positive”) to consider the overall negative and positive scores. The cut-off points were below -1, between -1 and 1, and above 1 [48].

All results were presented as changes in birth outcomes (95% confidence intervals per interquartile range (IQR) increase in metal concentrations. We also considered significance after adjusting for multiple testing. In order to be able to identify as many significant comparisons as possible while still maintaining a low false positive rate, False Discovery Rate (FDR) and its analog the *q* value are utilized implementing the Benjamini-Hochberg method [64]. Q value (adjusted *p* value) is the expected proportion of false positives among all features as or more extreme than the observed one. Since birth outcomes were correlated, we calculated *q* values treating each outcome as a family of tests. A cutoff of 0.1 for *q* value was used to further interpret the main results with greater confidence. Data were analyzed using R version 3.6.2 [65] and the clustering calculation was performed by using the R package “cluster” [66].

3. Results

3.1 Descriptive statistics

The demographic characteristics of 682 women in this analysis are summarized in **Table IV.1** and were described previously [41, 67]. Participants had a mean age of 27 years with approximately half of the women having a BMI less than 25kg/m^2 prior to pregnancy. The majority of women (62%) had private medical insurance and were employed (61%). Nearly half of them had annual household incomes less than $30,000 while 88% reported graduating from college or higher. The prevalence of current smokers was very low (1%). Very few (7%) of the women reported consumption of alcohol within the last few months. Mean gestational age was 38.9 (standard deviation=1.9) weeks for 682 singleton births included in this analysis, among which 61 (9%) were preterm and 36 (5%) were spontaneous preterm; the rates of SGA and LGA were both 10%.

Descriptive statistics (geometric mean [GM], geometric standard deviation [GSD], select percentiles) of blood metals were summarized in **Table IV.2** and were previously described in detail [6]. Levels of most metals in pregnant Puerto Rican women were higher than levels observed in nonpregnant women aged 18–40 years in the general U.S. population, except for Hg and Pb

[6]. There were weak to moderate correlations between different blood metal concentrations (Mn and Co, r=0.36; As and Hg, r=0.32; Cd and Co, r=0.33). Distributions of the ESSI, PSS, CES-D, and LES scales across demographic characteristics were included in **Table IV.2** and were described in detail previously [50]. Overall, most psychosocial variables were associated with lower SES indicators, such as unemployment, lower income, and lower education.

3.2 Clustering

The optimal number of clusters was two (k=2) for the k- means clustering analysis based on the elbow method, average silhouette method, and gap statistics method. Therefore, women in this study were divided into two groups, labeled as having "good" psychosocial status (N=526) and "poor" psychosocial status (N=156). **Figure IV.2** shows the mean of each standardized log-transformed score of depression (CES-D), perceived stress (PSS), social support (ESSI), and life events (LES). Note that women in the "good" psychosocial status cluster had a lower standardized mean score (<0) for depression and perceived stress and higher mean score (>0) for social support and life events, whereas women in the "poor" psychosocial status cluster had a higher mean score (>0) for depression and perceived stress and lower mean score (<0) for social support and life events.

We also calculated the geometric mean and geometric standard deviation of blood metal (loid) concentrations (ng/ml) among women within "good" and "poor" psychosocial status clusters and tested whether the metal concentrations vary between the two clusters (**Table IV.3**). There were no differences, except for Pb, where the concentrations were statistically higher in the "poor" psychosocial cluster compared to the "good" psychosocial cluster (p <0.001).

3.3 Main Analysis

Figure IV.3 presents the associations between blood metal concentrations and birth outcomes within "good" and "poor" psychosocial status clusters. The effect estimates, confidence intervals, and p values are also given in **Table IV.4**. Co was associated with shorter gestational age among both clusters of mothers having "good" and "poor" psychosocial status and the effect estimates did not vary between the two clusters (p for interaction =0.17). We found a statistically significant interaction between maternal blood Mn concentration and psychosocial status on gestational age

(*p* for interaction=0.01). Within the "poor" psychosocial status cluster, an interquartile range (IQR) increase in blood Mn concentration was associated with 5 days shorter gestational age (95% CI: -7.2, -1.9, $p<0.001$) whereas the association was not significant among mothers with "good" psychosocial status. After correcting for multiple testing, the associations between blood Mn and gestational age among "poor" psychosocial status cluster, as well as the interaction had *q*-values < 0.1 (**Figure IV.3**, **Table IV.4**), providing greater confidence in these associations. Similar interactions were observed for overall (*p* for interaction=0.05) and spontaneous preterm birth (*p* for interaction=0.09); there were strong associations between blood Mn and significantly increased odds of overall preterm birth (OR/IQR= 2.76, 95%CI: 1.25, 6.12, $p=0.01$, $q=0.06$) and spontaneous preterm birth (OR/IQR= 3.68, 95%CI: 1.17, 11.61, $p=0.03$, $q>0.1$) only among mothers classified with "poor" psychosocial status. Ni was associated with 0.43 and 0.33 times lower odds of preterm birth (95%CI=0.22, 0.81, $p=0.01$, $q=0.06$) and spontaneous preterm birth (95%CI=0.13, 0.83, $p=0.02$, $q>0.1$) among mothers with "poor" psychosocial status which was statistically different (*p* for interaction=0.01 and 0.02, respectively) compared to the null associations among mothers classified in the "good" psychosocial status cluster.

Although associations between blood Pb and blood Zn and preterm birth/gestational age were statistically significant among mothers having "good" psychosocial status but not significant within the "poor" psychosocial status cluster, the interaction terms for the differences were not statistically significant. Cu was significantly associated with higher odds of SGA (OR/IQR= 2.81, 95%CI=1.20, 6.57, $p=0.02$) among mothers classified in the "poor" psychosocial status cluster; however, the association was not found among mothers having "good" psychosocial status and this difference was statistically significant (*p* for interaction =0.01). An IQR increase in Ni was associated with 0.55 times lower odds of SGA only among mothers with "good" psychosocial status ($p<0.001$, $q=0.02$) and this protective effect was significantly different from the null effect observed within the "poor" psychosocial status cluster (*p* for interaction =0.04). Associations between the metal biomarkers and birthweight z-score and LGA were not statistically significant within either cluster. Results from GAM including metal concentrations as splines and the GAM output graphics showed that when the smoothing estimator is significant the observed associations within two clusters are linear (estimated degree of freedom=1), after adjusting for covariates (e.g.

Figure IV.4: Relationship between blood Mn concentration and preterm birth within "good" and "poor" psychosocial status clusters.)

3.4 Sensitivity Analysis

The results from additional analysis including income as a covariate were similar to the main analysis results we reported (**Table IV.5**). Excluding women with conditions such as preeclampsia and gestational diabetes also yielded similar results (data not shown). The binary birth outcome (preterm birth, SGA, LGA) models including interactions between metal concentrations, psychosocial cluster, and study visit failed to converge due to small sample size in each stratum. The results of models evaluating associations between metal concentrations and gestational age (continuous) by study visit and psychosocial clusters are presented in **Table IV.6** (Birthweight z-score models did not yield significant results, data not shown). The direction of the cluster-specific associations within each study visit remained the same. The interactions between metal concentrations and psychosocial cluster did not statistically vary between two study visits (three-way interaction *p* values>0.1). Results from additional analyses evaluating individual psychosocial scales as potential effect modifiers were included in **Table IV.7**, **IV.8**, **IV.9**, and **IV.10**. There were inverse interactions between Mn and depression score (*p* for interaction [gestational age] =0.05), perceived stress (*p* for interaction [overall preterm] =0.09) social support (*p* for interaction [gestational age, overall and spontaneous preterm birth] =0.09, 0.01, and 0.03). Mn concentration was associated with shortened gestation and higher odds of preterm birth only among women who had higher perceived stress and higher depression score, and lower social support.

4. Discussion

This study uses data collected in the PROTECT birth cohort to examine the modifying effect of overall psychosocial status on the relationships between blood concentrations of essential and non-essential metals and birth outcomes. We found increased odds of preterm birth (overall and spontaneous) associated with higher blood Mn concentration only among women with "poor" psychosocial status. Higher prenatal Cu was associated with increased odds of SGA among women concurrently at "poor" psychosocial status. Conversely, higher Ni was found to be associated with lower odds of preterm birth among women with "poor" psychosocial status, but lower odds of SGA among women having "good" psychosocial status.

Previous studies have suggested that elevated levels of essential metals Mn, Cu, and Ni may be associated with increased risk of intrauterine growth restriction [68], preterm delivery [11, 12, 69], and low birthweight [13, 14]. It is also well established that maternal psychosocial stress during pregnancy is associated with adverse birth outcomes [22-28]. Moreover, a growing body of recent epidemiological studies has reported the modifying effect of prenatal psychosocial stress on the associations between environmental chemical exposure, including metals (Pb, Cd, Mn, and Chromium [Cr]), and pregnancy outcomes [48, 70-75], and childhood developmental outcomes [32, 76-81].

Psychosocial stress scales were not associated with any birth outcomes in this cohort [48] or a prospective cohort of US women (TIDES study) that used similar psychosocial scales [82]. Therefore, it is unlikely that the modifying effects are attributable to mediation through psychosocial stress. However, it is possible that double hits could lead to a joint effect; a form of "double jeopardy" has been used to describe this potential interaction of environmental chemicals and stress [83, 84]. Chemical and psychosocial exposure may both interfere with complex mechanisms that impair individual resistance and capability to recover when there is more than one "hit" and eventually lead to exacerbation of physiological response to the cumulative "hits" [84-86].

Several important biological pathways have also been hypothesized for the interaction between environmental chemicals and psychosocial stress on the association with pregnancy outcomes. Oxidative stress and inflammation, two interrelated biological pathways, have generated attention as they are impacted by both prenatal metal exposure and the psychosocial status of the mother. One important finding we showed here in PROTECT is that associations between Mn and preterm birth were statistically significant and greater in magnitude among women with "poor" psychosocial status (OR/IQR=2.76, 95%CI=1.25, 6.12, p=0.01) but not among women with "good" psychosocial status (OR/IQR=1.12, 95%CI=0.75, 1.68, p=0.57, p for interaction=0.01). Cu was also associated with higher odds of SGA only among women with "poor" psychosocial status (OR/IQR= 2.81, 95%CI=1.20, 6.57, p=0.02, p for interaction=0.01). Although both Mn and Cu play an important role in many aspects of human physiology [87, 88], animal and human studies

found a relationship between elevated Mn and Cu levels and biomarkers of oxidative stress and inflammation [89-99]. Being at a "poor" psychosocial status during pregnancy may induce similar oxidative stress and inflammatory immune reactions to that of exposure to metals [100]. It is possible that oxidative stress induced by psychosocial factors can result in vulnerability at the mother fetus interface to metals, such as Mn and Cu. However, psychosocial variables have not been significantly associated with increased markers of oxidative stress in this cohort [49] but have in other cohorts [86].

The interactive effect of metal and psychosocial stress on birth outcomes may also be working through modulating fetal hypothalamic–pituitary–adrenal (HPA) activities [101]. A previous study on this cohort examining the relationship between maternal metal and hormone concentrations reported a strong positive association between Mn and corticotropin-releasing hormone (CRH) [102]. CRH is produced by the hypothalamus and placenta and has an important role in setting the biological clock of pregnancy duration. CRH is also produced by the hypothalamus in response to maternal stress to stimulate the production of cortisol, which plays a fundamental role during pregnancy and fetal development. Thus, it may be possible that the combined effect of an increase in CRH via Mn as well as an increase in cortisol via psychosocial stress caused an increase in allostatic load, leading to a significant decrease in gestational length. This hypothesis for metals in general is also supported by evidence from animal and human studies showing changes in glucocorticoid hormones (i.e., cortisol) by metals [103, 104] and psychosocial stress [105, 106], or both in unison [107, 108]. Several epigenome-wide association studies have linked neurotoxic metals, including Mn, to increased placental glucocorticoid receptors (majority on NR3C1) methylation [109-111]. Likewise, prenatal stress has been reported to contribute to the epigenetic alteration of the same receptors, both independently [112-114] as well as when combined with metal exposure [115, 116]. Taken together, epigenetic mechanisms may explain the interaction between metal and psychosocial stress on the disruption of fetal HPA axis functioning, which may result in adverse birth outcomes. Nevertheless, our results suggest that the prenatal mechanisms by which changes in gestational age and fetal growth are impacted by the co-exposure to metals and psychosocial stress need to be further explored.

It is worth noting that, contrary to our hypothesis, we observed protective modifying effects of being classified as having "poor" psychosocial status on the association between maternal blood Ni and preterm birth. The mechanism underlying this result is unclear as the health effects associated with prenatal Ni are sparsely investigated in the literature. While higher levels of blood Ni are generally reflective of women's exposure to Ni in air, water, or food [117], women can be occupationally exposed to Ni from certain workplaces/occupations, such as manufacturing of jewelry, medical devices, and stainless steel [118]. Although our previous research within PROTECT did not find significant and specific predictors or sources of the Ni exposure in this population [6], it is possible that there are unmeasured confounders and/or sources of Ni exposure among this population that were driving the results. Our findings on Ni may also be a chance finding warranting the need for future research to either support or refute this observation.

Blood Pb levels were significantly higher among women within the "poor" psychosocial status cluster compared to the "good" psychosocial status cluster. It is possible that Pb levels may influence the association of other metals in this study. Metals like Pb and Mn share transporters and targets in the body [119] and there are multiple mechanisms and pathways through which Pb and Mn may interact [120]. Therefore, the differential impacts of Mn in the two psychosocial clusters observed in this study may be attributed in part to possible additive interaction and effect modification between the Pb and Mn on gestational length. Our sample sizes were limited to detect such interactions, future studies are needed to consider the framework of metal dyshomeostasis and look for patterns in metal fluctuations across studies.

Perceived stress, life events, social support, and depression are highly interlinked in many populations [121, 122] including PROTECT [50]. Studies have reported an increase in the rate of depression with higher levels of perceived stress and stressful events [123, 124], and others have reported a protective effect of social support on depression [125, 126]. However, potential bidirectional pairwise associations are likely among these four psychosocial variables. For example, whereas some studies examined social support as a mediator between stress and maternal depression [127], others examined the mediating effect of stress on social support and maternal depression [128]. The first study reported a mediating and moderating effect of social support on stressful events and postpartum depressive symptoms in a Hong Kong study of 2,365 women

[127]. The second study reported a mediating effect of perceived stress in a longitudinal study on 1,316 U.S. women, such that lower levels of social support led to higher levels of perceived stress and higher rates of postpartum depression. Additional well-designed studies are needed to disentangle these relationships and the causal framework involved.

In this study, we had data on a total of four psychosocial scales that allowed us to capture different aspects of psychosocial well-being. We utilized a clustering method on the four psychosocial scales to divide women into two groups of having "poor" and "good" psychosocial status during pregnancy. Using a novel example of dimension reduction on the effect of modifier space, we then evaluated the modifying effect of this overall psychosocial status on the association between metals and pregnancy instead of examining each scale separately. This study is also highlighted by the quantification of interaction between metal exposure and psychosocial stress and an extensive panel of blood metal biomarkers that assessed essential metals, such as Cu, which have not been studied with non-chemical exposures in detail to date.

While our study is among the first to explore the interaction between prenatal metal exposure and psychosocial stress during pregnancy, there were a few limitations. Measurements in this study, including metal biomarkers, psychosocial variable scores, and covariates (i.e. pre-pregnancy BMI) may be affected by measurement error. Considering the potential for non-differential measurement error in exposures, covariates, and outcome variables, the effects were likely to be attenuated towards the null. Nonetheless, the repeated collection of blood samples enabled us to examine metal biomarkers at two time points across pregnancy to more fully characterize prenatal metals exposure. Repeated exposure measurements help reduce measurement error and provide greater statistical power, relative to studies with single time point measurements. Although we adjusted for a variety of covariates, possible residual or additional unmeasured confounders of metal exposure and/or psychosocial variables may be unaccounted for in our analysis. In addition, the small sample size in the "poor" psychosocial status cluster limited the assessment power of effects estimates in the cluster, therefore, future studies are needed to validate our findings. While this work studied the effects of multiple metals, other metals that are not explored in this study such as iron (Fe) may also interact with these metals because Fe-deficiency increases divalent metal transporters 1 (DMT1) that is responsible for the transport of Pb, Mn, and Cu [129]. Similarly,

other environmental exposures, including phthalates and PAHs, were not explored. Future work to investigate the interaction between a more extensive range of metals and multiple chemical mixtures and psychosocial stress is needed. Finally, as psychosocial variable distributions are bound to material and social factors in the countries/regions being studied, they may vary from this cohort to others. Therefore, our results may not be generalizable to the overall U.S. pregnant population or pregnant women populations in other countries.

5. Conclusion

We examined the interaction between environmental metals and maternal psychosocial status on birth outcomes among pregnant women in Northern Puerto Rico. We observed associations between Mn and increased odds of preterm birth (overall and spontaneous), Ni and decreased odds of preterm birth, and Cu and increased odds SGA, that were modified by whether a mother was at a "poor" psychosocial status during pregnancy. Our findings provide evidence for the modifying role of psychosocial status on the effect of prenatal metal exposure among pregnant women and further suggest prenatal stress and social support could be modifiable psychosocial assets that may help mitigate risk. This study also highlights the need for future research in this area to examine the effects of co-exposure to both environmental and psychosocial conditions, particularly during sensitive developmental stages.

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Figure IV.1 PROTECT cohort study design and sample size

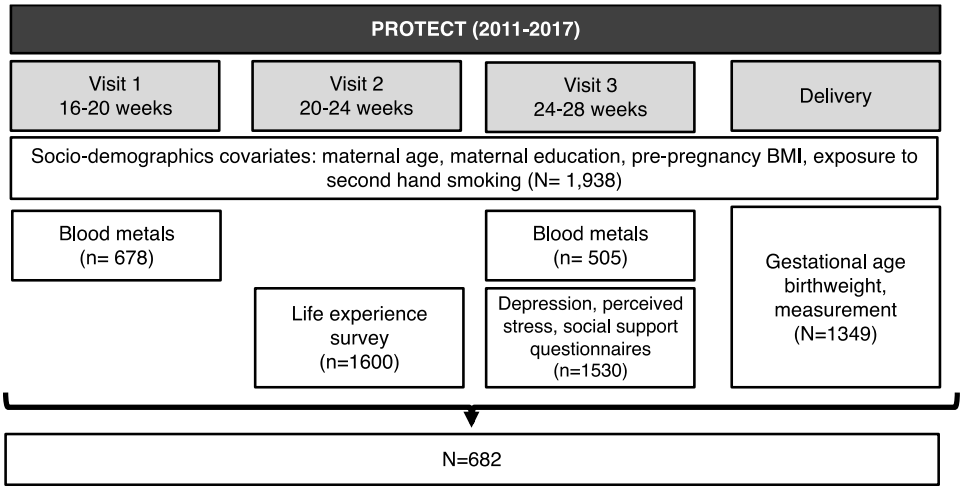


Table IV.1 Demographic characteristics of n = 682 pregnant women from Puerto Rico

Variable	**Mean (SD)**	
Maternal Age	26.8 (5.6)	
Characteristic	**Category**	**Count (Percent)**
Insurance Type	Private	425 (62.3%)
	Public (mi salud)	238 (34.9%)
	Missing	19 (2.8%)
Maternal Education	<=High school/GED	143 (21.0%)
	Some college or technical school	238 (34.9%)
	College degree	214 (31.4%)
	Master's degree or higher	87 (12.8%)
	Missing	0 (0.0%)
Household Income	<$10,000	185 (27.1%)
	≥$10,000 to <$30,000	191 (28.0%)
	≥$30,000 to <$50,000	141 (20.7%)
	≥$50,000	85 (12.5%)
	Missing	80 (11.7%)
Marital Status	Single	136 (19.9%)
	Married or living together	544 (79.8%)
	Missing	2 (0.3%)
Gravidity (# Pregnancies)	0	289 (42.4%)
	1	249 (36.5%)
	>1	143 (21.0%)
	Missing	1 (0.1%)
Pre-pregnancy BMI (kg/m^2)	≤25	372 (54.5%)
	>25 to ≤30	183 (26.8%)
	>30	127 (18.6%)
	Missing	0 (0.0%)
Infant Gender	Female	328 (48.1%)
	Male	352 (51.6%)
	Missing	2 (0.3%)
Employment Status	Unemployed	417 (61.1%)
	Employed	259 (38.0%)
	Missing	0 (0.0%)
Smoking	Never	581 (85.2%)
	Ever	93 (13.6%)
	Current	8 (1.2%)
	Missing	0 (0.0%)
Exposure to Second-hand Smoking	None	623 (91.3%)
	Up to 1 hour	18 (2.6%)
	More than 1 hour	41 (6.0%)
	Missing	0 (0.0%)
Alcohol Consumption	None	331(48.5%)
	Before pregnancy	299 (43.8%)
	Within the last few months	48 (7.0%)
	Missing	4 (0.6%)

Table IV.2 Blood concentrations of metal(loid)s (ng/ml) and psychosocial variables in 682 pregnant women from Puerto Rico

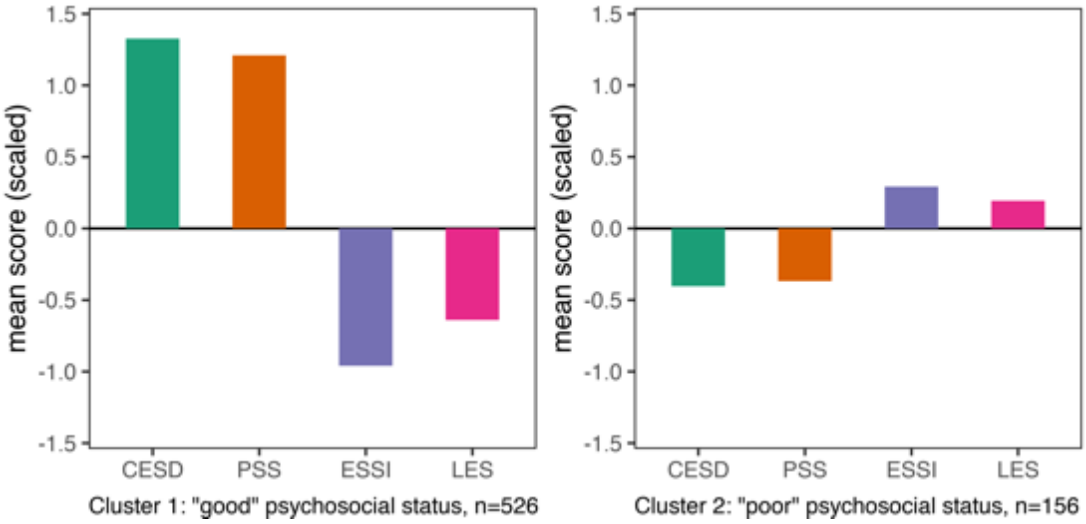
Metal (loid)[a]	LOD	% >LOD	GM	GSD	25%	50%	75%	95%	Max
Co	0.2	98.2	0.35	1.3	0.29	0.34	0.41	0.55	1.3
Cs	0.04	99.9	1.1	1.3	0.9	1.1	1.4	1.9	2.9
Cu	9	99.9	1592	1.2	1428	1616	1779	2133	3798
Mn	2	99.9	11.2	1.4	9.0	11.2	13.9	19.0	34.9
Ni	0.5	96.4	1.0	1.6	0.77	1.0	1.3	2.2	16.7
Zn	24	99.9	4736	1.2	4248	4780	5314	6269	8043
As	0.3	48.9	0.32	1.7	<LOD	<LOD	0.45	1.0	3.0
Cd	0.1	60.9	0.12	1.6	<LOD	0.11	0.16	0.27	0.9
Hg	0.2	99.9	1.2	1.7	0.85	1.2	1.7	3.1	5.4
Pb[b]	0.02	99.9	3.1	1.5	2.3	3.1	4.1	6.5	15.1
Psychosocial stress scales		**Range**	**Mean**	**Min**	**25%**	**50%**	**75%**		**Max**
CESD		0-48	11	0	5	9	15		48
PSS		0-40	14	0	8	13	18		39
ESSI		0-34	32	8	31	33	34		34
LES		-39-39	0	-35	-2	0	2		19

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); limit of detection (LOD); geometric mean (GM); geometric standard deviation (GSD); Center for Epidemiological Studies-Depression (CESD); Perceived Stress Scale (PSS); Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Social Support Instrument (ESSI); Life Experience Survey (LES).

[a] Includes blood metal concentrations for up to 2 repeated samples per woman (n = 1035 samples);

[b] Pb concentration unit is µg/dL

Figure IV.2 Cluster means of the four standardized psychosocial variable scales using k-means in the PROTECT data[ab]



Abbreviations: Center for Epidemiological Studies-Depression (CESD); Perceived Stress Scale (PSS); Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Social Support Instrument (ESSI); Life Experience Survey (LES).

[a] Y-axis (cluster means) represents the mean standardized psychosocial variable scale

[b] Cluster 1: "good" overall psychosocial status pattern with low depression score and perceived stress and high social support and overall positive life events; cluster 2: "poor" overall psychosocial status pattern with high depression score and perceived stress and low social support and overall negative life events

Table IV.3 Geometric mean and geometric standard deviation of blood metal (loid) concentrations (ng/ml) among women within "good" and "poor" psychosocial status clusters

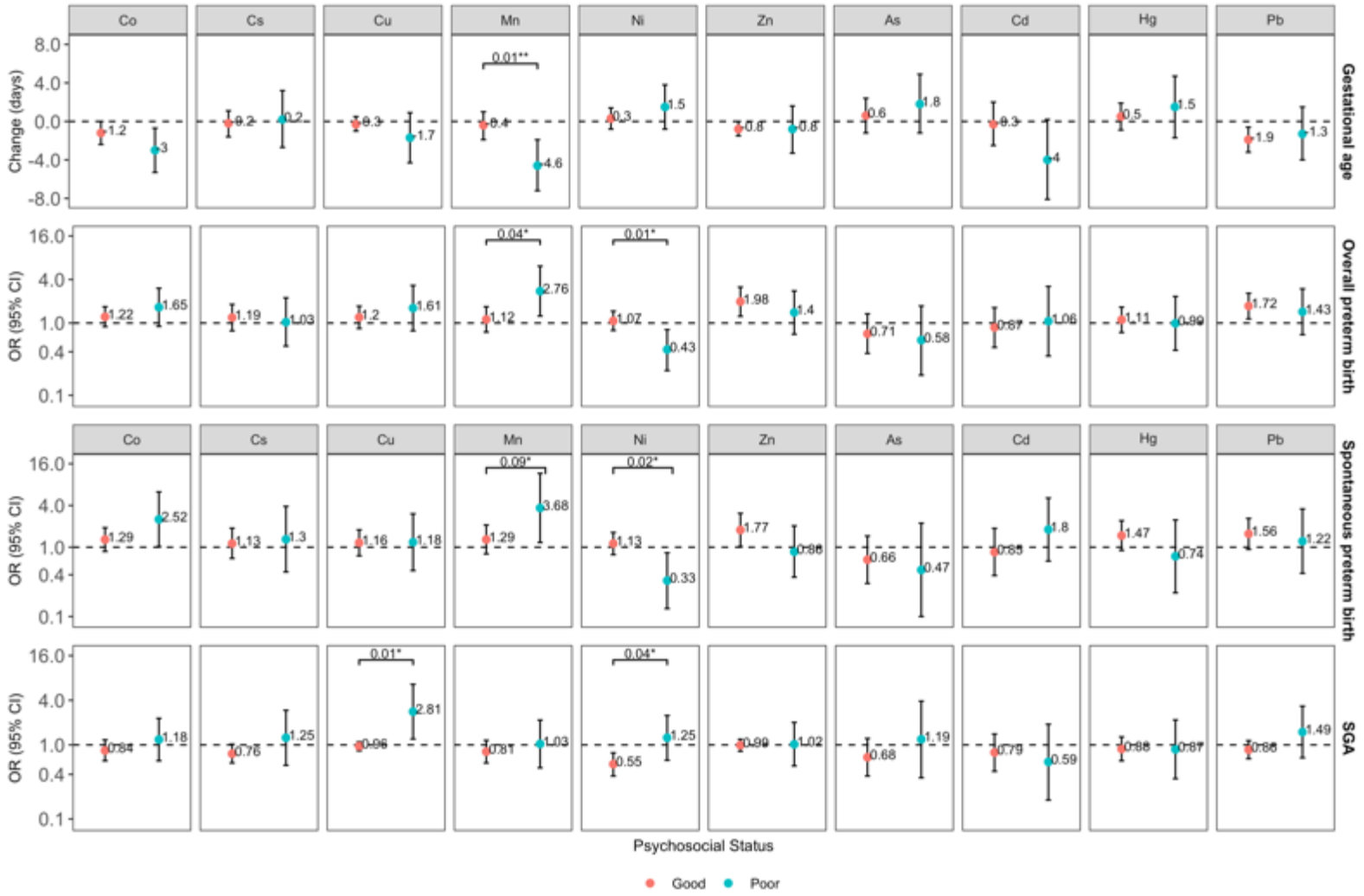
Metal ((loid)	Cluster 1: "good" psychosocial status		Cluster 2: "poor" psychosocial status		P value[b]
	GM	GSD	GM	GSD	
Co	0.36	1.4	0.37	1.3	0.14
Cs	1.2	1.4	1.2	1.3	0.31
Cu	1554	1.3	1614	1.2	0.14
Mn	11.0	1.4	11.6	1.4	0.16
Ni	1.1	1.8	1.1	1.7	0.36
Zn	4780	1.3	4760	1.2	0.82
As	0.37	1.8	0.38	1.7	0.25
Cd	0.12	1.6	0.13	1.8	0.11
Hg	1.1	1.9	1.1	1.8	0.68
Pb[a]	3.0	15.9	3.5	15.0	<0.001**

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); geometric mean (GM); geometric standard deviation (GSD).

[a] Pb concentration unit is µg/dL

[b] Two sample t-test to test for differences in log-transformed metal concentrations between the two clusters, ** indicates P value <0.05

Figure IV.3 Change in birth outcomes associated with geometric mean exposure biomarker concentration within two psychosocial status clusters. Effect estimates presented as changes in gestation or odds ratio (OR) for IQR increase in exposure biomarker concentration[a]. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking



Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb).
[a]As, Cd were compared between two categories of above LOD and below LOD.
*indicates *p* for interaction <0.1 considered significant for interaction metal*psychosocial status, ** indicates *p* for interaction <0.1 & *q* for interaction <0.1

Table IV.4 Change in birth outcomes associated with geometric mean exposure biomarker concentration within two psychosocial status clusters. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration[a]. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

Metals	**Gestational age**					**Birthweight z-score**				
	Cluster 1 ("good" psychosocial status)		**Cluster 2 ("poor" psychosocial status)**			**Cluster 1 ("good" psychosocial status)**		**Cluster 2 ("poor" psychosocial status)**		
	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	*Int p*	Change in z-score (95% CI)	*p* value	Change in z-score (95% CI)	*p* value	*Int p*
Co	**-1.2 (-2.4, 0.0)**	**0.05***	**-3 (-5.3, -0.7)**	**0.01****	0.17	0.1 (0.0, 0.2)	0.16	0.0 (-0.2, 0.2)	0.76	0.71
Cs	-0.2 (-1.6, 1.1)	0.72	0.2 (-2.7, 3.2)	0.87	0.77	0.1 (0.0, 0.2)	0.08	-0.1 (-0.4, 0.1)	0.37	0.12
Cu	-0.3 (-1.0, 0.5)	0.47	-1.7 (-4.3, 0.9)	0.20	0.30	0.0 (0.0, 0.1)	0.54	-0.1 (-0.3, 0.1)	0.26	0.21
Mn	-0.4 (-1.9, 1.0)	0.54	**-4.6 (-7.2, -1.9)**	**<0.001****	**0.01****	0.0 (-0.1, 0.2)	0.41	0.0 (-0.2, 0.3)	0.74	0.94
Ni	0.3 (-0.8, 1.4)	0.61	1.5 (-0.8, 3.8)	0.19	0.34	0.1 (0.0, 0.2)	0.08	0.0 (-0.2, 0.1)	0.65	0.24
Zn	-0.8 (-1.5, 0.0)	0.06	-0.8 (-3.3, 1.6)	0.51	0.96	0.0 (-0.1, 0.1)	0.89	0.0 (-0.2, 0.2)	0.88	0.92
As[a]	0.6 (-1.2, 2.4)	0.50	1.8 (-1.2, 4.9)	0.24	0.50	0.1 (-0.1, 0.3)	0.35	-0.1 (-0.4, 0.3)	0.62	0.38
Cd[a]	-0.3 (-2.5, 2.0)	0.82	-4.0 (-8.1, 0.2)	0.06	0.12	-0.1 (-0.3, 0.1)	0.39	0.3 (-0.1, 0.6)	0.12	0.17
Hg	0.5 (-0.9, 1.9)	0.51	1.5 (-1.7, 4.7)	0.35	0.56	0.0 (-0.1, 0.2)	0.45	0.1 (-0.2, 0.4)	0.43	0.68
Pb	**-1.9 (-3.2, -0.6)**	**0.004****	-1.3 (-4.0, 1.5)	0.38	0.67	0.1 (0.0, 0.2)	0.11	-0.1 (-0.3, 0.2)	0.47	0.18

Metals	**Preterm birth (overall)**					**Preterm birth (spontaneous)**				
	Cluster 1 ("good psychosocial status")		**Cluster 2 ("poor psychosocial status")**			**Cluster 1 ("good" psychosocial status)**		**Cluster 2 ("poor" psychosocial status)**		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	1.22 (0.89, 1.68)	0.21	1.65 (0.90, 3.03)	0.11	0.39	1.29 (0.87, 1.91)	0.20	**2.52 (1.02, 6.27)**	**0.05***	0.18
Cs	1.19 (0.78, 1.81)	0.42	1.03 (0.48, 2.23)	0.93	0.75	1.13 (0.69, 1.88)	0.62	1.30 (0.44, 3.88)	0.63	0.82
Cu	1.20 (0.84, 1.71)	0.32	1.61 (0.78, 3.31)	0.19	0.46	1.16 (0.75, 1.78)	0.51	1.18 (0.46, 3.03)	0.73	0.97
Mn	1.12 (0.75, 1.68)	0.57	**2.76 (1.25, 6.12)**	**0.01****	**0.04***	1.29 (0.80, 2.09)	0.29	**3.68 (1.17, 11.61)**	**0.03***	**0.09***
Ni	1.07 (0.79, 1.46)	0.65	**0.43 (0.22, 0.81)**	**0.01****	**0.01***	1.13 (0.78, 1.63)	0.53	**0.33 (0.13, 0.83)**	**0.02***	**0.02***
Zn	**1.98 (1.24, 3.14)**	**0.004****	1.40 (0.70, 2.78)	0.34	0.41	**1.77 (1.02, 3.08)**	**0.04***	0.86 (0.37, 2.03)	0.74	0.17
As[a]	0.71 (0.38, 1.34)	0.30	0.58 (0.19, 1.72)	0.32	0.74	0.66 (0.30, 1.45)	0.30	0.47 (0.10, 2.22)	0.34	0.70
Cd[a]	0.87 (0.46, 1.63)	0.66	1.06 (0.35, 3.21)	0.92	0.76	0.85 (0.39, 1.87)	0.69	1.80 (0.63, 5.12)	0.27	0.18
Hg	1.11 (0.74, 1.66)	0.63	0.99 (0.42, 2.33)	0.98	0.81	1.47 (0.90, 2.41)	0.13	0.74 (0.22, 2.47)	0.63	0.30
Pb	**1.72 (1.14, 2.58)**	**0.01****	1.43 (0.69, 2.97)	0.34	0.66	1.56 (0.93, 2.6)	0.09	1.22 (0.42, 3.56)	0.71	0.69

Table IV.4 Continued

Metals	SGA					LGA				
	Cluster 1 ("good" psychosocial status)		Cluster 2 ("poor" psychosocial status)			Cluster 1 ("good" psychosocial status)		Cluster 2 ("poor" psychosocial status)		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	0.84 (0.61, 1.17)	0.30	1.18 (0.61, 2.28)	0.62	0.37	1.13 (0.81, 1.57)	0.48	0.94 (0.57, 1.56)	0.81	0.56
Cs	0.76 (0.57, 1.02)	0.07	1.25 (0.53, 2.93)	0.61	0.28	1.13 (0.74, 1.70)	0.57	1.01 (0.52, 1.94)	0.98	0.77
Cu	0.96 (0.83, 1.10)	0.54	**2.81 (1.20, 6.57)**	**0.02***	**0.01***	1.01 (0.80, 1.27)	0.93	0.97 (0.56, 1.66)	0.90	0.88
Mn	0.81 (0.57, 1.15)	0.24	1.03 (0.49, 2.16)	0.94	0.57	0.94 (0.63, 1.40)	0.75	1.58 (0.85, 2.94)	0.14	0.16
Ni	**0.55 (0.38, 0.78)**	**<0.001****	1.25 (0.62, 2.49)	0.54	**0.04***	1.01 (0.73, 1.40)	0.95	0.93 (0.57, 1.52)	0.77	0.78
Zn	0.99 (0.82, 1.19)	0.90	1.02 (0.52, 2.01)	0.95	0.93	1.05 (0.77, 1.44)	0.76	1.01 (0.60, 1.69)	0.97	0.90
As[a]	0.68 (0.38, 1.22)	0.20	1.19 (0.36, 3.89)	0.77	0.41	0.91 (0.48, 1.72)	0.78	1.16 (0.46, 2.93)	0.75	0.67
Cd[a]	0.79 (0.44, 1.40)	0.42	0.59 (0.18, 1.90)	0.38	0.66	0.72 (0.38, 1.37)	0.32	1.16 (0.46, 2.92)	0.75	0.40
Hg	0.88 (0.61, 1.28)	0.51	0.87 (0.35, 2.17)	0.77	0.98	1.06 (0.71, 1.59)	0.78	1.49 (0.72, 3.10)	0.29	0.42
Pb	0.86 (0.65, 1.14)	0.30	1.49 (0.67, 3.33)	0.33	0.21	0.89 (0.64, 1.23)	0.49	1.10 (0.57, 2.10)	0.78	0.57

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestational age (SGA); large for gestational age (LGA).

[a] As, Cd were compared between two categories of above LOD and below LOD

**p* value <0.05 considered significant for effect estimates, **indicate *q* value <0.1& *p* value <0.05

*int *p* (interaction p value) <0.1 considered significant for interaction metal*psychosocial status, **indicate *q* value <0.1& *Int p* <0.1

Figure IV.4 Relationship between blood manganese (Mn) concentration and preterm birth among women within "good" and "poor" psychosocial status clusters, generated from generalized additive model of log-Mn concentration and preterm birth

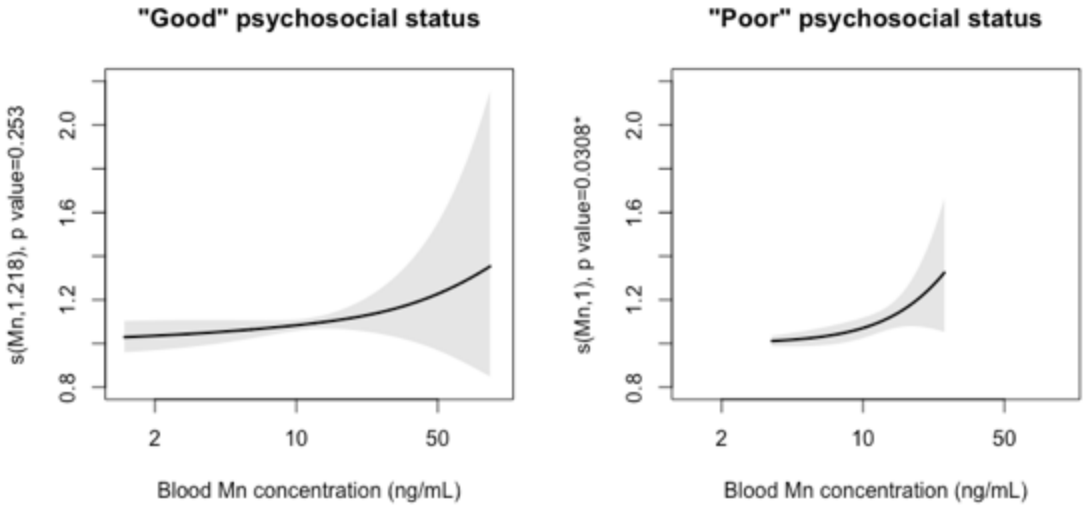


Table IV.5 Change in birth outcomes associated with geometric mean exposure biomarker concentration within two psychosocial status clusters. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentrationa. Models were adjusted for maternal age, maternal income, pre-pregnancy BMI, and exposure to secondhand smoking

Metals	**Gestational age**					**Birthweight z-score**				
	Cluster 1 ("good" psychosocial status)		**Cluster 2 ("poor" psychosocial status)**			**Cluster 1 ("good" psychosocial status)**		**Cluster 2 ("poor" psychosocial status)**		
	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	*Int p*	Change in z-score (95% CI)	*p* value	Change in z-score (95% CI)	*p* value	*Int p*
Co	-0.1 (-1.4, 1.3)	0.93	**-2.9 (-5.4, -0.5)**	**0.02***	**0.04***	0.0 (-0.1, 0.1)	0.51	0.0 (-0.2, 0.2)	0.89	0.85
Cs	-0.4 (-1.8, 1.0)	0.60	-0.2 (-3.6, 3.2)	0.92	0.92	0.1 (0.0, 0.2)	0.11	-0.1 (-0.4, 0.2)	0.49	0.21
Cu	-0.3 (-1.1, 0.5)	0.47	-2.4 (-5.2, 0.4)	0.09	0.15	0.0 (0.0, 0.1)	0.58	-0.2 (-0.4, 0.1)	0.21	0.17
Mn	-0.2 (-1.7, 1.3)	0.79	**-5.1 (-8.0, -2.3)**	**<0.001****	**0.003****	0.0 (-0.1, 0.2)	0.64	0.0 (-0.3, 0.2)	0.92	0.77
Ni	0.3 (-0.9, 1.6)	0.59	2.3 (-0.2, 4.7)	0.07	0.17	0.1 (0.0, 0.2)	0.02	0.0 (-0.2, 0.2)	0.84	0.22
Zn	-0.7 (-1.6, 0.1)	0.07	-0.6 (-3.3, 2.0)	0.64	0.94	0.0 (-0.1, 0.1)	0.98	0.0 (-0.2, 0.3)	0.70	0.72
As[a]	0.9 (-1.5, 3.2)	0.48	4.2 (-0.3, 8.7)	0.07	0.20	0.1 (-0.1, 0.3)	0.35	-0.1 (-0.5, 0.3)	0.55	0.33
Cd[a]	-0.3 (-2.8, 2.1)	0.80	-3.4 (-8.0, 1.1)	0.14	0.23	-0.1 (-0.3, 0.1)	0.43	0.3 (-0.1, 0.7)	0.15	0.10
Hg	0.5 (-1.1, 2.0)	0.57	1.8 (-1.7, 5.2)	0.32	0.50	0.0 (-0.1, 0.2)	0.60	0.1 (-0.2, 0.4)	0.50	0.69
Pb	**-1.9 (-3.3, -0.6)**	**0.01****	-1.6 (-4.5, 1.3)	0.28	0.83	0.1 (0.0, 0.2)	0.20	-0.1 (-0.3, 0.2)	0.59	0.30

Metals	**Preterm birth (overall)**					**Preterm birth (spontaneous)**				
	Cluster 1 ("good" psychosocial status)		**Cluster 2 ("poor" psychosocial status)**			**Cluster 1 (good psychosocial status)**		**Cluster 2 ("poor" psychosocial status)**		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	1.02 (0.72, 1.44)	0.93	1.56 (0.85, 2.88)	0.15	0.23	1.18 (0.78, 1.78)	0.43	2.32 (0.93, 5.75)	0.07	0.18
Cs	1.24 (0.80, 1.93)	0.33	0.96 (0.42, 2.16)	0.91	0.57	1.14 (0.69, 1.91)	0.61	1.27 (0.41, 3.97)	0.68	0.87
Cu	1.24 (0.86, 1.79)	0.24	1.63 (0.79, 3.34)	0.18	0.50	1.21 (0.78, 1.89)	0.39	1.20 (0.47, 3.09)	0.70	0.99
Mn	1.11 (0.74, 1.66)	0.62	**3.13 (1.35, 7.27)**	**0.01****	**0.03***	1.29 (0.80, 2.08)	0.30	**4.16 (1.22, 14.15)**	**0.02***	**0.08***
Ni	1.12 (0.81, 1.56)	0.49	**0.42 (0.22, 0.81)**	**0.01****	**0.01***	1.19 (0.80, 1.75)	0.39	**0.33 (0.13, 0.84)**	**0.02***	**0.01***
Zn	**2.04 (1.26, 3.31)**	**0.004****	1.36 (0.67, 2.77)	0.39	0.36	1.86 (1.05, 3.28)	0.03	0.85 (0.36, 2.00)	0.71	0.14
As[a]	0.74 (0.38, 1.42)	0.36	0.58 (0.19, 1.76)	0.34	0.72	0.71 (0.32, 1.59)	0.41	0.48 (0.10, 2.27)	0.35	0.65
Cd[a]	0.89 (0.46, 1.71)	0.73	0.95 (0.31, 2.92)	0.93	0.92	0.96 (0.43, 2.16)	0.93	3.57 (0.41, 31.12)	0.25	0.26
Hg	1.23 (0.80, 1.91)	0.35	1.10 (0.46, 2.64)	0.83	0.82	1.65 (0.97, 2.82)	0.07	0.82 (0.24, 2.78)	0.75	0.30
Pb	**1.75 (1.17, 2.64)**	**0.01****	1.35 (0.66, 2.78)	0.41	0.54	1.56 (0.94, 2.60)	0.08	1.16 (0.41, 3.29)	0.78	0.61

Table IV.5 Continued

Metals	SGA					LGA				
	Cluster 1 ("good" psychosocial status)		Cluster 2 ("poor" psychosocial status)			Cluster 1 ("good" psychosocial status)		Cluster 2 ("poor" psychosocial status)		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	0.80 (0.57, 1.13)	0.21	1.08 (0.56, 2.09)	0.81	0.43	1.07 (0.74, 1.54)	0.72	0.85 (0.49, 1.47)	0.57	0.50
Cs	0.78 (0.58, 1.05)	0.10	1.26 (0.53, 3.01)	0.60	0.30	1.23 (0.79, 1.93)	0.36	1.09 (0.51, 2.31)	0.83	0.77
Cu	0.96 (0.83, 1.12)	0.63	**2.81 (1.20, 6.57)**	**0.02***	**0.01***	1.07 (0.77, 1.48)	0.68	0.96 (0.53, 1.73)	0.89	0.75
Mn	0.82 (0.57, 1.17)	0.27	1.09 (0.52, 2.30)	0.82	0.50	0.95 (0.63, 1.45)	0.82	1.41 (0.73, 2.73)	0.31	0.33
Ni	**0.54 (0.38, 0.78)**	**0.001****	1.31 (0.64, 2.67)	0.46	**0.03***	1.06 (0.75, 1.49)	0.75	1.00 (0.58, 1.70)	0.99	0.86
Zn	1.02 (0.81, 1.30)	0.85	1.03 (0.51, 2.07)	0.93	0.99	1.10 (0.74, 1.63)	0.64	1.16 (0.64, 2.10)	0.63	0.89
As[a]	0.59 (0.32, 1.08)	0.09	1.15 (0.35, 3.80)	0.82	0.32	0.95 (0.49, 1.85)	0.89	0.80 (0.30, 2.15)	0.66	0.78
Cd[a]	0.72 (0.40, 1.31)	0.28	0.51 (0.16, 1.66)	0.26	0.61	0.71 (0.37, 1.38)	0.32	0.90 (0.33, 2.45)	0.84	0.70
Hg	0.81 (0.55, 1.20)	0.29	0.96 (0.38, 2.46)	0.94	0.74	1.03 (0.67, 1.59)	0.88	1.61 (0.71, 3.65)	0.25	0.34
Pb	0.87 (0.65, 1.16)	0.35	1.43 (0.65, 3.16)	0.38	0.25	0.91 (0.65, 1.29)	0.61	1.19 (0.59, 2.38)	0.63	0.51

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestational age (SGA); large for gestational age (LGA).

[a] As, Cd were compared between two categories of above LOD and below LOD

**p* value <0.05 considered significant for effect estimates, **indicate *q* value <0.1& *p* value <0.05

*int *p* (interaction p value) <0.10 considered significant for interaction metal*psychosocial status, **indicate *q* value <0.1& *Int p* <0.1

Table IV.6 Change in gestational age (days) associated with geometric mean exposure biomarker concentration within two psychosocial status clusters stratified by study visits. Effect estimates presented as changes for IQR increase in exposure biomarker concentrationa. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

Gestational age											
	Cluster 1 ("good" psychosocial status)					**Cluster 2 ("poor" psychosocial status)**					
	Visit 1		**Visit 3**			**Visit 1**		**Visit 3**			
Metals	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	*Int p (metal*visit)*	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	*Int p (metal*visit)*	*Int p (metal*visit*cluster)*
Co	-0.8 (-2.3, 0.7)	0.28	-0.5 (-2.2, 1.2)	0.57	0.80	-2 (-4.8, 0.7)	0.15	-2 (-5.2, 1.3)	0.23	0.98	0.47
Cs	-0.4 (-1.8, 1.1)	0.63	-1 (-2.8, 0.8)	0.27	0.58	-0.1 (-3.3, 3.1)	0.95	0.7 (-3, 4.4)	0.69	0.73	0.92
Cu	0 (-0.9, 0.8)	0.94	0 (-1.6, 1.6)	0.98	0.95	**-3 (-6, 0)**	**0.05***	-1 (-4, 2.1)	0.53	0.34	0.21
Mn	-0.5 (-2.1, 1)	0.50	0.2 (-1.8, 2.2)	0.86	0.58	**-2.7 (-5.7, 0.3)**	**0.08**	**-4.9 (-8.6, -1.3)**	**0.01***	0.35	0.59
Ni	-0.3 (-1.5, 0.9)	0.66	0.5 (-0.9, 1.9)	0.46	0.40	0.7 (-1.7, 3.2)	0.56	0.3 (-2.6, 3.2)	0.82	0.84	0.17
Zn	**-0.7 (-1.5, 0.1)**	**0.08**	**-1.8 (-3.6, 0)**	**0.05***	0.28	-1.2 (-4.1, 1.7)	0.41	-0.6 (-3.4, 2.2)	0.69	0.76	0.40
As[a]	1.1 (-1.3, 3.6)	0.37	-0.1 (-2.9, 2.7)	0.92	0.51	3 (-1.7, 7.8)	0.21	3.2 (-1.8, 8.3)	0.21	0.95	0.71
Cd[a]	-0.5 (-3, 2)	0.70	0.3 (-2.6, 3.1)	0.85	0.69	**-5.9 (-10.5, -1.2)**	**0.01***	0.1 (-5.3, 5.4)	0.98	0.10	0.21
Hg	0.3 (-1.2, 1.8)	0.73	-0.6 (-2.4, 1.2)	0.54	0.48	1.7 (-1.7, 5.1)	0.34	1.2 (-2.6, 4.9)	0.54	0.85	0.11
Pb	**-1.8 (-3.2, -0.4)**	**0.01***	**-1.7 (-3.6, 0.2)**	**0.08**	0.96	-1.1 (-4.4, 2.2)	0.51	-1 (-4.3, 2.4)	0.57	0.95	0.59

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb).

[a] As, Cd were compared between two categories of above LOD and below LOD

**p* value <0.05 considered significant for effect estimates

*int *p* (interaction p value) <0.10 considered significant for interaction

Table IV.7 Change in birth outcomes associated with geometric mean exposure biomarker concentration within two groups of high and low Depression score (CES-D). Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration[a]. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

Metals	**Gestational age**					**Birthweight z-score**				
	CES-D Depression Score (<16)		**CES-D Depression Score (>=16)**			**CES-D Depression Score (<16)**		**CES-D Depression Score (>=16)**		
	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	*Int p*	Change in z-score (95% CI)	*p* value	Change in z-score (95% CI)	*p* value	*Int p*
Co	**-1.4 (-2.5, -0.2)**	**0.02***	-2.3 (-4.7, 0.2)	0.07	0.69	0.0 (-0.1, 0.1)	0.36	0.1 (-0.1, 0.4)	0.17	0.47
Cs	-0.2 (-1.5, 1.1)	0.77	0.1 (-3, 3.2)	0.94	0.85	0.1 (0.0, 0.2)	0.12	-0.1 (-0.4, 0.1)	0.33	0.12
Cu	-0.4 (-1.2, 0.4)	0.32	-0.6 (-2.8, 1.5)	0.56	0.79	0.0 (0.0, 0.1)	0.44	-0.1 (-0.3, 0.1)	0.17	0.12
Mn	-0.8 (-2.2, 0.5)	0.23	**-4.3 (-7.1, -1.4)**	**0.004****	**0.05***	0.1 (-0.1, 0.2)	0.30	0.0 (-0.2, 0.2)	0.99	0.62
Ni	0.3 (-0.8, 1.4)	0.61	1.7 (-0.8, 4.2)	0.18	0.35	0.1 (0.0, 0.2)	0.13	0.0 (-0.2, 0.2)	0.90	0.40
Zn	-0.7 (-1.5, 0.1)	0.08	-1.3 (-3.4, 0.8)	0.23	0.58	0.0 (-0.1, 0.1)	0.82	0.0 (-0.2, 0.2)	0.97	0.91
As[a]	0.1 (-2.1, 2.3)	0.93	**5.7 (1.6, 9.7)**	**0.01****	**0.02***	0.0 (-0.2, 0.2)	0.80	0.0 (-0.3, 0.4)	0.82	0.94
Cd[a]	-0.4 (-2.6, 1.9)	0.76	-2.9 (-7, 1.1)	0.16	0.27	-0.1 (-0.3, 0.1)	0.52	0.2 (-0.1, 0.6)	0.18	0.13
Hg	0.3 (-1.2, 1.8)	0.73	1.4 (-1.1, 3.9)	0.29	0.44	0.0 (-0.1, 0.2)	0.47	0.1 (-0.1, 0.3)	0.38	0.66
Pb	**-2.0 (-3.3, -0.6)**	**0.004****	-0.9 (-3.6, 1.8)	0.50	0.50	0.1 (-0.1, 0.2)	0.29	0.0 (-0.2, 0.3)	0.79	0.82

Metals	**Preterm birth (overall)**					**Preterm birth (spontaneous)**				
	CES-D Depression Score (<16)		**CES-D Depression Score (>=16)**			**CES-D Depression Score (<16)**		**CES-D Depression Score (>=16)**		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	1.25 (0.92, 1.70)	0.16	1.54 (0.84, 2.82)	0.17	0.67	1.34 (0.91, 1.98)	0.14	1.83 (0.93, 3.60)	0.08	0.36
Cs	1.21 (0.80, 1.82)	0.37	0.91 (0.40, 2.09)	0.83	0.54	1.13 (0.69, 1.84)	0.62	0.88 (0.25, 3.03)	0.84	0.70
Cu	1.24 (0.86, 1.79)	0.24	1.48 (0.79, 2.77)	0.22	0.53	1.25 (0.79, 1.99)	0.34	1.15 (0.48, 2.77)	0.75	0.93
Mn	1.21 (0.83, 1.75)	0.33	**2.85 (1.14, 7.10)**	**0.02***	0.11	1.41 (0.90, 2.21)	0.13	3.12 (0.94, 10.39)	0.06	0.22
Ni	1.02 (0.76, 1.37)	0.91	**0.50 (0.25, 1.00)**	**0.05***	**0.07***	1.03 (0.71, 1.50)	0.86	0.49 (0.21, 1.17)	0.11	0.10
Zn	**1.95 (1.24, 3.05)**	**0.004****	1.51 (0.78, 2.94)	0.22	0.61	**1.77 (1.03, 3.02)**	**0.04***	0.90 (0.40, 2.05)	0.80	0.18
As[a]	0.77 (0.42, 1.42)	0.40	0.50 (0.16, 1.54)	0.23	0.51	0.66 (0.31, 1.41)	0.28	0.31 (0.05, 1.77)	0.19	0.44
Cd[a]	0.95 (0.52, 1.76)	0.88	0.69 (0.23, 2.13)	0.52	0.62	1.02 (0.47, 2.20)	0.97	1.29 (0.22, 7.44)	0.77	0.80
Hg	1.13 (0.74, 1.71)	0.57	1.02 (0.50, 2.11)	0.95	0.84	1.48 (0.92, 2.36)	0.10	0.80 (0.25, 2.58)	0.71	0.31
Pb	**1.65 (1.10, 2.50)**	**0.02***	1.53 (0.72, 3.27)	0.27	0.88	1.46 (0.87, 2.44)	0.15	1.34 (0.44, 4.08)	0.60	0.92

Metals	**SGA**					**LGA**				
	CES-D Depression Score (<16)		**CES-D Depression Score (>=16)**			**CES-D Depression Score (<16)**		**CES-D Depression Score (>=16)**		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	0.85 (0.62, 1.18)	0.35	1.07 (0.56, 2.04)	0.83	0.51	1.08 (0.78, 1.48)	0.66	1.08 (0.60, 1.93)	0.80	0.97
Cs	0.79 (0.59, 1.05)	0.11	1.15 (0.44, 3.00)	0.77	0.43	1.17 (0.81, 1.71)	0.40	0.81 (0.34, 1.91)	0.62	0.40
Cu	0.97 (0.83, 1.12)	0.66	2.09 (0.90, 4.85)	0.09	**0.08***	1.02 (0.82, 1.26)	0.88	0.86 (0.52, 1.40)	0.54	0.53
Mn	0.80 (0.57, 1.11)	0.18	1.24 (0.58, 2.63)	0.58	0.28	0.93 (0.65, 1.34)	0.70	**1.96 (1.02, 3.78)**	**0.04***	**0.05***
Ni	**0.61 (0.43, 0.86)**	**0.004****	0.89 (0.47, 1.68)	0.72	0.28	1.04 (0.76, 1.41)	0.82	0.87 (0.53, 1.42)	0.58	0.55
Zn	0.99 (0.83, 1.18)	0.92	1.06 (0.53, 2.13)	0.87	0.86	1.04 (0.79, 1.38)	0.78	1.05 (0.62, 1.76)	0.87	0.98
As[a]	0.71 (0.40, 1.25)	0.23	1.30 (0.40, 4.21)	0.67	0.36	0.74 (0.41, 1.36)	0.34	1.80 (0.64, 5.03)	0.26	0.15
Cd[a]	0.80 (0.45, 1.41)	0.43	0.64 (0.20, 2.03)	0.45	0.74	0.71 (0.39, 1.29)	0.26	1.40 (0.52, 3.79)	0.51	0.25
Hg	0.87 (0.59, 1.27)	0.47	0.95 (0.44, 2.04)	0.89	0.85	1.01 (0.69, 1.47)	0.95	1.73 (0.85, 3.52)	0.13	0.20
Pb	0.87 (0.64, 1.18)	0.36	1.30 (0.63, 2.67)	0.48	0.33	0.86 (0.63, 1.16)	0.33	1.28 (0.65, 2.55)	0.48	0.29

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestational age (SGA); large for gestational age (LGA).

[a] As, Cd were compared between two categories of above LOD and below LOD

*p value <0.05 considered significant for effect estimates, **indicate q value <0.1& p value <0.05

*int p (interaction p value) <0.10 considered significant for interaction metal*depression score, **indicate q value <0.1& *Int* p <0.1

Table IV.8 Change in birth outcomes associated with geometric mean exposure biomarker concentration within two groups of high and low perceived stress score (PSS). Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentrationa. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

Metals	**Gestational age**					**Birthweight z-score**				
	PSS-Perceived Stress (<75th)		**PSS-Perceived Stress (>=75th)**			**PSS-Perceived Stress (<75th)**		**PSS-Perceived Stress (>=75th)**		
	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	*Int p*	Change in z-score (95% CI)	*p* value	Change in z-score (95% CI)	*p* value	*Int p*
Co	**-1.4 (-2.6, -0.3)**	**0.02****	-1.9 (-4.2, 0.4)	0.10	0.78	0.1 (0.0, 0.2)	0.18	0.1 (-0.1, 0.3)	0.42	0.95
Cs	-0.3 (-1.7, 1.0)	0.64	0.5 (-2.1, 3.1)	0.70	0.57	0.1 (0.0, 0.2)	0.06	-0.2 (-0.4, 0.1)	0.15	**0.03***
Cu	-0.3 (-1.1, 0.5)	0.43	-1.3 (-3.4, 0.8)	0.22	0.37	0.0 (0.0, 0.1)	0.56	0.0 (-0.2, 0.1)	0.68	0.55
Mn	-0.8 (-2.2, 0.5)	0.24	**-3.3 (-5.9, -0.8)**	**0.01****	0.12	0.1 (0.0, 0.2)	0.26	0.0 (-0.2, 0.2)	0.78	0.73
Ni	0.4 (-0.7, 1.5)	0.53	1.0 (-1.1, 3.1)	0.36	0.66	0.0 (0.0, 0.1)	0.32	0.1 (-0.1, 0.2)	0.42	0.88
Zn	**-0.8 (-1.6, -0.1)**	**0.04***	-0.4 (-2.8, 1.9)	0.73	0.70	0.0 (0.0, 0.1)	0.58	-0.1 (-0.3, 0.1)	0.37	0.30
As[a]	0.8 (-1.5, 3.1)	0.49	2.9 (-0.8, 6.7)	0.13	0.35	0.0 (-0.1, 0.2)	0.63	0.0 (-0.3, 0.3)	0.93	0.86
Cd[a]	0.6 (-1.7, 2.9)	0.61	**-4.7 (-8.5, -0.8)**	**0.02****	**0.02***	-0.1 (-0.3, 0.1)	0.18	**0.4 (0.1, 0.8)**	**0.01***	**0.003****
Hg	0.3 (-1.3, 1.8)	0.75	1.2 (-1.0, 3.5)	0.27	0.43	0.1 (-0.1, 0.2)	0.44	0.1 (-0.1, 0.2)	0.56	0.91
Pb	**-2.0 (-3.4, -0.7)**	**0.003****	-0.4 (-3, 2.2)	0.77	0.27	0.0 (-0.1, 0.2)	0.47	0.1 (-0.1, 0.4)	0.21	0.43

Metals	**Preterm birth (overall)**					**Preterm birth (spontaneous)**				
	PSS-Perceived Stress (<75th)		**PSS-Perceived Stress (>=75th)**			**PSS-Perceived Stress (<75th)**		**PSS-Perceived Stress (>=75th)**		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	1.18 (0.88, 1.60)	0.27	**1.92 (1.03, 3.58)**	**0.04***	0.18	1.27 (0.84, 1.91)	0.25	**2.75 (1.25, 6.06)**	**0.01***	**0.07***
Cs	1.15 (0.76, 1.74)	0.52	1.09 (0.54, 2.19)	0.80	0.92	1.13 (0.67, 1.89)	0.64	0.91 (0.36, 2.31)	0.85	0.70
Cu	1.15 (0.80, 1.66)	0.45	1.83 (0.99, 3.38)	0.06	0.18	1.16 (0.73, 1.84)	0.53	1.49 (0.66, 3.33)	0.33	0.56
Mn	1.17 (0.80, 1.72)	0.41	**2.50 (1.15, 5.44)**	**0.02***	**0.09***	1.32 (0.82, 2.14)	0.25	**3.19 (1.16, 8.79)**	**0.02***	0.12
Ni	0.96 (0.70, 1.30)	0.78	0.70 (0.39, 1.27)	0.24	0.40	1.04 (0.70, 1.54)	0.84	0.55 (0.26, 1.15)	0.11	0.13
Zn	**2.16 (1.37, 3.42)**	**<0.001****	1.31 (0.67, 2.56)	0.43	0.17	**2.06 (1.17, 3.62)**	**0.01***	0.78 (0.38, 1.62)	0.51	**0.04***
As[a]	0.68 (0.36, 1.28)	0.23	0.72 (0.26, 1.98)	0.52	0.93	0.61 (0.28, 1.35)	0.23	0.44 (0.10, 1.91)	0.27	0.69
Cd[a]	0.70 (0.37, 1.30)	0.26	1.74 (0.57, 5.30)	0.33	0.16	0.78 (0.35, 1.71)	0.53	4.42 (0.52, 37.34)	0.17	0.13
Hg	1.18 (0.77, 1.82)	0.45	0.90 (0.48, 1.69)	0.74	0.51	1.60 (0.96, 2.67)	0.07	0.75 (0.29, 1.91)	0.55	0.17
Pb	**1.73 (1.14, 2.61)**	**0.01****	1.28 (0.63, 2.61)	0.50	0.47	**1.68 (1.00, 2.83)**	**0.05***	0.74 (0.28, 1.96)	0.54	0.15

Metals	**SGA**					**LGA**				
	PSS-Perceived Stress (<75th)		**PSS-Perceived Stress (>=75th)**			**PSS-Perceived Stress (<75th)**		**PSS-Perceived Stress (>=75th)**		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	0.85 (0.60, 1.19)	0.34	1.02 (0.60, 1.74)	0.95	0.58	1.15 (0.84, 1.57)	0.38	0.88 (0.51, 1.54)	0.66	0.40
Cs	0.75 (0.55, 1.01)	0.06	1.31 (0.61, 2.82)	0.49	0.16	1.24 (0.83, 1.85)	0.29	0.76 (0.39, 1.49)	0.42	0.21
Cu	0.95 (0.82, 1.10)	0.49	1.81 (0.97, 3.36)	0.06	**0.05***	1.00 (0.82, 1.22)	1.00	0.99 (0.66, 1.49)	0.97	0.97
Mn	0.83 (0.58, 1.18)	0.29	0.97 (0.56, 1.69)	0.91	0.64	1.00 (0.69, 1.45)	0.99	1.51 (0.83, 2.72)	0.17	0.26
Ni	**0.57 (0.39, 0.83)**	**0.003****	0.85 (0.51, 1.40)	0.52	0.19	0.98 (0.70, 1.36)	0.89	1.00 (0.66, 1.52)	1.00	0.94
Zn	1.00 (0.81, 1.23)	0.99	0.98 (0.58, 1.66)	0.93	0.93	1.16 (0.78, 1.73)	0.47	0.82 (0.50, 1.36)	0.45	0.29
As[a]	0.68 (0.37, 1.25)	0.21	1.09 (0.42, 2.82)	0.85	0.40	0.85 (0.46, 1.57)	0.60	1.20 (0.49, 2.94)	0.68	0.52
Cd[a]	0.84 (0.46, 1.55)	0.58	0.58 (0.22, 1.51)	0.26	0.52	0.60 (0.32, 1.13)	0.11	1.70 (0.65, 4.44)	0.28	**0.07***
Hg	0.87 (0.58, 1.31)	0.50	0.91 (0.50, 1.69)	0.78	0.91	1.10 (0.73, 1.66)	0.65	1.24 (0.72, 2.12)	0.44	0.69
Pb	0.95 (0.68, 1.33)	0.76	0.74 (0.39, 1.39)	0.35	0.48	0.84 (0.61, 1.14)	0.26	1.27 (0.68, 2.40)	0.45	0.24

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestational age (SGA); large for gestational age (LGA).

[a] As, Cd were compared between two categories of above LOD and below LOD

*p value <0.05 considered significant for effect estimates, **indicate q value <0.1& p value <0.05

*int p (interaction p value) <0.10 considered significant for interaction metal*perceived stress, **indicate q value <0.1& *Int* p <0.1

Table IV.9 Change in birth outcomes associated with geometric mean exposure biomarker concentration within two groups of high and low social support (ESSI). Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration[a]. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

Metals	**Gestational age**					**Birthweight z-score**				
	SS-Social Support (>25th)		**SS-Social Support (<=25th)**			**SS-Social Support (>25th)**		**SS-Social Support (<=25th)**		
	Change in days (95% CI)	*p* value	Change in days (95% CI)	*p* value	*Int p*	Change in z-score (95% CI)	*p* value	Change in z-score (95% CI)	*p* value	*Int p*
Co	**-1.4 (-2.6, -0.2)**	**0.02***	**-2.3 (-4.3, -0.2)**	**0.03***	0.52	0.1 (0.0, 0.2)	0.16	0.1 (-0.1, 0.2)	0.45	0.92
Cs	-0.2 (-1.5, 1.2)	0.80	-0.4 (-3, 2.1.0)	0.76	0.87	0.1 (0.0, 0.2)	0.21	0.0 (-0.2, 0.3)	0.73	0.76
Cu	-0.5 (-1.3, 0.3)	0.24	-0.2 (-2.3, 1.9)	0.85	0.84	0.0 (0.0, 0.1)	0.48	-0.1 (-0.3, 0.1)	0.36	0.27
Mn	-0.8 (-2.2, 0.6)	0.27	**-3.3 (-5.7, -0.9)**	**0.01****	**0.09***	0.0 (-0.1, 0.2)	0.41	0.1 (-0.1, 0.3)	0.42	0.79
Ni	0.0 (-1.1, 1.1)	0.98	1.9 (-0.1, 3.9)	0.06	0.11	0.0 (-0.1, 0.1)	0.41	0.1 (-0.1, 0.3)	0.32	0.65
Zn	-0.8 (-1.6, 0.0)	0.05	-0.4 (-2.7, 1.9)	0.72	0.76	0.0 (-0.1, 0.1)	0.91	0.1 (-0.1, 0.3)	0.54	0.59
As[a]	1.2 (-1.0, 3.5)	0.28	1.5 (-2.2, 5.2)	0.42	0.91	0.1 (-0.1, 0.3)	0.40	0.0 (-0.3, 0.3)	0.82	0.52
Cd[a]	-0.5 (-2.8, 1.8)	0.67	-3.4 (-7.2, 0.4)	0.08	0.20	-0.2 (-0.3, 0.0)	0.12	0.5 (0.1, 0.8)	0.01	0.00
Hg	0.9 (-0.6, 2.3)	0.24	-0.5 (-3, 2.1)	0.71	0.37	0.0 (-0.1, 0.2)	0.46	0.1 (-0.1, 0.3)	0.52	0.83
Pb	**-1.8 (-3.0, -0.5)**	**0.01****	-2.2 (-4.9, 0.6)	0.13	0.89	0.1 (0.0, 0.2)	0.17	0.0 (-0.2, 0.2)	0.96	0.56

Metals	**Preterm birth (overall)**					**Preterm birth (spontaneous)**				
	SS-Social Support (>25th)		**SS-Social Support (<=25th)**			**SS-Social Support (>25th)**		**SS-Social Support (<=25th)**		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	1.26 (0.92, 1.74)	0.15	1.56 (0.89, 2.75)	0.12	0.55	1.36 (0.90, 2.04)	0.14	1.94 (0.96, 3.92)	0.07	0.46
Cs	1.04 (0.71, 1.52)	0.83	1.74 (0.79, 3.86)	0.17	0.25	0.93 (0.60, 1.45)	0.76	2.61 (0.98, 6.96)	0.06	**0.06***
Cu	1.35 (0.94, 1.93)	0.10	1.05 (0.54, 2.03)	0.89	0.54	1.27 (0.79, 2.06)	0.33	1.03 (0.50, 2.12)	0.94	0.67
Mn	1.11 (0.75, 1.63)	0.60	**3.73 (1.54, 9.06)**	**0.003****	**0.01****	1.17 (0.72, 1.89)	0.52	**5.2 (1.62, 16.68)**	**0.01***	**0.03***
Ni	1.10 (0.82, 1.46)	0.52	**0.33 (0.16, 0.67)**	**0.002****	**0.002****	1.14 (0.79, 1.64)	0.50	**0.37 (0.16, 0.82)**	**0.01***	**0.01***
Zn	**1.93 (1.25, 2.99)**	**0.003****	1.30 (0.61, 2.77)	0.50	0.36	1.54 (0.89, 2.68)	0.12	1.23 (0.54, 2.79)	0.63	0.67
As[a]	0.77 (0.42, 1.43)	0.41	0.47 (0.15, 1.52)	0.21	0.46	0.61 (0.27, 1.38)	0.23	0.55 (0.14, 2.15)	0.39	0.90
Cd[a]	0.81 (0.44, 1.50)	0.50	2.00 (0.52, 7.76)	0.32	0.23	0.72 (0.32, 1.61)	0.42	0.66 (0.23, 1.87)	0.98	0.98
Hg	1.06 (0.71, 1.57)	0.78	1.25 (0.56, 2.79)	0.59	0.71	1.43 (0.84, 2.45)	0.19	1.04 (0.44, 2.46)	0.93	0.60
Pb	**1.53 (1.03, 2.27)**	**0.03***	2.33 (0.99, 5.49)	0.05	0.43	1.27 (0.74, 2.18)	0.38	2.50 (0.88, 7.06)	0.08	0.27

Metals	**SGA**					**LGA**				
	SS-Social Support (>25th)		**SS-Social Support (<=25th)**			**SS-Social Support (>25th)**		**SS-Social Support (<=25th)**		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*Int p*
Co	0.89 (0.63, 1.25)	0.49	0.91 (0.48, 1.72)	0.78	0.93	1.08 (0.76, 1.55)	0.66	1.09 (0.73, 1.62)	0.67	0.90
Cs	0.78 (0.58, 1.03)	0.08	1.11 (0.49, 2.54)	0.80	0.37	1.02 (0.70, 1.50)	0.91	1.27 (0.69, 2.35)	0.44	0.54
Cu	0.96 (0.83, 1.11)	0.61	**2.16 (1.01, 4.63)**	**0.05***	**0.04***	1.03 (0.81, 1.31)	0.81	0.84 (0.52, 1.34)	0.46	0.44
Mn	0.79 (0.55, 1.12)	0.19	1.13 (0.60, 2.13)	0.70	0.36	0.90 (0.63, 1.30)	0.58	**2.16 (1.07, 4.34)**	**0.03***	**0.03***
Ni	**0.67 (0.48, 0.93)**	**0.02***	0.65 (0.32, 1.32)	0.23	0.89	0.94 (0.69, 1.28)	0.69	1.18 (0.69, 2.00)	0.55	0.47
Zn	1.00 (0.83, 1.20)	0.96	0.98 (0.49, 1.98)	0.96	0.97	1.00 (0.82, 1.23)	0.97	1.32 (0.69, 2.53)	0.40	0.43
As[a]	0.93 (0.52, 1.66)	0.80	0.41 (0.13, 1.30)	0.13	0.22	1.19 (0.65, 2.18)	0.58	0.70 (0.27, 1.81)	0.46	0.36
Cd[a]	0.91 (0.51, 1.64)	0.76	0.41 (0.13, 1.28)	0.13	0.22	0.65 (0.36, 1.20)	0.17	1.67 (0.56, 4.96)	0.36	0.14
Hg	0.90 (0.61, 1.33)	0.60	0.85 (0.39, 1.85)	0.67	0.86	1.11 (0.77, 1.61)	0.58	1.32 (0.66, 2.62)	0.43	0.67
Pb	0.91 (0.67, 1.25)	0.57	0.98 (0.45, 2.13)	0.97	0.87	1.02 (0.71, 1.46)	0.93	0.75 (0.37, 1.53)	0.43	0.45

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestational age (SGA); large for gestational age (LGA).

[a] As, Cd were compared between two categories of above LOD and below LOD

*p value <0.05 considered significant for effect estimates, **indicate q value <0.1& p value <0.05

*int p (interaction p value) <0.10 considered significant for interaction metal*psychosocial status, **indicate q value <0.1& *Int* p <0.1

Table IV.10 Change in birth outcomes associated with geometric mean exposure biomarker concentration within three groups of overall negative, neutral, and positive life events (LES). Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration[a]. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

	Gestational age								
Metals	**LES-Life Events (negative)**			**LES-Life Events (neutral)**			**LES-Life Events (positive)**		
	Change in days (95% CI)	*p* value	*int p*[a]	Change in days (95% CI)	*p* value	*Int p*[b]	Change in days (95% CI)	*p* value	*Int p*[c]
Co	0.2 (-1.8, 2.3)	0.83	0.51	**-2.0 (-3.7, -0.3)**	**0.02***	0.12	-0.8 (-2.9, 1.3)	0.47	0.38
Cs	0.9 (-1.8, 3.7)	0.51	0.16	0.5 (-1.8, 2.8)	0.67	0.79	-1.4 (-3.3, 0.4)	0.13	0.18
Cu	-0.3 (-2.5, 1.8)	0.77	0.96	-0.9 (-2.7, 1.0)	0.35	0.78	-0.5 (-1.4, 0.5)	0.35	0.71
Mn	-1.0 (-3.4, 1.4)	0.42	0.97	-0.9 (-3.1, 1.4)	0.46	0.89	-0.9 (-3.0, 1.1)	0.38	0.86
Ni	1.3 (-1.0, 3.6)	0.27	0.30	1.6 (-0.1, 3.3)	0.06	0.73	-0.2 (-2.0, 1.5)	0.79	0.13
Zn	-0.6 (-2.8, 1.6)	0.60	0.98	-1.6 (-3.9, 0.7)	0.17	0.63	-0.6 (-1.5, 0.3)	0.20	0.48
As[d]	3.6 (-0.4, 7.6)	0.08	0.46	0.7 (-2.6, 3.9)	0.69	0.27	1.5 (-2.1, 5.1)	0.41	0.73
Cd[d]	-2.1 (-6.2, 1.9)	0.30	0.70	0.1 (-3.2, 3.4)	0.95	0.40	-1.1 (-4.8, 2.7)	0.58	0.64
Hg	0.5 (-2.4, 3.3)	0.76	0.90	1.0 (-1.0, 3.0)	0.31	0.72	0.2 (-2.3, 2.7)	0.86	0.60
Pb	-2.2 (-4.9, 0.4)	0.10	0.38	-2.5 (-4.7, -0.3)	**0.03***	0.75	-0.7 (-2.7, 1.3)	0.47	0.21
	Birthweight z-score								
	LES-Life Events (negative)			**LES-Life Events (neutral)**			**LES-Life Events (positive)**		
	Change in z-score (95% CI)	*p* value	*int p*[a]	Change in z-score (95% CI)	*p* value	*Int p*[b]	Change in z-score (95% CI)	*p* value	*Int p*[c]
Co	0.0 (-0.2, 0.2)	0.94	0.70	0.1 (0.0, 0.2)	0.09	0.28	0.0 (-0.1, 0.2)	0.63	0.50
Cs	0.1 (-0.2, 0.3)	0.60	0.84	0.1 (-0.1, 0.3)	0.22	0.73	0.0 (-0.1, 0.2)	0.68	0.52
Cu	0.0 (-0.2, 0.2)	0.97	0.99	0.1 (-0.1, 0.2)	0.44	0.64	0.0 (-0.1, 0.1)	0.95	0.48
Mn	0.1 (-0.1, 0.2)	0.59	0.47	0.1 (-0.1, 0.3)	0.20	0.67	0.0 (-0.2, 0.1)	0.63	0.22
Ni	-0.1 (-0.3, 0.1)	0.47	0.12	0.1 (-0.1, 0.2)	0.29	0.22	0.1 (0.0, 0.2)	0.12	0.72
Zn	0.0 (-0.2, 0.1)	0.81	0.82	0.0 (-0.1, 0.2)	0.60	0.61	0.0 (-0.1, 0.1)	0.98	0.64
As[d]	-0.1 (-0.4, 0.2)	0.42	0.15	0.0 (-0.2, 0.3)	0.82	0.44	0.2 (-0.1, 0.5)	0.20	0.43
Cd[d]	0.1 (-0.2, 0.5)	0.36	0.76	-0.1 (-0.4, 0.1)	0.40	0.21	0.1 (-0.2, 0.4)	0.59	0.33
Hg	-0.1 (-0.3, 0.2)	0.63	**0.07***	0.0 (-0.1, 0.2)	0.95	0.67	0.2 (0.0, 0.4)	**0.03***	0.11
Pb	0.1 (-0.1, 0.3)	0.31	0.45	0.1 (-0.1, 0.2)	0.46	0.81	0.0 (-0.1, 0.2)	0.92	0.61

Table IV.10 Continued

	Preterm birth (overall)								
Metals	**LES-Life Events (negative)**			**LES-Life Events (neutral)**			**LES-Life Events (positive)**		
	OR (95% CI)	*p* value	*int p*[a]	OR (95% CI)	*p* value	*Int p*[b]	OR (95% CI)	*p* value	*Int p*[c]
Co	1.44 (0.92, 2.24)	0.11	0.14	1.26 (0.86, 1.83)	0.24	0.59	0.87 (0.52, 1.46)	0.60	0.26
Cs	1.21 (0.63, 2.35)	0.57	0.76	1.08 (0.61, 1.90)	0.79	0.77	1.39 (0.77, 2.49)	0.27	0.51
Cu	1.13 (0.68, 1.87)	0.64	0.44	1.40 (0.88, 2.23)	0.15	0.65	1.60 (0.92, 2.77)	0.09	0.68
Mn	1.59 (0.95, 2.64)	0.08	0.54	1.10 (0.64, 1.90)	0.73	0.30	1.20 (0.70, 2.06)	0.51	0.77
Ni	0.86 (0.48, 1.55)	0.62	0.37	**0.56 (0.36, 0.88)**	**0.01***	0.20	1.19 (0.80, 1.75)	0.39	**0.01***
Zn	1.40 (0.81, 2.42)	0.22	0.22	**1.90 (1.03, 3.49)**	**0.04***	0.64	**2.46 (1.28, 4.72)**	**0.01***	0.38
As[d]	0.92 (0.35, 2.42)	0.86	0.35	0.89 (0.41, 1.93)	0.76	0.95	0.49 (0.20, 1.21)	0.12	0.33
Cd[d]	1.52 (0.55, 4.23)	0.42	0.15	1.11 (0.50, 2.45)	0.79	0.63	0.56 (0.23, 1.36)	0.20	0.26
Hg	1.45 (0.74, 2.83)	0.28	0.46	0.96 (0.59, 1.57)	0.88	0.35	1.04 (0.56, 1.91)	0.91	0.85
Pb	1.79 (0.98, 3.27)	0.06	0.31	**2.02 (1.18, 3.44)**	**0.01***	0.62	1.15 (0.65, 2.06)	0.63	0.13
	Preterm birth (spontaneous)								
	LES-Life Events (negative)			**LES-Life Events (neutral)**			**LES-Life Events (positive)**		
	OR (95% CI)	*p* value	*int p*[a]	OR (95% CI)	*p* value	*Int p*[b]	OR (95% CI)	*p* value	*Int p*[c]
Co	**1.67 (1.04, 2.69)**	**0.03***	0.26	1.15 (0.67, 1.96)	0.61	0.29	1.11 (0.53, 2.30)	0.78	0.90
Cs	1.38 (0.63, 3.04)	0.42	0.89	0.80 (0.37, 1.74)	0.57	0.32	1.25 (0.64, 2.43)	0.52	0.38
Cu	1.35 (0.69, 2.64)	0.37	0.93	1.25 (0.68, 2.32)	0.48	0.81	1.31 (0.66, 2.60)	0.44	0.89
Mn	**1.94 (1.07, 3.50)**	**0.03***	0.29	1.08 (0.56, 2.10)	0.81	0.24	1.12 (0.50, 2.50)	0.79	0.97
Ni	0.74 (0.42, 1.31)	0.30	0.07	**0.34 (0.17, 0.70)**	**0.003****	0.15	1.45 (0.94, 2.25)	0.09	**<0.001****
Zn	1.05 (0.57, 1.92)	0.88	0.13	1.59 (0.75, 3.38)	0.22	0.43	**2.60 (1.04, 6.51)**	**0.04***	0.44
As[d]	0.57 (0.19, 1.77)	0.33	0.94	0.78 (0.26, 2.33)	0.66	0.70	0.54 (0.17, 1.72)	0.30	0.65
Cd[d]	1.66 (0.49, 5.57)	0.41	0.31	1.54 (0.49, 4.83)	0.45	0.93	0.70 (0.22, 2.18)	0.54	0.33
Hg	1.47 (0.70, 3.10)	0.31	0.84	0.92 (0.46, 1.84)	0.82	0.36	1.64 (0.79, 3.42)	0.19	0.26
Pb	1.47 (0.76, 2.87)	0.26	0.66	1.90 (0.88, 4.13)	0.10	0.63	1.17 (0.57, 2.40)	0.67	0.38

Table IV.10 Continued

Metals	LES-Life Events (negative)			LES-Life Events (neutral)			LES-Life Events (positive)		
	OR (95% CI)	*p* value	*int p*[a]	OR (95% CI)	*p* value	*Int p*[b]	OR (95% CI)	*p* value	*Int p*[c]
SGA									
Co	1.27 (0.84, 1.91)	0.91	0.67	1.27 (0.84, 1.91)	0.15	0.42	1.27 (0.84, 1.91)	0.63	0.21
Cs	1.13 (0.67, 1.89)	0.98	0.48	1.13 (0.67, 1.89)	0.37	0.56	1.13 (0.67, 1.89)	0.16	0.93
Cu	1.16 (0.73, 1.84)	0.40	0.29	1.16 (0.73, 1.84)	0.49	0.74	1.16 (0.73, 1.84)	0.54	0.36
Mn	1.32 (0.82, 2.14)	0.13	**0.01***	1.32 (0.82, 2.14)	0.06	0.90	**1.32 (0.82, 2.14)**	**0.04***	**0.01***
Ni	1.04 (0.70, 1.54)	0.76	0.08	**1.04 (0.70, 1.54)**	**0.05***	**0.03***	**1.04 (0.70, 1.54)**	**0.04***	0.83
Zn	2.06 (1.17, 3.62)	0.94	0.87	2.06 (1.17, 3.62)	0.43	0.59	2.06 (1.17, 3.62)	0.73	0.43
As[d]	0.61 (0.28, 1.35)	0.72	0.25	0.61 (0.28, 1.35)	0.52	0.50	0.61 (0.28, 1.35)	0.21	0.56
Cd[d]	0.78 (0.35, 1.71)	0.77	0.64	0.78 (0.35, 1.71)	0.55	0.88	0.78 (0.35, 1.71)	0.34	0.71
Hg	1.60 (0.96, 2.67)	0.76	0.77	1.60 (0.96, 2.67)	0.78	0.93	1.60 (0.96, 2.67)	0.44	0.67
Pb	1.68 (1.00, 2.83)	0.07	0.07	1.68 (1.00, 2.83)	0.47	0.37	1.68 (1.00, 2.83)	0.44	0.29
LGA									
	LES-Life Events (negative)			**LES-Life Events (neutral)**			**LES-Life Events (positive)**		
	OR (95% CI)	*p* value	*int p*[a]	OR (95% CI)	*p* value	*Int p*[b]	OR (95% CI)	*p* value	*Int p*[c]
Co	1.18 (0.88, 1.60)	0.69	0.24	1.18 (0.88, 1.60)	0.81	0.64	1.18 (0.88, 1.60)	0.23	0.39
Cs	1.15 (0.76, 1.74)	0.38	0.48	1.15 (0.76, 1.74)	0.67	0.67	1.15 (0.76, 1.74)	0.99	0.75
Cu	1.15 (0.80, 1.66)	0.19	0.22	1.15 (0.80, 1.66)	0.72	0.20	1.15 (0.80, 1.66)	0.95	0.79
Mn	1.17 (0.80, 1.72)	0.86	0.37	1.17 (0.80, 1.72)	0.74	0.72	1.17 (0.80, 1.72)	0.32	0.53
Ni	0.96 (0.70, 1.30)	0.57	0.82	0.96 (0.70, 1.30)	0.13	0.14	0.96 (0.70, 1.30)	0.33	**0.08***
Zn	2.16 (1.37, 3.42)	0.75	0.81	2.16 (1.37, 3.42)	0.91	0.87	2.16 (1.37, 3.42)	0.92	0.96
As[d]	0.68 (0.36, 1.28)	0.93	1.00	0.68 (0.36, 1.28)	0.92	0.90	0.68 (0.36, 1.28)	0.93	0.89
Cd[d]	0.70 (0.37, 1.30)	0.32	0.20	0.70 (0.37, 1.30)	0.28	0.15	0.70 (0.37, 1.30)	0.41	0.98
Hg	1.18 (0.77, 1.82)	0.47	0.27	1.18 (0.77, 1.82)	0.17	0.16	1.18 (0.77, 1.82)	0.40	0.86
Pb	1.73 (1.14, 2.61)	0.97	0.52	1.73 (1.14, 2.61)	0.06	0.16	1.73 (1.14, 2.61)	0.39	**0.06***

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestational age (SGA); large for gestational age (LGA).

[a] interaction between negative and positive categories

[b] interaction between neutral and negative categories

[c] interaction between positive and neutral categories

[d] As, Cd were compared between two categories of above LOD and below LOD

**p* value <0.05 considered significant for effect estimates, **indicate *q* value <0.1& *p* value <0.05

*int *p* (interaction p value) <0.10 considered significant for interaction metal*life events categories, **indicate *q* value <0.1& *Int p* <0.1

Chapter V

Performance of Urine, Blood, and Integrated Metal Biomarkers in Relation to Birth Outcomes in a Mixture Setting

Abstract

Background: Studies on the health effects of metal mixtures typically utilize biomarkers measured in a single biological medium, such as blood or urine. However, the ability to evaluate mixture effects are limited by the uncertainty whether a unified medium can fully capture exposure for each metal. Therefore, it is important to compare and assess metal mixtures measured in different media in epidemiology studies. **Objective:** The aim of this study was to examine the mixture predictive performance of urine and blood metal biomarkers and integrated multi-media biomarkers in association with preterm birth. **Methods**: In our analysis of 847 women from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) study, we measured 10 essential and non-essential metals in repeated and paired samples of urine and blood during pregnancy. For each metal, we integrated exposure estimates from paired urine and blood biomarkers into multi-media biomarkers (MMBs), using intraclass-correlation coefficient (ICC) and weighted quantile sum (WQS) approaches. Using Ridge regressions, four separate Environmental risk scores (ERSs) for metals in urine, blood, MMB_{ICC}, and MMB_{WQS} were computed as a weighted sum of the 10 metal concentrations. We then examined associations between urine, blood, and multi-media biomarker ERSs and preterm birth using logistic regressions, adjusting for maternal age, maternal education, pre-pregnancy body mass index (BMI), and second-hand smoke exposure. The performance of each ERS was evaluated with continuous and tertile estimates and 95% confidence intervals of the odds ratio of preterm birth using area under the curve (AUC). **Results**: Pb was the most important contributor of blood ERS as well as the two integrated multi-media biomarker ERSs. Individuals with high ERS (3^{rd} tertile) showed increased odds of preterm birth compared to individuals with low ERS (1^{st} tertile), with 2.8-fold (95% CI, 1.49 to 5.40) for urine (specific gravity corrected); 3.2- fold (95% CI, 1.68 to 6.25) for blood; 3.9-fold (95% CI, 1.72 to 8.66) for the multi-media biomarkers composed using

ICC; and 5.2-fold (95% CI, 2.34 to 11.42) for multi-media biomarkers composed using WQS. The four ERSs had comparable predictive performances (AUC ranging from 0.64 to 0.68) when urine is examined with specific gravity corrected concentrations; the performances were also significantly better than the performance of urine ERS without accounting for specific gravity. **Conclusions**: Within a practical metal panel, measuring metals in either urine or blood may be an equally good approach to evaluate the metals as a mixture, but only when the urine measurements are corrected for urinary dilution. Applications in practical study design require validation of these methods with other cohorts, larger panels of metals and also within the context of other adverse health effects of interest.

1. Introduction

Exposure biomonitoring, which estimates human exposure by measuring chemical or other agents of interest or their metabolic products in different biologic media, such as blood and urine [1], has become a fundamental approach used in exposure assessment and environmental epidemiology [2]. With growing interest in the realistic scenario of studying the collective effects of environmental chemicals on humans, including metals [3-11], biomonitoring has become indispensable in studies of mixtures. Due to limiting factors such as financial cost and methodologic challenges, mixture studies based on biomarkers typically use a unified human specimen (i.e. blood, urine, etc.) to determine exposure to various chemicals [12-17]. While this approach may capture overall exposure to a class of chemicals with similar structure and pharmacokinetic properties, such as urinary phthalates and blood perfluorinated compounds (PFCs), it is more challenging to evaluate chemical classes such as metals. Because each metal possesses different pharmacokinetic properties, utilizing one medium for measuring metal mixtures may not represent exposure for each metal or accurately reflect overall human exposure. Moreover, for different metals, each medium may also represent a different window of exposure that provides important information in relation to the health outcome of interest.

A set of biomarkers reflecting integrated metal mixture information from multiple media not only reduces the error in the exposure estimation, but also captures different exposure sources and pathways. Thus, it may be appropriate to combine exposure from different media to assess human exposure to both single metal and metal mixtures. Previous studies have proposed different

techniques to integrate biomarkers of exposure to single chemicals, including confirmatory factor analysis [18, 19], structural equation models [19-21], and the derivation of multi-media biomarkers (MMBs) through mixture methods: non-negative matrix factorization (NMF), independent component analysis (ICA) and weighted quantile sum (WQS) regression [22]. A few studies have modeled metal mixtures measured in multiple matrices simultaneously and demonstrated that a combination of different metal biomarker factors may improve the prediction of health outcomes [23, 24]. Those studies have validated techniques to select the most important biomarker for each exposure individually, which has provided useful information for recommending a more suitable biomarker for a single metal. However, to our knowledge, no epidemiological study has evaluated the overall performance of metal mixtures measured in different media in association with health outcomes. Therefore, our goals were to assess whether data on metal mixture exposures measured using different media can be integrated and compare the performances of different matrices when measuring metal mixtures.

To achieve this goal, we conducted the following study. First, we proposed ways to integrate multi-media exposure information from biomarkers measured in different media. Second, we assessed the performance of metal mixtures measured in different media and the combined multi-media exposure as related to health outcomes. We chose adverse birth outcomes as our outcome of interest because exposure to metals impacts various biological pathways that contribute to adverse birth outcomes, including preterm delivery and low birthweight [4, 12, 25-37]. Limited mixture studies on this topic have mostly focused on metals measured in either urine or blood [4-8]. In the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) study, we measured a wide range of metals in paired urine and blood samples, which enabled us to compare the associations between adverse birth outcomes and urinary or blood metal mixtures, as well as integrated metal mixtures utilizing both matrices. We hypothesized that the use of urine, blood, and the integrated metal mixtures would demonstrate differing performance when modeling adverse birth outcomes, informing more efficient study designs for exposure assessment.

2. Methods

2.1 Study population

This study used data collected from 847 pregnant women participating in the PROTECT study, an ongoing, prospective birth cohort [38-41]. The PROTECT study was launched in 2010 with funding from the National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program to investigate Puerto Rico's high preterm birth rate and the extent of hazardous waste contamination on the island. PROTECT aims to explore environmental exposures and other factors contributing to preterm birth risk and other adverse birth outcomes in Puerto Rico.

Study participants were recruited at approximately 14 ± 2 weeks of gestation at seven prenatal clinics and hospitals throughout Northern Puerto Rico and followed until delivery. [38, 39]. Pregnant women included in the study were aged between 18 to 40 years, resided inside of the Northern Karst aquifer region, and were planning to deliver in the participating hospitals. Exclusion criteria have been described elsewhere [42]. Each woman participated in a total of up to three study visits during 18 ± 2 weeks, 22 ± 2 weeks, and 26 ± 2 weeks of gestation. At the initial visit, detailed information on medical and pregnancy history was collected. Nurse-administered questionnaires were used to gather information on housing characteristics, employment status, and family situation at an in-home visit (22 ± 2 weeks). Spot urine samples were collected from women at up to three visits and blood samples were collected during the first and third visits. A total of 847 women who delivered a live singleton birth had available data on 10 paired urine and blood metal biomarkers (collected at the same time point) as well as information on covariates (**Figure V.1**).

The research protocol was approved by the Ethics and Research Committees of the University of Puerto Rico and participating clinics, the University of Michigan, Northeastern University, and the University of Georgia. The study was described in detail to all participants, and informed consent was obtained prior to study enrollment.

2.2 Measurement of metals

Spot urine was collected in sterile polypropylene cups and aliquoted within one hour after collection, while blood samples were collected in metal-free whole blood tubes. All samples were frozen and stored at -80°C and shipped on dry ice. Analysis was performed at NSF International (Ann Arbor, MI, USA), where concentrations of 16 metals and metalloids were measured in both

urine and blood: arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), cesium (Cs), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), titanium (Ti), uranium (U), vanadium (V), and zinc (Zn); an additional 5 metals and metalloids were measured in urine only: molybdenum (Mo), platinum (Pt), antimony (Sb), tin (Sn), and tungsten (W). Metal(loid) concentrations were measured using inductively coupled plasma mass spectrometry (ICPMS) as described previously [4]. The laboratory selected the appropriate isotopes for the requested elements to best avoid interferences from high levels of carbon and chloride in the biological sample matrix. The ICPMS was calibrated with a blank and a minimum of 4 standards for each element of interest. An R^2 value of >0.995 was the minimum criterion for an acceptable calibration curve. The calibration curves were verified by initial checks at three calibration points within the curve. Continuing calibration checks and blanks after every 10 samples were also utilized throughout the analytical run to ensure the ICPMS system was maintaining acceptable performance. Urinary specific gravity (SG) was measured at the University of Puerto Rico Medical Sciences Campus using a hand-held digital refractometer (Atago Co., Ltd., Tokyo, Japan) as an indicator of urine dilution.

2.3 Preterm birth and auxiliary birth outcomes

All birth outcome data were extracted from medical records. We used the American Congress of Gynecologists (ACOG) recommendations to calculate gestational age at birth [43], as previously described in detail [44, 45]. In this study, preterm birth was defined as delivery < 37 completed weeks of gestation, as per common practice. Preterm birth can be classed into two groups, based on the clinical presentation of preterm delivery: medically indicated preterm birth (preterm births with preeclampsia, or with both artificial membrane rupture and induced labor) and spontaneous preterm birth (presentation of premature rupture of the membranes, spontaneous preterm labor, or both). In our analysis, the outcome of interest was overall and spontaneous preterm birth.

Other birth outcomes, including gestational age, and fetal growth outcomes [birthweight z-score, small for gestational age (SGA), and large for gestational age (LGA)], were also included in our analysis as auxiliary outcomes. INTERGROWTH-21st standard gestational age- and sex-specific birthweight z-scores were constructed and used in the analysis [46]. SGA and LGA births were

defined as below the 10th percentile and above the 90th percentile of birthweight z-scores, respectively.

2.4 Data pre-processing for statistical analyses

To account for urinary dilution, metal(loid) concentrations in urine were corrected for SG using the equation: $P_c = P[(SG_p - 1)/(SG_i - 1)]$; where P_c is the SG corrected biomarker concentration (ng/mL), P is the measured biomarker concentration, SG_p is the median urinary specific gravity in this population (1.019), and SG_i is the individual's urinary specific gravity. Metal concentrations below the limit of detection (LOD) were replaced by LOD/√2. Metals that were measured in paired media (urine and blood) and had at least 50% of samples with concentrations above the LOD in both matrices were included in statistical analysis.

Descriptive statistics were calculated for all exposures and outcome variables. We applied natural logarithmic transformation to all urinary and blood metals because the distributions were right-skewed prior to transformation. The geometric averages of participant concentrations across the visits were calculated for each urinary and blood metal. Spearman's rank correlations (r_s) were used for the analysis of the relationships between paired urinary and blood metal concentrations. Data were analyzed using R version 3.6.2. A schematic representation of the data accumulation and analytic procedure is also presented in **Figure 1**.

2.5 MMB composition

2.5.1 Integrating multi-media biomarker (MMB) using intraclass correlation coefficient (ICC)

Characterizing the within- and between-individual variation of measurements with parameters such as intraclass correlation coefficient (ICC) gives information on the reliability of the different media biomarkers. ICC is defined as the proportion of the total variance that is attributed to between-individual variability:

$$[1]\ \mathrm{ICC} = \frac{\sigma_b 2}{\sigma_b 2 + \sigma_w 2}$$

In epidemiological studies with repeated measurements, the ICC metric, ranging from 0 to 1, indicates reliability among multiple measurements of a quantity; when close to 0, ICC reflects

large variations between repeated measures, a.k.a. poor ability to distinguish between individuals with high and low exposure levels; when close to 1, the repeated measures are close to each other which reflects a good ability to discriminate between individuals with high and low exposure levels. In this unsupervised machine learning approach, we utilized the ICCs calculated from repeated measurements of urinary and blood metals [17] as weighting parameters to construct an integrated multi-media biomarker (MMB) separately for each metal using equations [2], [3]:

$$[2]\ w_{urine[ICC]} = \frac{\mathrm{ICC}_{urine}}{\mathrm{ICC}_{urine} + \mathrm{ICC}_{blood}}\ ;$$

$$[3]\ \mathrm{MMB}_{ICC} = w_{urine[ICC]}\mathrm{C}_{urine} + (1 - w_{urine[ICC]})\mathrm{C}_{blood}\ ,$$

where w is the weight of a medium and C is the metal concentration.

2.5.2 Integrating multi-media biomarker (MMB) using weighted quantile sum regression (WQS)

In addition to the unsupervised learning method, the amount of exposure information each biomarker carries can be quantified simultaneously based on the relationship of the exposure measured in a certain medium and health effect--a supervised approach. Therefore, the second approach for integrating urinary and blood biomarkers was weighted quantile sum regression (WQS), which models the body burden of quantiles of exposure. WQS estimates a set of weights, such that the linear combinations of the weights * quantile biomarkers have the highest association with the outcome [47] (equation [4]). Details of the WQS equation and annotations are previously described [48]. In our analysis, w_i is the unknown weight for the *i*th medium (1=urine, 2=blood, c=2), z represents a vector of adjusted covariates, and ϕ is a vector of regression coefficients for those covariates. By placing the constraints of the weight (w_i) estimates to be non-negative and sum to 1, the comparative values of urine and blood metals to multi-media biomarkers and the joint effect can be determined. The weights can then be used to quantify the contribution of each medium to the multi-media biomarker. In this supervised learning approach, we used WQS (100 bootstraps) to determine the association between each birth outcome and urinary and blood biomarkers of each metal, separately. Then we combined the weights to generate multi-media biomarkers using equation [5].

$$[4]\ g(\mu) = \beta_0 + \beta_1\left(\sum_{i=1}^{c} w_i q_i\right) + z'\phi \quad (0 \le w_i \le 1, \sum_{i=1}^{c} w_i = 1);$$

$$[5]\ \mathrm{MMB}_{WQS}=w_{urine[WQS]}\,\mathrm{C}_{urine}+w_{blood[WQS]}\mathrm{C}_{blood}\ .$$

2.6 Single-pollutant Analysis

Generalized linear models (GLM) were used to examine the associations between four types of metal biomarkers measured and composed (urinary, blood, MMB_{ICC}, and MMB_{WQS}) and birth outcomes. Separate models were used for each metal biomarker and outcome. The full models included the tertiles of metal biomarker concentrations and a final set of covariates that were selected based on a priori knowledge and whether their inclusion appreciably changed the effect estimates of metal exposure [49]. These covariates were maternal age, maternal education level, pre-pregnancy BMI, and exposure to second-hand smoking. Effect estimates and 95% confidence intervals were calculated for the highest versus the lowest tertiles of exposure to measure the risk stratification properties of individual metals and compare them to the collective effects of metal mixtures as described below.

2.7 Mixture Analysis

2.7.1 Construction of Environmental Risk Scores (ERSs) using Ridge regression

We constructed Environmental Risk Scores (ERSs) as weighted summary measures of the effects of metals where the weights were regression coefficients derived from models of the association between metal mixtures and the outcome of interest. We utilized Ridge regression to guide the weight of each metal in relation to preterm birth. Ridge regression is a regularized regression technique and it is one of the commonly used supervised machine learning solutions. Ridge is used to constrain the size of the estimated coefficients, and the objective function for a continuous outcome can be expressed as:

$$[6]\ \hat{\beta}_{Ridge}=\underset{(\beta_0,\beta)\in\mathbb{R}^{p+1}}{\arg\min}\ \sum_{i=1}^{n}(y_i-\beta_0-x_i^T\beta)^2+\lambda\|\beta\|_2^2\ ,$$

where $i=1,\ldots,n$ indexes the subjects, $x_i^T\in\mathbb{R}^p$ is the vector of p covariates for the ith subject, and y_i is the continuous health outcome for the ith subject. Ridge regression utilizes the regularization penalty parameter λ ($\lambda\in[0,\infty)$) to solve the multicollinearity problem and control

the shrinkage of the L2 penalty. Ridge regression decreases the complexity of the models and enforces the β coefficients to be lower without forcing them to be zero. This was ideal as our analytic purpose was to evaluate the same full set of metals as mixtures across different media and integrated biomarkers. Using Ridge regression with an underlying model including biomarkers of 10 metals and covariates, we performed 10-fold cross-validation and selected the value that minimized the cross-validated sum of squared residuals. Four separate ERSs for metals in urine, blood, MMB_{ICC}, and MMB_{WQS} were computed as a weighted sum of the 10 metal concentrations (C):

$$[7]\ ERS = \sum_{i=1}^{10} \beta_i C_i \ .$$

2.7.2 ERS models and evaluations

We further categorized ERSs by tertiles and refit the regression models with both continuous and categorical ERSs to examine its associations with preterm birth as well as the auxiliary birth outcomes. We conceptualized the ERSs as a weighted sum of metal exposure measured in urine, blood, and multi-media biomarkers composed by WQS and ICC methods, namely, ERS_{urine}, ERS_{blood}, $ERS_{MMB\text{-}WQS}$, and $ERS_{MMB\text{-}ICC}$. ROC (Receiver Operating Characteristics) curves were used to evaluate the preterm, spontaneous preterm birth, SGA, and LGA classification model performances of four ERSs. Specifically, the area under curve (AUC) of ROC were computed for quantifying and visualizing the biomarkers' classification accuracy for the above-mentioned binary outcomes. We used a bootstrap resampling (2000 iterations) to compute 95% confidence intervals of AUCs for different models [50] and to test the difference between AUCs (the ci.auc() and roc.test() functions in the pROC package in R [51]). For binary outcome models with ERS tertiles, we also computed the odds ratio (OR) for the highest tertile versus the lowest tertile to measure the risk stratification properties of ERS.

3. Results

3.1 Descriptive statistics

Demographic characteristics of the 847 women in this analysis are summarized in **Table V.1** and were described previously [41, 52]. Briefly, the cohort included women in their late 20s (median =27 years) and half of them had a BMI less than 25 kg/m^2 prior to pregnancy. The majority of women (57%) had private medical insurance, were non-smokers (86%) and very few (6%)

reported alcohol consumption within the last few months. More than half reported an annual household income of less than $30,000, while 44% had reported graduating from college or higher. **Table V.2** displays descriptive statistics, including geometric mean, geometric standard deviation, and selected percentiles, of 10 metal concentrations measured in the paired urine and blood samples, as well as Spearman correlation coefficients between two media for each metal. Most of the paired metal concentrations in the two matrices had a low but significant correlation, with Spearman correlation coefficient ranging from 0.07 to 0.43, while Mn, Ni, and Zn concentrations measured in urine and blood were not correlated. All the following results on urinary metals are presented for SG-corrected concentrations unless described otherwise.

3.2 MMB composition

The weights of urinary and blood metals in the composition of MMBs from ICC and WQS approaches are depicted in **Figure V.2**. As the ICC approach is based on an unsupervised learning method, the metal biomarker weights are the same across the birth outcomes. In contrast, the WQS approach is a supervised learning method, therefore, the weights constructed for each of the metal biomarkers were different for the respective birth outcomes. The corresponding urinary and blood weights (WQS) for each birth outcome are presented in **Table V.3,** while **Figure V.2** focuses on weights constructed from WQS models regressing preterm birth. For the majority of metals, blood was the main contributor to the MMBs from both ICC and WQS approaches. The blood weights for those metals were higher from the WQS approach than the ICC approach, except for Mn and Pb where the blood weights were higher from the ICC approach (60% and 88%) than the WQS approach (56% and 72%). In contrast, MMB for As was mostly attributed to urine from the WQS approach (95%).

3.2 ERSs

ERS weights derived from Ridge models regressing preterm and spontaneous preterm birth on metal mixtures are shown in **Figure V.3**. The values of the weights for all the outcomes are provided in **Table V.4**. The largest contributors to preterm birth ERS from urine mixture were Cd (-0.01), Ni (-0.008), and As (-0.006). For preterm birth ERSs constructed from the blood, MMB_{ICC}, and MMB_{WQS} mixtures, Pb and Mn were the largest positive weight contributors for all three. A similar weight distribution was observed for spontaneous preterm birth. The preterm birth ERSs

from each biomarker mixture were normally distributed and ranged from −0.06 to 0.04 for urine; −0.13 to 0.88 for blood; −0.001 to 0.11 for MMB_{ICC}; and 0.08 to 0.48 for MMB_{WQS}. For preterm birth ERSs, pairwise correlations among urine ERS and other ERSs were weak (r<0.2), whereas blood ERS had relatively higher correlations with the ERSs for MMB_{ICC} (r=0.88) and MMB_{WQS} (r=0.53). The weight distribution for spontaneous preterm birth ERSs were similar to overall preterm birth ERS weights.

3.3 Continuous ERSs and birth outcomes

The result from our primary analyses of continuous ERSs and preterm birth are presented in **Figure V.4.** To illustrate the difference in the urine biomarker performance between disregarding versus accounting for urine dilution, we reported the odds ratio associated with both uncorrected and SG-corrected urine ERSs. Therefore, the performance was compared between metal mixtures measured in uncorrected urine, SG-corrected urine, blood, MMB_{ICC}, and MMB_{WQS}. As shown in **Figure V.4**, all the ERSs were significantly associated with increased odds of preterm birth except for uncorrected urine metal ERS. Although not significant, the odds of preterm birth was 1.3 times higher for a subject in the 75th percentile of exposure, as determined via ERS for uncorrected urine metals, compared with a subject in the 25th percentile of exposure (95% CI: 0.94 to 1.86). The odds ratios for other ERS associations were greater than the odds ratio of uncorrected urine metal ERS, ranging from 1.81 (95% CI: 1.27 to 2.59) to 2.00 (95% CI: 1.45 to 2.75). Changes in auxiliary birth outcomes associated with ERSs are shown in **Table V.5**. When spontaneous preterm birth is regressed on the four ERSs, odds ratios were generally higher compared to the overall preterm birth models, ranging from 2.34 (95% CI: 1.53, 3.56) to 2.56 (95% CI: 1.58, 4.14). For fetal growth outcomes, all ERSs were significantly associated with lower birthweight z-scores; the associations between ERSs and SGA were stronger (OR: 1.54 to 1.99) than the associations between ERSs and LGA (OR: 1.32 to 1.51) (**Table V.5)**.

The *p* values in **Figure V.4** represent the significance of the test results for comparing the predictive performances (preterm birth) of different ERSs using AUC. While the consistent performance of SG-corrected urine, blood, and MMB biomarkers were observed, the uncorrected urine ERS showed lower prediction performance than SG-corrected urine (p=0.04), blood ERS (p=0.02), MMB_{ICC} ERS (p=0.003), and MMB_{WQS} ERS (p=0.07). This is visualized by the AUC

plots depicting the performance of different ERSs and preterm birth models (**Figure V.5**). **Figure V.5 (a)** shows that the area under the uncorrected urine ERS curve (AUC = 0.61; 95%CI = 0.54–0.68) is smaller than the blood ERS (AUC = 0.68; 95%CI = 0.62–0.74), MMB_{ICC} ERS (AUC = 0.67; 95%CI = 0.61–0.73), and MMB_{WQS} (AUC = 0.68; 95%CI = 0.62–0.74). **Figure V.5 (b)** shows that there are no obvious differences in the AUC between SG-corrected urine, blood, and two MMB ERSs. Predictive performances of ERS on other binary outcomes followed similar patterns; performances of SG-corrected urine, blood, and MMB biomarkers were comparable, with AUC ranging from 0.66 to 0.69 for spontaneous preterm birth, from 0.60 to 0.65 for SGA, and from 0.60 to 0.62 for LGA; uncorrected urine ERS showed substantially lower prediction performance for spontaneous preterm birth, but not SGA and LGA.

Because Pb was the most important contributor of preterm birth blood ERS as well as the two MMB ERSs, we conducted additional analyses excluding Pb from Ridge models while constructing and evaluating the performance of the ERSs. The effect estimates from this analysis for all four (urine, blood, MMB_{ICC}, MMB_{WQS}) ERS were attenuated compared to the primary analyses (**Table V.6**). Continuous blood ERS was no longer significantly associated with preterm birth (OR/IQR=1.03, 95% CI 0.77 to 1.37, p=0.83). The effect estimates for urine ERS was 1.73 (95% CI 1.22 to 2.43, p=0.002), MMB_{ICC} was 1.83 (95% CI 1.23 to 2.54, p<0.001), and MMB_{WQS} was 1.76 (95% CI 1.29 to 2.41, p<0.001). The AUCs for these ERSs without Pb are shown in Figure 5(c). Although the performance of SG corrected urine (blue line) and blood ERSs (red line) are comparable, the area is significantly smaller for the blood ERS model compared to the MMB_{ICC} (p=0.01) and MMB_{WQS} models (p=0.02).

3.4 Tertile metals, ERSs, and birth outcomes

ORs of preterm birth comparing the highest versus the lowest tertiles of individual metal biomarkers and ERSs are shown in **Figure V.6**. After adjusting for covariates, individual associations for Mn (MMBs), Ni (urine), Zn (blood, MMBs), Cd (urine), Pb (blood, MMBs), and odds of preterm were significant. Ni and Cd biomarkers were associated with lower odds of preterm birth while Mn, Zn, and Pb were associated with higher odds of preterm birth. For example, a subject in Ni tertile 3 had 0.76 times lower odds of preterm birth (95% CI, 0.57 to 1) compared with a subject in Ni tertile 1. In contrast, a subject in Pb tertile 3 had 1.53 times higher

odds of preterm birth (95% CI 1.14 to 2.06) compared with a subject in Pb tertile 1. As for ERS models, ORs of preterm birth ranged from 2.83 (95% CI, 1.49 to 5.40) for urine; 3.24 (95% CI, 1.68 to 6.25) for blood; 3.86 (95% CI, 1.72 to 8.66) for MMB_{ICC}; and 5.17 (95% CI, 2.34 to 11.42) for MMB_{WQS}, after controlling for the same set of covariates. These ORs from the mixture analysis were considerably stronger than those for individual metals. Both individual and mixture analysis results for spontaneous preterm birth and auxiliary outcomes can be found in **Tables V.5** and **V.7**.

4. Discussion

Epidemiologic studies aiming to determine the effects of environmental chemical mixtures on human health are growing rapidly. Due to limiting factors such as the financial cost and methodologic challenges, mixture studies based on biomarkers typically use a unified human specimen, such as blood or urine to determine exposure to various chemicals [12-17]. Although this approach applies well to chemicals with similar structure and pharmacokinetics, it is challenging to accurately describe metal mixtures using one unified medium. Each metal exhibits unique physiochemical properties and toxicokinetics, such as half-life, storage, or elimination rate from the body. As such, the preference for either blood or urine concentration as a better indicator is different across metals, often determined by the half-life of each metal and cost of measurement. For example, urinary concentration of As has often been used as an indicator of recent exposure because urine is the main route of excretion of most arsenic species [53, 54]. In contrast, blood is the preferred specimen for Pb, as blood Pb has a longer half-life and subsequently lower variability in the body compared to urine [55]. As for other metals such as Mn, Cu, and Cr, there is a lack of consensus in the literature as to which biomarker is the most consistent and valid. Previous mixture studies on prenatal metal exposures and birth outcomes measured metals in different media including urine [4, 56-58], whole blood [7, 59], cord blood [60], toenails [5], and teeth [61]. As mentioned above, each medium depicts biomarker levels in a particular body compartment that may have differential biological relevance and may not fully represent the best measure of internal dose for all the metals. Therefore, it is imperative that we understand how the choice of different media can impact the performance of analyzing chemical mixtures in relation to a specific health outcome. In this paper, we evaluated metals measured in urine and blood, as well as two integrated multi-media biomarker indices, in relation to birth outcomes among pregnant women.

We first applied supervised and unsupervised approaches to combine exposure information from multi-media (urine and blood) metal biomarkers. For each individual metal, the weights for urine and blood from both approaches were generally similar with a few exceptions, most notably with As. When applying the supervised approach with WQS where the relationship of urine and blood biomarkers with the health outcome (preterm birth) is considered, approximately 95% of the As association was driven by urinary As. This result indicates that urinary As is the more important predictor than the blood As in modeling preterm birth. However, the weight for urinary As from the ICC approach was much lower (42%). This difference is mainly due to a lower ICC (0.21) for the repeated measurements of urinary As in this study [17]. To our knowledge, only one previous study on pregnant women reported an ICC (0.16) for urinary As during pregnancy, which is similar to the ICC calculated in the present study [62]. These ICCs indicate weak reliability of urinary As during pregnancy, while reports on the general population demonstrated fair reliability of urinary As (ICC ranging from 0.45 to 0.49) over a longer period of time (1-2 years) [63, 64]. The discrepancy was possibly due to the physiological changes related to the pregnancy (i.e. metabolic changes, plasma volume expansion) [65-68], and unique environmental and behavioral factors such as dietary habits unique to this population.

Once we constructed MMBs, we used Ridge models to guide the weights of each metal biomarker in constructing the ERSs. Examining uncorrected metal mixture concentrations resulted in a significantly lower performance in urine ERS compared to the other ERSs (SG-corrected urine, blood, and the integrated MMBs) that had comparable level of performances amongst themselves. These results indicate that consideration of measuring metal mixtures in either urine or blood may be an equally good approach when correcting urinary metals for SG. Although the optimal urine concentration adjustment approach for metals remains uncertain [69-71], our findings underline the importance of considering urinary dilution when evaluating the health effects of a metal mixture measured in urine. This conclusion is supported by previous literature validating the improved robustness and reliability of physiological measures for correcting variation in urinary output [72-76].

In Ridge models using blood, MMB_{ICC}, and MMB_{WQS}, Pb was most strongly associated with preterm birth. This is consistent with our previous finding within this population where

concentrations of blood Pb among pregnant women in Puerto Rico (average=0.33 μg/dL) were well below the level of concern set by CDC, a blood level of 5 μg/dL for pregnant women [77]. Yet Pb was the most significant predictor of preterm birth [49]. Therefore, when we conducted the same analysis for all the biomarker mixtures excluding Pb, the association between blood ERS and preterm birth were no longer significant. The performance of blood ERS was also significantly lower than the MMB_{ICC} (p=0.01) and MMB_{WQS} (p=0.02) models. However, the performance of urine and blood ERSs was still comparable in that the addition of urine biomarker information significantly improved the performance of blood biomarkers but not vice versa. These findings shed light on the importance of studying Pb in metal mixtures, especially blood biomarkers, as the performance of blood ERS was mainly driven by the strong effect of Pb. The findings also warrant further studies of different metal panels in regard to mixture performance.

After analyzing individual and ERS tertiles with preterm birth, we observed a few significant but overall smaller effects corresponding to individual metals and stronger effects corresponding to the four combined ERSs, indicating the cumulative impact of the individual metals (**Figure 6**). This is consistent with previous literature where stronger cumulative effects of metals and environmental chemicals were reported when analyzed as mixtures [6, 78, 79]. After adjusting for covariates, the odds ratio of preterm birth comparing a subject in the higher end of overall metals exposure (ERS tertile 3) with a subject in the lower end (ERS tertile 1) were 2.8 for urine (95% CI, 1.49 to 5.40), 3.2 for blood (95% CI, 1.68 to 6.25), 3.9 for MMB_{ICC} (95% CI, 1.72 to 8.66), and 5.2 for MMB_{WQS} (95% CI, 2.34 to 11.42). Assuming these odds ratios quantify the potential for risk stratification of preterm birth, the integrated multi-media biomarker models resulted in a higher risk of preterm birth associated with overall metal exposure. From a risk stratification perspective, integration of urine and blood biomarkers that were derived from both ICC and WQS approaches improved the model performance in the mixture models compared to the sole urine or blood biomarker models. Although the confidence intervals for these odds ratio estimates were wider, the MMB integration, especially using the WQS approach, resulted in substantially higher effect estimate. This finding supports that while multiple measurements of the exposure mixture may measure metal body burden differentially, there may still be room for improvement for exposure measurement error structure and effect estimate when incorporating urinary and blood biomarker information.

Specific strengths of our study include its longitudinal design in which repeated urinary and blood biomarkers provided more accurate exposure information during pregnancy. This allowed us to quantify the temporal reliability of the two media measurements (ICCs), which were further used as weights to integrate the multi-media measure of exposure. Secondly, this study utilized data-driven machine learning approaches to 1) inform the composition of multi-media metal biomarkers measured in different media and 2) guide the construction of environmental risk scores for each medium reflecting the overall exposure to the metal mixture. Finally, this study evaluated the health effects of multiple metals simultaneously and compared the performance of different media biomarkers and integrated biomarkers in relation to adverse birth outcomes in the context of a mixture. The results lay the groundwork for future epidemiological studies on biomarker selection when examining the mixture effects of metals.

However, our study has several limitations. The relatively small sample size did not allow cross-validation within this population on ERS, which may have caused overfitting of ERS. Future studies with a larger sample size should implement training and test datasets to cross-validate ERSs. Studies with larger sample sizes are also needed to address potential improvement of models by including non-linear terms and interactions between metals and covariates that were not accounted for in the current analysis. Depending on the pharmacokinetics of each chemical within a family, mixture studies, in general, are challenged by the complications of combining chemical biomarkers where each may be representing a unique window of exposure. While our ERS estimates also suffered from this limitation, by combining multi-media exposure using both urine and blood biomarkers, we were able to reduce the measurement error in the mixture analysis to an extent. In addition, while we evaluated a mixture of 10 essential and non-essential metals, it is possible that other metals that were not assessed in our study affect the performance of urine or blood biomarkers. Future work is needed to expand the mixture evaluation to include more metals and other biospecimens including hair and saliva, as well as other adverse health outcomes because our results may not be generalizable to other outcomes of interest. Finally, while the mixture of metals in this study is representative of exposures experienced by Puerto Rican populations, it may not accurately reflect exposure profiles for other populations.

5. Conclusion

Our study used innovative methodology to provide new detailed insights into the individual and integrated associations of urinary and blood mixture biomarkers of metal exposure with birth outcomes. Our investigation demonstrates, within practical metal analytical panels, that measuring metals in either urine or blood may be an equally good approach to evaluate the metals as a mixture, but only when the urine measurements are corrected for urinary dilution. The results of our study elucidate the importance of considering the overall mixture performance of a certain medium. Future studies are needed to expand to evaluate the performance with different metal panels, media, health outcomes of interest, and methods to integrate exposure information, to further address how to most effectively study the health impacts of exposure to mixtures.

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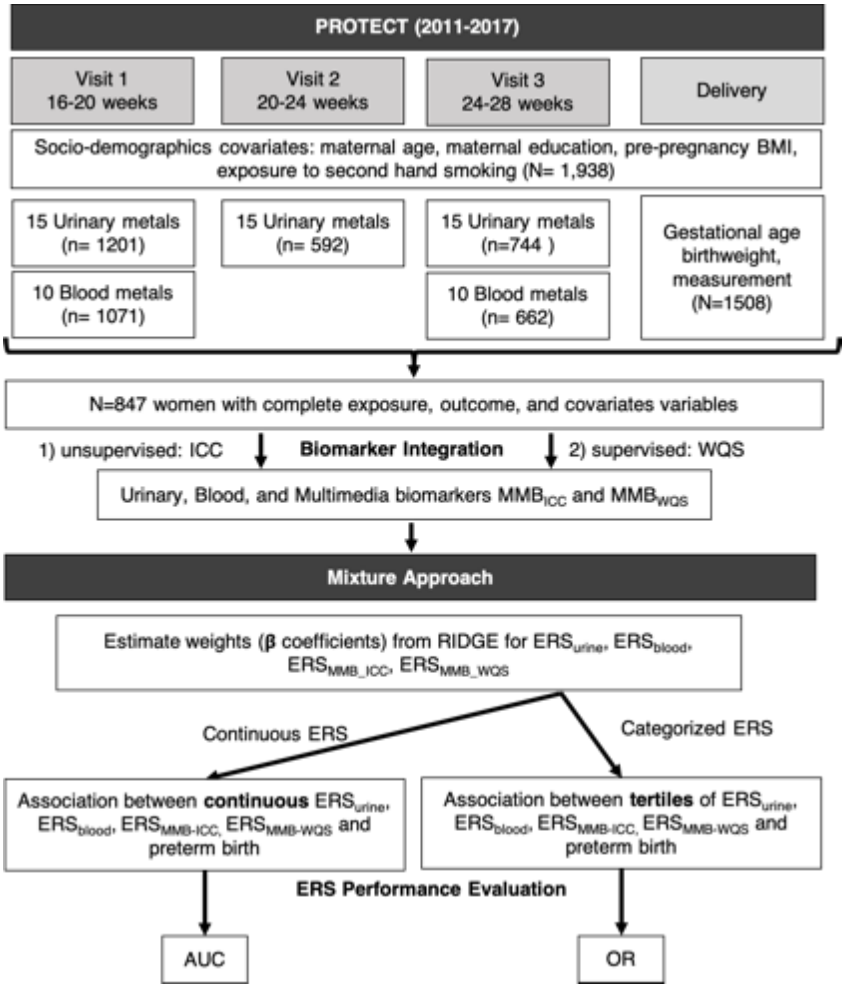
Figure V.1 Schematic plot of study design, sample size, and statistical methods for constructing and evaluating multi-media biomarker (MMB) and Environmental Risk Score (ERS)



Abbreviations: intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS); area under the curve (AUC); odds ratio (OR).

Table V.1 Demographic characteristics of study participants from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort (n=847)

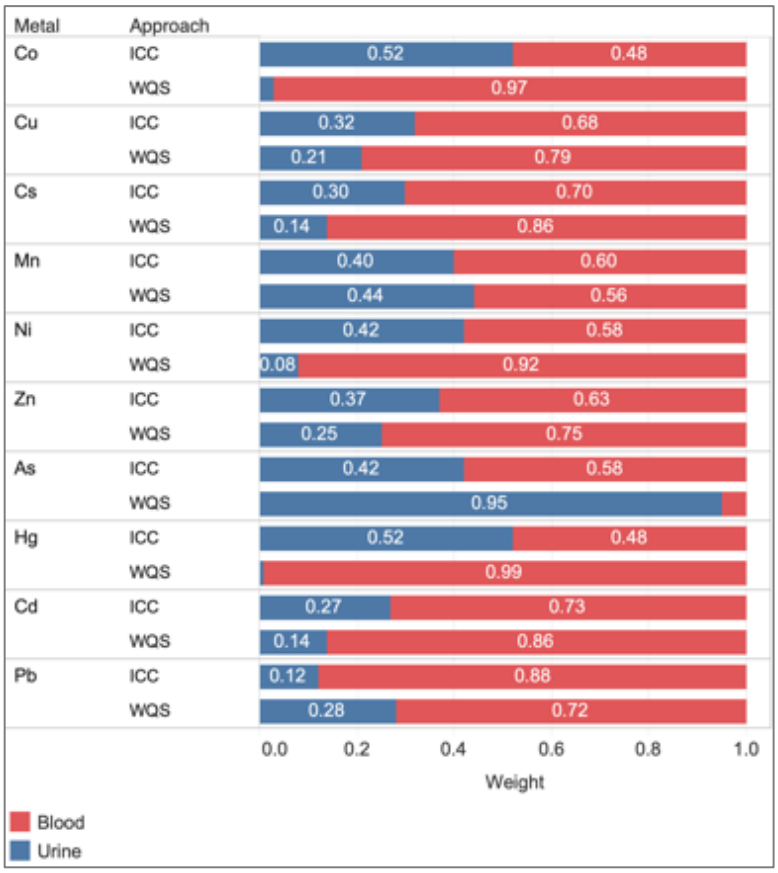
Variable	Mean (SD)
Maternal age at enrollment (years)	26.9 (5.6)
gravidity (# pregnancies)	2 (1)
Insurance type	N (%)
Private	480 (57%)
Public (Mi Salud)	281 (33%)
Missing	86 (10%)
Maternal education (years)	
High school/GED	185 (22%)
Some College or technical school	293 (35%)
College degree	268 (32%)
Masters degree or higher	101 (12%)
Missing	0 (0%)
Income status (US $)	
<$10,000	260 (31%)
≥$10,000 to <$30,000	212 (25%)
≥$30,000 to <$50,000	176 (21%)
≥$50,000	104 (12%)
Missing	95 (11%)
Marital status	
Single	163 (19%)
Married or living together	680 (80%)
Missing	4 (0%)
Gravidity (# pregnancies)	
0	361 (43%)
1	304 (36%)
>1	181 (21%)
Missing	1 (0%)
Pre-pregnancy BMI (kg/m^2)	
≤25	447 (53%)
>25 to ≤30	230 (27%)
>30	170 (20%)
Missing	0 (0%)
Smoking Status	
Never	726 (86%)
Ever	109 (13%)
Current	12 (1%)
Missing	0 (0%)
Exposure to second hand smoking	
None	772 (91%)
Up to 1 hour/day	30 (4%)
More than 1 hour/day	45 (5%)
Missing	0 (0%)
Alcohol consumption	
None	438 (52%)
Before pregnancy	354 (42%)
Within the last few months	50 (6%)
Missing	5 (1%)
Infant Sex	
Female	406 (48%)
Male	437 (52%)
Missing	4 (0%)

Table V.2 Distribution of 10 metal(loid)s in urine and blood (ng/ml) in 847 pregnant women from Puerto Rico in 2011–2017

Metal (loid)	Specimen	N	%>LOD	GM	GSD	25%	50%	75%		Max	r[d]
Co	Urine[a]	632	100	1.1	1.6	0.80	1.1	1.5	2.8	16.4	0.30**
	Blood[b]	948	98.2	0.35	1.4	0.29	0.34	0.41	0.57	1.6	
Cs	Urine[a]	632	100	5.5	1.4	4.4	5.6	6.9	9.8	18.4	0.43**
	Blood[b]	948	99.9	1.1	1.4	0.9	1.2	1.4	1.9	2.9	
Cu	Urine[a]	632	99.3	15.2	1.5	11.5	14.8	19.3	32.1	109	0.23**
	Blood[b]	948	99.9	1568	1.3	1408	1583	1756	2103	3798	
Mn	Urine[a]	632	100	1.0	2.4	0.83	1.2	1.6	2.6	6.7	-0.01
	Blood[b]	948	99.9	11.2	1.4	9.0	11.3	13.9	19.1	90.7	
Ni	Urine[a]	632	98.9	5.4	1.8	3.8	5.4	7.9	13.4	127	-0.06
	Blood[b]	948	96.4	1.0	1.6	0.79	1.0	1.3	2.3	22.8	
Zn	Urine[a]	632	100	309	2.1	204	330	521	878	2136	0.07*
	Blood[b]	948	99.9	4720	1.3	4288	4793	5322	6102	8043	
As	Urine[a]	632	100	11.3	2.2	6.6	11.0	18.3	41.4	281	0.26**
	Blood[b]	948	48.9	0.33	1.8	<LOD	<LOD	0.47	1.0	7.9	
Cd	Urine[a]	632	74.5	0.13	2.0	0.08	0.12	0.20	0.48	1.8	0.18**
	Blood[b]	947	60.9	0.12	1.6	<LOD	0.11	0.16	0.27	1.3	
Hg	Urine[a]	628	98.6	0.72	2.7	0.37	0.73	1.3	3.5	64.9	0.36**
	Blood[b]	948	99.9	1.2	1.7	0.85	1.2	1.7	3.0	10.6	
Pb[c]	Urine[a]	632	72.1	0.25	2.4	<LOD	0.26	0.44	1.1	4.6	0.28**
	Blood[b]	948	99.9	3.3	1.6	2.5	3.3	4.3	6.4	21.8	

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); limit of detection (LOD); geometric mean (GM); geometric standard deviation (GSD).
[a] Includes SG-corrected urinary metal concentrations for up to 3 repeated samples per woman (n = 1601 samples)
[b] Includes blood metal concentrations for up to 2 repeated samples per woman (n = 1217 samples)
[c] Blood Pb concentration unit is µg/dL
[d] Spearman correlation coefficient calculated for blood and urine metal concentrations; **P value for the Spearman test<0.01

Figure V.2 Bar graph of estimated urinary and blood biomarker weights for the MMBs using ICC approach and the WQS models of overall preterm birth. Larger weights indicate greater contributions of the original biomarkers to the MMBs



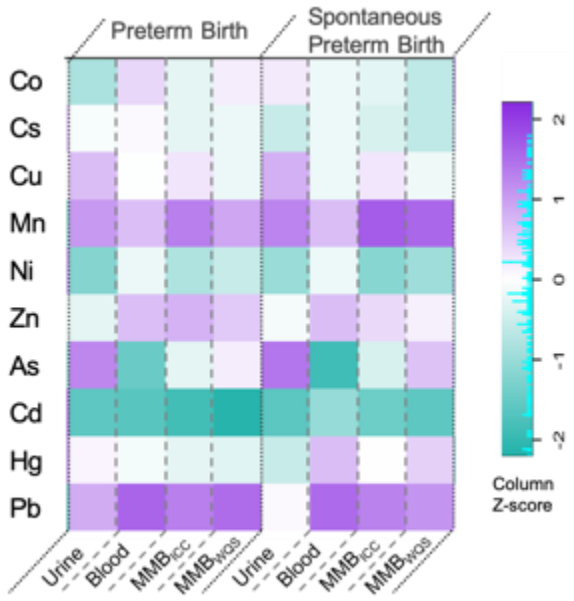
Abbreviations: multi-media biomarkers (MMBs); intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS); cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb).

Table V.3 Estimated biomarker weights for the MMBs using WQS approach regressing birth outcomes on urinary and blood biomarkers

	Gestational age		***Preterm birth***		***Spontaneous preterm birth***	
Metals	urine	blood	urine	blood	urine	blood
Co	0.15	0.85	0.03	0.97	0.32	0.68
Cs	0.13	0.87	0.14	0.86	0.89	0.11
Cu	0.16	0.84	0.21	0.79	0.76	0.24
Mn	0.36	0.64	0.44	0.56	0.00	1.00
Ni	0.21	0.79	0.08	0.92	0.29	0.71
Zn	0.08	0.92	0.25	0.75	0.72	0.28
As	0.94	0.06	0.95	0.05	0.05	0.95
Cd	0.90	0.10	0.14	0.86	0.64	0.36
Hg	0.18	0.82	0.01	0.99	1.00	0.00
Pb	0.36	0.64	0.28	0.72	0.01	0.99
	Birthweight z-score		***SGA***		***LGA***	
Metals	urine	blood	urine	blood	urine	blood
Co	0.13	0.87	0.00	1.00	0.97	0.03
Cs	0.83	0.17	0.54	0.46	0.10	0.90
Cu	0.78	0.22	0.70	0.30	0.16	0.84
Mn	0.34	0.66	0.55	0.45	0.30	0.70
Ni	0.51	0.49	0.29	0.71	0.60	0.40
Zn	0.92	0.08	0.94	0.06	0.08	0.92
As	0.98	0.02	0.69	0.31	0.58	0.42
Cd	0.33	0.67	0.92	0.08	0.99	0.01
Hg	0.53	0.47	0.42	0.58	0.77	0.23
Pb	0.29	0.71	0.21	0.79	0.21	0.79

Abbreviations: multi-media biomarkers (MMBs); weighted quantile sum regression (WQS); cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestational age (SGA); large for gestational age (LGA).

Figure V.3 Heat map of weights for each individual metal biomarker extracted by the Ridge regression models regressing birth outcomes on urinary, blood, and two integrated multi-media biomarkers (MMB)



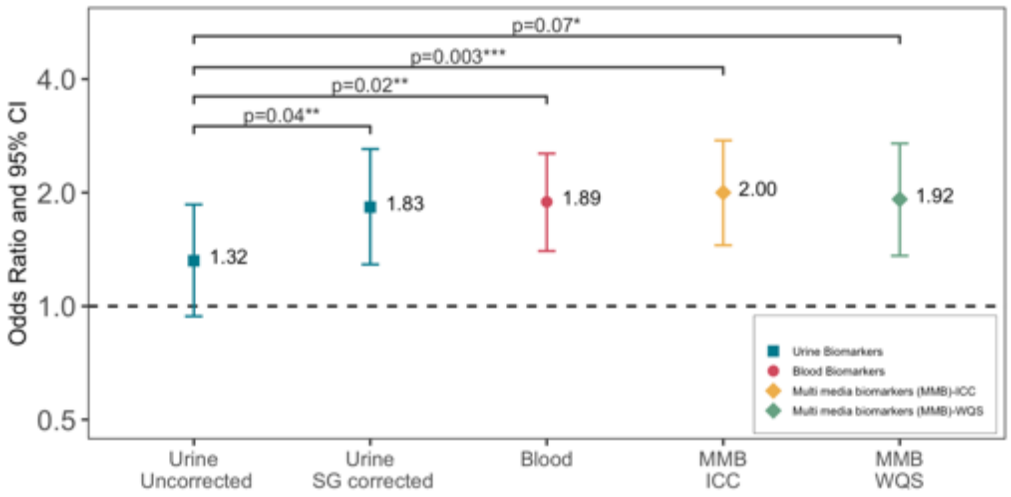
Abbreviations: multi-media biomarkers (MMBs); cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS).

Table V.4 Estimated environmental risk score (ERS) weights (regression coefficient) for metals from each birth outcome Ridge models. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

	Gestational age				***Preterm birth***				***Spontaneous preterm birth***			
Metals	urine	blood	MMB_{ICC}	MMB_{WQS}	urine	blood	MMB_{ICC}	MMB_{WQS}	urine	blood	MMB_{ICC}	MMB_{WQS}
Co	0.40	-0.12	0.062	-0.266	-0.006	0.009	0.000	0.006	-0.001	0.000	-0.002	-0.001
Cs	0.17	0.04	0.117	0.143	-0.002	0.005	0.000	0.003	-0.006	0.000	-0.003	-0.001
Cu	-0.64	-0.03	-0.134	-0.081	0.002	0.004	0.001	0.003	0.003	0.000	0.002	0.002
Mn	-0.31	-0.18	-0.528	-0.646	0.004	0.012	0.003	0.012	0.006	0.001	0.013	0.013
Ni	0.40	0.09	0.329	0.338	-0.008	0.002	-0.001	0.000	-0.009	0.000	-0.009	-0.003
Zn	-0.06	-0.09	-0.167	-0.201	-0.003	0.012	0.002	0.009	-0.003	0.001	0.003	0.004
As	-0.74	0.31	-0.103	-0.636	0.005	-0.013	0.000	0.006	0.007	-0.002	-0.003	0.007
Cd	1.12	0.04	0.457	0.525	-0.010	-0.015	-0.003	-0.013	-0.013	-0.001	-0.011	-0.007
Hg	0.76	0.10	0.465	0.405	-0.001	0.003	0.000	0.002	-0.006	0.001	0.000	0.006
Pb	-0.59	-0.37	-0.596	-0.827	0.003	0.023	0.003	0.017	-0.002	0.002	0.010	0.010
	Birthweight z-score				***SGA***				***LGA***			
Metals	urine	blood	MMB_{ICC}	MMB_{WQS}	urine	blood	MMB_{ICC}	MMB_{WQS}	urine	blood	MMB_{ICC}	MMB_{WQS}
Co	0.004	0.030	0.027	0.029	-0.175	0.001	-0.018	-0.001	-0.003	0.006	0.002	0.006
Cs	0.002	0.023	0.017	0.013	-0.112	-0.005	-0.022	-0.009	0.001	0.003	0.003	0.002
Cu	0.000	0.001	0.002	0.002	0.074	0.001	0.007	0.003	0.000	0.001	0.001	0.001
Mn	0.001	-0.010	-0.001	-0.003	-0.067	0.003	-0.001	0.000	0.001	0.003	0.003	0.004
Ni	0.003	0.012	0.019	0.026	-0.053	-0.002	-0.019	-0.009	0.002	0.001	0.003	0.004
Zn	-0.002	-0.002	-0.003	-0.013	0.035	0.001	0.005	0.001	-0.007	0.001	0.000	-0.009
As	-0.002	0.028	0.007	-0.008	0.063	-0.001	0.004	0.003	0.001	0.008	0.006	0.001
Cd	0.001	0.022	0.017	0.021	0.102	-0.007	-0.012	-0.004	0.003	0.002	0.004	0.005
Hg	-0.003	-0.014	-0.016	-0.020	0.022	0.000	0.004	0.002	-0.002	0.004	0.000	0.000
Pb	0.001	-0.006	-0.004	-0.003	-0.093	0.003	0.007	0.002	0.000	-0.005	-0.004	-0.005

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); small for gestational age (SGA); large for gestational age (LGA); multi-media biomarker (MMB); intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS).

Figure V.4 Odds ratio (OR) of preterm birth associated with Environmental Risk Scores (ERSs) constructed for uncorrected urine, SG-corrected urine, blood, and two integrated multi-media biomarkers (MMB) as well as including both SG-corrected urine and blood ERS. Effect estimates presented as OR for IQR increase in average exposure biomarker concentration. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking. P values are from tests comparing the area under the curves (AUCs), using a bootstrap method



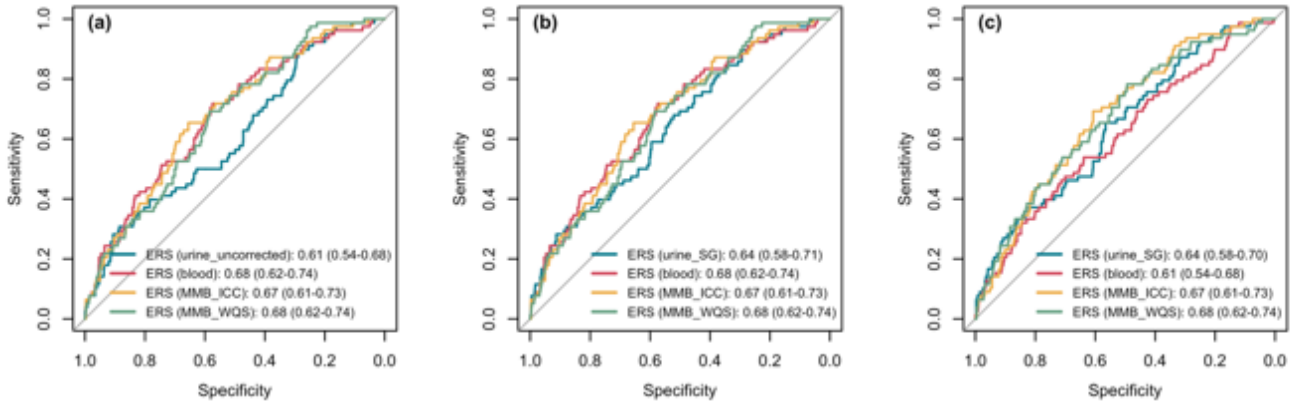
Abbreviations: specific gravity (SG); intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS).

Table V.5 Change in birth outcomes associated with Environmental Risk Score (ERS) constructed for uncorrected urine, SG-corrected urine, blood, and two integrated multi-media biomarkers (MMBs). Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

	Gestational age		***Preterm birth***		***Spontaneous preterm birth***	
ERSs	Change in days (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
$Urine_{uncorrected}$	-3.0 (-4.2, -1.8)	<0.001***	1.32 (0.94, 1.86)	0.11	1.64 (1.04, 2.57)	0.03**
$Urine_{SG}$	-3.1 (-4.4, -1.9)	<0.001***	1.83 (1.29, 2.61)	<0.001***	2.53 (1.58, 4.03)	<0.001***
Blood	-1.8 (-2.9, -0.8)	<0.001***	1.89 (1.40, 2.54)	<0.001***	2.34 (1.53, 3.56)	<0.001***
MMB_{ICC}	-2.2 (-3.3, -1.2)	<0.001***	2.00 (1.45, 2.75)	<0.001***	2.44 (1.55, 3.85)	<0.001***
MMB_{WQS}	-3.2 (-4.4, -2.0)	<0.001***	1.92 (1.36, 2.70)	<0.001***	2.56 (1.58, 4.14)	<0.001***
	Birthweight z-score		***SGA***		***LGA***	
ERSs	Change in z-score (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
$Urine_{uncorrected}$	-0.12 (-0.21, -0.02)	0.02**	1.84 (1.32, 2.57)	<0.001***	1.50 (1.10, 2.05)	0.01**
$Urine_{SG}$	-0.13 (-0.22, -0.03)	0.01**	1.99 (1.41, 2.81)	<0.001***	1.51 (1.11, 2.03)	0.01**
Blood	-0.15 (-0.24, -0.06)	0.001***	1.54 (1.10, 2.15)	0.01**	1.40 (1.05, 1.87)	0.02**
MMB_{ICC}	-0.16 (-0.26, -0.07)	<0.001***	1.64 (1.16, 2.30)	0.005***	1.32 (0.98, 1.79)	0.07*
MMB_{WQS}	-0.14 (-0.24, -0.05)	0.004***	1.70 (1.23, 2.36)	<0.001***	1.53 (1.11, 2.10)	0.01**

Abbreviations: specific gravity (SG); intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS); small for gestational age (SGA); large for gestational age (LGA).

Figure V.5 Area under the curves (AUCs) for preterm birth according to environmental risk score (ERS) constructed for urinary, blood, and two integrated multi-media biomarkers (MMB)



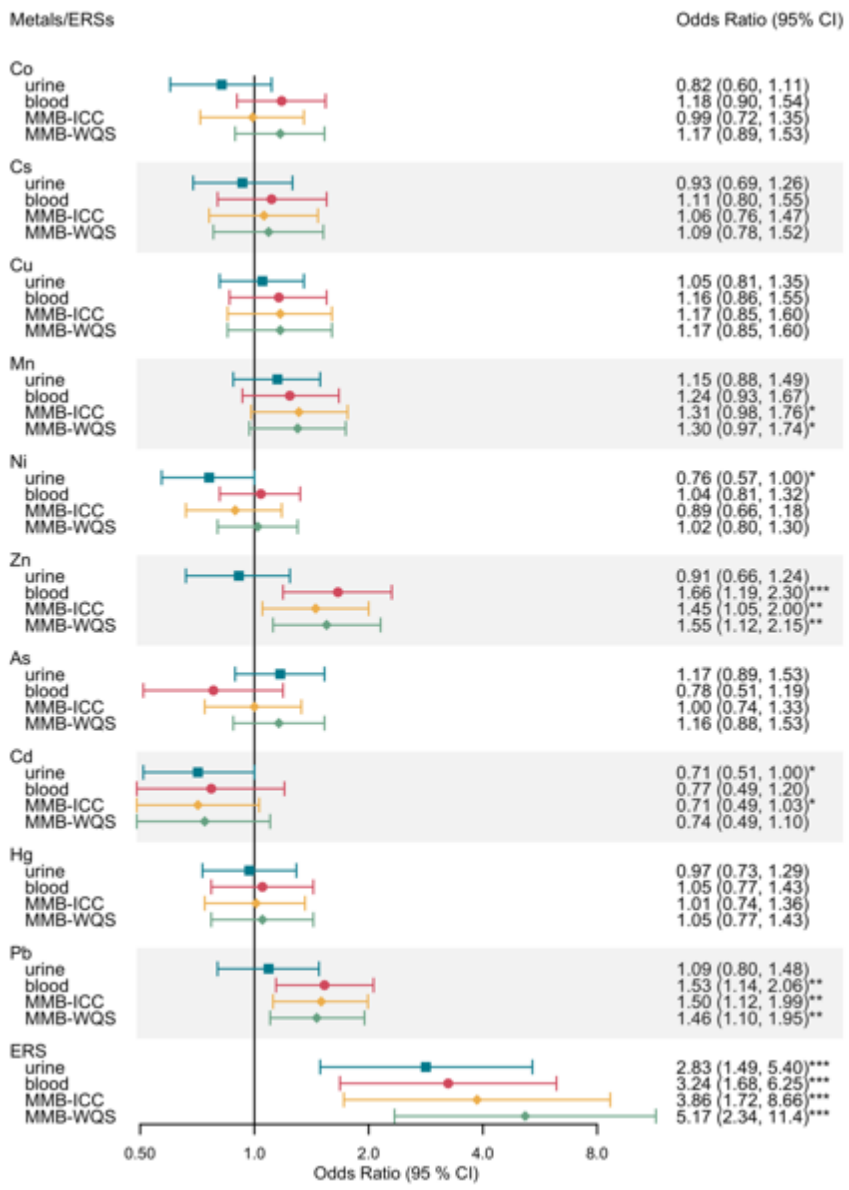
Abbreviations: specific gravity (SG); intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS).

Table V.6 Change in birth outcomes associated with Environmental Risk Score (ERS) constructed for SG-corrected urine, blood, and two integrated multi-media biomarkers (MMBs), excluding Pb in Ridge models. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

	Gestational age		***Preterm birth***		***Spontaneous preterm birth***	
ERSs	Change in days (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
$Urine_{SG}$	-3.0 (-4.3, -1.7)	<0.001***	1.73 (1.22, 2.43)	0.002***	2.65 (1.64, 4.28)	<0.001***
Blood	-1.4 (-2.4, -0.4)	0.01**	1.03 (0.77, 1.37)	0.83	2.19 (1.40, 3.42)	<0.001***
MMB_{ICC}	-1.9 (-3.0, -0.8)	<0.001***	1.83 (1.32, 2.54)	<0.001***	2.13 (1.35, 3.35)	0.001***
MMB_{WQS}	-2.2 (-3.4, -1.0)	<0.001***	1.76 (1.29, 2.41)	<0.001***	1.93 (1.25, 2.97)	0.002***
	Birthweight z-score		***SGA***		***LGA***	
ERSs	Change in z-score (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
$Urine_{SG}$	-0.13 (-0.22, -0.03)	0.01**	1.94 (1.36, 2.77)	<0.001***	1.52 (1.13, 2.06)	0.01**
Blood	-0.11 (-0.20, -0.02)	0.02**	1.52 (1.09, 2.12)	0.01**	0.97 (0.72, 1.31)	0.86
MMB_{ICC}	-0.16 (-0.26, -0.07)	<0.001***	1.58 (1.13, 2.21)	0.01**	1.23 (0.90, 1.69)	0.20
MMB_{WQS}	-0.16 (-0.25, -0.07)	<0.001***	1.59 (1.12, 2.25)	0.01**	1.45 (1.06, 1.97)	0.02**

Abbreviations: specific gravity (SG); intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS); small for gestational age (SGA); large for gestational age (LGA).

Figure V.6 Odds ratio (OR) comparing the highest versus the lowest tertiles of individual metals and environmental risk scores (ERSs) constructed for urine, blood, and two integrated multi-media biomarkers (MMB) mixtures. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking



Abbreviations: intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS); cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb).

Table V.7 Odds ratio (OR) comparing the highest vs. the lowest tertiles of individual metals and environmental risk scores (ERSs) constructed for urine, blood, and two integrated multi-media biomarkers (MMB) mixtures. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking

		Gestational age		***Preterm birth***		***Spontaneous preterm birth***	
Metals	Biomarkers	Change in days (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Co	$Urine_{SG}$	0.6 (-0.6, 1.8)	0.30	0.82 (0.60, 1.11)	0.20	0.95 (0.63, 1.43)	0.81
	Blood	-0.6 (-1.6, 0.5)	0.31	1.18 (0.90, 1.54)	0.24	0.95 (0.65, 1.37)	0.77
	MMB_{ICC}	0.0 (-1.2, 1.3)	0.97	0.99 (0.72, 1.35)	0.93	0.93 (0.61, 1.42)	0.74
	MMB_{WQS}	-0.5 (-1.6, 0.6)	0.34	1.17 (0.89, 1.53)	0.26	0.94 (0.65, 1.38)	0.76
Cs	$Urine_{SG}$	0.3 (-0.9, 1.4)	0.67	0.93 (0.69, 1.26)	0.66	0.77 (0.52, 1.15)	0.20
	Blood	0.0 (-1.2, 1.2)	0.95	1.11 (0.80, 1.55)	0.52	1.00 (0.66, 1.52)	0.99
	MMB_{ICC}	0.2 (-1.1, 1.4)	0.80	1.06 (0.76, 1.47)	0.72	0.90 (0.59, 1.37)	0.62
	MMB_{WQS}	0.1 (-1.1, 1.3)	0.86	1.09 (0.78, 1.52)	0.60	0.96 (0.63, 1.45)	0.83
Cu	$Urine_{SG}$	-0.7 (-1.7, 0.4)	0.20	1.05 (0.81, 1.35)	0.73	1.10 (0.79, 1.53)	0.56
	Blood	-0.3 (-1.0, 0.5)	0.48	1.16 (0.86, 1.55)	0.34	1.10 (0.76, 1.60)	0.62
	MMB_{ICC}	-0.5 (-1.4, 0.4)	0.27	1.17 (0.85, 1.60)	0.34	1.15 (0.76, 1.75)	0.51
	MMB_{WQS}	-0.4 (-1.3, 0.4)	0.34	1.17 (0.85, 1.60)	0.33	1.14 (0.75, 1.72)	0.54
Mn	$Urine_{SG}$	-0.6 (-1.6, 0.3)	0.17	1.15 (0.88, 1.49)	0.30	1.30 (0.90, 1.87)	0.16
	Blood	-1.0 (-2.1, 0.2)	0.11	1.24 (0.93, 1.67)	0.15	1.35 (0.93, 1.98)	0.12
	MMB_{ICC}	-1.2 (-2.3, -0.1)	0.04**	1.31 (0.98, 1.76)	0.07*	1.52 (1.04, 2.24)	0.03**
	MMB_{WQS}	-1.1 (-2.2, -0.1)	0.04**	1.30 (0.97, 1.74)	0.07*	1.52 (1.04, 2.23)	0.03**
Ni	$Urine_{SG}$	0.4 (-0.7, 1.5)	0.46	0.76 (0.57, 1.00)	0.05*	0.70 (0.48, 1.01)	0.06*
	Blood	0.3 (-0.7, 1.2)	0.59	1.04 (0.81, 1.32)	0.77	0.92 (0.66, 1.29)	0.64
	MMB_{ICC}	0.5 (-0.6, 1.6)	0.36	0.89 (0.66, 1.18)	0.41	0.74 (0.50, 1.10)	0.14
	MMB_{WQS}	0.3 (-0.6, 1.3)	0.53	1.02 (0.80, 1.30)	0.89	0.90 (0.64, 1.26)	0.53
Zn	$Urine_{SG}$	-0.1 (-1.4, 1.2)	0.88	0.91 (0.66, 1.24)	0.54	0.89 (0.58, 1.35)	0.57
	Blood	-0.5 (-1.3, 0.2)	0.17	1.66 (1.19, 2.30)	0.003***	1.35 (0.88, 2.08)	0.16
	MMB_{ICC}	-0.5 (-1.4, 0.3)	0.21	1.45 (1.05, 2.00)	0.02**	1.22 (0.80, 1.86)	0.36
	MMB_{WQS}	-0.5 (-1.3, 0.3)	0.20	1.55 (1.12, 2.15)	0.01**	1.28 (0.84, 1.97)	0.25
As	$Urine_{SG}$	-1.2 (-2.3, -0.1)	0.03**	1.17 (0.89, 1.53)	0.27	1.30 (0.92, 1.85)	0.14
	Blood	1.1 (-0.4, 2.5)	0.16	0.78 (0.51, 1.19)	0.24	0.49 (0.26, 0.93)	0.03**
	MMB_{ICC}	0.7 (-0.6, 2.1)	0.75	1.00 (0.74, 1.33)	0.98	0.92 (0.62, 1.36)	0.67
	MMB_{WQS}	0.4 (-1.1, 1.9)	0.04**	1.16 (0.88, 1.53)	0.29	1.28 (0.90, 1.84)	0.17
Cd	$Urine_{SG}$	1.5 (0.2, 2.7)	0.02**	0.71 (0.51, 1.00)	0.05	0.54 (0.34, 0.88)	0.01**
	Blood	0.1 (-1.5, 1.8)	0.87	0.77 (0.49, 1.20)	0.25	0.86 (0.48, 1.55)	0.62
	MMB_{ICC}	0.0 (-1.2, 1.3)	0.30	0.71 (0.49, 1.03)	0.07*	0.69 (0.42, 1.14)	0.15
	MMB_{WQS}	-0.5 (-1.6, 0.6)	0.59	0.74 (0.49, 1.10)	0.14	0.77 (0.45, 1.31)	0.34
Hg	$Urine_{SG}$	1.1 (-0.1, 2.2)	0.07*	0.97 (0.73, 1.29)	0.84	0.80 (0.54, 1.18)	0.27
	Blood	0.4 (-0.8, 1.6)	0.48	1.05 (0.77, 1.43)	0.76	1.32 (0.87, 2.01)	0.20
	MMB_{ICC}	0.9 (-0.3, 2.1)	0.13	1.01 (0.74, 1.36)	0.97	1.00 (0.67, 1.51)	0.99
	MMB_{WQS}	0.4 (-0.8, 1.6)	0.53	1.05 (0.77, 1.43)	0.76	1.31 (0.87, 1.99)	0.20
Pb	$Urine_{SG}$	-0.4 (-1.7, 0.8)	0.50	1.09 (0.80, 1.48)	0.59	0.92 (0.60, 1.39)	0.68
	Blood	-1.6 (-2.8, -0.5)	0.01**	1.53 (1.14, 2.06)	0.01**	1.57 (1.07, 2.29)	0.02**
	MMB_{ICC}	-1.5 (-2.6, -0.4)	0.01**	1.50 (1.12, 1.99)	0.01**	1.51 (1.04, 2.19)	0.03**
	MMB_{WQS}	-1.5 (-2.6, -0.4)	0.01**	1.46 (1.10, 1.95)	0.01**	1.43 (0.98, 2.10)	0.06*
ERS	$Urine_{SG}$	-3 (-4.3, -1.7)	<0.001***	1.73 (1.22, 2.43)	0.002***	2.65 (1.64, 4.28)	<0.001***
	Blood	-1.4 (-2.4, -0.4)	0.01**	1.03 (0.77, 1.37)	0.83	2.19 (1.4, 3.42)	<0.001***
	MMB_{ICC}	-1.9 (-3, -0.8)	<0.001***	1.83 (1.32, 2.54)	<0.001***	2.13 (1.35, 3.35)	0.001***
	MMB_{WQS}	-2.2 (-3.4, -1)	<0.001***	1.76 (1.29, 2.41)	<0.001***	1.93 (1.25, 2.97)	0.002***

Table V.7 Continued

Metals	Biomarkers	***Birthweight z-score***		***SGA***		***LGA***	
		Change in z-score (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Co	$Urine_{SG}$	0.07 (-0.02, 0.17)	0.13	0.66 (0.47, 0.93)	0.02	0.89 (0.65, 1.20)	0.44
	Blood	0.10 (0.02, 0.19)	0.02	1.02 (0.77, 1.36)	0.87	1.23 (0.94, 1.60)	0.13
	MMB_{ICC}	0.12 (0.02, 0.22)	0.02	0.80 (0.57, 1.10)	0.17	0.98 (0.74, 1.29)	0.87
	MMB_{WQS}	0.11 (0.02, 0.20)	0.01	0.98 (0.73, 1.30)	0.88	1.19 (0.92, 1.55)	0.18
Cs	$Urine_{SG}$	0.04 (-0.05, 0.14)	0.39	0.77 (0.56, 1.05)	0.10	1.03 (0.77, 1.38)	0.83
	Blood	0.08 (-0.02, 0.17)	0.12	0.83 (0.63, 1.09)	0.17	1.10 (0.81, 1.50)	0.55
	MMB_{ICC}	0.08 (-0.02, 0.18)	0.11	0.77 (0.57, 1.04)	0.08	1.17 (0.87, 1.56)	0.30
	MMB_{WQS}	0.05 (-0.04, 0.15)	0.28	0.75 (0.54, 1.03)	0.07	1.05 (0.78, 1.41)	0.76
Cu	$Urine_{SG}$	0.01 (-0.08, 0.09)	0.88	1.07 (0.83, 1.40)	0.60	1.01 (0.79, 1.29)	0.93
	Blood	0.01 (-0.05, 0.07)	0.68	1.09 (0.83, 1.44)	0.54	1.04 (0.83, 1.29)	0.75
	MMB_{ICC}	0.02 (-0.06, 0.09)	0.67	1.13 (0.82, 1.54)	0.46	1.05 (0.83, 1.33)	0.68
	MMB_{WQS}	0.01 (-0.08, 0.10)	0.78	1.11 (0.83, 1.48)	0.49	1.02 (0.79, 1.33)	0.86
Mn	$Urine_{SG}$	0.02 (-0.06, 0.09)	0.68	0.89 (0.72, 1.10)	0.30	1.03 (0.82, 1.30)	0.78
	Blood	-0.01 (-0.11, 0.08)	0.79	1.13 (0.84, 1.52)	0.43	1.09 (0.81, 1.46)	0.59
	MMB_{ICC}	0.00 (-0.09, 0.09)	0.94	0.99 (0.74, 1.31)	0.93	1.00 (0.78, 1.28)	0.98
	MMB_{WQS}	0.00 (-0.09, 0.09)	0.99	1.01 (0.75, 1.36)	0.94	1.09 (0.82, 1.46)	0.54
Ni	$Urine_{SG}$	0.05 (-0.04, 0.13)	0.28	0.82 (0.62, 1.09)	0.17	1.08 (0.82, 1.42)	0.58
	Blood	0.05 (-0.03, 0.13)	0.19	0.92 (0.72, 1.18)	0.50	1.04 (0.82, 1.31)	0.76
	MMB_{ICC}	0.08 (-0.01, 0.17)	0.08	0.81 (0.60, 1.09)	0.17	1.11 (0.86, 1.43)	0.44
	MMB_{WQS}	0.09 (-0.01, 0.18)	0.08	0.78 (0.56, 1.08)	0.13	1.10 (0.82, 1.49)	0.52
Zn	$Urine_{SG}$	-0.04 (-0.14, 0.06)	0.45	1.02 (0.73, 1.43)	0.89	0.77 (0.58, 1.04)	0.09
	Blood	0.00 (-0.06, 0.07)	0.90	1.07 (0.81, 1.40)	0.64	1.07 (0.82, 1.38)	0.63
	MMB_{ICC}	0.00 (-0.07, 0.06)	0.90	1.07 (0.81, 1.41)	0.64	1.00 (0.83, 1.21)	0.99
	MMB_{WQS}	-0.04 (-0.14, 0.06)	0.46	1.03 (0.74, 1.45)	0.84	0.78 (0.58, 1.05)	0.11
As	$Urine_{SG}$	-0.03 (-0.12, 0.06)	0.48	1.09 (0.82, 1.45)	0.57	1.04 (0.79, 1.36)	0.79
	Blood	0.10 (-0.02, 0.22)	0.11	0.97 (0.66, 1.43)	0.87	1.25 (0.88, 1.79)	0.21
	MMB_{ICC}	0.03 (-0.07, 0.12)	0.56	1.04 (0.77, 1.40)	0.81	1.11 (0.86, 1.43)	0.43
	MMB_{WQS}	-0.03 (-0.12, 0.06)	0.50	1.09 (0.81, 1.47)	0.57	1.04 (0.79, 1.37)	0.77
Cd	$Urine_{SG}$	0.02 (-0.08, 0.12)	0.69	1.18 (0.85, 1.63)	0.33	1.14 (0.84, 1.54)	0.40
	Blood	0.09 (-0.04, 0.23)	0.18	0.73 (0.45, 1.17)	0.19	1.06 (0.70, 1.61)	0.79
	MMB_{ICC}	0.07 (-0.04, 0.18)	0.20	0.88 (0.60, 1.27)	0.49	1.04 (0.76, 1.42)	0.80
	MMB_{WQS}	0.07 (-0.04, 0.18)	0.22	0.91 (0.63, 1.31)	0.61	1.11 (0.80, 1.55)	0.52
Hg	$Urine_{SG}$	-0.06 (-0.15, 0.03)	0.20	1.06 (0.79, 1.42)	0.71	0.92 (0.69, 1.22)	0.54
	Blood	-0.05 (-0.15, 0.05)	0.34	1.02 (0.75, 1.40)	0.90	1.13 (0.84, 1.52)	0.42
	MMB_{ICC}	-0.07 (-0.16, 0.03)	0.17	1.05 (0.77, 1.44)	0.76	1.10 (0.83, 1.45)	0.52
	MMB_{WQS}	-0.07 (-0.16, 0.03)	0.17	1.05 (0.77, 1.43)	0.75	1.01 (0.75, 1.36)	0.96
Pb	$Urine_{SG}$	0.02 (-0.08, 0.12)	0.69	0.83 (0.59, 1.15)	0.26	1.02 (0.75, 1.37)	0.92
	Blood	-0.01 (-0.1, 0.08)	0.82	1.13 (0.83, 1.54)	0.44	0.88 (0.67, 1.14)	0.33
	MMB_{ICC}	-0.01 (-0.1, 0.08)	0.87	1.10 (0.81, 1.48)	0.54	0.87 (0.68, 1.10)	0.25
	MMB_{WQS}	0 (-0.09, 0.09)	0.96	1.05 (0.78, 1.41)	0.76	0.89 (0.68, 1.17)	0.40
ERS	$Urine_{SG}$	-0.13 (-0.22, -0.03)	0.01**	1.94 (1.36, 2.77)	<0.001***	1.52 (1.13, 2.06)	0.01**
	Blood	-0.11 (-0.2, -0.02)	0.02**	1.52 (1.09, 2.12)	0.01**	0.97 (0.72, 1.31)	0.86
	MMB_{ICC}	-0.16 (-0.26, -0.07)	<0.001***	1.58 (1.13, 2.21)	0.01**	1.23 (0.90, 1.69)	0.20
	MMB_{WQS}	-0.16 (-0.25, -0.07)	<0.001***	1.59 (1.12, 2.25)	0.01**	1.45 (1.06, 1.97)	0.02**

Abbreviations: cobalt (Co); cesium (Cs); copper (Cu); manganese (Mn); nickel (Ni); zinc (Zn); arsenic (As); cadmium (Cd); mercury (Hg); lead (Pb); intraclass correlation coefficient (ICC); weighted quantile sum regression (WQS); small for gestational age (SGA); large for gestational age (LGA).

Chapter VI

Conclusions

Summary of findings

This dissertation presents findings from four studies investigating the relationships between metal exposures and adverse birth outcomes. **Figure VI.1** illustrates the main findings and significance of this dissertation. Using novel study designs, I evaluated the predictors of metal profiles measured in different media and their association with adverse birth outcomes, as well as the interactions between metals and maternal psychosocial status. The results provide strong evidence for the relationship between environmental metal exposure during pregnancy and increased risk of adverse pregnancy and fetal growth outcomes, and also suggest a possible role of psychosocial status in modifying these relationships. While additional epidemiological investigations are required, the present dissertation work has potential implications for public health policies and infrastructure design changes aimed at reducing the rates of adverse birth outcomes and their social and economic burdens.

Predictors of maternal metal exposure. The investigation in Aim 1 characterized the metal(loid) biomarker variation over time and between matrices and explored important exposure sources and predictors among 1040 study participants from the PROTECT cohort. Distributions, trends, correlations, predictors of urinary and blood metal(loid) concentrations were assessed. Levels of blood Pb in pregnant Puerto Rican women were particularly low (GM=0.33 μg/dL) when comparing across other studies of pregnant women and all blood samples in our study had Pb concentration lower than the level of concern set by CDC for pregnant women (5 μg/dL) [1, 2]. Correlations for urine:blood metal pairs were weak to moderate. This is consistent with our knowledge that the circulating level and excreted level may not be correlated and often are representing different windows of exposure. Urinary concentrations of metal(loid)s were significantly different between the three visits for Co, Cs, Cu, Mo, and Zn. Reported use of shaving cream, shampoo, and other hair products among women was associated with lowered Cu, Hg, Mn,

and Zn concentrations while fish and rice consumption were associated with an increase in urinary As and blood Hg concentrations. Iron and folic supplement intake was associated with elevated urinary Cs, Mo, and Sb concentrations.

Maternal blood metal exposure and adverse pregnancy outcomes. Aim 2 assessed the individual and collective effects of maternal blood metal(loid)s on adverse birth outcomes in 812 pregnant women in the PROTECT cohort. The analysis revealed that maternal blood Pb, even at very low levels, was most strongly associated with increased risk of preterm birth and shorter gestational age. When analyzed as a mixture, odds ratios were greater in magnitude and more precise compared to the odds ratio estimates from the single pollutant models. Mixture analysis also provided evidence that Pb, Zn, and Mn are likely key exposures during pregnancy contributing to adverse birth outcomes. The stratified analysis by study visits of sample collection revealed potential windows of susceptibility for adverse birth outcomes, especially for overall and spontaneous preterm birth. In samples collected at median 22 weeks of gestation, increases in blood Pb and Zn were associated with a twofold increase in the odds of overall and spontaneous preterm birth, while an association with similar magnitude was observed between Hg and the odds of spontaneous birth at median 26 weeks of gestation. Additionally, an infant sex-specific interaction was noted for Zn and gestational age, which could be indicative of vulnerability for women carrying a female fetus. We did not detect any significant associations for birthweight or large for gestational age.

Interaction between psychosocial status and metal exposure. Aim 3 examined the extent to which overall psychosocial status, characterized by depression, perceived stress, social support, and life events, modified the association between metal exposure and adverse pregnancy outcome in 682 women from the PROTECT study. The examination of two clusters of women being in "good" and "poor" psychosocial status reported significant and strengthened associations in the presence of "poor" psychosocial status. Specifically, women with "poor" psychosocial status had stronger associations between blood Mn concentration and gestational age and preterm birth. The association between Cu and small for gestational age was also statistically significant only among women having "poor" psychosocial status.

Evaluating the performance of urine, blood, multimedia metal mixtures. For Aim 4, we focused on a subset of 847 PROTECT participants with paired urine and blood measurements of 10 metals. First, integrated exposure estimates, multi-media biomarkers (MMBs), were composed from paired urine and blood biomarkers. Then, the mixture predictive performance of urine and blood metal biomarkers, and integrated multi-media biomarkers, were evaluated using associations between environmental risk scores (ERSs) and preterm birth. The ERSs constructed for urine biomarkers correcting for urinary dilution, blood biomarkers, and multi-media biomarkers had comparable predictive performances, and they all substantially outperformed urine ERS when using uncorrected urine metal concentrations, which do not account for urinary dilution.

Integration of findings

Together, the results from the four aims of this dissertation research showed significant evidence of associations between metal exposure and adverse birth outcomes, some of which are modified by maternal psychosocial stress. Some of the essential and non-essential metals presented consistent associations that were supported by results from limited previous studies, while some associations were less understood in the previous literature. Nonetheless, several overarching themes emerged from this dissertation:

*Pb exposure is crucial in metal mixtures, even at low levels***.** Findings from all four aims strongly suggest that Pb was the most important metal for the determination of true impact of metal mixtures on pregnancy. All of the women in the PROTECT cohort had blood Pb levels well below the level of concern set by CDC, a blood level of 5 µg/dL for pregnant women [1, 2]. Moreover, the average blood Pb concentration among Puerto Rican women (3.3 µg/dL) was particularly low compared to the levels reported in other existing literature on pregnant women. And yet, mixtures analyses (Aim 2 and Aim 4) revealed that blood Pb level was the most important predictor of increased risk of preterm birth, when accounting for the effect of other metals. Excluding Pb from metal mixtures in the analysis significantly lowered the impact of mixture on preterm birth, suggesting that the mixture effect was driven mainly by the strong effect of Pb. Aim 3 shed light on another notable finding on Pb. Even though the effect of Pb on birth outcomes did not differ by overall psychosocial well-being of the mothers, Pb was the only metal that was significantly higher

among women within the "poor" psychosocial status group compared to the "good" psychosocial status group. It is possible that this is due to unmeasured common cause(s) of high Pb exposure and "poor" psychosocial status and/or the potential of Pb exposure impacting psychosocial health. Together, these findings suggest that not only Pb exposure has its own dominant contribution to the effect of metal mixtures, but it may also influence the association of other metals. Therefore, in the case of Pb, this dissertation highlights the importance of considering Pb and the framework of metal dyshomeostasis in metal mixture studies. Furthermore, this dissertation provide evidence for the need to reduce Pb exposure as much as possible for all pregnant women.

Considering both predictors and modifiers of metal exposure for mitigation strategies. Aim 1 and Aim 3 results suggest that interventions through predictors and modifiers of metal exposure should be put in place to alleviate the effects of metals on pregnant women. Aim 1 identified that diet, water sources, and smoking were predictors and sources of metal exposures, which suggests that reduction of exposure may be achieved by modifying consumer behaviors and the household environment. Identified sources of exposure can also inform techniques and tools for reducing the actual exposure, including infrastructure designs. In Aim 3, the strengthened effects of metals on adverse birth outcomes among women with poor overall psychosocial health give insights to modifiable psychosocial assets that may help mitigate risk. Knowledge on psychosocial modifiers can help us integrate useful mental health resources to provide for expecting mothers. From a chemical and psychosocial stress interaction perspective, by identifying pregnant women who are at higher risk (who are at "poor" psychosocial status) at early visits, care providers can provide mental health resources for them. This identification can also help target groups to focus more attention on reducing actual chemical exposure through primary sources of different metal exposure. These suggestions are especially relevant today as the island is still recovering from the catastrophic damages caused by Hurricane Maria, a Category 5 Hurricane, hitting Puerto Rico in late 2017. While the hurricane spared no one, leaving residents homeless and short of electricity, water, and food supply, the unique vulnerabilities of particular groups, including pregnant women, were exposed. Environmental contamination conditions exacerbated by the flooding and the destruction

of buildings, combined with the elevated levels of stress as a result of Hurricane Maria created an especially vulnerable state for pregnant women. Psychosocial scale data used in this dissertation were collected prior to Hurricane Maria and therefore do not reflect the difference between pre- and post-hurricane. Nonetheless, findings from this dissertation suggest that interventions should be initiated to alleviate the effects of chemical and psychosocial stressors on pregnant women, in addition to the continuing recovery efforts on the island.

Importance of considering windows of vulnerability and effect modification by fetal sex. Limited studies have measured and/or compared metal(loid) concentrations at different times during pregnancy and explored window specific or sex-specific association among existing cohorts assessing the impact of environmental chemicals and adverse birth outcomes among pregnant women [3-7]. The PROTECT study design enables the assessment of the differences in associations by study visit and fetal sex. In this dissertation, we provided evidence that the association between study visits and fetal sex, manifested differential associations between metals and birth outcomes. Firstly, in Aim 1, we studied the variability of metal(loid)s across pregnancy and reported that the concentrations of some metals varied across three visits, which may due to various factors including a metal's unique physiochemical properties and toxicokinetics, the changes in fetal and maternal nutrient supply [8], and the metabolic changes such as variation in glomerular filtration rate [9, 10] and plasma volume expansion [11]. In Aim 2 and Aim 3, we explored whether the associations of metals with birth outcomes differed between study visits. Aim 2 revealed positive and robust associations between the metals such as Pb and Zn that are mainly driven by associations in an earlier visit. In contrast, Aim 3 showed that the interaction of metals and psychosocial status on birth outcomes did not statistically vary by visits. The inconsistent findings from Aim 2 and Aim 3 were likely due to the small sample size in the "poor" psychosocial status cluster in Aim 3, limiting the assessment power of effects estimates. Therefore, we suggest that elevated levels at particular gestational ages may play a critical role in the association between metal(loid)s and adverse birth outcomes. In Aim 2, we also provided evidence for a heightened vulnerability to metal exposure for female fetuses compared with male fetuses; one of the main predictors of

preterm birth, Zn, showed a significant association with increased odds of preterm birth among women carrying female fetuses, while the association were null among women carrying male fetuses. In order to fully test the hypothesis of window specific vulnerability and effect modification of fetal sex, larger longitudinal cohorts with repeated measurements are needed.

Urine and blood biomarkers highlight different prediction performances. In the PROTECT study, repeated measures of metal(loid) concentrations in both blood and urine samples enabled us to characterize the reliability and predictive performance of metal exposures measured in different biological matrices. Results from Aim 1 and Aim 4 provide useful information on metal biomarker selection to future epidemiology studies of birth outcomes. More specifically, Aim 1 concluded that blood measurements of metals such as Cu, Zn, Mn, Hg, and Pb presents higher reliability and abundance compared to urine measurements. This conclusion can inform single-pollutant studies on recommending a more suitable biomarker for a single metal. Aim 4 examined the predictive performance of metal exposures measured in different biological matrices in a mixture setting because there is growing interest in the realistic scenario of studying the collective effects of environmental chemicals on humans, including metals [4, 12-19]. Our investigation in Aim 4 demonstrates, within practical metal analytical panels, that measuring metals in either urine or blood may be an equally good approach to evaluate the metals as a mixture, but only when the urine measurements are corrected for urinary dilution. These findings provide evidence that exposure assessment in urine and blood may inform us of different biological relationships under single metal and metal mixture scenarios. However, future expanded studies are needed to evaluate the performance with different metal panels, media, health outcomes of interest, and methods to integrate exposure information, to further address how to most effectively study the health impacts of exposure to metals.

Future research questions

Employing an innovative study design and novel statistical methodologies, this dissertation adds to the growing evidence on the risk of metals exposure on adverse birth outcomes. However, future studies are required to build on results reported here in several areas:

Mechanisms of action and mediation analysis. As this study suggests that metal exposures may be a contributor to adverse birth outcomes, additional research is necessary to explore the mechanisms and pathways through which metals are impacting pregnancy. Exposure to metals impacts various biological pathways associated with adverse birth outcomes: (1) one of the leading proposed mechanisms for metal toxicity is oxidative stress, defined as the homeostatic imbalance between cellular oxidants and availability of antioxidants to favor oxidation [20, 21]. Oxidative stress plays an important role in the development of many adverse birth outcomes, including preeclampsia, preterm birth, and intrauterine growth restriction [22-26]. The levels of oxidative stress biomarkers, such as 8-iso-prostaglandin F2α (8-iso-PGF2α), increase during pregnancy and peak at delivery [27], suggesting that this mechanism plays an important role in normal childbirth. Previous human studies have shown positive associations between higher levels of oxidative stress biomarkers (8-iso-PGF2α) and preterm birth [28-32]. A recent analysis in the PROTECT study also suggested that elevated levels of 8-iso-PGF2α and its metabolite are associated with higher odds of overall preterm birth, and particularly spontaneous preterm birth [33]. Several in vivo and in vitro studies have linked metal exposure with increased formation of reactive oxygen species (ROS) [34, 35]. The excessive ROS can induce oxidative stress and cause damage to cells, leading to the release of lipid peroxidation products into circulation [20]. Elevated biomarkers of oxidative stress in association with exposure to heavy metals, including lead (Pb), arsenic (As), and cadmium (Cd), have been reported [36-40]. (2) Reproductive hormones also play an important role in maintaining pregnancy; in turn, disruption of the complex interplay between hormones may lead to adverse effects during gestation. A number of metals, including Cd, Hg, As, and Pb, are reproductive toxicants and suspected endocrine disruptors [41-44]. Evidence suggests that metals can influence reproductive hormone levels through several pathways, including hormone synthesis, regulation, transport and metabolism, and/or interference with receptors [45-52]. These results suggest that the mechanism of action for metals affecting pregnancy may also be through disrupting reproductive hormones. (3) Epigenetic changes could also play a role in this pathway as well [53, 54].

Longitudinal studies such as PROTECT with data on molecular markers of these potential mediators provide an opportunity to explore the biological mechanisms and pathways through which prenatal exposure metals are influencing the length of gestation and fetal growth. The role of these potential underlying mechanisms should be investigated more closely as mediation analysis will provide an additional step towards establishing a causal pathway.

Comprehensive evaluation of metal and psychological stressor interactions. Our research findings also provide motivation for future work investigating other adverse health effects resulting from the interaction between metal and psychosocial stressors. While this dissertation demonstrates that the effects of metals on adverse birth outcomes vary by maternal psychosocial status, the interaction between metal and psychosocial status may also contribute to other known outcomes associated with metals, including reproduction, neurodevelopmental outcomes, and sexual development. Future research is required to elucidate more comprehensive interactive relationships between metals and psychosocial factors on reproductive and child health.

Expanding mixture analysis. Considering the prevalence and daily exposure of environmental contaminants in the island, an important next step is to assess the collective effect of different chemical mixtures in relation to adverse birth outcomes. The mixture approaches and models established in the present work using metal biomarkers may serve as a useful tool for a larger mixture analysis including additional chemical families, such as phenols, phthalates, metals, and particulate matter altogether.

Public health impact and innovation

The rate of preterm births in the U.S. has been increasing over the last few decades and is associated with many chronic health conditions and developmental disabilities. Over the decades, Puerto Rico has been experiencing an unusually high rate of preterm birth, as well as environmental and financial burdens. PROTECT, a large prospective longitudinal cohort study in Puerto Rico, is exploring how environmental exposures during pregnancy are potentially associated with preterm birth. With repeated longitudinal data on chemical panels, PROTECT provided a unique opportunity to study the causal relationships between environmental pollutants

and preterm birth in this at-risk population, and to test more realistic exposure profiles' impact on preterm birth.

This work produced important insights into the levels of prenatal exposure of Puerto Rican pregnant women to metals, as well as helping to fill knowledge gaps about the individual and collective effects of prenatal metals exposure on adverse birth outcomes and the interaction between chemical and psychosocial stressors. Evaluation of chemical mixtures measured in different media will inform more efficient study designs for exposure assessment. Improved understanding of environmental and other factors that contribute to preterm birth, together with developing sustainable technologies to remove contamination, will have a direct impact in Puerto Rico. The metal exposures targeted for this analysis are ubiquitous in the U.S. and elsewhere around the world, making the findings of this study broadly applicable to pregnant women and newborns worldwide.

Overall Conclusion

The present dissertation utilized innovative approaches to provide important evidence on the impact of a class of chemicals, metals, on adverse birth outcomes. The dissertation includes the first study to characterize the profile, trends, and predictors of metal exposure among pregnant women in Puerto Rico. Incorporating the full richness and structure of the repeated data across pregnancy, important predictors and sources of a large panel of metals were identified which can provide potential guidelines for food choice and behavioral changes during pregnancy and also inform engineering designs that may help reduce metal exposure. In this dissertation, more realistic exposure profiles were analyzed both individually and collectively as a mixture which provided evidence that selected metals including Pb, Zn, Mn are associated with adverse birth outcomes. A novel example of dimension reduction on the effect of modifier space with clustering revealed that the presence of "poor" psychosocial status is strengthening the negative effect of some metals, especially manganese, on gestational length. This finding complements current research regarding how environmental and psychosocial stressors interact in their contribution to birth outcomes. Together with knowledge on the predictor and sources of metal exposures, the findings also help identify the appropriate mitigation strategies and interventions necessary to reduce the burden of metal-related adverse pregnancy outcomes. Data-driven, unsupervised and supervised machine

learning approaches were also utilized to integrate exposure to metals measured in different media and evaluate the performance of different metal mixture biomarkers; the findings demonstrate, within practical metal analytical panels, that measuring metals in either urine or blood may be an equally good approach to evaluate the metals as a mixture, but only when the urine measurements are corrected for urinary dilution. Future expanded studies are needed to evaluate the performance with different metal panels, media, health outcomes of interest, and methods to integrate exposure information, to further address how to most effectively study the health impacts of exposure to metals. A significant overall finding from all the aims in this dissertation is that even though Pb level among this population is particularly low Pb exposure measured in blood was the most important predictor of preterm birth among all the metals we examined. Our study supports the recent suggestion from the scientific community on lowering guideline levels for Pb to protect pregnant women and their children. Altogether, this dissertation advances our understanding of the impact of metal exposure on adverse pregnancy and fetal growth outcomes.

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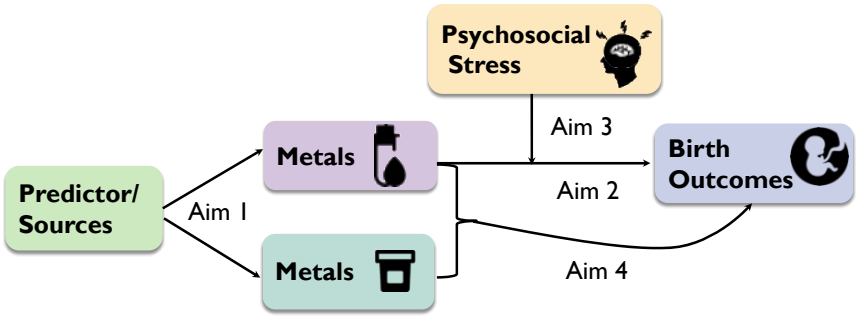
Figure VI.1 Overview of Research Aims and Main Findings

Aim 1	Aim 2	Aim 3	Aim 4
Exposure predictors: ➢ Fish, rice-As ➢ Fish-Hg ➢ Public water-Pb ➢ Smoking-Cd ➢ Supplement-Cs, Mo, and Sb	➢ Pb at low levels / below current guidelines may be associated with preterm birth. ➢ Some essential metals such as Mn and Zn may be harmful at higher levels.	➢ Women were clustered to two groups of having "good" vs "poor" psychosocial status ➢ Adverse associations between Mn and preterm birth and Cu and SGA were stronger in the presence of poor psychosocial status	➢ Measuring metals in either urine or blood may be an equally good approach to evaluate the metals as a mixture, but only when the urine measurements are corrected for urinary dilution

Overview of Research Aims and Main Findings



Impact and Significance

- Fills a critical gap in literature by reporting evidence of metal mixture influencing birth outcomes.
- Findings suggest that reduction of exposure may be achieved by modifying consumer behaviors, household environment.
- Modifiable psychosocial assets that may help mitigate risk
- Data-driven machine learning approaches to inform the selection of biomarker sets in the context of a mixture